UNIVERSITÀ DI PARMA

UNIVERSITA' DEGLI STUDI DI PARMA

DOTTORATO DI RICERCA IN

"NEUROSCIENZE"

CICLO XXXI

Genetic and environmental influence on neurocognitive and behavior development: the importance of multiple methodologies and time dependent in children and adolescents with chronic and severe pathologies

Coordinatore:

Chiar.mo Prof. Vittorio Gallese

Tutore:

Chiar.ma Prof.ssa Johanna MC Blom

Dottoranda: Chiara Colliva

Anni 2015/2018

INDEX

CHAPTER 1: study framework, aims and outline

1.1 Study framework

Paediatric chronic diseases are often defined by the emergence of significant neurocognitive and emotional challenges that influence the present as well as the future burden of disease. Innovative discoveries from other fields, such as, imaging techniques and mathematical modelling together with graph analysis have led to new conceptual thinking resulting in increasingly explanatory and predictive models which offer a more realistic image of the strengths and needs of these young patients. Especially, approaches such as the research domain cluster approach and network analysis may help to improve our comprehension concerning the temporal character of the development of neurocognitive problems which is fundamental when choosing the optimal scheme for surveillance and monitor within-subject dynamic change and vulnerability over time. Does the time frame differ for different diseases, different cognitive domains or their association? Could it be that deficits in specific domains, such as, mental flexibility and the planning of an action or thought first of all depend on a deficit in a more central (hub) or earlier developing (initially more diffuse) domain of functioning? If so, therapy directed at problems related to the most central domain(s) would constitute the more rational approach because this individualized therapy automatically carries over to other domains that depend on this more central function. Epilepsy, asthma, and certain types of pediatric tumours are among the most common chronic or severe paediatric disorders. Evidence suggests that these pathologies might have a direct psychobiological link to behavioural and emotional functioning. While such comorbidities are traditionally thought to arise predominantly from the effects of recurrent clinical difficulties, iatrogenic effects of medications, and adverse psychosocial reactions to chronic or severe illness, a growing body of evidence points to the importance of genetic predisposition and environmental factors.

The potential genetic, as well as the molecular pathways that might be involved include the sensitivity to context of the child or adolescent, that is, the correct functioning of the hypothalamic pituitary adrenal axis (HPA-axis). This, in combination with other genetic susceptibility factors that involve the dysregulation of a number of functional networks, such as, dopaminergic, serotoninergic, and folate related pathways, confers resilience or vulnerability to children with chronic or severe diseases with respect to the development of disease or treatment related neurocognitive or emotional and behavioural problems.

Furthermore, there is a growing trend to comprehend the brain as a complex interplay of structural and functional networks. Perceiving the brain as a network provides a theoretical framework to better understand, for example, the widespread cognitive problems and structural brain abnormalities found in these diseases.

1.2 Identifying those at risk: a multi-domain approach using genetic polymorphisms and network analysis

WHO: It is becoming increasingly clear that there is a key role for predisposing genetic and environmental influences in differentially mediating neurocognitive and emotional risk in the child and adolescent with chronic or severe diseases. We Know that many factors influence the pattern and the magnitude of the response of the child or adolescent, including gender, the age of onset, the duration of treatment, the type of treatment, the developmental stage of the child, and the psychosocial environment of the child. The question why some children suffering from chronic or severe disease are more likely than others to develop neurocognitive and behavioural problems in the face of similar levels of trauma or environmental exposure is one of the most critical problems that need an urgent reply. In our study we consider children with chronic diseases such as asthma and epilepsy, and children in follow-up for acute lymphoblastic leukemia and various types of solid tumors. Overall, these are different and among the most important diseases affecting the paediatric age. While they present commonalities and differences, together they provide a group of patients in which to study common underlying mechanisms of susceptibility to adversity and to try and gain a better understanding of predisposing factors using innovative multidisciplinary strategies in the field of paediatrics neuroscience.

WHY: Children with chronic or severe diseases such as asthma and epilepsy, and paediatric cancer survivors may carry vulnerabilities and symptoms related to the interaction between the individual, the disease and their context; often these vulnerabilities are not correctly identified and persist in time, causing them to be more at risk to and stray on their correct development trajectory**.** Thus, children diagnosed with asthma and epilepsy, and paediatric cancer survivors are more susceptible to the effects of stress caused both by the disease itself and the familial environment. The Research Domain Criteria (RDoC) approach embraced by the NIMH provides the right approach and allows for the integration of many levels of information (from genomics and circuits to behaviour and self-reports) to explore basic dimensions of functioning that span the full range of human behaviour from normal to abnormal. The inclusion and assessment of neural biomarkers, as a comprehensive set of genes, involved in both neuronal development and repair as well as in the formation of functional networks, may provide an additional tool to predict the susceptibility of these young patients to the negative progression of their disease.

In this context, we studied polymorphisms related to neurotransmitters, such as serotonin and dopamine, neural development and plasticity (Brain-Derived Neurotrophic Factor, BDNF), enzymes (Methylenetetrahydrofolate Reductase, MTHFR) and hormones (Glucocorticoids, FKBP) known to be involved in regulating emotion and cognition, as well as the physical functions of children and adolescents. All polymorphisms considered together create an individual and unique profile of the capability of these

young patients to respond adequately to the demands posed by the burden of each specific disease, its treatment and the distress it causes and consequently may help to predict compromised coping when confronted with chronic or repetitive stressful life events.

The serotonin transporter protein (5-HTT), which terminates the action of serotonin by facilitating its reuptake from the synapse, appears to be part of the pathway leading to psychiatric disorders. Carrying a short allele of the serotonin transporter polymorphism (5-HTTLPR) while experiencing repeated exposure to stressful environments is linked to elevated risk for anxiety and depression: short-allele carriers compared with non-short-allele carriers display enhanced negative emotional reactivity to stressors. The link between development and neuronal dysfunction is provided mainly by neurotrophins, in particular the Brain Derived Neurotrophic Factor (BDNF). Because of its role in neuro-differentiation, BDNF has been the focus of research examining early adverse care, brain development and behavioral outcomes, and has been identified as one of the key neural signals orchestrating synaptic plasticity. Individuals carrying the Met polymorphism display decreased activity-dependent BDNF secretion from neurons, leading to impairment in learning, working memory, and sustained attention and increased depressive symptoms when compared to Val/Val carriers (BDNF Val66Met). Furthermore, the pleiotropic role of Catechol-O-methyltransferase (COMT) on dopamine catabolism in the prefrontal cortex (PFC) provides the basis for the association between cognitive and emotional processes. The Val158Met variant is linked to pre-frontal mediated cognition (e.g., executive function and working memory and attentional control) as well as to emotional regulation. In particular, Met alleles are associated with more efficient patterns of prefrontal cortical activation and superior cognitive performance. Among COMT val158met genotypes, Met/Met is linked to the lowest level of COMT enzyme activity and yields the highest level of extracellular dopamine in the PFC. The Met allele is also considered to index risk for psychiatric disorders due to a hypothesized inflexibility in switching set, which ultimately results in low resiliency. We included Methylenetetrahydrofolate Reductase (MTHFR C677T) polymorphisms known to influence DNA methylation. Epigenetic mechanisms such as DNA methylation are thought to work at the interface between genes and the environment as they are sensitive to changes in environmental stimuli, since, differential DNA methylation patterns have been associated with the effects of a range of (negative) life experiences. Moreover, HPA axis sensitization and alterations in glucocorticoid receptor regulation and functioning are thought to play an important role in the reactivity to stress, and impairments in this system have been widely implicated in psychiatric disorders such as anxiety. FKBP5 (rs1360780 and rs3800373) is a key factor in the physiological stress response, shaping neuroendocrine reactivity as well as coping behaviour. This then lends strong support to the concept emerging from studies of FKBP5 as important factor governing gene–environment interactions relevant for the ethology of stress related emotional and cognitive disorders. Furthermore, allele-specific changes in FKBP5 methylation are associated with the response to treatment and treatment related side effects suggesting a critical role for HPA axis related genes.

WHAT: We use a longitudinal study including a network approach and neural biomarkers that differentiate the sensitivity of an individual to its context. The network approach permits us to study clearly the different neurodevelopmental domains that can give a complex and exhaustive view of clinical, familiar and molecular outline, from which the group of patients can be considered up to the single individual.

WHEN: If symptoms of cognitive and emotional problems in children diagnosed with and treated for asthma, epilepsy or pediatric cancer are the late stage results of disease and therapy, are we missing the right time to intervene or prevent long-term negative outcome? In this light, specific genetic polymorphisms linked to, 1 neurocognitive or emotional endo-phenotypes, 2 brain and HPA-axis functioning and 3 the development of function, may represent useful biomarkers and offer an additional tool to help predict early who is more susceptible to cognitive and emotional late effects and thus give us more time to plan appropriate therapeutic interventions and organize **regular risk based follow-up** with specific attention to the management of those most fragile.

Thus, using signs together with symptoms, that is, objective indicators along with behavioural symptoms and screening indices will provide more quantitative measures of deficits avoiding the exclusive use of clinical rating scales.

Furthermore, fractioning problems in various subtypes based on specific patterns of cognition, emotion and behavior variables together with a more objective risk assessment may help improve the understanding of disease and treatment related (neurodevelopmental) toxicities.

1.2 aims and objects

The central question that drives this research, is why some children are more susceptible than others to develop alterations in neurocognitive outcomes in the face of same disease, same treatment, same gender or age? Based on the theorical approach suggested by the Research Domain Criteria model (RDoC), various domains, from genes to behavior, should be considered to explain this variability and to generate an individual treatment plan, especially considering a long-term perspective (Casey et al.,2014).

Neurocognitive functions are a collection of processes fundamental for the individual to adapt to daily life and reaching his own goals. Problems in these processes aggravate the burden of disease and compromise the Quality of Life children with chronic or severe diseases and their families. The most commonly used analysis models are not exhaustive to resolve the needs of latent underlying vulnerabilities that may aggravate the patient's psychopathological picture. Given that difficulties in neurocognitive functions reflect the interaction of various domains, from the body, the mind and the environment, it is necessary to go beyond the simple classification of symptoms and consider various domains of neurodevelopment, studying their interaction and how they evolve over time and focus on the individual to organize the care of children with chronic or severe illnesses.

We used three theoretical models to structure the research: one is RDOC; second is the Single Case experimental Design (SCED) and 3 the Network analysis approach. Together they allowed analyzing the role of various domains such as genes, environmental and constitutive factors on the neurocognitive development in children with Epilepsy, Asthma and pediatric cancer survivors starting from each child individual profile. Finally, we belief that such a combined approach will help to organize intervention programs that focused on the care and needs of the child and their family.

The objective of this thesis is three-fold. First, by means of routinely available clinical information, we investigated if children with asthma, epilepsy or pediatric cancer survivors, were more at risk for executive function and emotional or behavioral deficits compared to normative data. The second aim of this thesis concerned the development of prediction models related to disease, environment, neural and psychosocial factors in these patient groups. To that aim, we used traditional multivariate analysis. Third, we studied whether a network analytical approach could increase our understanding of the pathological mechanisms underlying pediatric asthma, epilepsy, pediatric cancer and possibly contribute to the diagnostic process and focus of treatment. More specifically, we characterized functional network alterations to investigate how global and specific network changes coincide with clinical signs and symptoms.

In brief, the specific **aims** of this longitudinal, prospective and observational study are:

1. Analyze the neurocognitive and behavioral profile of children with epilepsy, asthma and pediatric cancer survivors and how it changes over time
2. Analyze the different patterns in psychosocial context in relation to each pathology
3. Analyzed polymorphism profiles to determinate if and which polymorphism is more relevant for each disease
4. Verify which factors may be predictors of compromised domains of cognitive and behavioral function and draw up a risk profile
5. Introduce a network approach to study the interaction of the various domains that may be used to direct the development of effective intervention models.

outline

Chapter 2

This chapter displays the introduction of the thesis, it is divided in:

- Part I, Theoretical framework. In this part the theoretical models that create the framework supporting the study are described
- Part II, Pathologies. Here is reported the description of pathologies investigated, with a specific reference to neurocognitive characteristics
- Part III, Genes. Here are presented which genes and single nucleotide polymorphism we have examined and why.
- Part IV: environment and neurodevelopment. The study of SNPs must be organize considering the interaction Gene X Environment and the neurodevelopment trajectory of the child where periods alternate whit different characteristics of plasticity.

Chapter 3

Materials and methods. Including the description of participants' group, procedures and analysis used

Chapter 4

Results. In this part all relevant results emerged are reported

Chapter 5

Discussion and summary. Finally, the main findings from this thesis are summarized and discussed. Finally, a vision is given on how future (networks) studies can be used to be of additional value in clinical practice.

CHAPTER 2: Introduction

2.1 The Theoretical Framework

The Research Domain Criteria

In recent years he National Institute of Mental Health (NIMH) Research Domain Criteria (RDoC) project has develop a research classification system for psychopathology based upon various dimensions of neurobiology and observable behavior (Insel et al., 2010). The research reported here adopt the RDoC model and highlights the compatibility of the dimensional approach with extant research on brain and behavioral development and demonstrates how the application of a neurodevelopmental perspective can extend and enrich RDoC.

The National Institute of Mental Health instituted the RDoC project in early 2009. This framework for research explicates fundamental biobehavioral dimensions that cut across current heterogeneous categories of mental disorders. Through a series of workshops involving scientific leaders in a number of fields, a **matrix** consisting of five primary domains was developed: negative valence, positive valence, cognitive systems, systems for social processes, and arousal/modulatory systems. Within each of these domains, a core set of individual constructs was identified, and the ongoing goal is to optimize and elaborate these constructs across levels (units) of analysis including behavior, neurobiology, and genes (Casey et al.,2014).

There are three primary guiding principles of RDoC with respect to research. First, RDoC calls for a **dimensional rather** than a diagnostic or categorical system to more easily link brain circuits to specific behaviors and symptoms. Second, rather than beginning with a diagnostic group and attempting to discover its **neurobiological basis**, RDoC begins with our current understanding of behavior-brain relationships and relates it to clinical phenomenology. Third, different levels of analysis (molecular, circuit, behavior, symptom) in RDoC instantiate dimensional constructs that are presumed to underlie core symptoms of mental disorders. These constructs (e.g., fear, attention, memory, arousal) will rarely bear one-to-one relationships with traditional categories of disorders. In this context, a developmental trajectory is the progression of brain and behavioral changes across age and time that may be linear or nonlinear (Casey et al., 2006). Identification of developmental trajectories lays the groundwork for understanding how development occurs, which systems develop before or after others, and how these systems differentially interact across development (Karmiloff-Smith et al.,2014). To this purpose, we have to focus on the phenotype and start from here. The better we are cable to capture is individual strengths and needs. In this context it is important to analyze without losing the individual differences that define the relationships between the domains giving relevance to the individual, therefore we have adopted as model

of study the single case experimental design in order to identify difficulties, strengths and needs of each patient.

Single-case experimental designs: analyzing without losing differences

The Single-case experimental designs (SCED) is a useful method in clinical research practice to investigate individual client progress (Maric et al., 2015). This method can help the clinical researcher to investigate whether an intervention works as compared with a baseline period or another intervention type, and to determine whether symptom improvement is clinically significant (Krasny-Pacini and Evans, 2018). Unlike randomized clinical trials (RCTs) in which a group is compared to another, in a SCED study the comparison takes place or in the subject itself or among subjects. In SCED studies, typically there is a comparison between two periods of time, known as phases; it starts from a reference phase that serves by comparison for the following phases (Smith et al., 2012). Furthermore, the RCTs include a large number of participants and use the assignment of random groups to create similar groups of study in terms of potential confounding variables; it is difficult, however, to identify all of them confounding variables unlike the SCED studies, in which each participant acts as its own control which allows for a more adequate control many confounding variables (Lobo et al., 2017). In this way patient is placed in the center considering all the characteristics and differences that often get lost in other types of research; therefore, it is functional for the purpose of constructing structured interventions and targeted over time for each patient. But how study and define the underling mechanisms or factors that are predictors of what domain re most susceptible to change? Therefore, we introduce a network approach analysis to define connection between the studied domains.

Network analysis: a new window of opportunities for research

Networks, where nodes denote entities and links denote associations (Borsboon and Cramer, 2013, Pessoa, 2018), provide a unified representation for a variety of complex systems, from social relationships to molecular interactions. In an era of big data, network analysis has been proved useful in biological applications such as predicting functions of domains, guiding the design of experiments, and discovering biomarkers of diseases. We have applied network analysis to define underlying causes and interaction between neurocognitive domain functions of pediatric diseases. To examine the interrelationships among diverse cognitive abilities is to conceptualize neurocognition as a network in which cognitive skills are arranged in a balance between integration and segregation (Garcia-Ramos, et al., 2015). This will almost certainly lead to more effective strategies for prevention and treatment of diseases because understanding the connections underling the domains we can explicit domain who are more compromised or more susceptible to change and direct therapy efficiently (Fried et al., 2017). Neurocognition is not simply the summation of abilities but is a dynamic network in which one cognitive, emotional, behavior domain

augments skills in a different domain. In doing so, we hypothesize that patterns of neurodevelopment in pediatric patients with chronic or severe disease, will reveal different networks that facilitate the study of these pathologies. Graph theory may provide a new way to look and analyze the architectural organization of cognitive function, as defined by the network formed by the interrelationships between multiple cognitive abilities and domains and allows permits the evaluation not only of grouping of cognitive modules but also the participation of cognitive functions/domains within the entire cognitive architecture. For this reason, cognitive networks may provide novel insights into longitudinal changes in cognitive structure, especially in regard to the abnormal conformation that may be driven by pathological such as epilepsy. Even though cognitive networks are not individualized measures (i.e., they arise from group-wise correlations), they can be used to infer how individual test metrics may be related to the overall cognitive network under investigation.

2.2 The pathologies

Pediatric Epilepsy

Epilepsy, as report by the International League Against Epilepsy (ILAE) of 2017, is a chronic disorder of the brain that affects people worldwide. It is characterized by recurrent seizures, which are brief episodes of involuntary movement that may involve a part of the body (partial) or the entire body (generalized) and are sometimes accompanied by loss of consciousness and control of bowel or bladder function. Epilepsy is one of the world's oldest recognized conditions, with written records dating back to 4000 BC. Approximately 50 million people worldwide have epilepsy, making it one of the most common neurological diseases globally and by data transmitted by the World Health Organization (WHO) nearly 80% of the people with epilepsy live in low- and middle-income countries. The most people respond positively to treatment but remain about 30% that are resistant. Also, from the WHO data of 2018 it is reported as often still patients and their families are subject to stigma and discrimination.

As Know, seizure episodes are a result of excessive electrical discharges in a group of brain cells. Different parts of the brain can be the site of such discharges. Seizures can vary from the briefest lapses of attention or muscle jerks to severe and prolonged convulsions. Seizures can also vary in frequency, from less than 1 per year to several per day. One seizure does not signify epilepsy and up to 10% of people worldwide have one seizure during their lifetime (WHO 2018), while epilepsy is defined as having 2 or more unprovoked seizures. Temporary symptoms occur, such as loss of awareness or consciousness, and disturbances of movement, sensation (including vision, hearing and taste), mood, or other cognitive functions.

Epilepsy is the most frequent chronic neurologic condition in children (Pearl, 2018). Once a diagnosis of epilepsy is established, syndrome classification is considered such that the best clinical management plan can be established. At the first level, the seizure type is described as generalized, localization related, or undetermined. The second level classifies seizures according to whether they are idiopathic, symptomatic, or cryptogenic. At the final tier of classification, a specific syndrome is assigned. Specific epilepsy syndromes represent a complex of signs and symptoms that define a unique epilepsy condition. In this study the focus of our attention is mainly on two types of cryptogenic epilepsy: Benign Rolandic Epilepsy; Temporal Lobe epilepsy and Epileptic Dysphasia.

In pediatric epilepsy the age of onset is one of the most important risk factors to consider both in terms of the pathology and its neuro-cognitive complications. The growing brain is particularly vulnerable due to the great plasticity that characterizes the first years of life and certain successive periods. In this sense epilepsy is a disturbing factor that, even in the absence of structural alteration, is able to slow down or even interrupt the normal growth trajectory of the areas in which the discharge is localized and spread. In confirmation of this, Hermann et al. (2002) have shown that patients who develop epilepsy at an early age have more cognitive problems than those who develop epilepsy later (Hermann et al.,2002; Hermann et al.,

2016). With respect to consider neuro-cognitive complications, sexual differences are evident when considering the types of psychiatric and behavioral co-morbidities that accompany epilepsy. As for the adult, even in the pediatric field, in the female it is easier to find anxiety disorders and depression while in males more frequent attention disorders and aggressive behavior are observed.

Benign Rolandic Epilepsy (RE)

Rolandic epilepsy occurs in about 15% of all casas of pediatric epilepsy (Bell et al., 2011) and is the most common of partial epilepsy in childhood. Usually, BRE occurs in children between 5 and 11 years of age, with seizures during sleep. The episodes are characterized by clonic movements starting from the mouth and tend to spread to the entire hemilateral face; they are often accompanied by guttural noises, salivation and difficulty speaking. The crisis can be single or repeat itself after a short time, while remaining always short-lived. With regard to frequency, events are generally rare: most patients (60-70%) have between 2 and a maximum of 10 life-time crises (Kolb et al.,2014). The EEG aspect in the inter-critical phase is characterized by the presence of single-pointed or single-limbed slow-wave tips in small groups on the central and central-temporal region that tend to increase during sleep. It is a form of benign epilepsy that tends to disappear with age, usually within puberty. However, in these children often learning and cognitive difficulties remains (Kolb et al.,2014). Although epilepsy affects both genders, some differences have long been known to highlight. Epidemiologically, epilepsy has a higher incidence in males than females. Gender differences are also confirmed in other aspects such as the susceptibility of the brain to develop crisis. Thus, if on the one hand the male sex has greater susceptibility, the female sex, on the other hand, more frequently experiences fluctuations in the time of this predisposition, particularly in relation to the phases of the menstrual cycle (Janszky, 2004; Perrucca et al., 2014)

Temporal Lobe Epilepsy (TLE)

Rappresent the most common type of focal epilepsy, and in most cases emerge before ten years of age. Many heterogeneous idiopathic forms exist but from the clinical point of view, we can distinguish two main forms: a mesial and a laterial form. The first is characterized by gustatory and olfactory visceral aura followed by motor stop, absence or afasia. The second one is characterized by a prevalent visual aura, hallucinations and dystonia (Hermann et al., 2002; Bell et al., 2011; Blair 2012). As regards the associated comorbidities, due to the site, cognitive problems mainly concern anterograde memory. However, recent studies have shown that other functions, such as language and executive functions, not strictly attributable to temporal lobes, are compromised as well. Finally, involvement of the temporal lobe is often associated with psychiatric problems such as major depression, anxiety disorders and psychosis.

The structural alterations of temporal epilepsy and their possible correlation with cognitive disorders immediately represented an interesting field of research. Most of the studies focused on the alterations in the hippocampus, the site of origin of mesial temporal epilepsy. Recently, thanks also to the advent of fMRI, it was possible observed that the anatomical alterations were not confined only to this area but extended to other regions (Blair, 2012). In particular, many studies showed that in patients with early onset of temporal epilepsy, alterations affect other regions; the volumetric reduction affects zones both ipsilateral to the epileptogenic focus but also contralateral; not only temporal but also extra-temporal. While the primary focus would remain confined to the hippocampus, structural alterations have been established in other areas, influencing other regions and their respective connections; according to some authors, it is the reduction of white matter in these regions that explains many of the cognitive problems that distinguish this form of epilepsy and that cannot be linked to the temporal region (Hermann et al., 2002; Bell et al.,, 2011).

Epileptic Dysphasia

Epileptic dysphasia should be considered along a spectrum and is a group of conditions with overlapping signs and symptoms (Sheffer, 2000; Tasai et a., 2013). The prevalence of the epilepsy dysphasia spectrum is unknown, most of the conditions in the spectrum are rare. A key feature of these conditions is impairment of language skills (speaking, reading, and writing) although in a minor way. Another feature of this disorder is the presence of certain patterns of abnormal electrical activity in the brain, which are detected by electroencephalogram (EEG). Many people with conditions in this spectrum go on to develop epilepsy. The conditions in the epilepsy disphasia spectrum, which all begin in childhood may include Landau-Kleffner syndrome (LKS), epileptic encephalopathy with continuous spike-and-wave during sleep syndrome (ECSWS), autosomal dominant rolandic epilepsy with speech dyspraxia (ADRESD), intermediate epilepsy-aphasia disorder (IEAD), atypical childhood epilepsy with centrotemporal spikes (ACECTS), and childhood epilepsy with centrotemporal spikes (CECTS) (Lesca et al.,2013).

Treatment

Drug therapy should only be initiated after confirming the diagnosis of epilepsy and should be, at least in the early stages, based on the use of a single drug. However, when it is not possible to achieve crisis control after some attempts, is advisable to start a poly-therapy with the aim of enhances crisis control. However, not all patients with epilepsy are candidates for pharmacological treatment: it is estimated that 60-70% of epileptic children may become "seizure-free" with a low-moderate dose of drug administered as monotherapy or even without treatment (Kwan and Brodie, 2001; Raspall-Chaure et al.,2008). Common drugs for partial or tonic-clonic seizures include carbamazepine or oxacarbamazepine, phenytoin, topiramate, valproate (valproic acid) and ethosuximide. For many types of childhood epilepsy there are no

randomized trials that demonstrate the efficacy of one drug compared to others: only Oxcarbazepine is supported by level A of evidence of efficacy for the treatment of focal seizures (Raspall-Chaure et al.,2008). The patients considered in this study are mainly treated with Carbamazepine and Oxcarbazepine for focal forms, and Valproate, if the form is secondarily generalized. In reference to these drugs, the literature does not show serious associated cognitive effects, even if Valproate can in some cases alter attention, visuo-motor functions and psychomotor speed while Carbamazepine has a very safe profile, so much so that it is often taken as a yardstick in comparison with other drugs (Donati et al., 2006). The most frequent side effects are somnolence, vertigo, ataxia, diplopia, but above all cognitive problems such as memory problems, attention / vigilance, psycho-motor speed and behavioral problems (Park and Kwon 2008). In general, these alterations are more evident using old-generation drugs among which Phenobarbital is the most toxic. Its effects on language can persist even after the end of treatment. In contrast, among the newer drugs (felbamate, gabapentin, lamotrigine, tiagabine, topiramate, vigabatrin), only Topiramate is considered more related to the development of side effects in the cognitive sphere (Park and Kwon 2008).

Neurocognitive profile

When a child is diagnosed with epilepsy, treating the disease should not be the only goal. While we have been able to treat children with epilepsy frequently resulting in satisfactory crisis control, we have not been so good at reducing both short and long-term morbidity or disability developed by some of these patients (Fastenau et al., 2009; Reilly et al., 2014; Alfstad et al., 2016; Yuen et al., 2018). The nature of the disability is often cognitive or behavioral (Kavanaugh et al., 2015), which significantly affects the course of development as well as their quality of life and constitutes a major burden of disease (Fayed et al., 2015; Schraegle and Titus, 2016).

Children and adolescents diagnosed with epilepsy, even the more benign types, often display compromised executive functioning (EF) which are primarily mediated by the prefrontal cortex (Kernan et al., 2012; Longo et al., 2013). Moreover, the effects of the disease and its treatment on physical and mental health depend on the brain areas that are developing, reorganizing or declining (Kellermann et al., 2015). A significant proportion of children and adolescents develop clinically meaningful deficits in attention, information processing speed, executive functions such as working memory (WM) (Hermann et al., 2016; MacAllister et al., 2016; Nickels et al., 2016; Schraegle and Titus, 2016). Especially attention and WM play a fundamental transversal function in various domains of executive function and are particularly associated with the availability and activity of dopamine (Cools et al., 2011; Eriksson et al., 2015).

Pediatric Asthma

Asthma is a chronic disease characterized by recurrent attacks of breathlessness and wheezing (Gelfand, 2009; Ascher and Pearce, 2014; Wingrove, 2016), which vary in severity and frequency. Symptoms may occur several times in a day or week in affected individuals, and for some people become worse during physical activity or at night. During an asthma attack, the lining of the bronchial tubes swells, causing the airways to narrow and reducing the flow of air into and out of the lungs. Recurrent asthma symptoms frequently cause sleeplessness, daytime fatigue, reduced activity levels and school and work absenteeism. Asthma has a relatively low fatality rate compared to other chronic diseases (Ascher and Pearce, 2014).

The fundamental causes of asthma are not completely understood. The strongest risk factors for developing asthma are a combination of genetic predisposition with environmental exposure to inhaled substances and particles that may provoke allergic reactions or irritate the airways, such as: indoor allergens (for example house dust mites in bedding, carpets and stuffed furniture, pollution and pet dander); outdoor allergens (such as pollens); tobacco smoke; chemical irritants and air pollution.

Other triggers can include cold air, extreme emotional arousal such as anger or fear, and physical exercise. The increased prevalence observed in metropolitan areas with respect to rural ones and, overall, in industrialized countries, highlighted the role of air pollution in asthma inception (Ferrante and La Grutta, 2018). According to World Health Organization (WHO), 235 million people worldwide suffer from asthma and it is the most common chronic disease among children (WHO 2018): asthma is among the top 20 chronic conditions for global ranking of disability-adjusted life years in children; in the mid-childhood ages 5–14 years it is among the top 10 causes (Ascher and Pearce, 2014). There are striking global variations in the prevalence of asthma symptoms in children, with up to a 13-fold differences among countries. Although asthma symptoms are more common in many high-income countries (HICs), some low- and middle-income countries (LMICs) also display high levels of asthma symptom prevalence (Ascher and Pearce, 2014; Soriano et a., 2015). At the same time, many LMICs with large populations showed enhanced prevalence, suggesting that the overall world burden is increasing, and that therefore global disparities in asthma prevalence are decreasing (Ascher and Pearce, 2014). The costs of asthma, (where they have been estimated) are relatively high. The global burden of asthma in children, including costs, needs ongoing monitoring using standardised methods (Ascher and Pearce, 2014). Asthma is one of the main causes of hospitalization which are particularly common in children aged < 5 years with increased prevalence that has been increased during the last two decades, mostly in LMICs (Ferrante and La Grutta, 2018). Indirect costs are usually higher in children than in older patients, including both school and work-related costs. Intangible costs are unquantifiable, since they are related to impairment of quality of life, limitation of physical activities and study performance (Ferrante and La Grutta, 2018).

The implementation of strategies aimed to early detect asthma thus providing access to the proper treatment has been shown to effectively reduce the burden of the disease.

This study focused on two asthma phenotypes:

- Allergic asthma
- Non-allergic asthma

The main factors, as reported in the Global Initiative for Asthma (GINA) 2018, to consider making a diagnosis of asthma are:

- history of variable respiratory symptoms
- other investigations as Skin prick test and dosage of IgE
- Measuring lung function by spirometry composed of the following:

1. Forced expiratory vital capacity (FVC): is the volume of air that can forcibly be blown out after full inspiration, measured in litre
2. Forced expiratory volume in 1 s (FEV1): is the volume of air that can forcibly be blown out in the first 1 second, after full inspiration (GINA 2018). Values between 80% and 120% of the average value are considered normal. Values above 70% are considered lightly compromised; between 60-69% are defined moderate; between 50-59% are mildly compromised; between 35-49 severe and below 35% are considered as highly severe.
3. FEV1/FVC (FEV1%) is the ratio of FEV1 to FVC. In healthy children this should be approximately >90% (0.90). In obstructive diseases (asthma, COPD, chronic bronchitis, emphysema) FEV1 is diminished because of increased airway resistance to expiratory flow; the FVC may be decreased as well, due to the premature closure of airway in expiration, just not in the same proportion as FEV1 (for instance, both FEV1 and FVC are reduced, but the former is more affected because of the increased airway resistance).,
4. A derived value of FEV1% is FEV1% predicted, which is defined as FEV1% of the patient divided by the average FEV1% in the population for any person of the same age, height, gender, and race.
5. MEF stands for maximal (mid-)expiratory flow and is the peak of expiratory flow as taken from the flow-volume curve and measured in litres per second. Values above the 70% are considered normal.

While an important tool, often spirometry is poor at discriminating between children with asthma and those with airway obstruction due to other conditions. Normal spirometry in a child, especially when asymptomatic, does not exclude the diagnosis of asthma. FEV1 is often normal in children with persistent asthma (Fitzpatrick et al., 2011). Reduced FEV1 alone does not indicate that a child has asthma, because it is observed in other lung diseases. As well, a significant increase in FEV1 (>12% from baseline) after

administering a bronchodilator (e.g. 4 puffs of salbutamol 100 mcg/actuation) indicates that airflow limitation is reversible and supports the diagnosis of asthma. In children with asthma, it is also predictive of a good lung function response to inhaled corticosteroids. However, an absent response to bronchodilators does not exclude asthma (BTS, SIGN, Edinburgh, 2012).

The classification of asthma severity has fundamentally changed over the past years. Traditionally, the degree of severity (intermittent or permanent mild, moderate, severe) was based on symptoms and the use of rescue inhalers and limited lung function. Severity therefore is no longer a snapshot but is established retrospectively after a treatment period of several months and can change over time and it results from the therapy step that is required to achieve asthma control.

There are many unknown features in the natural course of the disease. For example, it is not known whether patients with initially mild disease progress to experiencing moderate to severe disease or if it remains mild, and whether severe asthma begins early and remains severe. Patients with persistent disease have diminished lung function, and there is an inverse correlation between lung function and disease severity (Gelfand, 2009). Although most individuals with asthma have mild to moderate disease, about 5 to 10% have severe disease that is refractory to treatment with currently available medications (Gelfand, 2009). Little is known about the age-related differences in the characteristics of asthma, especially regarding severe asthma in children and adults. Children are **more likely to be male,** to be more sensitive to the suppressive effects of glucocorticoids, and to have less impaired lung function. Only 28% of the children is classified as having severe persistent asthma based on FEV1 data, and more than 40% as having mild persistent disease. (Gelfand, 2009; Fitzpatrick et al., 2011; Giulbert et al., 2014). Asthma in early life may also differ from asthma in childhood. The first is characterized by parental asthma, a history of eczema, and early allergic sensitization. The infants appear less responsive to inhaled glucocorticoids, and the inflammatory response, if present, may be neutrophilic in nature. The 80 to 90% of children with asthma has allergic asthma, eosinophilic inflammation, some changes consistent with airway remodelling, and a generally good response to inhaled glucocorticoids (Gelfand, 2009; Wingrove, 2016). A child's social environment may play a critical role in the development and severity of asthma (Pedersen et al.,2010). Stress in family or other primary caregivers during the first year of life is associated with an atopic profile and wheeze in infants and is also associated with asthma at age 6–8 years (Wright et al., 2005; Kozyrskyj et al.,2008). Maternal distress in early life may play a role in the development of childhood asthma, especially if the distress continues beyond the postpartum period (Kozyrskyj et al., 2008; Pederson et al.,2010).

Treatment

Whereas some children with asthma have intermittent symptoms that are improved with short-acting bronchodilators, many have persistent symptoms requiring daily treatment with inhaled corticosteroids (ICS) (Fitzpatrick et al., 2011). Children with severe asthma are differentiated by ongoing symptoms and airway inflammation despite treatment with high doses of ICS and other controller medications.

Short-acting β2 agonists: Treatment of choice for intermittent and acute asthma episodes in children, very young children and for preventing exercise-induced asthma. The presence of exercise induced bronchospasm is, however, an indication to start regular preventive treatment with ICS or a Leukotriene receptor antagonists (LTRA). Salbutamol, the most commonly used drug, has a favorable safety and efficacy profile also in patients aged 2–5 years (Fitzpatrick et al., 2011)

inhaled corticosteroids (ICS): A first-line treatment for persistent asthma because it reduces the frequency and severity of exacerbations. This occurs mainly by down regulation of pro-inflammatory proteins. Also, corticosteroids seem to reverse components of the asthma-induced structural changes (airway remodelling), including the increased vascularity of the bronchial wall (Hossny et al.,2016). At high doses (i.e. >500 mcg pro die for Fluticasone or Beclometasone; >1000mcg/die for Budesonide), oral candidiasis may arise. ICS use has also been linked with effects on growth, bones, hypothalamic–pituitary–adrenal (HPA) axis function and the eyes. (Covar et al.,2003; Wolthers and Heuk, 2004; Allen, 2005). Studies demonstrate that ICS may cause suppression of the HPA axis and adrenal suppression may occur with increasing doses. Even in children whose growth does not appear to have been affected, adrenal suppression cannot be ruled out (Dunlop et al.,2004; Priftis et al.,2006). Currently, no studies can provide a good basis for practice recommendations; however, HPA axis function evaluation should be cheched by specialists.

Leukotriene receptor antagonists (LTRA): An alternative first-line treatment for persistent asthma. Evidence supports use of oral montelukast as an initial controller therapy for mild asthma in children, as it provides bronchoprotection, and reduces airway inflammation as measured by nitric oxide levels in some preschool children with allergic asthma (Bacharier et al.,2018).

Omalizumab: is a recently introduced monoclonal antibody that binds to IgE. It is licensed for children 12 years of age and older with severe, allergic asthma and proven IgE-mediated sensitivity to inhaled allergens. In such patients, omalizumab reduces the risk of severe exacerbations (Walters and Walters, 2003; Huffaker and Phipatanakul, 2015). Omalizumab is administered via subcutaneous injection every 2–4 weeks, depending on patient weight and total serum IgE level.

Neurocognitive profile

Cognitive deficits associated with asthma are often global, with strongest effects on broader measures involving academic achievement and executive functioning (EF), but with additional impact on processing speed, global intellect, attention, visuospatial functioning, language, learning, memory and developmental psychopathologies (Annett, 2000; Airf, 2010; Irani et al., 2017; Sonney and Insel, 2018). Severity of asthma may be a key moderator, with higher cognitive deficits associated with the most severe asthma profile. Not only, cognitive burden appears greatest in asthma patients who were younger, males, from low socioeconomic backgrounds, and from racial/ethnic minorities (Irani et al., 2017). Difficulties in EF are also associated to poor medication adherence that require remembering to take the medications despite competing demands, planning, organizing and self-regulating (Sonney and Insel, 2018). Addition studies have shown that children with asthma are at an increased risk for **behavioral problems** (Annett, 2000; Arif, 2010; Fasmer et al., 2011). However, children with asthma, despite their level of severity, scored between two thirds and one standard deviation below the normative value on a measure of attention assessing impulse control when compared to children without asthma (Koinis-Mitchell et al.,2009). The presence of neurocognitive deficits may interfere, not only with the normal adaptation to environment, but also on the asthma self-management and may affect how children perceive the severity of their symptoms (Koinis-Mitchell et al.,2009). Children with asthma, not only display cognitive impairment but also have a higher prevalence of **anxiety and depressive disorders** compared to children with other chronic illnesses or to children healthy (Koinis-Mitchell et al.,2009; Arif, 2010). Some reviews showed that in child/adolescent populations with asthma, up to one third met criteria for comorbid anxiety disorders and depressive symptoms (Morrison et al., 2002; Ortega et al., 2004; Klinnert et al.,2008; Arif, 2010, Katon, et al., 2004), especially in children with moderate or severe asthma and with poor asthma control (Arif, 2010). Some authors report that maternal and child anxiety is highest immediately prior to an asthma attack and may be a learned response to anxiety- provoking stimuli associated with asthma attacks (e.g., difficulty breathing) (Friedman, 2010; Taha, 2017). Additionally, a link between higher levels of global **internalizing symptoms** and childhood asthma has been shown (Gillaspy et al., 2002; McQuaid, Kopel et al., 2001; Ortega et al., 2002. As with the relations between stress, anxiety, and asthma status, is it possible that the stress associated with asthma increases the likelihood of the development of difficulties in neurocognitive domain? How these factors are related? The presence of emotional and behavior symptoms may influence children's tendency to negatively skew their asthma symptoms. Emotional and neurocognitive disfunction may also affect children's tendency to misperceive physiological sensations of anxiety as symptoms of asthma (Koinis-Mitchell et al.,2009).

The data regarding the effects of ICS on behavioral changes are conflicting data (De Vries et al., 2008) although there is reason to suspect an indirect effect of exogenous steroids on the developing nervous

system. We hypothesize a **dysregulation of the HPA axis** involving 1) the mechanism that involves the use of ICS and their mechanism of action, 2) the asthma attack in itself, 3) environmental factors such as: parents' smoking habits, low birth weight, intermittent hypoxia, sleep disturbance and low socioeconomic status and 4) genetic factors. Together they are responsible for this important comorbidity because could contribute negatively to the child's health and his cognitive development as well (Strachan, 2000; O'Brien and Gozal, 2002; Goodwin, 2007; Sommey and Insel, 2018; Irani et al.2017; Hajek et al., 2005). The cognitive task that appear the most compromised in children with asthma is the cognitive flexibility (Taha, 2017). In sum, the clinical need to assess cognition in individuals with asthma is underscored but it is important to take into consideration the comorbidity between asthma and neurocognitive difficulties, even in terms of EF and mental problems, to reduce the potential negative impact of asthma on later academic performance but more important to prevent difficulties in children in achieving their life goals.

Acute Lymphoblastic Leukemia (ALL)

Acute Lymphoblastic Leukemia (LLA) is the most common malignant neoplasm in children; it accounts for 25% of the tumor pathologies diagnosed in the pediatric population. Leukemia refers to a group of malignant neoplastic pathologies in which genetic abnormalities of a hematopoietic stem cell give rise to an unregulated clonal proliferation, such that the progeny derived from it has an advantage in terms of growth compared to the normal cell population due to increased proliferative activity and reduced death by apoptosis (Kliegman et al.,2016). This results in an alteration of the hematopoietic function of the bone marrow which ultimately leads to bone marrow failure. In particular, the term LLA contains a heterogeneous group of neoplastic pathologies characterized by an important number of characteristic genetic abnormalities, characterized according to the lymphocytic lineage of origin in B or T form, which represents 75% of the leukemia diagnosed in pediatric age; the peak incidence of LLA is between 2 and 5 years of age, with a prevalence for males (Kliegman et al.,2016). The improvement in prognosis in terms of survival over the last 30 years is one of the most important successes of clinical oncology and has constituted a paradigm of how patient survival can be constantly improved through cooperative clinical trials; thanks to the current treatment regimens and treatment protocols developed, today we have achieved a 5-year survival of over 90% (Cooper and Brown, 2015). With the achievement of these success rates, however, there has been a growing increase in the long-term effects deriving from the therapy that patients undergo for a period of 2 years. The administration of cytotoxic drugs has been related to various side effects that occur in the short term and in the long term (Kliegman et al.,2016). In LLA survivors, a series of long-term morbidities have been highlighted, each related to certain drugs, which may affect different organs systems, including brain which lead to including neurocognitive disorders and other neurological toxicity outcomes, cardiac dysfunction, alterations of the skeletal system i.e. reduced bone mineralization, cataracts, obesity and metabolic syndrome, neuromuscular disorders, and the risk of developing second malignant neoplasms (Silverman, 2014). In particular, preventive treatment of the Central Nervous System (systemic, intrathecal and radiotherapy) is considered to be responsible for long-term neurocognitive damage (Harila et al.,2009). Moreover, late effects have been shown in adults now considered long-term survivors of pediatric LLA, which include high risk of developing metabolic syndrome, cardiovascular risk factors (including abdominal obesity, hypertension, insulin resistance) and higher prevalence of chronic medical conditions, among which problems related to mental health are particularly relevant (Silverman, 2014). Given the continuous increase in the number of children who survive the disease, thus becoming long-term survivors, it is therefore fundamental to fully understand the costs of therapy in physical, cognitive, emotional, and behavior domain of function in an attempt to mitigate the long-term consequences of the disease treatment itself (Silverman, 2014).

Treatment

Acute Lymphoblastic Leukemia therapy is risk-based: the approach based on risk stratification such that the intensity of therapy is modulated on the basis of the risk of treatment failure. Patients with features considered favorable will receive less toxic treatment regimens, whereas more aggressive treatments will be reserved for patients who present indicators that qualify them as high risk. A particularly important independent predictor is the response to initial therapy: the suboptimal response to the initial induction phase, understood as the failure of induction or minimal residual positive disease, is an indicator of a greater risk of therapeutic failure (Cooper and Brown, 2015). In particular, LLA therapy is characterized by 4 key moments, which account for the organization in blocks of the therapeutic process: induction to remission, consolidation, maintenance and therapy directed to the SNC. The most recent therapeutic protocols applied at the Italian pediatric hemato-oncology are the protocols AIEOP LLA 2000, closed since 2009, and AIEOP BFM LLA 2009, closed in 2017, international co-operative protocols for the treatment of children and adolescents affected by LLA. The protocols are not reported here but they are structured in phases where the main drugs used are: Methotrexate (also intratecal); Corticosteroids and Vincristine. The introduction of Cranial Radiotherapy (CRT) in prophylaxis and for the treatment of diseases in charge of central Nervous System (CNS), resulted an important positive prognostic improvement of patients with acute lymphoblastic leukemia. However, Cranial Radiotherapy is responsible for severe adverse effects, including neurocognitive deficits and behavioral abnormalities that may lead to poor results in achieving academic goals, working life and relationship life in LLA survivors (Cheung and Krull, 2015). Therefore, current therapies tend to avoid its use.

Metrotexate: Patients with LLA undergo repeated exposure to methotrexate administered in different ways, orally, intravenously, and intrathecally. It acts as a folate antagonist reversibly inhibiting the dihydrofolatoreductase (DHFR), a fundamental enzyme for the conversion of dihydrofolate to tetrahydrofolate, or the main circulating form of folate necessary for the synthesis of nucleic acids and for the methylation of numerous elements such as neurotransmitters, histones and phospholipids (Krull et al., 2013). It also determines an accumulation of homocysteine. Homocysteine and its metabolites also act as excitatory amino acids, thus causing excitotoxic death of neuronal cells (Kamdar et al., 2011). Methotrexate therapy in children has been associated with different degrees of neurocognitive impairment, among which the reduction of the IQ, the decline of visuo-motor functions and scholastic and academic difficulties, especially in the field of mathematical skills. In these patients the problems related to attention difficulties and processing speed are particularly frequent. An emerging explanation for these difficulties is related to a reduction in the integrity of the white matter, in particular in the frontal and parietal regions and in the fronto-striatal tract, observing in patient's long-term follow-up (more than two years after the end of the therapy) (Cheung et al., 2016).

Prendisone and Desametasone: despite the fundamental role of these drugs, they present multiple side effects when administered in a chronic manner or at high doses, including hyperlipemia, hypertension, insulin resistance and hyperglycemia. They also exert important effects on body composition and bone structure (Moricke et a., 2016). Moreover, particularly evident are the adverse effects in the neuropsychiatric area that are acutely manifested (Drozdowicz and Bostwick, 2014) whit long term consequences, such as: elevation of mood: of variable entity, variable in a spectrum from mild euphoria to maniacal state, insomnia, depression, psychotic frank states.

The prolonged exposure to corticosteroids, being able to inhibit the use of glucose by nerve cells, has been associated with reduced plasticity of hippocampal neurons; Functional magnetic resonance imaging (fMRI) studies have also demonstrated an alteration of hippocampal and prefrontal activation patterns during tasks requiring memory recall (Edelmann et al., 2013; Warris et al., 2014).

Vincristine (VCR): is a Vinca alkaloid that exerts a cytotoxic effect during phase M of the cell cycle, blocking mitosis by inhibiting the intracellular synthesis of tubulin, necessary for the completion of cell divisions; as such, it is administered at different stages of antiblastic therapy. VCR is characterized by a series of side effects including leucopenia, thrombocytopenia and anemia, nausea and vomiting (Protocollo AIEOP BFM 2009); the main side effect, however, is represented by neurotoxicity.

Neurocognitive profile

In LLA survivors neurocognitive, neurosensory and neurological deficits are frequently observed. The impairment of neurocognitive functioning is a late effect that is widely associated in the literature with encephalic irradiation; it is expressed in terms of learning deficits, reduction of IQ, compromise in the domains of executive functions, attention and concentration, speed of memory processing, and visuo-motor integration (Robison and Hudson, 2014). Neurocognitive deficits may emerge late, even up to 10 years from the end of the therapy; some studies suggest a decline in cognitive functions over time. It has been hypothesized that this is linked to white matter processes, which could expose adult subjects to an increased risk of dementia with early onset (Harila et al., 2014). Adverse effects typically emerge within 1-2 years of therapy, but some studies have shown that cognitive impairment may also occur late, up to 10 years after the end of treatment. Despite the broad agreement on the neurotoxicity of this therapy, and although a dose-dependent relationship is has shown the effect of cumulative exposure to radiation therapy and neurocognitive outcomes, the existence of a dose-threshold associated with the damage remains controversial. Emerging relates the impact of radiation therapy with age, cranial RT dose and relative risk of developing neurocognitive impairments and academic functioning was documented. Moreover, an increasing trend has been identified of the impairment in various domains of executive functions as time increases from the diagnosis (Krull et al., 2013). Female sex is also a significant risk factor

for the impairment of intellectual functions and academic functioning in relation to CRT (Krull et al., 2013). One of the main objectives related to the elimination of radiation therapy in the treatment of LLA in children is linked to the attempt to reduce long-term neurocognitive outcomes; however, it is now known that CNS-directed therapies introduced as a substitute for CRT, such as chemotherapy with intrathecal MTX and administered at high intravenous doses and steroid therapies, are not without risks of reporting late sequences that interfere with functioning neurocognitive.

Pediatric solid tumor (PWST)

Pediatric solid tumors differ considerably from those of the adult by prognosis, histology, tumor site and classification methods. For children, the International Classification of Pediatric Tumors (International Classification of Childhood Cancer (ICCC) (Steliarova-Foucher et al., 2005) is based on the morphology of the neoplasm and consists of 12 main groups:

• I. Leukemias, myeloproliferative diseases, and myelodysplastic diseases;

• II. Lymphomas and reticuloendothelial neoplasms;

• III. Central nervous system and miscellaneous intracranial and intraspinal neoplasms

• IV. Neuroblastoma and other peripheral nervous cell tumors.

• V. Retinoblastoma.

• VI. Renal tumors.

• VII. Hepatic tumors.

• VIII. Malignant bone tumors.

• IX. Soft tissue and other extraosseous sarcomas.

• X. Germ cell tumors, trophoblastic tumors, and neoplasms of gonads

• XI. Other malignant epithelial neoplasms and malignant melanomas.

• XII. Other and unspecified malignant neoplasms.

However, pediatric solid tumors account for only 30% of all pediatric tumors s (La Vecchia et al., 2010). In Italy, thanks to therapeutic advances and improvements in the diagnostic field, 5-year survival from the time of diagnosis is 82% in children and 86% in adolescents with long-term survival (15 years) only slightly inferior (Passion et al., 2013). Solid tumors account for about 30% of all pediatric tumors. This study examined seven patients with some of the most common solid tumors in the pediatric population. In particular, the neoplasms of the patients in the study are:

• **Wilms tumor**. Wilms tumor, also called nephroblastoma, is the most common early renal malignant neoplasm in pediatric age. All therapies for this tumor have limitations and advantages, but with similar outcomes (Kliegman et al., 2012) The most active chemotherapy drugs for Wilms' tumor are actinomycin-D, vincristine and adriamycin. Patients follow the AIEOP TW2003 Protocol.

• **Neuroblastoma**. Neuroblastoma is a neoplasm derived from neural crest cells that then give rise to the sympathetic nervous system and the medullary part of the adrenal gland and is the most common solid extracranial tumor of childhood (Kliegman et al., 2012). Tumors of the sympathetic nervous system correspond to 5 -7% of pediatric tumors and about 96% of these are neuroblastoma (Jankovic et al.,2012). For neuroblastoma in the earliest stages, then confined to its place of origin, surgery is sufficient, while in the more advanced stages chemotherapy is associated (Protocol AIEOP NB Unresectable 2000).

• **Germ cell tumor at gonadal (ovary) and extragonadal sites**. Germ cell tumors (TCG) are rare tumors and represent approximately 2-3% of malignant neoplasms in pediatric age, with wide frequency variations based on the age range considered.

• **Lymphomas** (Hodgkin and non-Hodgkin). Lymphomas are a heterogeneous group of neoplasms of the lymphoreticular system. The two main categories of lymphoma are Hodgkin's lymphoma (Hodgkin Lymphoma, HL) and non-Hodgkin's lymphoma (Non-Hodgkin Lymphoma, NHL) presenting different clinical manifestations and treatments (Jankovic et al.,2012). Hodgkin's lymphoma (HL) is a neoplasm of the lymphoreticular system and accounts for 6% of childhood neoplasia; is more common among adolescents and accounts for 23% of tumors and 71% of all lymphomas in the 15-19-year-old age group. It is rare in children under 10 years. The incidence is higher in girls than boys. The 42% of non-Hodgkin's lymphomas are Burkitt's lymphomas. The incidence is higher in male children, Non-Hodgkin's lymphomas are highly associated with: Wiskott-Aldrich syndrome, severe combined immunodeficiency syndrome, and Bloom syndrome (Jankovic et al.,2012).

• **Soft tissue sarcomas**. Soft tissue sarcomas represent 6% of all childhood malignant neoplasms. The rate of incidence standardized by age on the European population of 10.6 cases per million children per year. The most frequent histological type is rhabdomyosarcoma, with 43% of cases, followed by fibrosarcoma, with 17% of cases; unspecified sarcomas represent 12% of cases (Jankovic et al.,2012).

• **Histiocytosis.** Histiocytosis of childhood is a heterogeneous group of pathologies that have in common the increased proliferation or accumulation of cells of the monocyte-macrophage system, which originates from the bone marrow. Their systematic classification is based on histopathological aspects. Langerhans cell histiocytosis (LCH) is characterized by a proliferation of mononuclear dendritic cells with local or diffuse infiltration of the organs.

Treatment

Antineoplastic drugs used in the treatment of these type of tumors are molecules that inhibit cell replication and neoplastic growth. Antitumor chemotherapeutic agents are subdivided, according to their

mechanism of action, into different classes: alkylating; platinum coordination compounds; antimetabolites; plant alkaloids; tumor antibiotics; other compounds (Cellerino et a., 2011).

The drugs most likely to cause Central Nervous System (CNS) toxic effects are:

- antimetabolite drugs, especially methotrexate and 5-fluorouracil
- alkylating agents, especially cyclophosphamide and ifosfamide;
- antibiotics including anthracyclines;
- vinca alkaloids as vincristine and vinblastine

Some of these drugs are administered more frequently in patients with non-cerebral solid tumors as the first line: methotrexate, alkylating agents e vincristine (Newton, 2012)

The treatment of each type of tumor follows a specific therapeutic protocol in which drugs are alternated, depending on the staging, the area and the risk:

- Protocol AIEOP TW2003 used for Wilms tumor
- Protocol AIEOP NB Unresectable 2000 for the neuroblastoma treatment
- Protocol AIEOP TCG2004 used for Germ cell tumor at the gonadal (ovary)
- Protocol AIEOP LH2004 followed for the treatment of Hodgkin's Lymphoma
- protocol AIEOP LNH97 for the cure of Non- Hodgkin's Lymphoma
- Protocol AIEOP RMS2004 for the treatment of rhabdomyosarcoma
- Protocol AIEOP LCHIII98 for the treatment of Langerhans cell histiocytosis (LCH)

Having previously mentioned the other drugs here I report briefly:

Alkylating drugs : the most the neurotoxic are alkylating drugs which perform their action by acting directly on the DNA, altering its structural integrity and function through an alkylation reaction: the drug forms a covalent bond with nucleophilic structures present in the cells, especially at the level of the nitrogen molecule in position 7 of the DNA guanine, resulting in an incorrect coupling with a thymine base; other molecules such as RNA and cytoplasmic proteins may also be alkylated (Ralhan and Kaur, 2007). The different alkylating subclasses show differences with respect to target macromolecules, mode of administration, toxic effects and use. Cyclophosphamide is the most used alkylating agent in clinical practice and is used in various childhood neoplasms such as Wilms tumor, neuroblastoma and retinoblastoma. Ifosfamide is an analog of cyclophosphamide, it is a particularly active drug on soft tissue sarcomas, lymphomas and testicular tumors and is only administered intravenously (Cellerino et al.,2011).

Intensity of Treatment Rating Scale (ITRS)

Current treatments for pediatric tumors vary widely in their intensity and are closely related to the type, stage, risk group and whether it is an initial diagnosis or a recurrence. However, despite different diagnoses and therapeutic protocols, it is possible to share patients for treatment intensity. This parameter allows us to assess the impact that the disease has on the patient and on the family and is very important for the purpose of structuring a personalized follow-up path for each patient.

The Intensity of Treatment Rating Scale (ITRS) is a psychometrically reliable and valid means for classifying the intensity of treatment of pediatric tumors from the least intensive to the more intensive in relation to the treatment modalities and the patient's stage / risk (Kazak et al., 2012).

The ITRS is composed by **intensity levels** and **content items**. The intensity levels consist of four treatment intensity categories which are:

• level 1: fewer intensive treatments;

• level 2: moderately intensive treatments;

• level 3: very intensive treatments;

• level 4: extremely intensive treatments.

The content items consist of 43 different pathologies and/or treatment modalities, each classified according to one of the four intensity levels. (Table 1, Appendix B) For example, Level 4, which is an extremely intense treatment, includes treatments such as hematopoietic stem cell transplantation (HSCT) or chemotherapy for acute myeloid leukemia (LMA). Treatment intensity assessments need to be updated periodically due to changes in treatment approaches and protocols. The ITRS-3.0 of 2011 is the latest version that replaced the previous ITRS-2.0 and is the one used in our study. (Kazak et al., 2012)

Neurocognitive profile

Risk factors associated with neurocognitive decline include age, duration and duration of the intensity of the treatment: younger age at treatment and greater intensity of therapy have been associated with worse neurocognitive outcomes, resulting, with much probability, from a greater vulnerability of the developing nervous system to cytotoxic agents. In particular, adverse effects are believed in younger patients may be related to toxic effects to newly synthesized myelin, that is metabolically more active and less stable (Reddick et al., 2014). Recent studies have shown that some areas like attention and concentration, working memory, speed of information processing, executive functions, visuospatial skills, psychomotor functions and verbal skills are more sensitive to chemotherapy (Anderson and Kunin-Batson, 2012).

Together these pathologies have in common a CNS susceptibility, study the role of polymorphisms may help to define the relevance of a system to cognition and behavioral with respect to a more general or primary domain of function.

2.3 Genes matter

The study of the Single Nucleotide Polymorphisms (SNPs)

Neurocognitive and executive functions drive our behavior and permit us to achieve our goals in daily life. These complex processes are largely mediated by cortical function and modulated by inputs directly related to mechanism of neuronal plasticity and repair, neurotransmitter availability, and the neuro- and behavioural toxicity of context. The ability of neurotransmitters, as serotonin and dopamine, hormones, as glucocorticoids, or neurotoxin as homocysteine, to modulate executive and neurocognitive function allows for adaptation in cognitive and emotional behaviour in response to changes in the environment (Logue and Gould, 2014). Because of the important role these factors play in regulating our nervous systems, changes in these systems can also have a severe impact on neurocognitive and executive function. Polymorphisms in genes associated with these factors may be associated with phenotypic differences in executive and neurocognitive functions. According to the NIMH Research Domain Criteria (RDoC) this study describes key dimensional constructs underlying mental function across multiple units of analysis, from genes to observable behaviours. The pathology studied in this thesis are all, in different way, sensitive to the context being of genetic, developmental or psychosocial nature. Therefore, we hypothesized that some polymorphisms related to these contexts might be important predictors of the susceptibility of the patient. Also, polymorphisms that regulate neurotransmitters availbility may have a different relevance over time. This chapter describes specific genes in order to better understand if they can be potential indicators (protective or predictive). The study of the SNPs must be considered in an environmental and development context, considering each child in his/her development trajectory.

Given this, the study focused on:

- the serotonin transporter gene-linked polymorphic region (5-HTT LPR; SLC6A4) as indicator of "**Serotonin System**" functionality;
- the Val108/158Met polymorphism of catechol-O-methyltransferase gene (COMT Val158Met; rs4680) as indicator of "**Dopamine System**";
- the Val66Met polymorphism of Brain Derived Neurotrophic Factor (BDNF Val66Met; rs6265) as indicator of **neuronal plasticity** and connection between Serotonin and Dopamine Systems;
- the polymorphism 677 of the methyl tetrahydro folate reductase (MTHFR C677T) as indicator of **folate pathways** involved in methylation processes; nucleotide synthesis; regulation of homocysteine levels and oxidative phenomena
- polymorphisms of FK506 Binding Protein 5 for its regulating role in **hypothalamic-pituitary-adrenal** (**HPA) axis** and glucocorticoids receptor

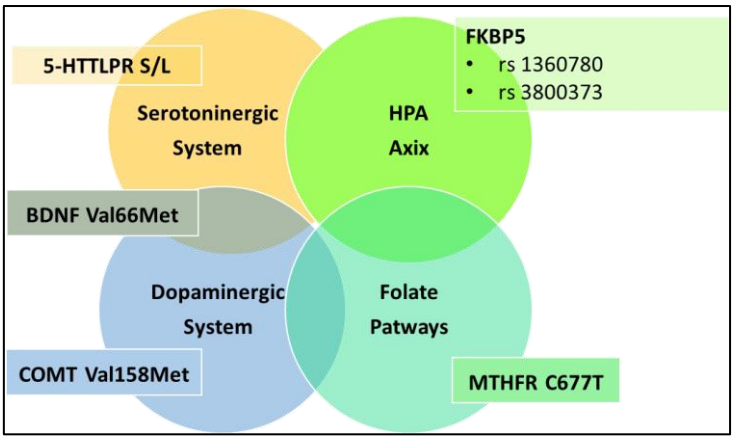


Figure 1: SNPs as potential indicators (protective or predictive) of the domains functioning

The Serotonin System: 5-HTT LPR

The regulation of serotonin plays an important role both in the regulation of the processes related to the emotional sphere and those related to the side of the executive functions. Both interact with the environment, because it is known that repeated stressful events can exacerbate underlying vulnerabilities

Serotonin appears already at 5 weeks of gestational age, is one of the first neurotransmitters involved during embryonic development exerting a particular trophic role for Central Nervous System (CNS), as well as for several other organ systems. Serotonergic projections have been identified in all the CNS regions, but are particularly present in the circuits that make-up the limbic system, responsible for regulating mood and behavior (Kenna et al., 2012). Serotonin is, therefore, a critical factor for the development of the brain regions responsible for the processing of emotions; as such, it has been repeatedly linked to various psychiatric conditions, such as anxiety, depression, antisocial behavior and addictions (Nordquist et al., 2010).

Once released, serotonin remains localized at the level of the intrasynaptic space until it is re-internalized at the level of the presynaptic side by the 5-HTT transporter, the main responsible for the re-uptake of serotonin, which is the main mediator of serotonergic transmission conclusion removing serotonin from the intrasynaptic space. The minority share that escapes re-uptake is rapidly inactivated by monoamine oxidase.

The serotonin transporter is encoded by the SCL6A4 gene located on chromosome 17 (17q11.1-q12) and is characterized by a promoter region that possesses a functional insertion-deletion polymorphism of two repeated elements of 22 base pairs. Such polymorphism results in two possible allelic variants: the variant L, and the variant S. The possible phenotypes related to the serotonin transporter are therefore:

• Homozygous for the S (SS) allele

• Heterozygote (SL)

• Homozygous for the L (LL) allele

The S allele is characterized by a reduced transcription efficiency, which results in a lower activity of the transporter in serotonergic re-uptake. It is considered a "risk allele" as it has been associated with increased amygdala reactivity leading to anxious personality traits, major depressive syndrome, suicide attempts and bipolar disorder. The increased transcription efficiency of the allele L, instead, is considered protective against depressive disorder (Kenna et al., 2012).

Several neuroimaging studies have documented the fundamental role of the serotonergic system in stress response (Caspi et al., 2003). In S allele carriers was found an increased amygdala reactivity to threat stimuli, both in terms of intensity and rapidity of activation, compared to LL homozygotes (Caspi et al.,2010) Figure 2). The S allele has also been associated with a reduction in the thickness of gray matter at the level of the medial prefrontal cortex, responsible for the superior control of the emotional component, as well as with a marked reactivity of the hypothalamic-pituitary-adrenal axis in response to stimuli of nature threatening. This increase in reactivity seems to affect only the activation caused by a stressful stimulus, not affecting the baseline activation levels (Caspi et al.,2010). The S carriers appear to be particularly sensitive to the quality of their environment, such that experiencing negative environments is associated with significantly worse psychological health (Belsky and Pluess, 2009; Hankin et al., 2015).

Essential is the interaction between genotype and environment (G x E, gene x environment), which determines the influence of stressful life episodes on the risk of depression. In a famous study, Caspi et al, showed a positive correlation between the number of stressful events and the probability of developing depression, statistically higher in subjects carrying the S allele compared to the homozygous subjects LL (Lenroot et al., 2005). In particular, Caspi et al showed that subjects carrying at least one copy of the S allele had an increased risk of suffering from psychiatric negative consequences following exposure to stress, consequences measured here in terms of symptoms associated to depression (Gelernter, 2014). An important correlation with the number an intensity of stressful events is also identified (Caspi et al.,2003).

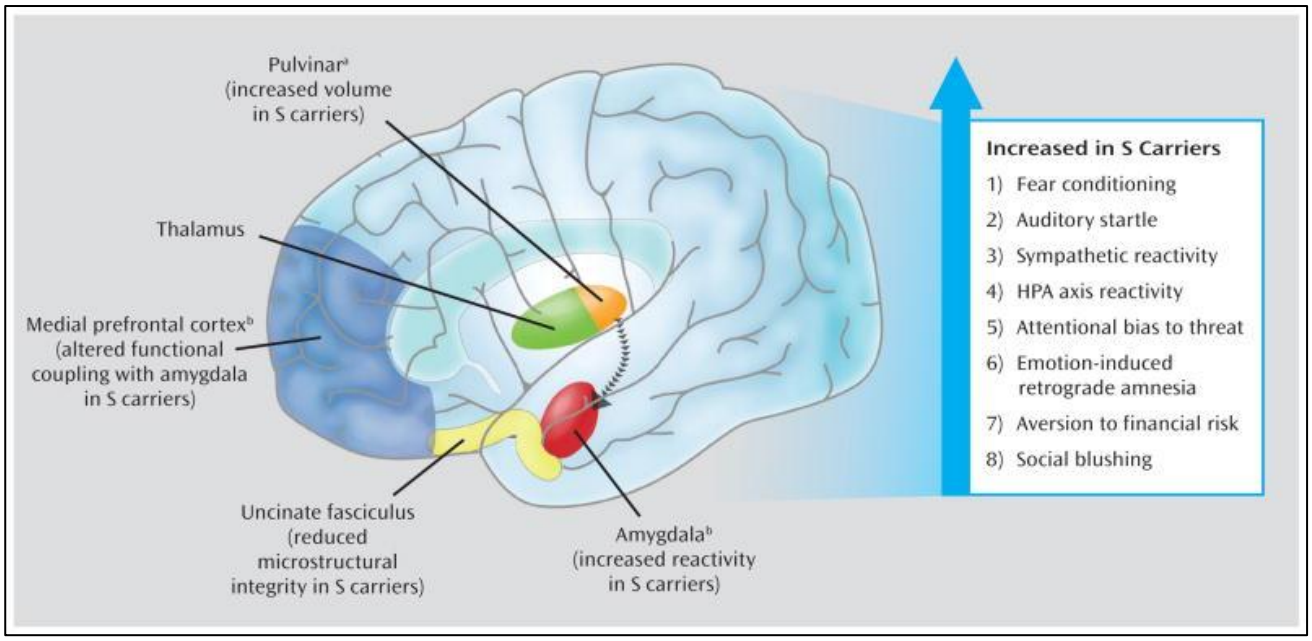


Figure 2**:** The *5HTT LPR* affect**s** neural circuitry (Caspi et al.,2010)

The Dopamine System: COMT Val158Met

The Catecol-O-Methyltransferase enzyme is an important enzyme responsible for the degradation of dopamine at the cortical level determining prefrontal dopaminergic flows (Meyer-Lindenberg and Weinberger, 2006).

The COMT gene is located on chromosome 22 (22q11.2); the Val158Met functional polymorphism encodes for the substitution of a valine with a methionine which interferes with the thermal stability of the protein product, such that the enzyme encoded by the Met allele presents an activity equal to 25% of the peptide containing valine. The mutation determines a significant reduction of the enzymatic activity in the carriers of the Met allele, with an increase of extracellular dopamine levels. The relationship between dopamine level and cognitive functions is characterized by an "inverted U" form, where both dopaminergic activity levels below and above the optimal level interfere with the activities of cortical region and are predictive of poor performance during tasks that evaluate cognitive functions (Dumontheil et al.,2011). This effect depends on the different dopamine role in different brain areas and to the dopaminergic receptor population present (D1 or D2). Therefore, the Val158Met polymorphism has a pleiotropic effect with respect to cognitive functions and emotional stability and assumes a different meaning in an evolutionary view (Mier et al., 2009). The role of dopaminergic signaling in fronto-striatal networks is crucial for cognitive control, in flexibility and in cognitive stability. Cognitive stability means the ability to retain cognitive representations, whereas cognitive flexibility implies the ability to update such mental representations in response to new information. This paradigm, widely demonstrated in adults, has recently been the subject of research even in childhood. In particular, the elevated basal dopamine levels characteristic of Met carriers is associated with a better performance in terms of cognitive stability, while

the reduced levels of prefrontal dopamine (characterizing the carriers of the more active Val variant) were associated with better performance in terms of cognitive flexibility. It is therefore evident that fronto-striatal signaling contributes to individual differences in terms of stability and cognitive flexibility even in the child and adolescent; a balanced budget in terms of stability and flexibility is critical, because (Makant et l.,2014):

- an excess of inflexible cognitive representations determines perseverative behavior

- susceptibility to new information can lead to excessive distractibility or impulsivity

The effect of COMT has also been studied in relation to the response to stressful and traumatic episodes in children. In particular, COMT variants were related to hippocampal activation. The likelihood of developing depression or PTSD (Post-Traumatic Stress Disorder) after exposure to adverse life episodes (such as loss of a parent, divorce, financial problems) or during stressful periods is indeed higher in carriers of the Met allele (Van Rooij et al., 2016).This suggests that increased hippocampal activation constitutes a mechanism to cope with stressful situations by relying more on the contextual information provided by the hippocampus for behavior regulation; hippocampal recruitment may therefore have the significance of improving the ability to use information from the surrounding environment to guide behavior and could increase the resilience of Val / Val individuals who have been exposed to traumatic events during early life stages (Van Rooij et al., 2016). Imaging studies have also documented reduced hippocampal and prefrontal volumes in subjects exposed to traumatic episodes during childhood (Van Rooij et al., 2016). Both these regions are fundamental for inhibition function, which corresponds to the ability to suppress behavior that is no longer necessary or that has become inappropriate in relation to the demands of the environment; it is mediated by the prefrontal ventro-medial cortex (vmPFC) and it is damaged in various neuropsychiatric disorders.

An “age x genotype” relationship has also been identified, such that the COMT genotype exercises its effect in a variable way during development. Analyzing the performance in terms of working memory, a trajectory has been documented: homozygous Met/Met individuals pass from a poorer performance during childhood to a more efficient one from mid adolescence onwards (Fig. 10)), where the transition between these two phases is at the age of 10.2 years. This has allowed to demonstrate that the effects of the COMT genotype in the executive and behavioral functions are not considered static during development but have a dynamic character such that the advantages associated the Met genotype only emerge after 10 years of age (Dumontheil et al.,2011)

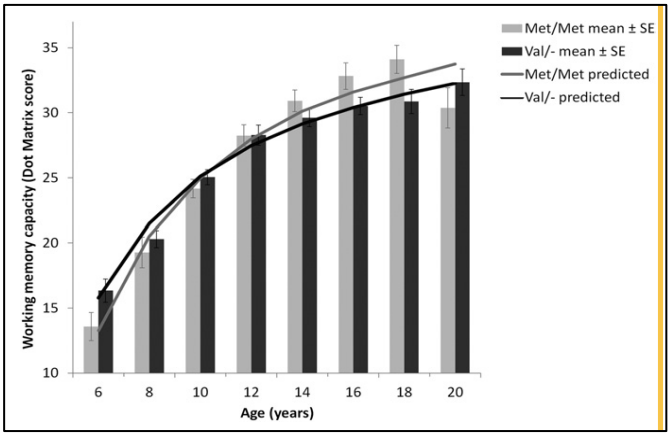


Figure 3: Working memory evolution during development in Met/Met and Val carries (Dumontheil et al.,2011)

Folete Patway: MTHFR C677T

MTHFR catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (Fig. 6), which is the main circulating folate form; as such, folates participate in the processes of nucleotide synthesis and remodeling of homocysteine to methionine, with vitamin B12 as cofactor in the latter process. The normal catalytic activity of MTHFR is therefore crucial for maintaining the circulating pool of folate and methionine, as well as preventing homocysteine accumulation (Botto et al., 2000). Moreover, its important role in the replication of the DNA molecule, as an enzyme limiting the synthesis of nucleotide compounds, makes MTHFR an important target in the field of oncology, pneumology and neurology. The corresponding gene is located on chromosome 1 (1p36.3) and two common variants have been described: the C677T allele and the A1298C allele. The study is focusd on the C677T variant, as responsible for the most important reduction in the catalytic activity of MTHFR. (Botto et al.,2000)

The C677T variant is characterized by a point mutation which determines the conversion of cytosine (C) into thymine (T), which is followed by the amino acid substitution of alanine with valine. The mutated gene codes for a thermolabile enzyme with a reduction of activity at temperatures of 37 ° or higher. Subjects homozygous for TT have levels of enzymatic activity reduced by 50-60% at a temperature of 37 ° C; heterozygous subjects (CT) present levels of enzymatic activity intermediate between those of homozygotes TT and CC.

Both homozygous (TT) and heterozygous (CT) genotypes are associated with reduced concentrations of folate and higher homocysteine concentrations compared to wild-type homozygotes (CC). This represents important factors in the early stages of pregnancy, both are risk factors for the appearance of neural tube

defects, among which the most common are spina bifida, anencephaly and encephalocele (Kirk et al.,2004). The C677T allele, therefore, represents a risk factor for the appearance of neural tube defects. However, this association must be considered from the perspective of a G x E relationship since it seems to vary depending on the nutritional status of the mother at the time of pregnancy (Botto et al.,2000). There are several mechanisms that have been identified as possible mediators of homocysteine neurotoxicity, including the reduction of the synthesis of neurotransmitters and the accumulation of metabolites that cause excitotoxic death of neuronal cells (Bhatia et al., 2015). Both these mechanisms have also been related to the clear association between hyperhomocysteinemia and depression: on the one hand, there is the reduced synthesis of neurotransmitters such as dopamine and serotonin, classically associated with the depressive phenotype, on the other the potential neurotoxic effect of elevated homocysteine levels, which is mainly involved on dopaminergic neurons (Bathia et al.,2015). Also, recent studies have identified an association between polymorphisms of genes involved in the folate pathway, attention difficulties reported by parents and alteration of direct neurocognitive measures in long-term survivors of LLA (Kull et al., 2013). Among these subjects, treated with the contemporary protocols involving the use of chemotherapy alone and in particular of methotrexate administered intrathecally, only for the carriers of polymorphisms associated with the reduction of the enzymatic activity of MTHFR (C677T but also A1298C) attention problems, reported by parents, were particularly relevant. It has been hypothesized that polymorphisms and therapy act synergistically by interfering with the folate pathway, determining both the reduction of tissue folate levels and hyperhomocysteinemia; homocysteine and excitatory amino acids could in fact accumulate and exert a double toxicity, acting at the endothelium level and sensitizing neurons to oxidative stress, ultimately causing the death of neuronal cells and the diminished myelin synthesis observed in these patients (Kamdar et al., 2011). Furthermore, MTHFR plays a role in the methylation of substrates, in fact it seems that MTHFR genotype influences the methylation of the COMT promoter gene, directly influencing the dopamine metabolism and consequently having a direct effect on the executive functions that are kinked to dopamine availability (Roffmann et al., 2008).

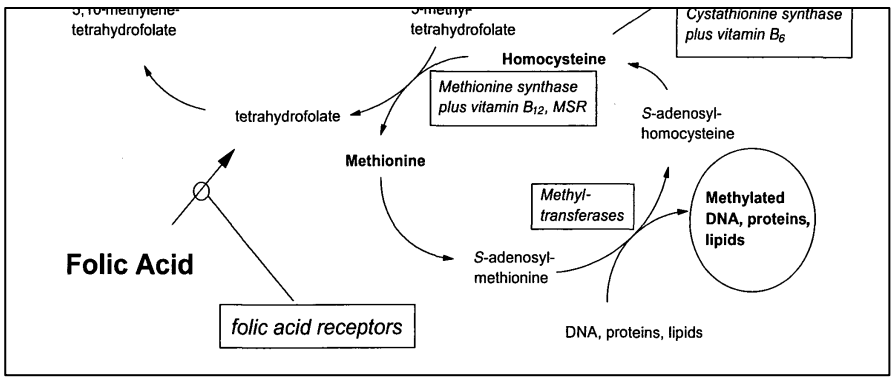


Figure 4: Folate Pathway: the role of MTHFR in folate and homocysteine metabolism (Botto et al., 2000)

Neuroplasticity: BDNF Val66Met

Brain-derived neurotrophic factor (BDNF) has a primary role in neuronal development, differentiation and plasticity in both the developing and adult brain. A single-nucleotide polymorphism in the proregion of BDNF, termed the Val66Met polymorphism, results in deficient subcellular translocation and activity-dependent secretion of BDNF, and has been associated with impaired neurocognitive function in healthy adults and in the incidence and clinical features of several psychiatric disorders (Notaras et al.,2015). It is expressed throughout the Central Nervous System, in particular in the prefrontal cortex (PFC) and the hippocampus, where it has long-term effects modulating hippocampal plasticity and learning from this employee (Cadwell et al.,2013). The Met allele has been able to interfere with the stimulation-dependent release of BDNF, interfering with its intracellular distribution. The stimulation of BDNF secretion, dependent from synaptic activity, plays a critical role in synaptic plasticity as well as in the neural circuits at the base of memory and learning. Therefore, it has been hypothesized that the Val66Met variant could interfere with hippocampal function and episodic memory (Egan et al., 2003).

The association between this polymorphism and enhanced susceptibility for the development of depression has been identified in an inconsistent manner (Cadwell et al., 2013), although a reduction in BDNF levels in subjects with depression has been documented (Marvsak et al., 2016). The Met allele has been associated with symptoms related to depression and traits of anxious personality, especially in the case of subjects who have a history of traumatic events. Infant traumas and BDNF genotype have been shown to interact in predicting structural changes in limbic regions involved in emotional control. Alterations in the development of limbic circuits could therefore lead to situations of emotional dysregulation, up to frankly pathological conditions. In this perspective, the BDNF genotype is believed to have a central role in influencing vulnerability, or resilience, to traumatic and stressful events (Marvsak et al., 2016). It has been hypothesized that it can influence the coping strategies of the individual, i.e. those strategies aimed at coping with the consequences of negative experiences that occur in the environment in which the child develops (Cadwell et al.,2013). A reduction of gray matter volumes was highlighted in the lateral portions of the frontal lobes, with peak values localized at the bilateral dorsolateral prefrontal cortex (DLPFC) (Pezawas et al., 2004). This has been related to the poorer performance documented in tests related to working memory in subjects carrying the Met allele. Form Thi evidence emerges therefore that the Val66Met variant is able to exert a significant effect on performance in terms of memory and executive functions (Savitz et al.,2003). Although the Met allele has been in multiple studies identified as a susceptibility factor to the development of depressive symptoms, the Val allele cannot however be considered as a "protective allele" in a univocal manner. BDNF could be considered not as a factor of vulnerability, but as a factor of plasticity able to favor the onset of depression on condition that particular challenges are presented in relation to the environment. We could therefore hypothesize that Val / Val

homozygotes may derive the greatest advantages from a favorable environment but that, due to their more pronounced plasticity, they may be more vulnerable to the effects of an environment with negative connotations. In contrast, in subjects carrying the Met allele, decreased plasticity could make them intrinsically more likely to develop depression but, at the same time, make them less susceptible to the influence of events, be they positive or negative, in determining outcome cognitive or behavioral type (Caldwell et al., 2013).

The HPA axix regulation: FKBP5

The gene encoding FK506 binding protein 51 (*FKBP5*), located on chromosome 6p21.31, is a highly interesting target for GxE as it is considered a shared etiological factor underlying stress-related disorders (Zannas and Binder, 2014; Daskalakis and Binder, 2015). The *FKBP5* protein, through the inhibition of glucocorticoid receptors activity (Figure 5), promotes the homeostatic regulation of the hypothalamic-pituitary-adrenal (HPA) axis, the principal biological mechanism of the stress response (Binder, 2009). Several *FKBP5* single nucleotide polymorphisms (SNPs) have been robustly associated with individual differences in the stress response of healthy adults (e.g., prolonged recovery period of the HPA axis after exposure to a stressor and increased glucocorticoid receptor resistance; (Binder, 2009)). Polymorphisms of the gene encoding this co-chaperone have been shown to associate with differential up-regulation of FKBP5 following GR activation and differences in GR sensitivity and stress hormone system regulation. Alleles associated with enhanced expression of FKBP5 following GR activation, lead to increased GR resistance and decreased efficiency of the negative feedback of the stress hormone axis. This results in a prolongation of stress hormone system activation following exposure to stress. The dysregulated stress response, then, might be a risk factor for stress-related psychiatric disorders. *FKBP5* variability has also been shown to be associated with heightened amygdala reactivity in the context of emotional neglect, increased attentional threat bias and differences in hippocampal shape (Binder, 2009; White et al.,2012), and with anxiety-proneness trait levels (Shibuya et al., 2010). Not only FKBP5 appears to appear to be a molecular amplifier of the stress response but also can influence many pathways implicated in neuronal function, synaptic plasticity, autophagy, and DNA methylation (Matosin et al., 2018). While psychiatric disorders are not single-gene diseases, FKBP5 may serve as an example for a molecular target at the stress-feedback neural interface that could help us understand mechanisms of risk and resilience underling psychopathology, especially in the context of adverse life events, that may extend to other targets (Rein, 2016).

GxE studies highlight the importance of investigating the FKBP5 as a susceptibility gene. On the basis of the diathesis-stress model, it would be predicted that individuals carrying the risk alleles would show higher scores on the anxiety-depression measures when they experienced more negative Life Events, as compared with participants that are homozygous for the non-risk alleles. In the differential-susceptibility model, the

same individuals carrying the risk alleles would also show lower anxiety-depression levels when exposed to more positive Les, as compared with participants homozygous for the non-risk alleles (Pèrez-Pèrez et al.,2018). We investigated the extent to which FKBP5 moderates the connectivity within and between networks implicated pathology as in Astma, Epilepsy, and pediatric cancer survivors by assessing executive function, emotional and behavior and psychosocial risk. Different studies have reported various risk alleles of FKBP5, we focus on two single-nucleotide polymorphisms (SNPs) in the FKBP5 gene region that have been repeatedly associated with increased risk for enhanced stress responses. The selection of these specific alleles is: (a) rs3800373 (A/C), for its role in increasing FKBP5 protein levels, impairing HPA negative feedback following stressors, and increasing psychopathology risk following trauma (Binder et al.,2008; Zimmerman et al.,2011); and (b) rs1360780 (C/T) that can reduces basal cortisol levels, augments risk for PTSD and depression, and allele risk carries have an altered perception of threat stimuli (Xie et al.,2010; Zimmerman et al.,2011; Boscarino et al., 2012).

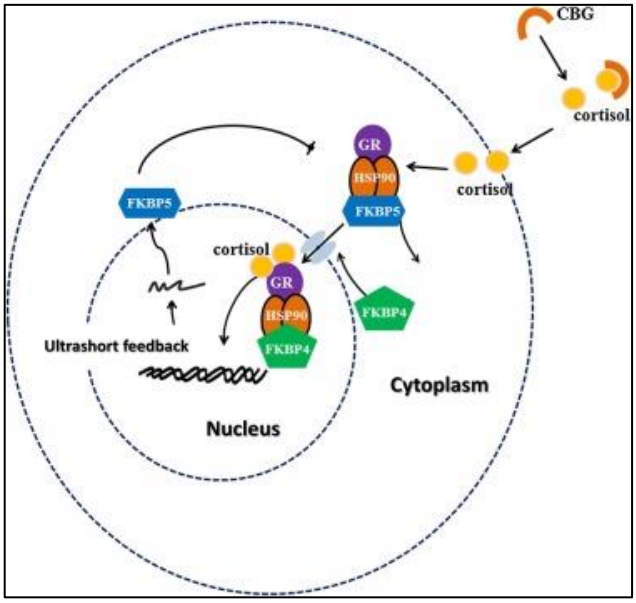


Figure 5: FKBP5 regulation on the glucocorticoid receptor (GR) function. It has been established that one of the major functions of FKBP5 is to act as a regulator of glucocorticoid receptor function through the association with heat shock protein 90 (HSP90). When HSP90-GR is bound to the FKBP5, it has a lower affinity for GR ligand (De la Cruz-Cano, 2017).

2.4 Environment and neurodevelopment

While for some pathologies there is a direct and univocal correlation between genotype and phenotype, for others it is very difficult to establish such correlation and the phenotype results as a consequence of a long cascade of molecular events involving a variable number of genes and environmental factors (Albert, 2011). The role of genetic polymorphisms is therefore part of a more complex habit that includes complex and multifactorial diseases**.** The presence/ absence of polymorphism is not a risk or protective factor, but it is necessary to consider such polymorphisms in the context of gene and environment interaction (G x E). This interaction occurs when the effect of exposure to a toxic or protective environment. Unlike the genotype-phenotype approach that associates monogenic causes with pathological conditions, the approach assumes that toxic environment cause disturbance and the presence of mutation influence the susceptibility to such environment (Capsi and Moffit, 2006). It is important to consider these polymorphisms, these genetic variants, not alone but in the context of an environment that can be more or less favorable (Ellis et al., 2011).

The plasticity of neural systems is, therefore, a complex process, genetically determined, sequential and time-dependent. It is long-lasting and is strictly regulated not only by intrinsic factors, but it is also influenced by extrinsic factors such as the environment and experience (Hochberg et al., 2011; Casey et al., 2005; Ian et al., 2017; Ismail et al., 2017). In addition to genetic factors (genetic blueprint), very important is the role of epigenetics in modulation of the development of the nervous system. In the pathologies that we consider the nervous system presents some fragility due to different causes, but it is always a brain in continuous growth and modification. Neuroplasticity involves fundamental changes in neurogenesis, migration of neuronal cells, formation of synapses and functional and structural networks (in natal and postnatal period) that lead to development and adaptation to constant changes in the external environment. These biological processes are subject to genetically programmed periods, limited in time, defined critical or *sensitive periods*, during which the nervous system is more susceptible to change. From this it emerges that all critical periods are sensitive periods but not all sensitive periods are critical periods. Furthermore, it is important to note that critical and sensitive periods are not absolute and differ in temporal and spatial profiles between the various neural subtypes (Nelson, 2000). Recent studies investigate whether these sensitive and critical periods may be a potential window of opportunity for neuromodulatory interventions. What has been said is only a theoretical reference to what is known in the literature but severe and chronic pediatric diseases such as asthma, epilepsy and pediatric tumors must therefore be considered in this framework of neurodevelopment where alternating periods of greater plasticity alternate. Events in these periods play a fundamental role in defining the development trajectory of these children. Thus, a child, in a sensitive growth period, such as early childhood or the beginning of adolescence, is to where to face a chemotherapy, or asthma attacks or an epileptic seizure onset, its development trajectory can suffer of some modifications; in this context the genes what role play? The

presence of some polymorphisms can play an important role as additive factors of vulnerability. More important, how all these phenomena (disease, treatment, age, gender, genetic) interacts on the neurodevelopmental trajectory?

CHAPTER 3: Material and Methods

We enrolled 126 children between 2 to 16 years old in a longitudinal prospective study from the Pediatric Unit of University Hospital "Azienda Ospedaliero-Universitario" Policlinic of Modena. 42 patients were diagnosed with Epilepsy, 63 patients were diagnosed with Asthma, 12 patients were Lymphoblastic Acute Leukemia survivors (LLA) and 9 patients were children with solid tumors survivors (PWST, children with solid tumor). Data shown here refer at patients examined in two times: at the beginning of the research (T0) and one year later (T1).

General eligibility criteria for the study were: (1) clinical diagnosis of asthma, or cryptogenic epilepsy, or to be a pediatric cancer survivor; (2) absence of comorbidities that can influence cognition (ES: ADHD; autism; Rett syndrome, etc..); (3) absence of motor disabilities (ES: Cerebral Palsy); (4) good comprehension of the Italian language.

Demographic and clinical data was collected via a semi-structured interview and by medical records, these included age of onset, treatment, presence of comorbidities, caregivers age, caregivers' highest degree of education, primary language, ethnicity, close relatives with chronic or mental diseases, the course of pregnancy, type of delivery and the most important early development milestones as first speech and steps.

Each child has been assigned, at the time of enrollment, a unique identification code consisting of two letters (MO) indicative of the center of reference, a letter indicating the belonging group, a letter indicating sex (M or F) and a progressive number. A detailed communication of the objectives and methods of the study was provided by the responsible of the research before participation.

The Ethics Committee of Modena approved the protocol (Reference: 13/EE/0157); informed written consent and age-appropriate assent (from 11 years on) was obtained from all young participants and their parents.

3.1 Participants

The Epilepsy group:

The sample consisted of 42 patients between 5 and 12 years of age. Patients were diagnosed with "cryptogenic epilepsy" (Di Rosa et al., 2013), and recruited from the pediatric neurology clinic of the University Hospital "Azienda Ospedaliero-Universitario Policlinico di Modena". Specific inclusion criteria were: (a) absence of brain lesions or genetic and metabolic disorders that could justify the onset of epilepsy; (b) absence of comorbid conditions known to affect cognition, (c) absence of one of other diseases included in the general research. Epilepsy classification, according to International League Against

Epilepsy (Engel, 2006), was made by a neurologic work-up, based on the most pertinent clinical characteristics and included an electroencephalogram (EEG). Patients were divided in 4 groups: 1 Rolandic Epilepsy; 2 Temporal Epilepsy; 3 Epileptic Dysphasia; 4 Other, including one patient diagnosed with previous West's Syndrome, one patient diagnosed with frontal lobe epilepsy and one patient with uncertain epileptogenic focus. The type of treatment was divided in 5 groups based on the active substance: 1 Carbamazepine and Oxcarbazepine, 2 Valproate, 3 Levetiracetam, 4 Topiramate, and 5 Out of Therapy.

In epilepsy group boys represent 69% (n= 29) of the sample and girls are 31% (n=13). Many participants are Caucasian (90.5%, n= 38) with an age of less than 10 years (72.3%, n=30) and in most cases the onset of pathology is before 5 years old (52.4%, n=22). The prevalent type of epilepsy is Rolandic that represent 50% of cases (n=21), followed by Temporal lobe epilepsy (28.6% n=12) and Epileptic Dysphasia (11.9% n=5). The 9.5% of patients (n=4) has a diagnosis of Learning Disorder.

We note a good control of the symptomatology with 64.3 % (n=27) hasn't got crisis in the last year but the 35.7% has not achieved total crisis control though under treatment, however all patients report an improvement in symptoms. The almost total of patients is under therapy with at least one drug (n=35; 83.3%), just 5 patients are out of therapy. The most common treatment is Oxcarbamazepine (33.3%, n=14) or Carbamazepine (30.9% n=13), 11.9 % (n=5) takes Levetiracetam, 9.5% (n=4) takes Valproate and 2.4% (n=1) takes Topiramate.

Regarding early childhood of patients, 97,6 % (n=41) report a good course of pregnancy and 28.6% (n=12) had a caesarian section. Just 2 patients (4.8%) report the presence of febrile seizure during childhood. Most children don't have any retard on child milestone development: 88% (n=37) report fist speech within 3 years old, 93% (n=39) started walk within 18 months old and just 4 patients reported difficulties on the sphincter control after 3 years old (9.5% n=4).

The mean age of mothers is 41 years (M=41.2; SD= 4.4) (table2), the mean age of fathers is 44 years old (M=44.1; SD=5.2). Most caregivers, considering mothers and fathers together, (42.9%; n=36) doesn't have the high school degree and just 19% (n=16) has a highly education. In 38.1% (n=16) of mothers doesn't have the high school diploma, 40.5% (n=17) holds diploma and just 21,4% (n=9) received a superior education as a master's or bachelor's degree. Fathers' profile is similar: 47.6% (n=20) doesn't have a high school diploma, 35.7% (n=15) has the diploma and just 16.7% (n=7) holds a superior education. The majority (92.8% n=39) of caregivers is in a stable relationship and has a job occupation (85,7% n=36 for mothers and 97.6% n=41 for fathers' group). The most common family unit composition is 4 members (66.7% n=28), 6 families (14.3%) are composed by 3 members and 8 families have almost 5 members (19%). In a high percentage of families, 71.4% (n=30), is reported the presence of relatives with chronic health

condition that grave on the family, in particular: 23.8% (n=10) has chronic disease which the most prevalent is Diabetes (n=5); 19% (n=8) has neurologic disease such as Dementia, and finally in 26.2% (n=11) reveals the presence of relatives with metal disease where Depression is the most prevalent.

Sample Characteristics	**Epilepsy N=42**	
	N	%
Male	29	69
female	13	31
Age (Mean; SD)	8.8 ± 2.4	
Age < 7 years old	14	33.3
8< age > 10 years old	16	39
Age > 10,6 years old	12	28.6
Age at diagnosis <5 years old	22	52.4
Rolandic Epilepsy	21	50
Temporal Lobe Epilepsy	12	28.6
Epileptic Dysphasia	5	11.9
Other	4	9.5
Presence of Learning Disorders	4	9.5
Complete seizures control	27	64.3
Not complete seizures control	15	35.7
1-TOLEP (oxcarbazepine)	14	33.3
1-TEGRETOL (carbamazepine)	13	30.9
2-DEPAKIN (valproate)	4	9.5
3-KEPPRA (levetiracetam)	5	11.9
4-TOPAMAX (topiramate)	1	2.4

5-out of therapy	5	11.9
Patient with one treatment	35	83.3
Patient with 2 treatment	2	4.8

Table 1: Epilepsy patients characteristics

Early development	N	%
Regular course of pregnancy	41	97.6
Caesarian section	12	28.6
breastfeeding	28	66.6
Artificial milk feeding	14	33.4
Positive history of febrile seizures	2	4.8
Speech before 3 years old	37	88
Speech after 3 years old	5	11.9
First steps before 18 months	39	93
First steps between 18 and 24 months	2	4.8
First steps after 24 months	1	2.4
Sphincter control after 3 years old	4	9.5

Table 2: Early childhood and developmental information

Demographic information	N	%
Caucasian ethnicity	38	90.5
Arabian ethnicity	4	9.5
Other ethnicity	0	0
Mother age (Mean, SD)	41.2±4.4	
Father age (Mean, SD)	44.1±5.2	
Family members ≤ 3	6	14.3
Family members = 4	28	66.7
Family members > 4	8	19
Parents in a stable relationship	39	92.8
Mother occupation	36	85.7
Father occupation	41	97.6
Mothers' education level < high school diploma	16	38.1
Mothers' education level High school diploma	17	40.5
Mothers' education level bachelor's or master's degree	9	21.4
Father's education level < high school diploma	20	47.5
Father's education level High school diploma	37.5	15
Father's education level bachelor's or master's degree	16.7	7
Parents education level < high school degree	36	42.9

Parents education level High school degree	32	38.1
Parents education level bachelor’s or master's degree	16	19
Relatives with chronic condition	30	71.4

Table 3: Demographic information

The Asthma group:

Asthma patients are enrolled from the pediatric Broncho-pneumology clinic from the Pediatric Unit of University Hospital "Azienda Ospedaliero-Universitario Policlinico" of Modena. We enlist 63 patients from 5 to 16 years old with a diagnosis of Asthma according to Global Initiative for Asthma (GINA 2018). Specific inclusion criteria were: (a) have a diagnosis of asthma (Allergic or Non-allergic), (b) absence of comorbid conditions known to affect cognition, (c) absence of one of other diseases included in the general research. Patients were divided in 4 groups by intensity of pathology; 1 Intermittent; 2 Mild; 3 Moderate; 4 Severe. Treatment was grouped in 4 subgroups based on the dose of inhaled steroid for die: 1) dose pair to 500 mcg/die; 2) dose > 500 mcg/die; 3) use of Leukotriene modifiers; 4) monoclonal antibody therapy (Horak et al., 2016).

Demographic and nosography information (table 4) are collected by clinical reports and by a semi-structured interview with caregivers. Most patients are Caucasian (66.7%, n=42), 15 patients are Arabian (23.8%) and 9.5% (n=6) patients are of Hispanic or African nationality. Males exceed females with 76.2% (n=48) versus 23.8% (n=15); the mean age of participants is 10 years old (M=10,08 ± 2.8) and 73% (n=46) received the diagnosis before 5 years old (mean age of onset is 4,2 ± 2.8). Most children suffer of Moderate Asthma (42.9% n=27) followed by children with Light Asthma (36.5 n=23), 15.9% of cases (n=10) have a diagnosis of Severe Asthma and just 3 patients (4.8%) have Light Intermittent Asthma. 5 children (7.9%) have a diagnosis of Learning Disorder.

All in all, patients have a good control of asthma symptoms (87.3%, n=55) just in 12.7% (n=8) control is absent though under treatment. In 39.7% (n=25) takes fluticasone, 39.7% (n=25) takes fluticasone in association with salmeterol, 23.8% (n=15) takes montelukast; 6.3% (n=4) takes budesonide and formoterol in association; 5 patients (7.9%) are under Omalizumab periodic treatment and 4 patients (6.3%) assume others drug as well as antihistamines. Many patients follow a therapy based on one drug (74.6%, n=47), the 22.2% take daily 2 drugs and just 2 patients received 3 treatments. In this analysis is excluded the use of salbutamol as needed or as premedication. The 88.9% of patients (n=56) has at least one allergy and the 30.2% (n=19) suffers of dermatitis.

About the early childhood course, the most of patients haven't any problem in reaching the main evolutionary stages (table 4). From the data emerges that in 85.7% of cases (n=54) the global course of pregnancy was good, in 14.3% (n=9) records early labor before the 37 weeks of gestation of which: 1 case (1.6%) born at 33 weeks, 5 cases (79%) born at 35 weeks, 2 cases (3.2%) born at 36 weeks, 1 (1.6%) case born at 37 weeks. All in all, 11 cases (17.5%) did caesarian section. May children were breastfed (71.4% n=45) and not reported retard in the stages of development of walking and language: speech before 3 years old in 95.2% (n=60) and first steps before 18 months in 92% (n=58). It was not possible to collect

some information on early childhood for two patients as they were adopted after three years old. Regarding the asthma group we have also looked for some factors deriving from the environment in which the child lives (Table 6) as possible environmental risk factors and evaluated also in subsequent analyzes: in 22.2% (n=17) of cases children live in urban areas while 66.7% (n=42) live in rural one; 23.8% (n=15) is exposed to passive smoking by parents and relatives; 30.2% (n=19) lives in houses with humidity and the 19% (n=12) has animals at home.

As reported in Table 7, mothers' and fathers' mean age is respectively 41 years old (M= 41.5 ± 6.40) and 45.3 (M= 45.3 ± 5.9). 61.9% (n=39) of mothers and 95.2% (n=60) of fathers works regularly. Considering the education level of mother and fathers together, 48.4% (n=61) have a high education level as a master's degree but in 4.7% (n=6) caregivers have an elementary or less education level. The 4.8% (n=3) of mothers report they have no recognized level of education, 27% (n=17) doesn't have high school diploma, the 47.6% (n=30) have obtained the diploma and 20.6% (n=13) have received a higher education level and has a bachelor's or a master's degree. 6.3% (n=4) of fathers doesn't have any school certification, the 33.3% (n=21) obtained the middle school diploma, 49.2% (n=31) have a high school diploma and just 11.1% (n=7) get higher graduations. All in all, 90.5% (n=57) of caregivers are married or in stable relationship. The family composition is various: 15 families (23,8%) are composed by 3 members, 28 (44.4%) have 4 members and 20 (31.7%) are composed at least by 5 members. Also, in asthma group the percentage of relatives with chronic diseases is high: 65.1 % (n=41).

Sample characteristics	Asthma (n=63)	
	N	%
Male	48	76.2
female	15	23.8
Age (Mean; SD)	10.1 ± 2.8	
Age < 7 years old	11	17.5
8< age > 10 years old	25	39.7
Age > 10,6 years old	27	42.9
Age at diagnosis <5 years old	46	73
Intermittent Asthma	3	4.8
Mild Asthma	23	36.5
Moderate Asthma	27	42.9
Severe Asthma	10	15.9
Presence of Learning Disorders	5	7.9
Good asthma crisis control	55	87.3
Not complete asthma crisis control	8	12.7
Fluticasone + salmeterol	25	39.7
fluticasone	25	39.7
montelukast	15	23.8
Omalizumab	5	7.9
Budesonide+ formoterol	4	6.3
Others	4	6.3
Patient with one treatment	47	74.6

Patient with 2 treatments	14	22.2
Patient with 3 treatment	2	3.2
Patient out of therapy	0	0
Dermatitis	19	30.2
Allergies	54	85.7

Table 4: Asthma patients characteristics

Early developmental stages	N	%
Early labor	9	14.3
Good course of pregnancy	54	85.7
Caesarian section	11	17.5
breastfeeding	45	71.5
Artificial milk feeding	18	28.5
Speech before 3 years old	61	96.8
Speech after 3 years old	0	0
First steps before 18 months	58	92.1
First steps between 18 and 24 months	2	3.2
First steps after 24 months	0	0
Sphincter control after 3 years old	2	3.2

Table 5: Early developmental stages in asthma group

Environmental exposure	N	%
Urban areas	14	22.2
Smoke	15	23.8
Humidity	19	30.2
Animals	12	19

Table 6: Important environment factors that can aggravate asthma disease

Demographic information	N	%
Caucasian ethnicity	42	66.7
Arabian ethnicity	15	23.8
Other ethnicity	6	9.5
Mother age (Mean, SD)	41.5 ± 6.40	
Father age (Mean, SD)	45.3 ± 5.9	
Family members ≤ 3	15	23.8
Family members = 4	28	44.4
Family members > 4	20	31.7
Parents in a stable relationship	57	90.5
Mother occupation	39	61.9
Father occupation	60	95.2
Mothers' education level < high school diploma	20	31.7
Mothers' education level High school diploma	30	47.6
Mothers' education level Bacherlor's or Mester's degree	13	20.6
Father's education level < high school diploma	25	39.7
Father's education level High school diploma	31	49.2
Father's education level Bacherlor's or Mester's degree	7	11.1
Parents education level:	6	4.7

< high school degree		
Parents education level: High school degree	45	35.7
Parents education level: Bachelor’s degree or Master's degree	61	48.4
Relatives with chronic condition	41	65.1

Table 7: Demographic information in asthma group

Acute Lymphoblastic Leukemia group

In the study, were selected 12 patients responding to inclusion criteria followed for acute lymphoblastic leukemia by the Oncohematology Unit of the Polyclinic Hospital-University of Modena and currently in remission. The observation times here reported are related to the stop therapy (SFU) and one or two years from the stop therapy (SFU+1). All the patients were treated according to the AIEOP LLA 2000 and AIEOP BFM 2009 protocols and were divided into 3 risk classes: standard, intermediate and high risk, for the modulation of the therapy based on clinical, genetic and immunophenotypically criteria.

Form collected information emerge that boys are double (66.7% n=8) respect girls (33.3% n=4); half of the sample is 10 years and 6 months old (50% n=6) with a mean age of 10 years (M=10.5, DS=5.6). The mean age of pathology's onset is 7 years and 3 months (M=7.3 SD=5.4) and 50% of the children received the diagnosis before 5 years old. The age of onset of males is earlier (M=5,5 years old) than in girls (M= 8 years old). According to the risk protocol, patients are equally distributed in standard (33% n=4), intermediate (33.3% n=4), and high-risk group (33.3% n=4). Just one patient (8.3%) reports pathologies before the diagnosis as allergies and atopy. In 58.3% (n=7) of cases report recurrent diseases after the period of illness as well as problem on concentration (n=2), headache (n=3), epistaxis (n=1) and stomach ache (n=1). depending on the risk protocol, all patients did, in different doses; Methotrexate; Prednisone; Dexamethasone; Vincristine.

About personal anamnesis data, in all of patients report a good pregnancy course and 2 caesarian section (16.7%). In the 50 % of cases report breastfeeding and a normal achievement of the evolutionary stages, just in 2 cases (16.7%) report first steps after 18 months old.

All patients are Caucasian ethnicity and mother mean age is 44.5 years old (M=44.5, SD= 5.6) and father mean age is 47.5 (M=47.5, SD=7.7). All together the most of parents (58.3% n=14) has a degree of education lower than the high school diploma, 33.3% (n=8) has high school diploma and just 2 (8.3%) has a bachelor's or a master's degree. In particular in 50% of mothers doesn't have diploma, t41.7% (n=5) have a high school diploma and just 1 have a master's degree. In fathers' group 66.7% (n=8) doesn't achieve high school diploma, the 25% (n=3) has diploma and 1 (8.3%) has a high grade of instruction. All in all, 100% of father and the 66.7% of mothers works regularly. All caregivers (100% n=12) is married or in a stable relationship. About the composition of the family nucleus most family are composed by 4 members (41.7% n=5) or 5 members (41.7% n=5) and 16.7% (n=2) count for 3 members. In 91.7% (n=11) of families reveals the presence of relatives with chronic diseases (50% n=6) of which 41.7% (n=5) cases of depression.

Sample characteristics	Children with Acute Lymphoblastic Leukemia n=12	
	N	%
Male	8	66.7
female	4	33.3
Age (Mean, SD)	10.5 ± 5.6	
Age < 7 years old	1	8.3
8< age > 10 years old	5	41.7
Age > 10,6 years old	6	50
Age at diagnosis <5 years old	6	50
Standard risk protocol	4	33.3
Intermediate risk protocol	4	33.3
High risk protocol	4	33.3
Presence of Learning Disorders	2	16.6
Problems before the diagnosis	1	8.3
recurrent disorders after the disease	7	58.3

Table 8: ALL general characteristics

Early developmental stages	N	%
Good course of pregnancy	12	100
Caesarian section	2	16.7
breastfeeding	6	50
Artificial milk feeding	6	50
Speech before 3 years old	12	100
Speech after 3 years old	0	0
First steps before 18 months	10	83.3
First steps between 18 and 24 months	2	16.7
First steps after 24 months	0	0
Sphincter control after 3 years old	12	100

Table 9: Early developmental stages in ALL group

Demographic information	N	%
Caucasian ethnicity	12	100
Othe ethnicity	0	0
Mother age (Mean, SD)	44.5± 5.6	
Father age (Mean, SD)	47.5 ± 7.7	
Family members ≤ 3	2	16.7

Family members = 4	5	41.7
Family members > 4	5	41.7
Parents in a stable relationship	12	100
Mother occupation	8	66.7
Father occupation	12	100
Mothers' education level < high school diploma	6	50
Mothers' education level High school diploma	5	41.7
Mothers' education level Bacherlor's or Mester's degree	1	8.3
Father's education level < high school diploma	8	66.7
Father's education level High school diploma	3	25
Father's education level Bacherlor's or Mester's degree	1	8.3
Parents education level < high school degree	14	58.3
Parents education level High school degree	8	33.3

Parents education level bachelor's or master's degree	2	8.3
Relatives with chronic condition	11	91.7

Table 10: Demographic characteristics in ALL group

Patients with solid tumor group

We included 9 patients responding to inclusion criteria followed for solid pediatric cancer by the Oncohematology Unit of the Polyclinic Hospital-University of Modena and currently analyzed at the stop therapy and after 1 year form the first time point. All the patients were treated according to specific pharmacology protocols based on the type of tumor (protocol AIEOP TW2003, protocol AIEOP NB Unresectable 2000, protocol AIEOP TCG2004, protocol AIEOP LH2004, protocol AIEOP LNH97, protocol AIEOP RMS2004, protocol AIEOP LCHIII); according to this, the patients were classified by the Intensity of treatment rating scale (ITRS) (Kazak, 2012) that divides treatments into four operationally defined levels of intensity based on diagnosis, stage, and treatment data from the medical record and completed by pediatric oncology specialists. Level 1 is Least Intensive Treatments, Level 2 is Moderately Intensive Treatments, Level 3 is Very Intensive Treatments and Level 4 is Most Intensive Treatments.

Boys represent the minority of the sample with 22.2% of cases (n=2) respect to girls that are 77.8% (n=7). The mean age of patients is 10.5 years (M=10.5, SD=5.6), the 22.2% (n=2) has less than 7 years old; the 33.3% (n=3) has between 8 and 10 years old and 44.4% (n=4) has more than 10 years and 6 months old. The mean age of onset is 7.3 years old (Mean=7.3, SD=5.4) and about half of the sample received the diagnosis before 5 years (44.4% n=4). All in all, none patients have a diagnosis of learning Disorder. Regarding the grouping by ITRS treatment: just 1 patient (11.1%) is placed in the low level of intensity, 3 patients (33.3%) received a moderately intensive treatment, 3 patients (33.3%) received a very intensive treatment and 2 patients (22.2%) fall in the higher level. The type of treatment varies according to the but most of patients taken Anthracyclines (88.9% n=8) and Vinca alkaloids specially Vincristine (77.8% n=7), Alkylating agents as Phosphamide (66.7% n=6). In 44.4% (n=4) received Corticosteroid and/or Platinum derivates, just one patient (11.1%) taken Methotrexate.

In 66% of cases report a regular pregnancy, only three cases (33.3%) have had a premature birth. More than half of the cases (55.5% n=5) were born by cesarean delivery and were nursed with artificial milk (66.6% n=6). Breastfeeding in 33% of cases. The acquisition of language is altered only in one case (11.1% n=1) in which the first words are recorded after the three years of age. The acquisition of walk is late in a single patient (11.1% n=1) while delays in sphincter control occur in two cases (22.2% n=2).

All participants are Caucasian, Mothers' mean age is 42.5 years old (mean 42.5, SD=9.5) while fathers' mean age is 45.7 (Mean 45.7, SD= 8.4). The 33.3% of mothers (n=3) don't achieve the high school diploma, 44.4% (n=4) has high school diploma and 22.2% (n=2) has a bachelor's or a master's degree. About fathers, 44.4% (n=4) doesn't have a high school degree, 44.4% (n=4) has only a high school diploma and just one (11.1%) have a bachelor's or a master's degree. All in all, the education level of caregivers just 3 parents (16.7%) have a high education level. In 88.9 % report being married or in a stable relationship. The

occupation between man and women is different: the 100% of fathers works regularly while 66.7% of mothers do not. Most of families are composed by 4 members (55.5% n=5), 2 families (22.2%) are composed by 3 members and 2 (22.2%) are composed of 5. Out of nine families, there are 11 relatives with chronic diseases, 4 of whom (44.4%) have illnesses such as anxiety and depression.

Sample Characteristics	**Patients with solid tumor n=9**	
	N	%
Male	2	22.2
female	7	77.8
Age (Mean, SD)	10.5 ± 5.6	
Age < 7 years old	2	22.2
8< age > 10 years old	3	33.3
Age > 10,6 years old	4	44.4
Age at diagnosis <5 years old	4	44.4
Least Intensive Treatments	1	11.1
Moderately Intensive Treatments	3	33.3
Very Intensive Treatments	3	33.3
Most Intensive Treatments	2	22.2
Anthracycline	8	88.9
Vinca alkaloids	7	77.8
Alkylating agents	6	66.7
Corticosteroids	4	44.4
Platinum agents	4	44.4

Radiotherapy	3	33.3
Antimetabolites (Metrotrexate)	1	11.1
Presence of Learning Disorders	0	0

Table 11: Patients characteristics in PWST group

Early developmental stages	N	%
Good course of pregnancy	6	66.7
Caesarian section	5	55.5
breastfeeding	3	33.3
Artificial milk feeding	6	66.7
Speech before 3 years old	9	100
Speech after 3 years old	0	0
First steps before 18 months	8	88.9
First steps between 18 and 24 months	1	11.1
First steps after 24 months	0	0
Sphincter control after 3 years old	2	22.2

Table 12: Early developmental stages in PWST

Demographic information	N	%
Caucasian ethnicity	9	100
Othe ethnicity	0	0
Mother age (Mean, SD)	42.5 ± 9.5	
Father age (Mean, SD)	45.7 ± 8.4	

Family members ≤ 3	2	22.2
Family members = 4	5	55.5
Family members > 4	2	22.2
Parents in a stable relationship	8	88.9
Mother occupation	6	66.7
Father occupation	9	100
Mothers' education level < high school diploma	3	33.3
Mothers' education level High school diploma	4	44.4
Mothers' education level Bacherlor's or Mester's degree	2	22.2
Father's education level < high school diploma	4	44.4
Father's education level High school diploma	4	44.4
Father's education level Bacherlor's or Mester's degree	1	11.1
Parents education level: < high school degree	7	38.8
Parents education level: High school degree	8	44.4
Parents education level: Bachelor's degree or Master's degree	3	16.7
Relatives with chronic condition	11	61.1

Table 13: Demographic characteristics in PWST group

3.2 Measures

Evaluations were carried out at the Unit of Pediatrics of the University Hospital "Azienda Ospedaliero-Universitaria Policlinico" of Modena, during the meetings of the duration about 30-45 minutes, which the first part was dedicated at the collection of some medical history information and the dialogue with children and parents, followed by the second one for the administration of tests and questionnaires. At the end of the meeting, a sample of buccal cells was taken by buccal swab: a totally non-invasive modality and therefore suitable for use in the pediatric population. Different precautions to ensure non-contamination of the sample have been put in place, such as wait at least 30 minutes after the last meal and rinse the oral cavity before sampling. The tampon was applied on the mucosa of the inner surface of the cheek, performing rubbing from top to bottom and repeating the operation 20 times per side, in order to withdraw a sample sufficient to guarantee DNA extraction. The tampon was therefore placed in a sterile container and signed with the patient's identification code, to ensure its unique attribution and complete anonymity. The sample was and stored at a temperature of 4 °C by using ice waiting for transfer to the laboratory, where storage at -20 °C it has allowed the conservation pending the extraction of DNA and subsequent analysis by PCR (Polymerase Chain Reaction) method.

The following test and questionnaires were illustrated and submitted to the parents:

1. Behavior Rating Inventory of Executive Function (BRIEF) and Pre-school version (BRIEF-P)

2. Strengths and Difficulties Questionnaire (SDQ 4-16)

3. Psychological Assessment Tool (PAT)

Assessment of executive functions

Executive Functioning (EF) was tested by indirect assessment (Kavanaugh et al., 2015; Nichols et al., 2015). Scores were standardized based on a group of typically developing children adjusted for gender and age. Indirect assessment of EF was evaluated using the Behavior Rating Inventory of Executive Function parent version (BRIEF and BRIEF-P). This dual mode allows us to check different areas of executive functions and compare the performances of the children with respect to what the parents report.

1. Behavior Rating Inventory of Executive Function (BRIEF)

Recipients: Parents of children from 6 to 18 years old

Execution time: 5-10 minutes

Evaluation of: Inhibition, Flexibility, Self-control, Working memory, Planification ability, Sustained attention

Parents completed the parent-report version of the Behavior Rating Inventory of Executive Functioning, Parent-Report (BRIEF). The BRIEF is a short questionnaire of 86 items that assesses aspects of executive functioning based on a child's or adolescent's (from 6 to 18 years old) observable behaviors and abilities in daily life. It is a norm-referenced rating inventory that assesses EF in relation to the performance of everyday tasks, across multiple domains. It includes three higher order index scores of executive functions, as well as several lower order functions. The higher order domains include the **Global Executive Composite score** (GEC); **the Behavioral Regulation Index** (BRI), which is the superordinate scale encompassing the Inhibition, Shift, and Emotional Control subscales; as well as the **Metacognition Index** (MI), which includes the subscales Initiation, Working Memory, Planning and Organization, Organization of Materials, and Self-Monitoring. The BRIEF has excellent psychometric properties (Gioia et al., 2013; Gioia et al., 2014) and has proven to be sensitive to executive function deficits in children and adolescents with chronic or severe diseases in several studies (MacAllister et al., 2012; Kavanaugh et al., 2015; Triplett et al., 2015; Maiman et al., 2017). The 8 scales that measure different aspects of executive functioning are:

1. Inhibit: measures the inhibitory control of the child, or the ability to control his own impulses and stop behavior no longer appropriate at a given time.

2. Shift: measures the child's ability to move quickly from a task, a problem or activity to another based on requests.

3. Flexibility is required in order to do this cognitive and skill in directing and focusing attention from one situation to another. This function is of fundamental importance in solving problems; if it is altered, the child will tend to persevere in attempts to address a problem using the same approach, despite this proves to be ineffective.

3. Emotional control: measures the child's ability to modulate emotional responses. One poor emotional control can manifest itself both as emotional and as emotional lability emotional explosiveness; the child may also present exaggerated or inadequate reactions aged in response to minor events.

4. Working memory: working memory allows to keep information available useful for completing a task for as long as necessary. It is therefore essential to complete multi-step activities, complete sequences of actions or calculate and follow complex instructions. Children with difficulty in this area show difficulty in remembering even the simplest things and often forgetting what they owe take when asked to retrieve an item,

5. Plan and Organize: assess the child's ability to cope with immediate or future demands. The "planning" component measures the child's ability to anticipate future events, ask themselves

objectives and determine the method and the means to achieve them. The component "Organization" refers instead to the ability to obtain and order material and adequate information from this perspective. Children with difficulty in this domain tend to approach the problems in a casual way, often being overwhelmed by the excessive load of information, and often delay in responding to external requests.

6. Initiate: measures the child's ability to start an activity, generate an idea or a problem-solving strategy independently and without external stimuli. Children with problems that involve this function frequently show difficulties in starting school tasks or activities in general and need encouragement from external people.

7. Organization of Materials: assess the regularity and order in work, play and spaces dedicated to the child (such as desks, wardrobes, ...) and the ability to keep their objects clean, tidy and organized. Children with difficulties in this domain often present problems in completing tasks and activities due to the unavailability of the instruments in their possession.

8. Monitor: measures the child's tendency to control the work done and to evaluate the changes that his actions or behaviors cause in the environment and in the people around them. Problems concerning this ability are manifested by errors of distraction, failure to verify the tasks performed in the end, inability to predict the consequences of their behavior or actions.

The 8 clinical scales are combined to form two partial indices:

1. BRI Index (Behavioral Regulation Index): assesses the child's ability to modulate emotions and behaviors through appropriate inhibitory control.

2. MI Index (Metacognition Index): measures the child's ability to initiate, plan, organize and sustain a problem-solving activity over time.

The final score (GEC) incorporates the eight scales and is used as an overall assessment of the child's executive functions.

Age norm-referenced T scores (M = 50; SD = 10), the scores obtained in the different sub-scales make it possible to classify patients into 3 categories:

• values <50 indicate patients in a normal range;

• values between 50 and 65 indicate the presence of subclinical difficulties and place patients in the borderline group;

• values> 65 indicate the presence of clinically significant problems and place the patient in the critical group; values above 65 do not necessarily indicate the presence of a disorder of executive functions: each value should be contextualized and evaluated in relation to the patient's clinic.

2. Behaviour Rating Inventory of Executive Function pre-school version (BRIEF-P)

Recipients: Parents of children from 2 to 5 years old

Execution time: 5-10 minutes

Evaluation of: Inhibition, Flexibility, Self-control, Emergent Metacognition, Working memory, Planification ability, Sustained Attention

BRIEF-P is a questionnaire addressed to parents of preschoolers consisting of 63 questions that allow to evaluate the executive functions (Gioia et al.,2014); the teacher version is also available. It presents differences with respect to the BRIEF, lacks some scales like Initiate, Organization of Materials and Monitor. It is also composed by a Global Executive Composite score (GEC), that is an overarching summary score of all scales; the inclusion of scores obtained in Inhibit and Emotional Control subscales generate the Inhibitory Self-Control Index (ISCI), that evaluate the children skill to moderate his emotion and reactions. Flexibility Index (FI) is composed of the Shift and Emotional Control subscales measure the children ability to adaptive to situations and Emergent Metacognition Index (EMI), which includes the Working Memory with Plan and Organize subscales, assesses the child's ability to use the information obtained to guide his / her behavior and choices to effectively solve a problem. Age norm-referenced T scores (Mean=50; SD=10) are computed, whereby higher T scores indicate more difficulties and T score ≥65 indicates high clinical significance (Maiman et al., 2017). The levels of impairment are the same of BRIEF.

Assessment of Emotional Competence and Behavior

Strengths and Difficulties Questionnaire (SDQ 4-16)

Recipients: parents of children over the age of 4 to 16

Time of administration: 5-10 minutes

Evaluation of: emotionality, pair relationship, Hyperactivity, behavior, social competence

The SDQ is a self-compiling questionnaire (Goodman et al., 1998; Goodman et al.,2010) and is a valuable screening tool to assess the presence or risk of developing emotional or behavioral disorders. Is one of the most widely used screening instruments for these purposes. The SDQ consists of 25 items equally divided

across five scales measuring emotional symptoms, conduct problems, hyperactivity-inattention, peer problems, and prosocial behavior (Stone at al., 2015). The questionnaire explores 5 domains:

1. Emotional problems

2. Conduct problems

3. Hyperactivity and inattention

4. Peer relationship problems

5. Prosocial behaviours (Excluded from Total Difficulties score)

The self-report version of the SDQ is suitable for young people aged between 11-16 however this varies depending on the young person's level of understanding and literacy. At each domain corresponding a score that places the patient in three possible levels: *Normal, Borderline* and *Clinic* (Table 14). The sum of the first 4 domains generate a final score called *Total Difficulties score* (www.sdqinfo.com). In both tests the version equipped with "Impact Supplement" was used with 5 questions on the total distress and on perceived social difficulties.

SDQ 4-16	**Normal**	**Borderline**	**Clinic**
Total Difficulties score	0-13	14-16	17-40
Emotional problems	0-3	4	5-10
Conduct problems	0-2	3	4-10
Hyperactivity and inattention	0-5	6	7-10
Peer relationship problems	0-2	3	4-10
Prosocial behaviours	6-10	5	0-4

Table 14: normative data of SDQ version compiled by parents

Assessment of Psychosocial Risk

1. Psychological Assessment Tool (PAT 2.0)

Recipients: families of children with chronic or severe diseases

Time of administration: 5-10 minutes

Evaluation of: Profile the psychosocial risk

The Psychosocial Assessment Tool, (PAT), is a brief parent report screener of psychosocial risk in pediatric health. Using a social ecological framework, PAT allows for identification of a family's areas of risk and resiliency across multiple domains (e.g., family structure and resources, family problems, social support, child problems, acute stress, sibling problems). PAT is based on the Pediatric Psychosocial Preventative Health Model (PPPHM; Kazak, 2006) which provides a tri-level determination of family risk (Universal, Targeted, Clinical) based on the total PAT score. Level of risk has implications for treatment recommendations to support family adaptation and address problems (Pai et al., 2008; Kazak, A. et al., 2006). The rationale of the psychosocial investigation derives from the need to consider the environmental context in which the child is inserted, not only the purely medical aspect. Family life may be profoundly affected by the diagnosis of chronic or severe illness in a young child even if this is influenced by the gravity. Chronic or severe illness such as asthma, epilepsy or pediatric cancer generates challenges in the child, including coping with the symptoms, such as pain or shortness of breath, and generates specific demands on the parents. These demands include illness and treatment monitoring, care, and ensuring financial stability. This tool permit to us to recognize the impact of different types of stressor, from social status, child and brother's characteristics to family's beliefs may constitute an important piece of assessment that can influence the prognosis and the course of the developmental trajectory of the child with chronic disease.

The test provides a global framework of the psychosocial context and consists of 7 different subscales:

1. family structure and resources;

2. social support;

3. child problems;

4. problems of relatives;

5. family problems;

6. reaction of parents to stress;

7. beliefs of the family.

Within each subclass, each item is evaluated in a dichotomous manner (risk = 1, not risk = 0). The total score of the PAT is associated significantly with situations of acute distress for parents, problems of behavior of the child and situations of family conflict: this makes it, therefore, an excellent screening tool. The following cutoffs categorize (Kazak et al., 2011; Kazak et al., 2012) family psychosocial risk scores: (a) Scores <1 fall in the universal risk category. Families that score in the universal category tend to be resilient and have multiple psychosocial resources. As a result, these families may experience transient distress in

response to the challenges of a chronic disease. In most pediatric chronic illness populations, most of families will score in the universal category (Kazak et al., 2012); (b) Scores between 1 and 2 fall in the targeted category. Families that score in the targeted category are typically experiencing acute psychosocial distress in response to the illnesses and have some risk factors (Pai et al., 2011, 2014). (c) Scores >2 are considered to fall in the clinical risk category. A small number of families tend to score in the clinical risk category. These families are typically experiencing high levels of distress and multiple psychosocial risk factors (Pai et al., 2011, 2014).

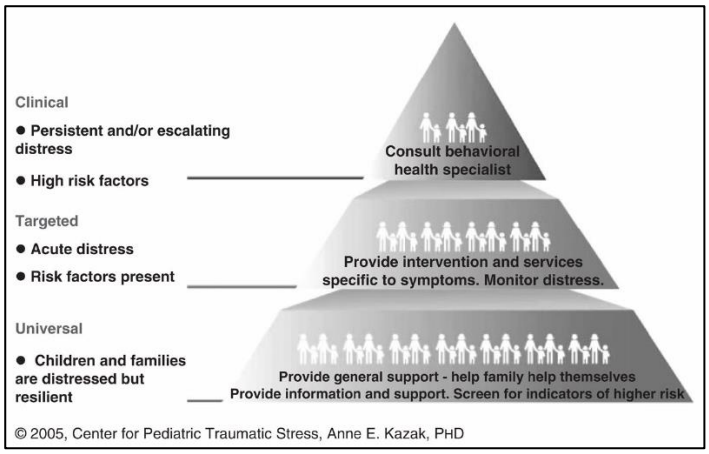


Figure 6: General representation of PAT risk distribution (Kazak 2005)

Assessment of Single Nucleotide Polymorphisms

The analysis was performed at the Department of Biomedical, Metabolic and Neuroscience Sciences of Modena, 3rd floor, Section of Pharmacology (supervisor Prof. Tascedda). DNA has been obtained starting from the buccal cells using the High Pure PCR Template Preparation Kit (Roche Applied Science, Germany). Once the DNA was extracted it was stored at -20 ° C. The use of buccal swabs for the detection of polymorphisms is a technique that lends itself well to the pediatric population as extremely fast, accurate, well tolerated and above all non-invasive. DNA analysis was performed by PCR-restriction fragment length polymorphism assays. The PCRs were carried out in SimplyAMP^{TM} thermal cycler (Thermo Scientific, USA) in a reaction volume of 50 µl containing 100 ng of genomic DNA, 0.3 mM of each primer, 0.2 mM of each dNTP, 1.5mM of MgCl2 solution and 1.25 units GoTaq® DNA polymerase (Promega). Below are the different genotyping protocols.

Methyl-tetra-hydro-folate reductase (MTHFR) C677T genotyping protocol

The primers (forward: 5’ GAAGGTGCAAGATCAGAG 3’; reverse: :5’ GAAGGTGCAAGATCAGAG 3’) were used to amplify a 232 bp genomic DNA fragment (Soleimani et al., 2016) that contains the polymorphic site

recognized by the restriction enzyme *HinfI* (*SibEnzyme*). The PCR reaction mixture, after a denaturation of 2 minutes at 95°C, was amplified by 35 PCR cycles: 95 ° C for 30 seconds, 58 °C for 30 seconds, 72 ° C for 1 minute, 72 ° C for 5 minutes, followed by an extension to 72 ° C for 5 minutes. After amplification 10 ul of PCR mixture was incubated with 20U of the restriction enzyme *HinfI* (SibEnzyme), in a final volume of reaction of 50 ul. The incubation was performed Over Night at 37 ° C, followed by the enzyme inactivation at 80 °C for 20 minutes. The electrophoretic run was carried out by 2.5% Agarose gel in TBE 1X and stained with DNA stain (Atlas). The homozygotes (CC) generate fragments of 199 bp and of 33 bp, while the homozygotes TT fragments weight 165 bp, 34 bp and 33 bp; heterozygotes (CT) generate fragments of 199 bp, 165 bp, 34bp and 33 bp.

Catecol-O-methyl-transferase (COMT) Val158Met genotyping protocol

The COMT Val158Met polymorphism was determined by the following PCR primers: forward 5'-TCGTGGACGCCGTGATTCAGG-3' and reverse 5'-AGGTCTGACAACGGGTCAGGC-3' (as previously described in Tahara et al., 2009), used to amplify a 217 bp fragment. The reaction mixture was denatured at a temperature of 95 °C for 2 minutes, and then amplified by 35 PCR cycles: 95 °C for 30 seconds, 68 °C for 30 seconds, 72 °C for 1 minute, 72 °C for 5 minutes, followed by an extension to 72 °C for 5 minutes. The PCR products of COMT contained the polymorphic site recognized by 10U *NlaIII* restriction enzyme (New England Biolabs). Homozygotes for COMT158Val generated fragments of 136 and 81bp, heterozygotes produced 136, 96, 81, and 40 bp fragments and homozygotes for COMT158Met generated 96, 81, and 40 bp fragments. Genotypes were analyzed after electrophoretic run on 3% agarose gel in TBE 1X with DNA stain (Atlas).

Brain Derived Neurotrophic Factor (BDNF) Val66Met genotyping protocol

The primers (forward: 5 'CCCCATGAAAGAAGCAAACA 3'and reverse: 3' TTTGTCTGCTGCCGTTACC 5 ') were used to amplify, by PCR, a 402 bp genomic DNA fragment containing the polymorphic site recognized by the restriction enzyme *NlaIII* (New England Biolabs) (Harris et al.,2010). The reaction mixture at first was denatured at a temperature of 95 °C for 2 minutes, and then amplified by 35 PCR cycles: denaturation 30 seconds at 95°C, annealing 30 seconds at 60 °C, extension 1 minute at 72 °C, followed by final extension to 72 °C for 5 minutes. The PCR mixture were incubated Over Night at 37 °C with 10 units of the enzyme *NlaIII*, in a final volume of 20 ul, followed by enzyme inactivation for 20 minutes at 65 °C. The PCR products were separated by 2% Agarose gel electrophoresis in TBE buffer 1X. Val homozygotes generate a 245 bp DNA fragment, while the Met homozygotes generate a 168 bp fragment; the heterozygotes generate fragments respectively of 245 and 168 bp.

Serotonin-transporter-linked polymorphic region (5-HTT LPR) genotyping protocol

DNA samples were amplified by PCR, using the forward primer (5'-ATGCCAGCACCTAACCCCTAATGT-3') and reverse primer (5'-GGAACCGCAAGGTGGGCGGGA-3') (Richardson et al.,2008). The PCR protocol involved preheating the samples at 95°C for 2 minutes, followed by 35 cycles of denaturation 30 seconds at 95°C, annealing 30 seconds at 64 °C, extension 1 minute at 72 °C, followed by a final extension to 72 °C for 5 minutes. Long and Short alleles were resolved on a 2% agarose gel with expected band at 375 bp for short allele and 419 bp for the long one.

FKBP5 binding protein 5 gene (FKBP5 rs3800373, rs1360780) genotyping protocol

FKBP5 was genotyped using assays for SNPs rs3800373, rs1360780 (Scheuer et al., 2015) pre-designed TaqMan SNP genotyping assays (Applied biosystems by Thermo Fisher Scientific, USA; assay number: C_8852038_10 and C_27489960_10) with amplification in Lightcycler 480 version 1.5 (Roche Applied Science, Germany) analyzing the endpoint fluorescence. PCR thermal cycling conditions were as follows: one cycle at 95 °C for 10 min followed by 40 cycles at 95°C for 15 s and 60 _C for 1 min. The genotyping protocol was performed according to our previous studies (Fujii et al.,2012). If a genotype for either gene or SNP could not be determined after the first run, then it was repeated up to two times. The call rates for the four FKBP5 SNPs ranged from 99.8% to 100%.

3.3 Statistical and Network Analysis

Statistical analysis

Chi-square (for categorical variables) and Mann-Whitney U (for continuous variables) tests were conducted to examine differences groups. The primary outcomes are neurobehavioral function and neurocognitive performance, as measured by the parent-rated BRIEF and SDQ and direct neurocognitive tests, respectively. For the BRIEF, raw scores for each individual domain, as well as global indices, were transformed into age- and gender- adjusted T-scores based on representative normative data provided by the BRIEF manual. Effort was taken by examiners to ensure survivors' parents completed every item on the BRIEF. Missing responses within the BRIEF were handled according to the test manual: missing responses for one or two items that contribute to a scale raw score were assigned a score of 1 when calculating the scale raw score. Neurocognitive scores were transformed into the respective standard scores in accordance to directions provided by the test manuals. Comparison of neurobehavioral and neurocognitive measures between survivors and the normative sample was conducted using one-sample t-test, adjusted for false discovery rate (FDR). Generalized linear modeling (GLM) was employed to examine the association between various polymorphisms and survivors' neurobehavioral and neurocognitive standard scores.

Statistical models were adjusted for age of diagnosis and years of illness, as these variables are known to be associated with functional outcomes. Comparison between survivors with versus without risk alleles of 5HTTLPR, COMT MTHFR and BDNF was conducted using GLM, adjusting for years since diagnosis. Unadjusted BRIEF raw scores were employed to avoid problems of multicollinearity as T-scores are age-adjusted. Statistical significance was defined as a P value of less than 0·050 and all statistical tests were two-sided. All analyses were conducted in SPSS version25 (SPSS, Chicago). Fisher's exact test was performed for relation of univariate analysis and binomial logistic regression analysis was used to analyze the influence of polymorphisms on neurocognitive and emotional function.

Developmental change was investigated in the organizational conformations of cognitive and emotional networks

Network analysis

In order to describe the interrelationship between different cognitive and behavioral domains, a correlation matrix was computed between the performance of different tests, separately for both groups, i.e., PWST and ALL. We evaluated the correlation coefficient between each possible pair of tests. A weighted adjacency matrix was then constructed for all groups where each link represented the correlation coefficient between the tests in the corresponding row and column.

Visual representation of network structure

Two-dimensional graphs were reconstructed to define the overall structure of cognition in each group. The data were exported to the software Gephi (http://gephi.github.io/) and displayed using a Force Atlas algorithm (attraction strength = 10, repulsion strength = 100, gravity = 30). To preserve the most important relationships and to improve visualization of the network structure, graphs were reconstructed using only links above the 70% percentile of weight in each group, i.e., links below the 70% percentile were given weight = 0, while the remaining links maintained their original weights.

Using Gephi, the community structure of each network was calculated, and each node was coded in accordance with module participation, whereby nodes with a strong interrelationship were arranged within a module and nodes with a relative lower relationship were arranged outside the module.

CHAPTER 4: Results

We have considered 4 groups of pathologies among the most relevant in the pediatric field for the effects on the central nervous system (CNS). Thinking about epilepsy, the difficulties related to neurocognitive functions derive from a direct involvement of the CNS which is in some sense malfunctioning. When the disease manifests, however, we do not know exactly how long this “malfunction” started to influence the brain function and becomes a stable condition in the child's development. Thinking about children with asthma, which is one of the most common diseases in the pediatric age, we see cognitive problems that reflect the effect of the symptoms of the pathology due to lack of sleep, frequent hypoxia, the involvement of the HPA axis, for treatment. Thinking about ALL and solid tumors, these are not chronic but severe diseases that involve CNS for their exposition to neurotoxic factors. Together, have in common the compromising communication of CNS. We do not verify directly that, but we see the neurocognitive and executive function that are the real skills requiring a good communication between CNS areas as well as mood and behavior. All these phenomena are influenced by the child’s genes and by the interaction between genetic, developmental and environmental factors**.** In table 15 is reported the polymorphism distribution in all samples, following data analyzed for each pathology.

First, we analyze the executive function, behavioral/emotional domain for each pathology; after we report the change over time in executive and behavior/emotional domains; we consider which factor may be predictors on these domains in each pathology and finally we analyze the role of single nucleotide polymorphism studied domain and over time. To better understand the connections between functions in each disease we applied finally a network analysis approach.

GENOTYPE		EPILEPSY (n=41)		ASTHMA (n=63)		CANCER (n=20)		TOTAL (n=124)	
		N	%	N	%	N	%	N	%
5HTT-LPR	*LL*	16	*39*	14	*23*	5	*25*	35	*28*
	SL	19	*46.3*	39	*62*	12	*60*	70	*56*
	SS	6	*14.6*	10	*16*	3	*15*	19	*15.3*
COMT Val158Met	*VV*	13	*31.7*	18	*28.6*	5	*25*	36	*29*
	VM	22	*53.7*	35	*55.5*	9	*45*	66	*53*
	MM	6	*14.6*	10	*15.9*	6	*30*	22	*18*
BDNF Val66Met	*VV*	27	*65.9*	41	*65.1*	11	*55*	79	*63.7*
	VM	13	*31.7*	20	*31.7*	9	*45*	42	*33.9*
	MM	1	*2.4*	2	*3.2*	0	*0*	3	*2.4*
MTHFR C677T	*CC*	16	*39*	20	*31.7*	10	*50*	46	*37*
	CT	23	*56.1*	27	*42.9*	7	*35*	57	*46*
	TT	2	*4.9*	16	*25.4*	3	*15*	21	*16.9*
FKBP5 rs1360780 C/T	*CC*	18	*43.9*	33	*52.4*	13	*65*	64	*51.6*
	CT	17	*41.5*	23	*36.5*	7	*35*	47	*37.9*
	TT	6	*14.6*	7	*11.1*	0	*0*	15	*12*
FKBP5 rs3800373 A/C	*AA*	18	*43.9*	32	*50.8*	13	*65*	63	*50.8*
	AC	19	*46.3*	15	*23.8*	7	*35*	41	*65.1*
	CC	4	*9.8*	13	*20.6*	0	*0*	17	*13.7*

Table 15: Single nucleotide polymorphism distribution

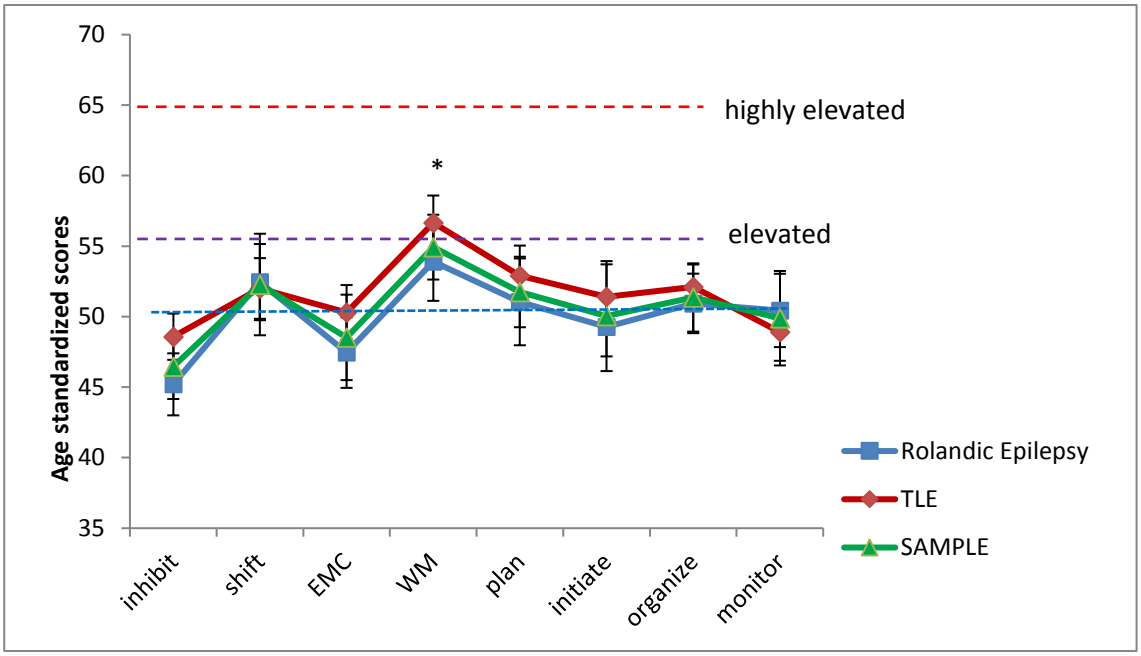
4.1 Patients with Epilepsy

OBJECT 1: cumulative prevalence of adverse neurocognitive, emotional/behavioral and psychosocial outcomes by exposure and diagnosis.

The children recruited in this study are routinely seen by pediatric neurologists in our clinic but not seen for not strictly epilepsy related problems. Executive function and behavioral difficulties associated with epilepsy were assessed using various tests. The data reported here include those from 3 questionnaires, namely The BRIEF, SDQ and the child related sub domain of the PAT. To comprehensively evaluate cognition and emotion, these tests were administered to 42 patients which measures reflect performance across the domains of executive function, behavior and emotion and psychosocial function of the family. Test scores were standardized according to age and gender norms where available. No main effect of age nor gender was observed, although the proportion of boys was higher in this sample. Note that using age and gender would have created collinearity because the scores were already adjusted for age and gender. Instead, the number of years the child suffered from epilepsy had a main effect (p<,000) and correlated significantly with some domains of executive function: with an increase in the years of illness, the domains inhibit ($p<0.05$), and the capacity to organize ones thought and actions ($p<0.05$) were more compromised. Thus, we considered the group as a whole in order to establish whether other factors, such as disease related or environmental factors were linked to or could predict compromised cognitive and behavioral outcome in this group of patients with years of illness as a covariate.

Executive Function in children with epilepsy

Children with epilepsy as a group did not differ on the subscales of the BRIEF with respect to normative data provided by the test manual. Only the domain of executive function Working Memory (WM) (t= 3,510, p<001, 95%CI 2,59-9,64) was compromised .



RE v TLE								
RE v NORM								
TLE v NORM								

Figure 7: Comparison epilepsy patients gruoups tested by BRIEF test. No mainly differences between diagnosis, the function more compromised is Working Memory and TLE group performes worse.

As formerly explained, executive functions are cognitive skills that are important for regulating behavior and for achieving goals. Our group of patients contained two main categories of patients with considerable heterogeneity across children, even within diagnostic categories. Therefore, we considered whether type of diagnosis affected EF, using two clusters of patients and hypothesized that distinct types of diagnosis, rolandic epilepsy or temporal lobe epilepsy, result in shared profiles of EF-related difficulties and the similarities in their behavioral problems, which might be related to specific patterns of brain organization that distinguish these groups. This then allowed us to relate differences in disease and treatment related factors and genetic factors. Diagnosis was compared on questionnaire measures of behavioral problems linked to everyday EF difficulties (BRIEF) and behavioral emotional problems (SDQ). A comparison of these measures indicated no oversall main effect of diagnosis. However for the BRIEF, children with RE (rolandic epilepsy) had more problems with working memory. Children with TLE (temporal lobe epilepsy) showed a similar pattern with WM slightly worse but remaining in the elevated performing range.

Behavioral and emotional problems in children with epilepsy

No overall main effect wasf ound regarding behavioral and emotional difficulties. Children diagnosed with RE and TLE did not differ from the norm nor from each other. This suggests that as a group children with epilepsy do not suffer from more emotional and behavioral problems than the norm.

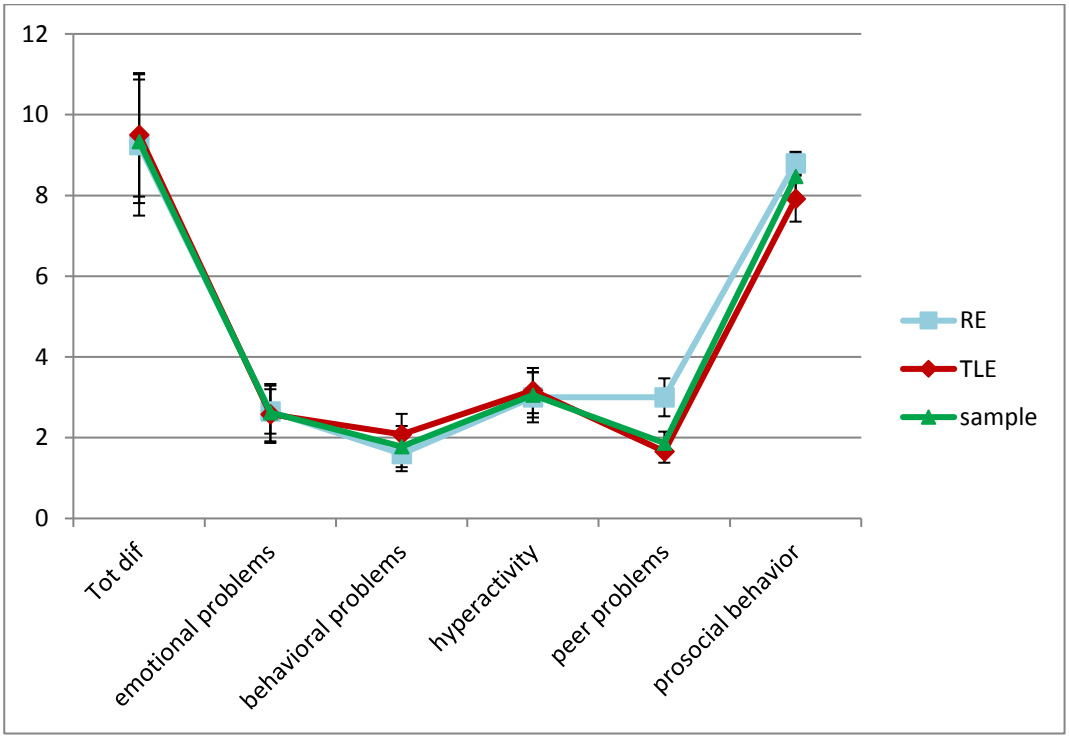


Figure 8: No differences beetween the two diagnosis group analyzed with SDQ

Executive function in patients diagnosed with epilepsy: changes over time

Patients in TLE group display worse changes over time specially on emotional control competence and monitor ability (Figure 9). Altogether, one year later do not have positive changes.

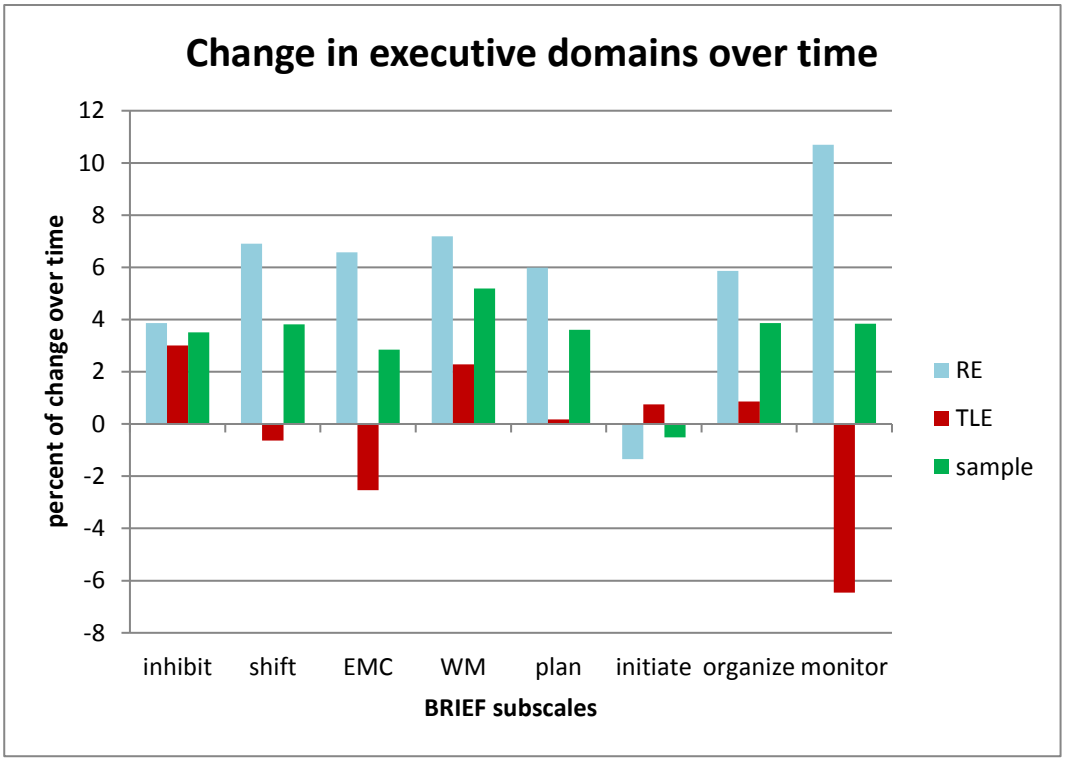


Figure 9: Change in EF domain over time, patients in TLE group present worse changes respect other group.

Behavior and emotional domains in patients diagnosed with epilepsy: changes over time

Children with TLE and children with RE display different emotional and behavior difficulties with major concern on emotional problem where RE get worse over time. Other functions as prosocial behavior and peer problems do not display changes (Figure 10).

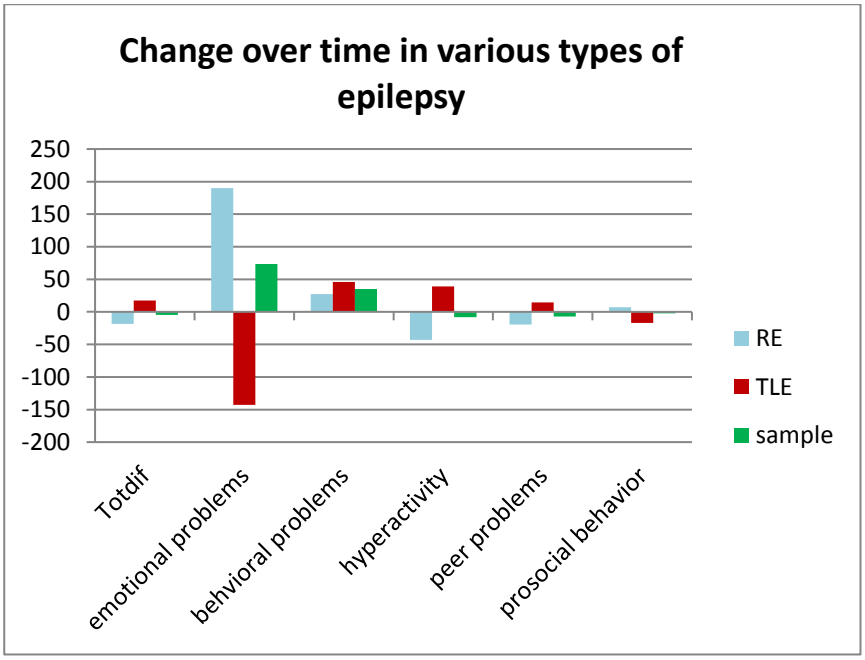


Figure 10: change over time in emotional and behavior domains tested with SDQ

OBJECT 2: Possible patient related predictive factors of neurocognitive deficits in children with epilepsy.

Disease related predictors: treatment, crisis control, adolescent age

Treatment did not affect EF nor did treatment interfer with behavior and emotional capacities. Crises control, that is having not more than one crises per year, had a significant main effect on EF domains. ($F(8,21)=2,499$ $p< 0.05$ Wilks $\Lambda = .523$), as well as being an adolescent or not($F(8,21)=3,179$ $p< 0.05$ Wilks $\Lambda = .452$) . Futhermore there was a significantl interaction between the control of crise and adolescent age, with crises being more diabling in adolescent patients than in children ($F(8,21)=3,816$ $p< 0.01$ Wilks $\Lambda = .408$). Poor crises control significantly affected domains such as inhibt, shift, working memory, plan, and initiate ($p<0.05$) of BRIEF test, whereas adolescent age significantly compromised the domains, inhibit, shift, plan, and initiate. Together they comprimised all domains of executive function.

Crises control

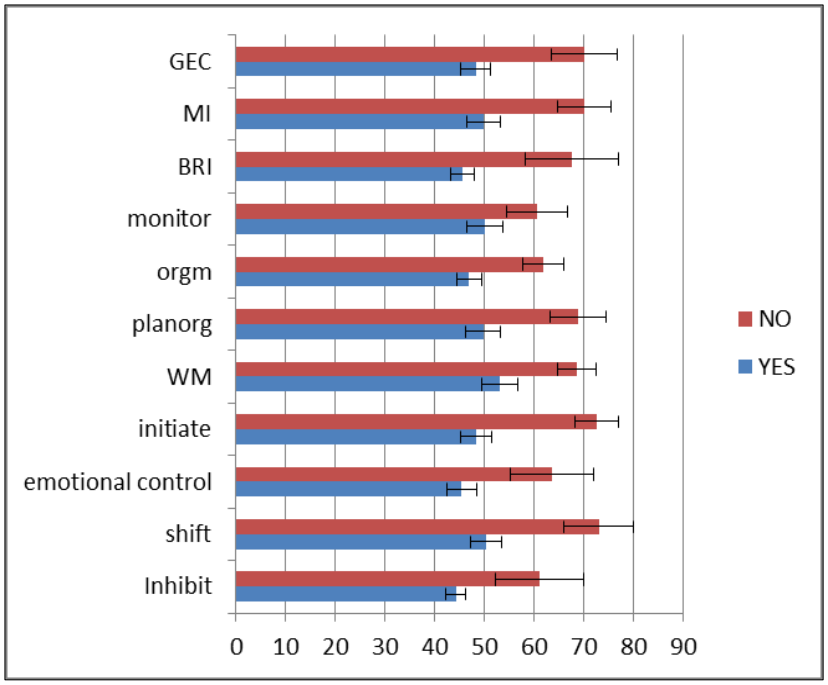


Figure 11: Children who don't achieve a complete control of seizures with the treatment performe worse than others, in some cases performance reaches scores that are in the clinical range.

With respect to behavioral and emotional domains, no main effect for crises control was observed ($F(5,28)=1,384$ $p> 0.05$ Wilks $\Lambda = .808$), only a main effect for adolescent age($F(5,28)=2,842$ $p< 0.05$ Wilks $\Lambda = .523$). However, a significant interaction was presen($F(5,28)=4,109$ $p< 0.01$ Wilks $\Lambda = .577$). In the absence of good control, children displayed significantly compromised function in all domains of of behavioral and emotional function, especially in inteaction with being of adolescent age or not, where adolescents were more compromised .

OBJECT 3: Genes as potential predictors of the severity of deficits in children with epilepsy

Effects of MTHFR C677T and COMT Val158Met polymorphisms with executive function

The targeted genetic variants were selected a priori on the basis of the literature and/or hypothesized associations with executive function and attention. Because neurocognitive impairment in children with epilepsy, and especially EF, is potentially mediated by genes regulating neurotransmitter availability and by folate depletion and related metabolic processes, we included SNPs of genes known to alter folate concentrations (MTHFR) as well as dopamine availability (COMT). Overall, when controlled for age, allelic expression of the MTHFR gene influenced EF in everyday life functioning, $F(8,22)=2,573$, $p< 0.05$ Wilks $\Lambda = .517$, partial $\eta 2$ .483 and power .794. Patients with at least one T allele of the MTHFR gene displayed significant difficulties especially with respect to WM $F(1,29)= 5,921$ $p<0.05$ partial $\eta 2= .170$ with power .655. Plan and Organize and Shift (mental flexibility) $p<0.05$. Allelic variation of the COMT gene was not predictive of EF overall nor did COMT allelic expression differentially affects various domains of the BRIEF. Thus, only variation in the allelic expression of the MTHFR but not COMT moderated working memory related to everyday tasks as reported by the parents.

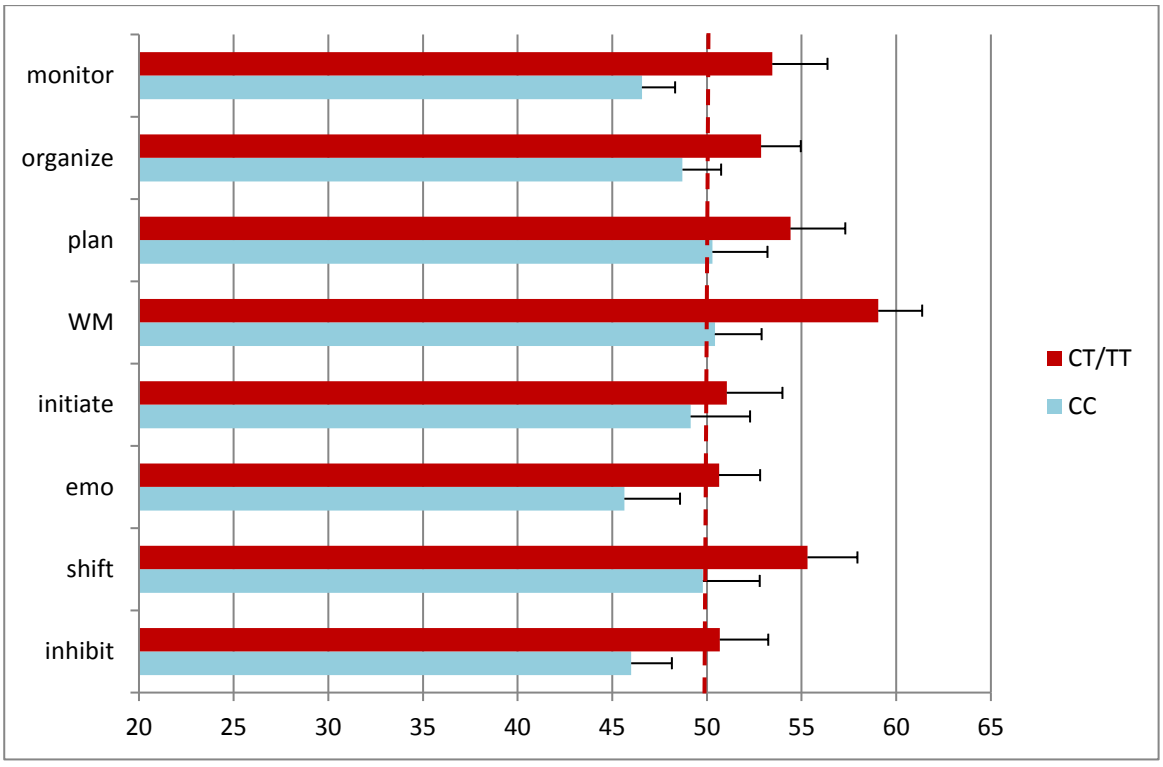
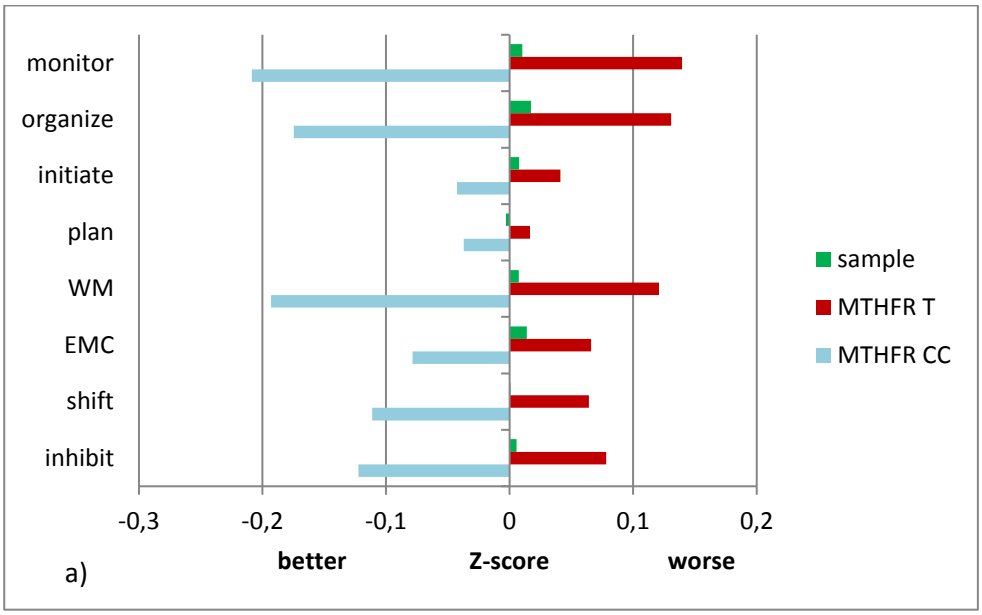


Figure 12: Tested with BRIEF, children carriers of T allele of MTHFR gene are worse performance in all executive function domains especially on shift, working memory, plan and monitor that are above the mean.

We analyzed the relationship between polymorphisms in folate-related genes and effects on executive function, behavioral and emotional problems. We examined alleles of the methylenetetrahydrofolate reductase or MTHFR gene that vary enzyme activity, altering the availability of the methyl donor and thus changing the efficiency of methylation. We hypothesized

that alleles of the MTHFR gene would influence behavior and executive functioning tasks. The MTHFR TT or CT genotype appeared to be correlated with more behavioral toxicity and T carriers performed worse on all scales (figure 13 a,b). COMT alone did not affect EF in a substantial way but seems to have an addictive effect associated with MTHFR (figure 14).



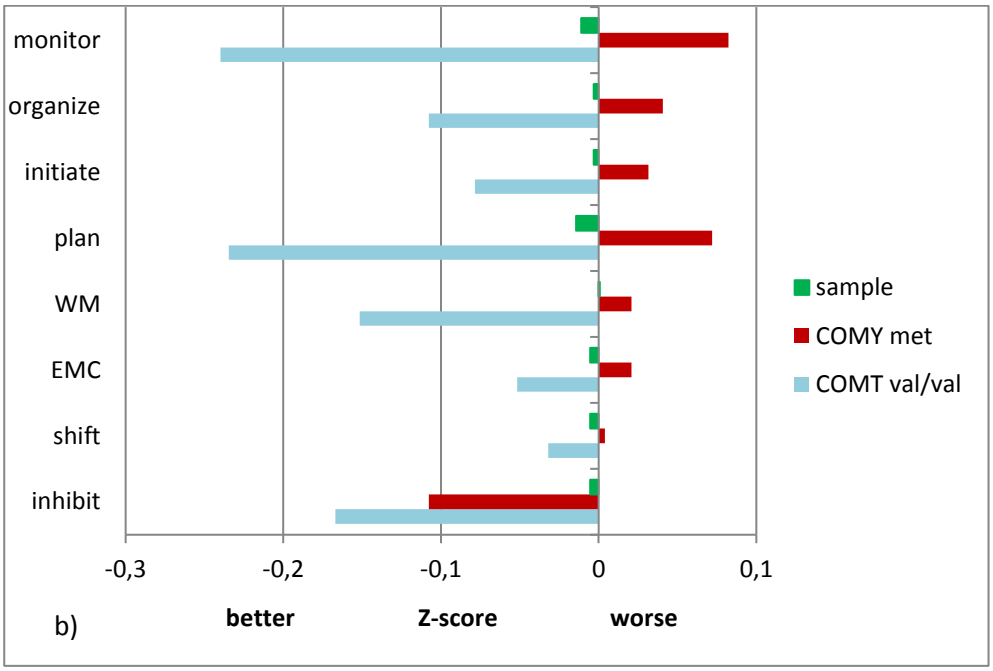


Figure 13 a and b: MTHFR T carriers performe worse than others over time; COMT Met carries are more compromised than Val/Val but not in significant way.

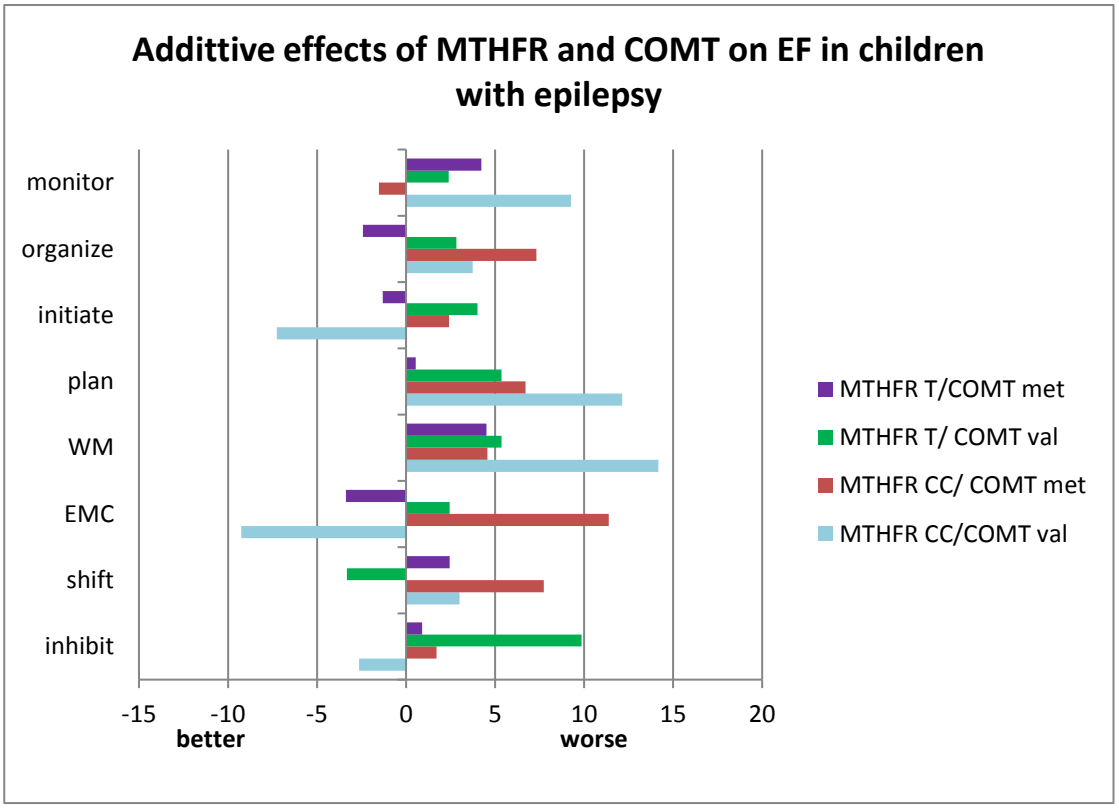


Figure 14: In children with epilepsy MTHFR and COMT have an additive effect: in presence of MTHFR CC alleles and COMT Met allele children performed worse in almost all domain. A possible explanation for this interaction falls on MHTR function on DNA Methylation: it can alter the availability of COMT promoter gene in this way can influence the COMT synthesis and the Dopamine availability.

Effects of polymorphism over the time

Over time patients CC carriers on MTHFR C677T appears to get worse respect T carriers

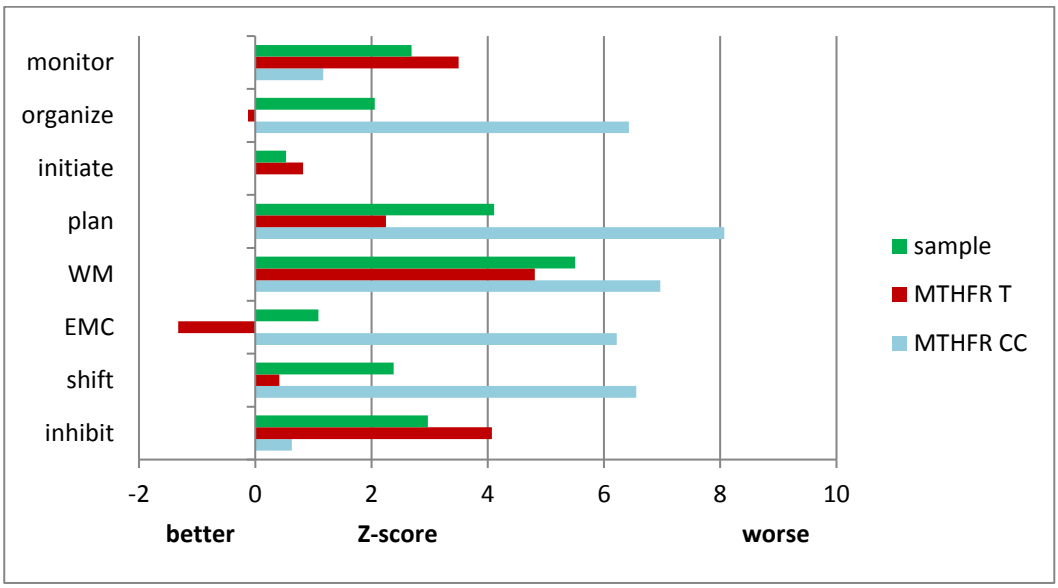
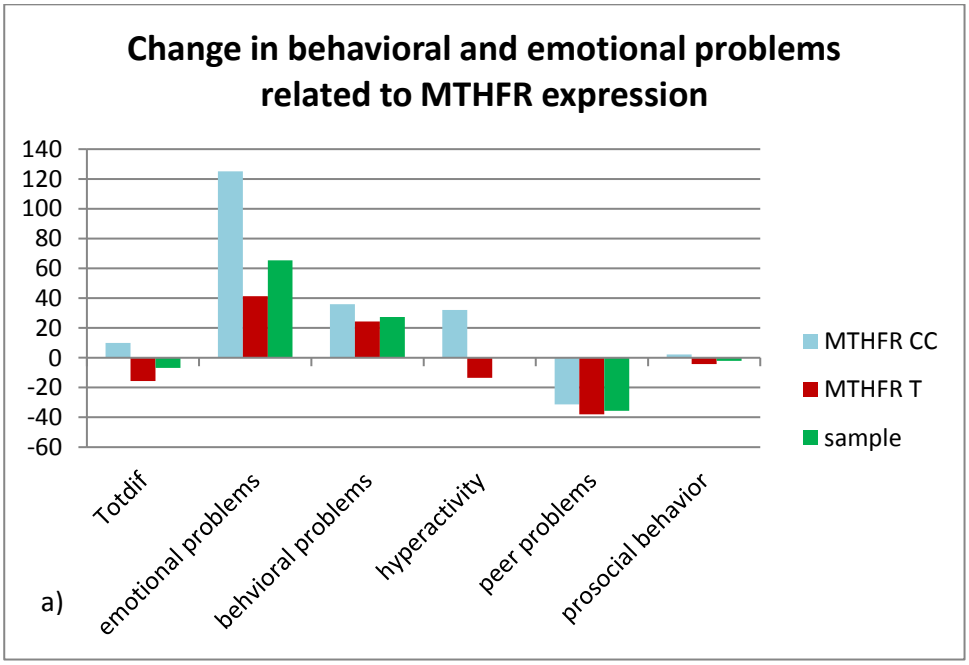


Figure 15: Effects of polymorphism over the time in executive function

Effects of MTHFR C677T and COMT Val158Met polymorphisms with emotional and behavior domains

Analyzing emotional and behavioral functions SNPs over time have a different effect: CC carriers on MTHFR and Met carriers on COMT are more compromised especially on emotional problems despite other domains.



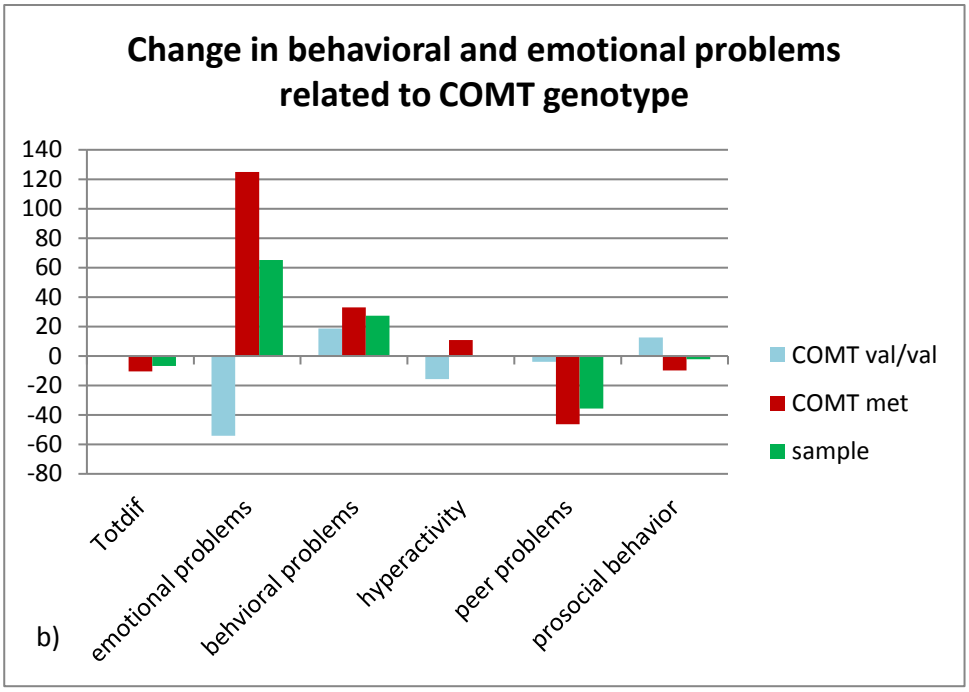
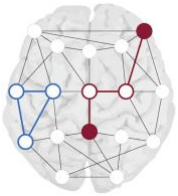


Figure 16 a and b: effects of COMT Val158Met and MTHFR C677T on change over time in emotional and behavior domain tested with SDQ

4.2 Patients with Asthma



OBJECT 1: cumulative prevalence of adverse neurocognitive, emotional/behavioral and psychosocial outcomes by exposure and diagnosis.

Executive Function in children with asthma

The children recruited in this study are routinely seen by specialist clinicians but not seen for strictly asthma related problems.

Executive function and behavioral difficulties associated with asthma were assessed using various direct and indirect tests. The data reported here include those from 3 questionnaires, namely the BRIEF and SDQ. We included 8 subscales of the BRIEF, measuring behavior problems related to inhibition, shifting, emotional control, initiation, working memory, planning/organizing, organization of materials, and monitoring. To comprehensively evaluate cognition and emotion, a battery of neuropsychological tests was administered to 63 patients which measures reflect performance across the domains of executive function, behavior and emotion and psychosocial function of the family. These test scores were standardized according to age and gender norms. No main effect of age nor gender was observed, although the proportion of boys was higher in this sample. Note that using age and gender would have created collinearity because the scores were already adjusted for age and gender.

On the other hand, the number of years the child suffered from asthma correlated significantly with some domains of executive function tested by BRIEF: with an increase in the years of illness, the domains inhibit ($p<0.05$), Emotional Control ($p<0.05$), Working Memory ($p<0.05$), Plan and Organize ($p<0.01$), and Initiate ($p<0.05$) were more compromised. Thus, we considered the group as a whole in order to establish whether other factors, such as disease related, or environmental factors were related or could predict compromised cognitive and behavioral outcome in this group of patients with years of illness as a covariate.

Children with asthma as a group did not differ on the subscales of the BRIEF with respect to normative data provided by the test manual (Figure 17). Interestingly, with respect to the capacity to plan, initiate, organize, and monitor a thought or action our patient group performed slightly better, a pattern that was observed for emotional control as well.

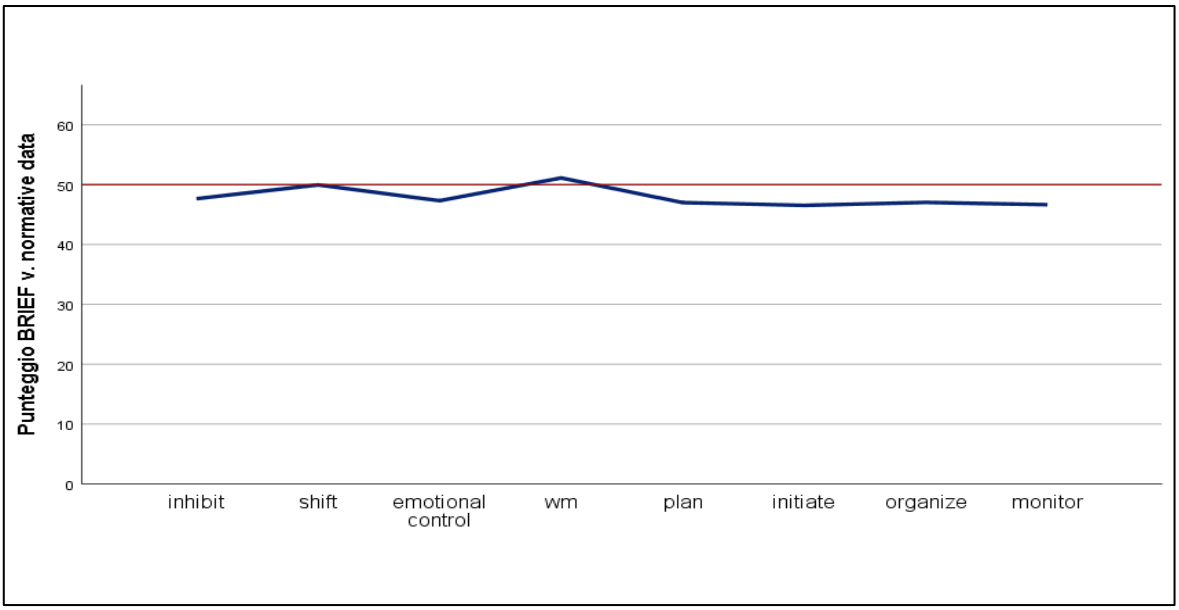


Figure 17: Overall, patients with asthma do not have fell in executive function domain

Executive functions are cognitive skills that are important for regulating behavior and for achieving goals. there is also considerable heterogeneity across children, even within diagnostic categories. This study took a data-driven approach to identify distinct clusters of children with common profiles of EF-related difficulties, and then identified patterns of brain organization that distinguish these data-driven groups. The current study used a data-driven community clustering approach to identify and group children by the similarity of their behavioral problems. Children with similar profiles of EF-associated behavioral problems were grouped which allowed us to relate these profiles to differences in disease and treatment related factors, the influence of the environment, and genetic factors. Data-driven subgrouping can provide the practical advantage of clearly defined groups of children with highly similar behavioral problems. In turn, this may help with identifying the pathophysiological mechanisms associated with those shared difficulties. We detected two distinct clusters: Cluster 1 was composed of 18 patients (18/63) characterized by having elevated mean scores on all BRIEF subscales (>55), with Shift and WM especially compromised. Cluster 2 contained 45 patients (45/63) which were characterized by extreme good performance in all domains of executive function. Thus, a score above 55 or substantially below 50 predicted the overall performance of the child**.** Clusters were compared on questionnaire measures of behavioral problems linked to everyday EF difficulties (BRIEF) and behavioral emotional problems (SDQ). A comparison of these measures indicated significant differences between the groups. For the BRIEF, children in Cluster 1 (low performers) had more problems with working memory. Children in Cluster 2 (high performers) showed a similar pattern with shift and WM slightly worse but remaining in the high performing range (Figure 18).

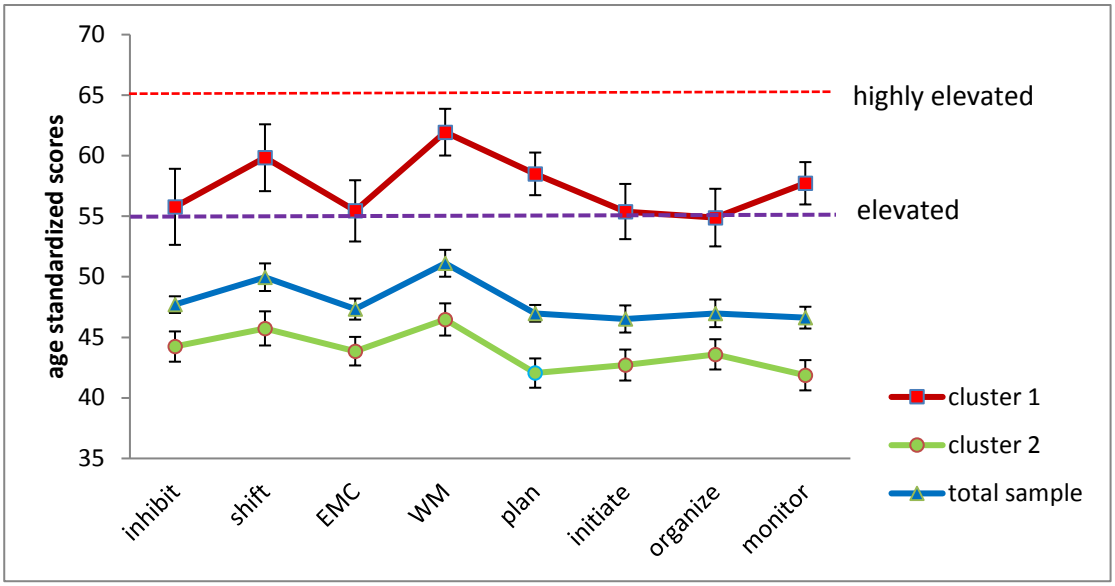


Figure 18: Subgroups showed differences in measures of Executive Function by BRIEF

Behavioral and emotional problems in children with asthma

We used data driven clustering and because EF underlie both cognitive as well as behavioral and emotional capacities, we analyzed whether the cluster structure based on EF, also holds for Behavioral and emotional functioning. The hypothesis was tested that in children and adolescents with asthma belonging to the cluster of compromised executive function, behavioral and emotional domains were compromised as well. Patients belonging to the cluster of high performers, performed better than patients belonging to the cluster of compromised executive function, with respect to overall behavioral and emotional problems ($F(1,62) = 6,068$, $p<.05$). This effect seems to especially related to differences regarding enhanced behavioral problems ($F(1,62) = 10,303$, $p<.01$) and hyperactivity ($F(1,62) = 4,343$, $p<.05$) in the low performing group (Figure 19).

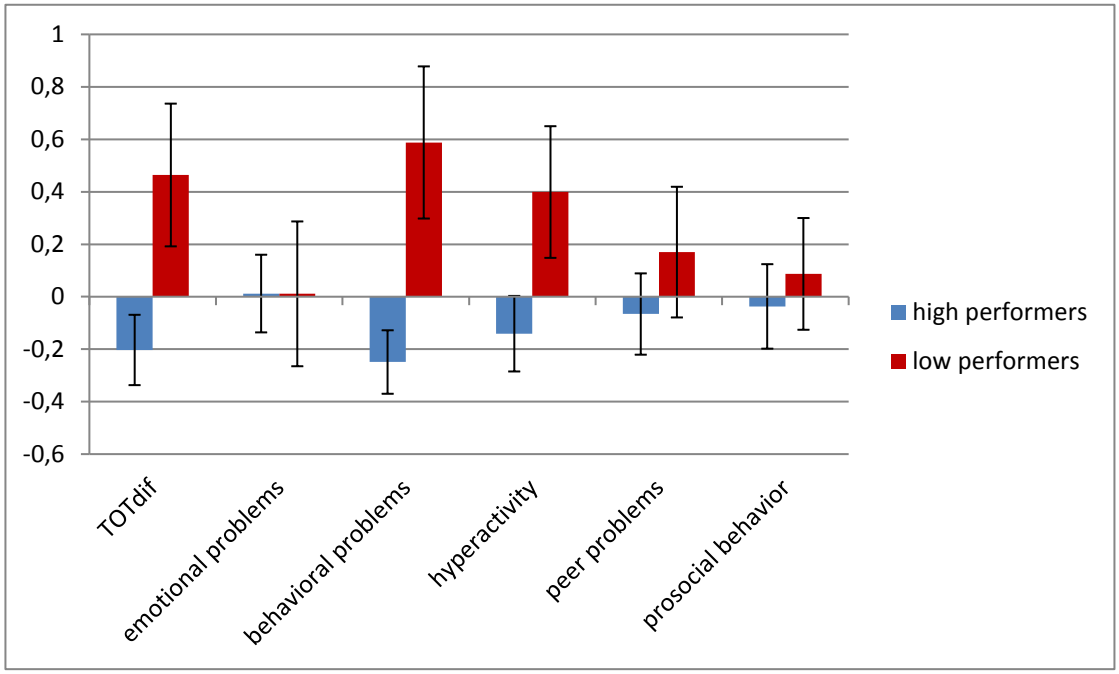


Figure 20: Comparison between Cluster 1 and 2 on SDQ items. Children more compromised on EF domain reflect this difficulty on behavior and emotional domain.

Executive function in patients diagnosed with asthma: changes over time

We test children in two time point, T1 is named the first one and T2 the second one year later. High responders do not display marked changes within a one year period, while children with difficulties in various domains tend to worsen. We examined whether genetic background may be part of the driving force behind this difference. Especially, SHIFT and PLAN as well as the subordinate functions INITIATE, ORGANIZE and MONITOR, the average, displayed a 10 point deterioration over time (Figure 21).

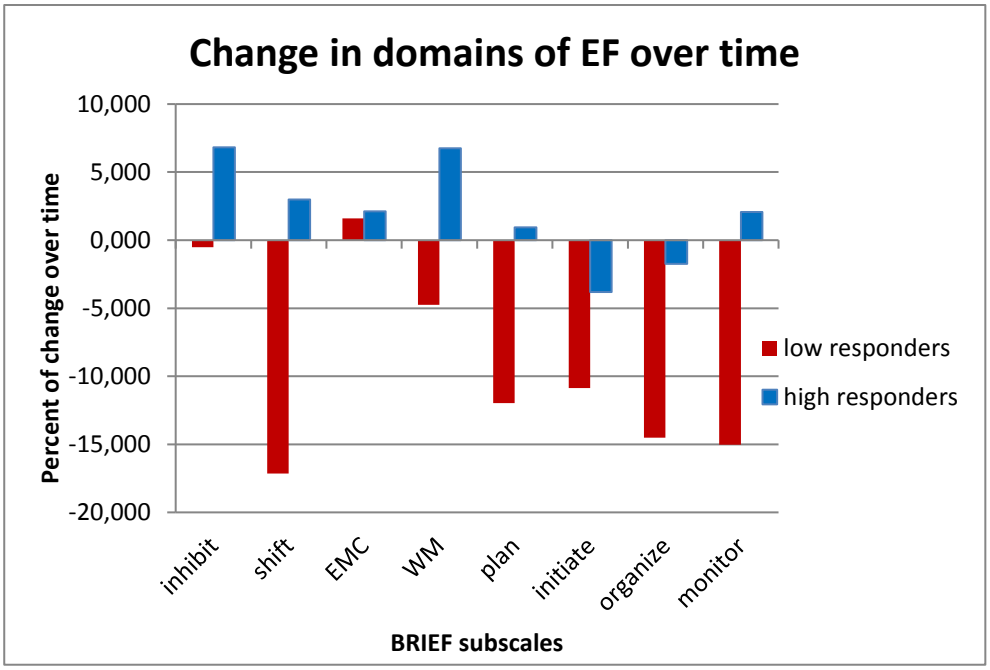


Figure 21: Differences between Cluster 1 and 2 in EF over time. Children belonging to Cluster low responders (Cluster 1) performed worse one year after the first evaluation.

OBJECT 2: Possible patient related predictive factors of neurocognitive deficits

Predictors in executive function domains

We wanted to establish whether environmental (such as smoke, humidity, city area, animals at home) and pathological factors related to asthma (as presence of dermatitis, allergies, rhino congestion, bronchitis) that can aggravate pathological findings, influenced executive functions. To see which of these factors may had an additive role on the impairment of neurocognitive, emotional and behavioral functions.

Allergies, dermatitis and upper airway respiratory problems

Allergies and dermatitis did not predict executive functions in children with asthma. Next, we assessed whether upper airway respiratory problems, rhino congestion (RHINO) and bronchitis (BRONCHO) should be considered important factors related to asthma capable of predicting compromised EF tested by BRIEF in these patients. Rhino congestion predicted compromised capacity regarding initiate a thought or action (INITIATE) but not in a significant way, while RHINO significantly predicted a worsening of the capacity to MONITOR one's thoughts or actions. However, though reliably predicting whether a child was compromised or not on these functions (83% and 79% respectively), they explained only about 11 to 12 % of the variance. Thus, they should be considered minor additional predictors.

Variabili nell'equazione[a]

		B	S.E.	Wald	gl	Sign.	Exp(B)	95% C.I.per EXP(B) Inferiore	Superiore
Fase 1[b]	RINOCONG(1)	1,496	,786	3,627	1	,057	4,465	,957	20,823
	BRONCHITI(1)	,047	,753	,004	1	,950	1,048	,240	4,590
	Costante	-2,411	,640	14,181	1	,000	,090		

a. tempo = tempo 1

b. Variabili inserite nella fase 1: RINOCONG, BRONCHITI.

Variabili nell'equazione[a]

		B	S.E.	Wald	gl	Sign.	Exp(B)	95% C.I.per EXP(B) Inferiore	Superiore
Fase 1[b]	RINOCONG(1)	1,518	,724	4,395	1	,036	4,563	1,104	18,867
	BRONCHITI(1)	-,338	,721	,219	1	,639	,713	,174	2,932
	Costante	-1,960	,558	12,332	1	,000	,141		

a. tempo = tempo 1

b. Variabili inserite nella fase 1: RINOCONG, BRONCHITI.

The presence of BRONCHITIS was not a significant predictor. However, the odds rate of compromised function in EF domains, such as, WORKING MEMORY, PLAN, INITIATE and ORGANIZE OF MATERIALS indicates that children with bronchitis are around 3 times more likely to have these functions reduced.

Environment related predictors

We tested whether environmental factors such as cigarette smoke (SMOKE), a humid environment (HUMIDITY), the presence of animals in the household (ANIMALS) or living in the city area (CITY) and Cluster, as defined through community assignment, were able to predict deficits in individual domains of function. We included years of illness as covariate because it significantly correlates with performance in the EF domains, SHIFT, EMC, WM, PLAN, INITIATE, and MONITOR. The longer the disease, the worse the performance.

Below, for each sub-domain of the executive functions which are the main predictors:

1. INHIBIT

Inhibit, a measure of cognitive control, was reliably predicted by our model (Table XXXXXX), Chi square (6)= 19.910, p<.001. The model explained 53% (R^2 Nagelkerke) of the variance in this cognitive domain and correctly classified 93% of the cases. Among the predictors, cluster, SMOKE and living in the city were the most important contributors. Children in Cluster 1 perform differently. While not being exposed to smoke

results in better function, living in the city enhances the risk of compromised performance more than 5 times. Consequently, smoking and living in more crowded places with more pollution predicts significant worsening.

Variabili nell'equazione

		B	S.E.	Wald	gl	Sign.	Exp(B)	95% C.I.per EXP(B) Inferiore	Superiore
Fase 1[a]	yearsillness	,179	,151	1,403	1	,236	1,196	,889	1,610
	Numero cluster del caso (1)	-3,073	1,186	6,712	1	,010	,046	,005	,473
	FUMO(1)	,032	1,134	,001	1	,977	1,033	,112	9,527
	UMIDITA'(1)	20,339	8199,244	,000	1	,998	681154107,3	,000	.
	ANIMALI(1)	,370	1,608	,053	1	,818	1,448	,062	33,833
	CITTA'(1)	1,693	1,297	1,703	1	,192	5,436	,428	69,102
	Costante	-22,716	8199,244	,000	1	,998	,000		

a. Variabili inserite nella fase 1: yearsillness, Numero cluster del caso, FUMO, UMIDITA', ANIMALI, CITTA'.

2. SHIFT

Shift is measure of the mental flexibility. The model significantly (Chi square (6) = 15,501, p<.05) explained 33,8% (R^2 Nagelkerke) of the variance in mental flexibility. Patients classified as having problems were identified correctly 77,2% of the cases. Cluster was the most important predictor together with the presence of a **humid** environment.

Variabili nell'equazione

		B	S.E.	Wald	gl	Sign.	Exp(B)	95% C.I.per EXP(B) Inferiore	Superiore
Fase 1[a]	yearsillness	,060	,103	,333	1	,564	1,061	,867	1,299
	Numero cluster del caso (1)	-2,292	,768	8,898	1	,003	,101	,022	,456
	FUMO(1)	,345	,822	,176	1	,674	1,413	,282	7,079
	UMIDITA'(1)	1,136	,835	1,849	1	,174	3,113	,606	15,999
	ANIMALI(1)	,444	,964	,212	1	,645	1,559	,236	10,312
	CITTA'(1)	-,144	,760	,036	1	,850	,866	,195	3,841
	Costante	-1,019	1,514	,453	1	,501	,361		

a. Variabili inserite nella fase 1: yearsillness, Numero cluster del caso, FUMO, UMIDITA', ANIMALI, CITTA'.

3. EMOTIONAL CONTROL

The model significantly (X^2 = 22. 847 p<.001) explained 62,9% of the variance in emotional control observed in children and the prediction was correct in 89,2% of the cases. Fewer years of illness, and not being exposed to SMOKE and HUMIDITY as well as belonging to cluster 1, markedly predicted better emotional control. Also, living in the CITY enhanced the odds of reduced emotional control.

Variabili nell'equazione

		B	S.E.	Wald	gl	Sign.	Exp(B)	95% C.I.per EXP(B) Inferiore	Superiore
Fase 1[a]	yearsillness	,452	,271	2,780	1	,095	1,572	,924	2,675
	Numero cluster del caso (1)	-5,529	2,471	5,004	1	,025	,004	,000	,504
	FUMO(1)	-3,787	1,957	3,744	1	,053	,023	,000	1,050
	UMIDITA'(1)	-2,914	1,659	3,085	1	,079	,054	,002	1,402
	ANIMALI(1)	-1,710	1,730	,977	1	,323	,181	,006	5,374
	CITTA'(1)	1,276	1,526	,699	1	,403	3,583	,180	71,300
	Costante	1,481	2,428	,372	1	,542	4,396		

a. Variabili inserite nella fase 1: yearsillness, Numero cluster del caso, FUMO, UMIDITA', ANIMALI, CITTA'.

4. WORKING MEMORY

Working memory is one of the most important and basic executive functions which underlies learning. Our model reliably predicted compromised function (X^2 = 38. 320 p<.000) accurately in 91,5 % of the cases and explained 71,5% of the variance (R2 Nagelkerke .715). Problems related to working memory were worse in the presence of SMOKE in low performers. Having animals around improved working memory.

Variabili nell'equazione

		B	S.E.	Wald	gl	Sign.	Exp(B)
Fase 0	Costante	-1,030	,301	11,717	1	,001	,357

Variabili nell'equazione

		B	S.E.	Wald	gl	Sign.	Exp(B)	95% C.I.per EXP(B) Inferiore	Superiore
Fase 1[a]	yearsillness	,175	,170	1,066	1	,302	1,192	,854	1,662
	Numero cluster del caso (1)	-4,514	1,260	12,828	1	,000	,011	,001	,130
	FUMO(1)	-2,900	1,228	5,574	1	,018	,055	,005	,611
	UMIDITA'(1)	-,026	1,149	,000	1	,982	,975	,103	9,261
	ANIMALI(1)	1,127	1,424	,627	1	,428	3,087	,190	50,269
	CITTA'(1)	,797	1,233	,418	1	,518	2,218	,198	24,838
	Costante	,939	2,310	,165	1	,684	2,558		

a. Variabili inserite nella fase 1: yearsillness, Numero cluster del caso, FUMO, UMIDITA', ANIMALI, CITTA'.

5. PLAN

The integrity of the capacity to plan of a thought or action was significantly predicted by our model (X2 = 38. 320 p<.000) and explained 55% of the variance being correct in 93% of the case. While affected predominantly by cluster, environmental factors did not majorly influence PLAN.

Variabili nell'equazione

		B	S.E.	Wald	gl	Sign.	Exp(B)
Fase 0	Costante	-1,674	,363	21,238	1	,000	,188

Variabili nell'equazione

		B	S.E.	Wald	gl	Sign.	Exp(B)	95% C.I.per EXP(B) Inferiore	Superiore
Fase 1[a]	yearsillness	,183	,153	1,430	1	,232	1,201	,890	1,620
	Numero cluster del caso (1)	-3,802	1,262	9,079	1	,003	,022	,002	,265
	FUMO(1)	-1,020	1,130	,814	1	,367	,361	,039	3,304
	UMIDITA'(1)	-,064	1,149	,003	1	,956	,938	,099	8,920
	ANIMALI(1)	-,569	1,533	,138	1	,710	,566	,028	11,411
	CITTA'(1)	-,525	1,028	,261	1	,609	,591	,079	4,436
	Costante	,452	2,210	,042	1	,838	1,572		

a. Variabili inserite nella fase 1: yearsillness, Numero cluster del caso, FUMO, UMIDITA', ANIMALI, CITTA'.

6. INITIATE

Belonging to cluster 2 significantly compromised INITIATE. No environmental factors independently worsened the capacity to initiate a though or action. The model, however significantly, explained 35 % of the variance in this domain, and was correct in 81 % of the time.

Variabili nell'equazione

		B	S.E.	Wald	gl	Sign.	Exp(B)	95% C.I.per EXP(B) Inferiore	Superiore
Fase 1[a]	yearsillness	,169	,127	1,787	1	,181	1,184	,924	1,518
	Numero cluster del caso (1)	-2,119	,940	5,085	1	,024	,120	,019	,758
	FUMO(1)	-,054	,951	,003	1	,955	,947	,147	6,112
	UMIDITA'(1)	1,212	1,204	1,013	1	,314	3,361	,317	35,613
	ANIMALI(1)	-,877	1,108	,626	1	,429	,416	,047	3,649
	CITTA'(1)	-,478	,896	,284	1	,594	,620	,107	3,591
	Costante	-1,402	1,951	,517	1	,472	,246		

a. Variabili inserite nella fase 1: yearsillness, Numero cluster del caso, FUMO, UMIDITA', ANIMALI, CITTA'.

7. ORGANIZE MATERIALS

Those patients classified as being part of cluster 2 performed worse when compared to those belonging to the cluster of high performers (B = -3.471, p<.05 CI .002-.476). Also, living in the city (CITY) represented a heightened risk for compromised function in this domain. Together, the model predicted 59% of the variance in this domain and classified the patient in the correct risk group, 91% of the time.

Variabili nell'equazione

		B	S.E.	Wald	gl	Sign.	Exp(B)	95% C.I.per EXP(B) Inferiore	Superiore
Fase 1[a]	yearsillness	-,129	,145	,784	1	,376	,879	,661	1,169
	Numero cluster del caso (1)	-3,471	1,392	6,218	1	,013	,031	,002	,476
	FUMO(1)	-,986	1,093	,814	1	,367	,373	,044	3,179
	UMIDITA'(1)	20,503	7860,041	,000	1	,998	802280915,8	,000	.
	ANIMALI(1)	-1,738	1,379	1,587	1	,208	,176	,012	2,627
	CITTA'(1)	-1,945	1,130	2,962	1	,085	,143	,016	1,310
	Costante	-16,211	7860,041	,000	1	,998	,000		

a. Variabili inserite nella fase 1: yearsillness, Numero cluster del caso, FUMO, UMIDITA', ANIMALI, CITTA'.

8. MONITOR

The variables in the equation lead to a significant model to predict MONITOR which accounted for 66,3% of the variance. Patients were classified 92,7 percent in the correct risk group. Cluster significantly predicted the risk of compromised function in this domain, with those classified in cluster 2 performing worse than patients in cluster 1.

Variabili nell'equazione

		B	S.E.	Wald	gl	Sign.	Exp(B)	95% C.I.per EXP(B) Inferiore	Superiore
Fase 1[a]	yearsillness	,189	,164	1,335	1	,248	1,208	,877	1,664
	Numero cluster del caso (1)	-4,405	1,261	12,211	1	,000	,012	,001	,145
	FUMO(1)	,040	1,216	,001	1	,974	1,041	,096	11,277
	UMIDITA'(1)	,894	1,223	,534	1	,465	2,444	,222	26,862
	ANIMALI(1)	,165	1,729	,009	1	,924	1,179	,040	34,975
	CITTA'(1)	-,849	1,128	,566	1	,452	,428	,047	3,902
	Costante	-,716	2,438	,086	1	,769	,489		

a. Variabili inserite nella fase 1: yearsillness, Numero cluster del caso, FUMO, UMIDITA', ANIMALI, CITTA'.

Next, we explored whether disease and environment factors were correlated or predictive of behavioral and emotional differences.

<u>Predictors in behavioral and emotional domains</u>

Disease related predictors

We considered as disease related factors the asthma severity, the type of treatment and the spirometer values. The severity of asthma (SEVERITY) index indicated that belonging to a higher risk group significantly predicts overall behavioral and emotional problems. The more severe the asthma, the more the total number of behavioral and emotional problems. Overall severity explained 26% of the problems ($p < .05$). Also, therapies other than 500 mcg/die cortisone or Montelukast correlated with more behavioral and

emotional problems. Emotional problems were predicted by the type of therapy (β= .152, p<.05 CI .028-.998) and the model explained almost 30% of the problems. Children treated with medication other than 500 mcg/die cortisone or Montelukast displayed more severe problems.

Anomalies related to spirometer performance did not predict enhanced overall difficulties.

However, belonging to more moderate forms of asthma (β= .479, p<.05 CI .028-.998) and spirometer anomalies (β= .697, p<.01 CI .028-.998) significantly predicted enhanced behavioral problems and explained 23% and 34 % of the variance in behavioral problems respectively.

Similarly, hyperactivity was predicted by the severity of asthma and the type of therapy. Belonging to a more severe form of asthma significantly predicted enhanced hyperactivity (β= .396, p<.05 CI .006-.785).

Thus, among the disease and treatment related factors asthma severity and exposure to high levels of corticosteroids or Omalizumab seem to be predictive of behavioral problems and hyperactivity.

Environment related predictors

The environmental predictors we considered, SMOKE, UMIDITY, ANIMALS, and CITY were not predictive of behavioral and emotional problem. Except for hyperactivity, where being exposed to smoke in the household significantly predicted enhanced hyperactivity ((β= .732, t= 2.629 p<.01 CI .0174-1.289).

Hence, environmental factors overall, play a minor role in predicting behavioral and emotional problems.

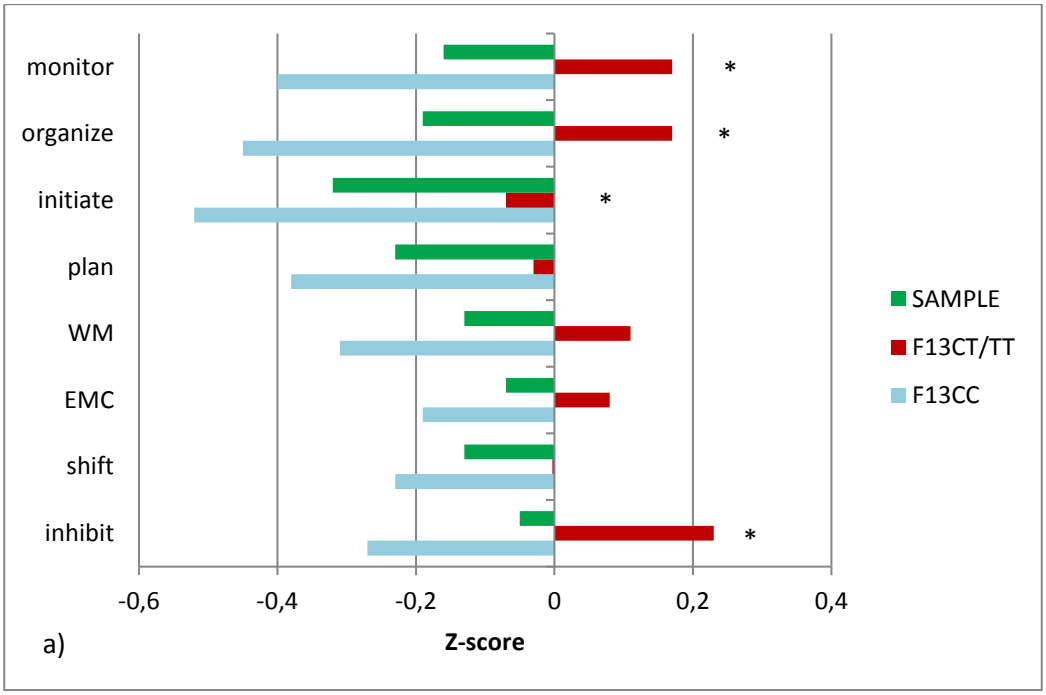
OBJECT 3: Genes as potential predictors of the severity of deficits in children with asthma

Genetic factors as predictors on executive function in children with asthma

In patients with asthma, belonging to high or low performers was predictive of problems in executive function: children belonging to the cluster characterized by substantial EF problems had an odd ratio of 19,758 of performing worse than the high performing cluster, and an OR of almost 10 in the presence of the FKBP5 3800373 risk allele.

Of all genetic polymorphisms, only FKBP5 rs3800373 and FKBP5 1360780 predicted differences in behavioral and emotional domains of function. More specifically, children carrying at least one C allele, that is, rs3800373 (F38) AC or CC carriers, displayed significantly more problems in the capacity to monitor actions than children homozygous for AA (F (1,54)= 4,289 p<.05). Similarly children having at least one T allele, performed significantly worse, that is, had higher scores than children homozygous for the C allele of rs1360780 (F13) (figure 22 a,b). Domains, such as, the capacity to monitor, organize and initiate a though or action as well as the capacity to control impulses (INHIBIT) were most compromised (p<.05). Together, the model, using F38 and F13 as predictors, explained 45 % of the variance with respect to EF in a significant way (p<.001).

As a result, glucocorticoid receptor functional integrity seems to be predictive of enhanced problems in executive function in children diagnosed with asthma.



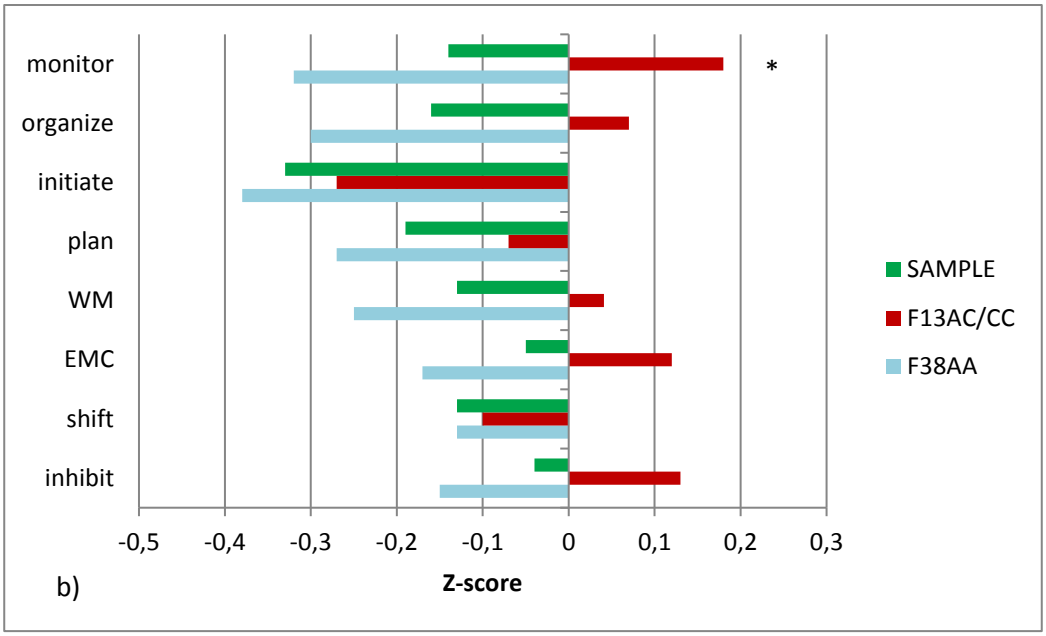
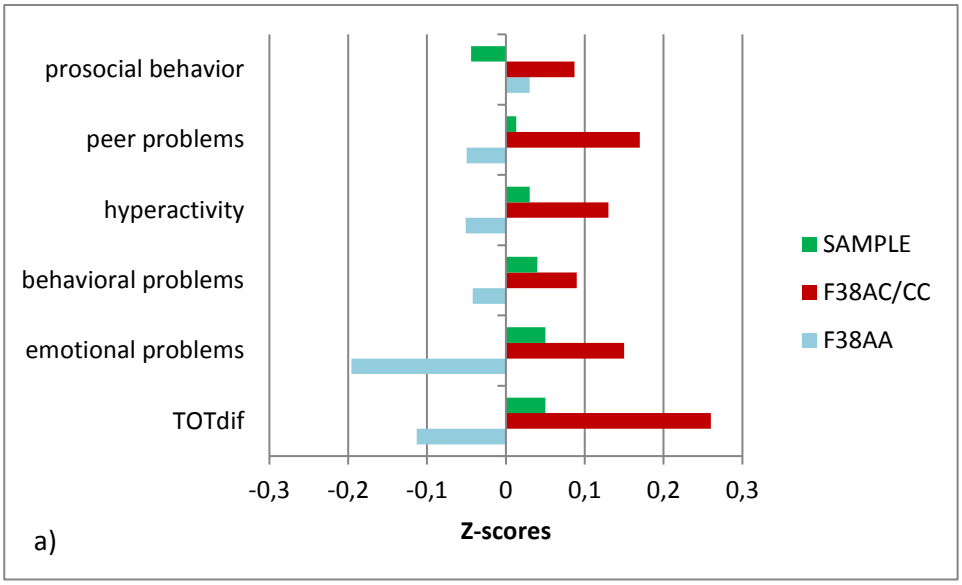


Figure 22 a and b: FKBP5 polymorphism effect on executive function domains

Genetic factors as predictors behavioral and emotional problems in children with asthma

The genetic polymorphisms considered in this group of patients, were 5HTT LPR, COMT Val158Met, BDNF Val66met, MTHFR C677T and FKBP5 (rs3800373 A/C and rs1360780 C/T). Of all genetic polymorphisms, only which related to the FKBP5 gene, predicted differences in behavioral and emotional domains of function. More specifically, children carrying at least one C allele, that is, rs3800373 AC or CC carriers, displayed significantly more problems than children homozygous for AA ($F(1,54) = 4{,}289$ $p<.05$). Similarly children having at least one T allele, performed significantly worse, that is, had higher scores than children homozygous for the C allele of rs1360780 (figure 23 a,b). Together, the model, using rs3800373 and rs1360780 as predictors, explained 45 % of the variance with respect to emotional problems in a significant way ($F(2,53) = 22{,}365$ $p<.001$).

Hence, glucocorticoid receptor functional integrity seems to be predictive of emotional problems in children diagnosed with asthma.



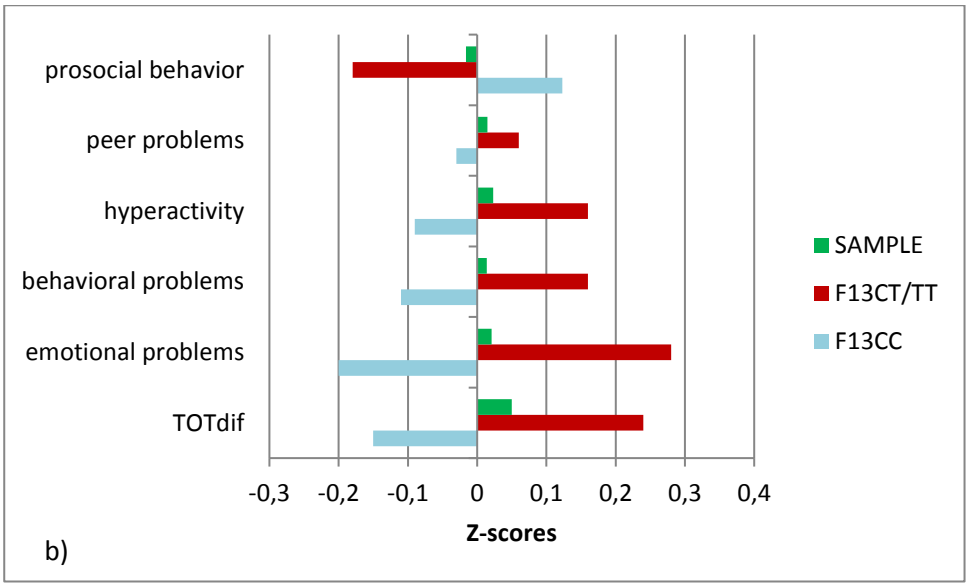


Figure 23 a and b: in the part a is reported how the presence of mutate allele on rs3800373 polymorphism influence the emotional and behavior domains. The part b report similarly how the rs1360780 polymorphism has a negative impact on almost all domains.

Remarkably, of the 32 patients rs3800373 homozygous for AA, 65.6% (N=21) belonged to cluster 1, high performers while 34,4 % belonged to the cluster of low performers. Within the cluster of low performers, however, markedly more patients were homozygous for AA (17,7% vs 11,3%). Similarly, more rs1360780 CC carriers 18,7 % than T carriers belonged to the cluster of low performers. This suggests that the cluster of low performers is characterized by patients with different genotypes, with those heterozygous or homozygous for the risk alleles, being more at risk for compromised emotional problems and overall behavioral and emotional difficulties. Also, prosocial behaviors were less developed in the latter. When analyzing the predictive value of polymorphisms, we observed that polymorphisms of were predictive of difficulties in some domains of EF, especially the domain related to mental flexibility.

We tested the hypothesis that because FKBP5 polymorphisms are predictive of some of these vulnerabilities, they may drive the resistance to change of children that have scores in the high range, that is children that are either scoring in the elevated range (>55) or in the clinical range (≥ 65). Data show that patients with at least one C allele of rs1360780, are more compromised in most domains of EF at T1 (figure 24a). At one year into the study (T2), children did not improve substantially, which suggests that this polymorphism might confer resistance to change. Similarly, patients with at least one C allele of FKBP5 rs3800373, performed worse when compared to patients homozygous for the C allele. However, this genotype seems to confer less resistance and we observed a slight but noticeable improvement, especially in the domain of mental flexibility (SHIFT) (figure 24 a b).

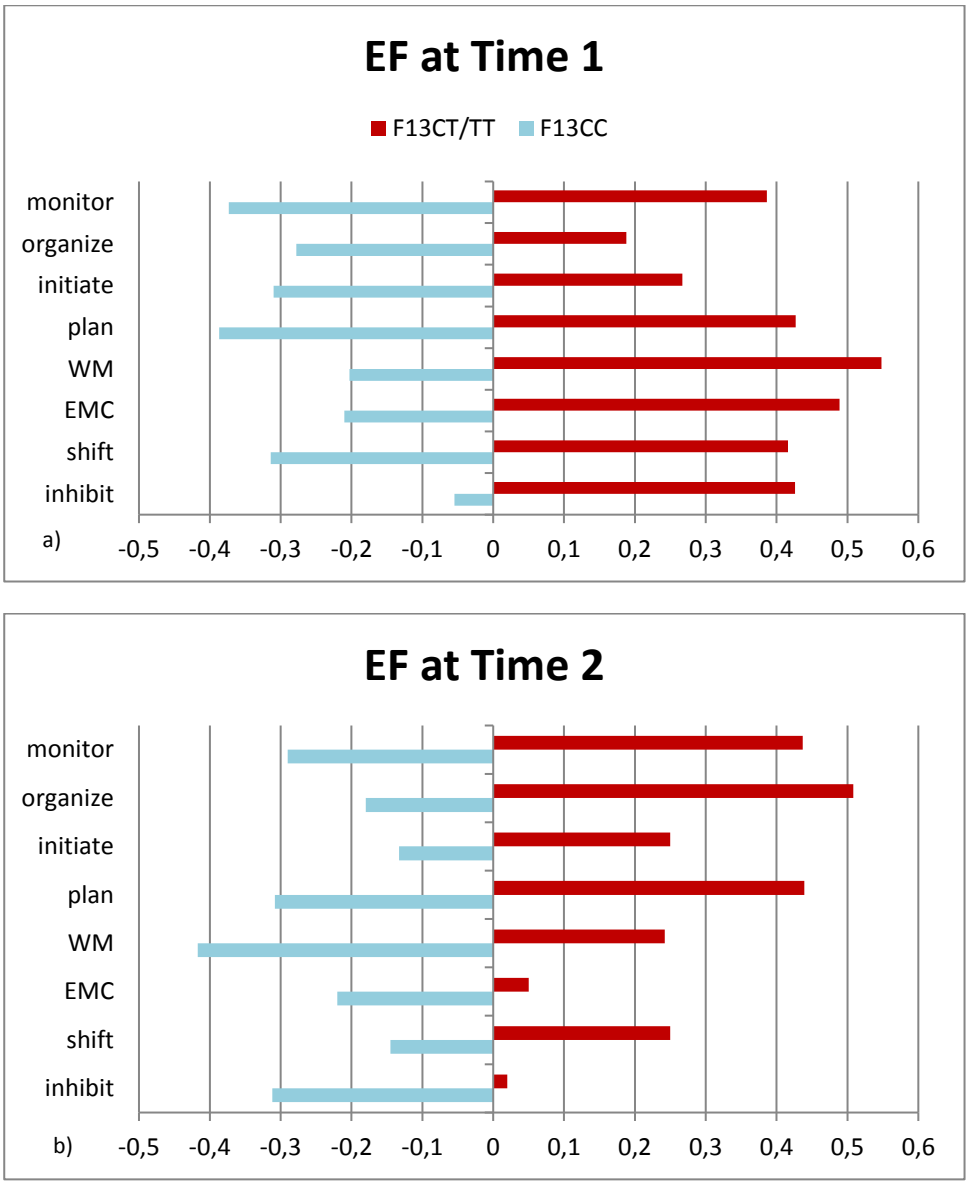
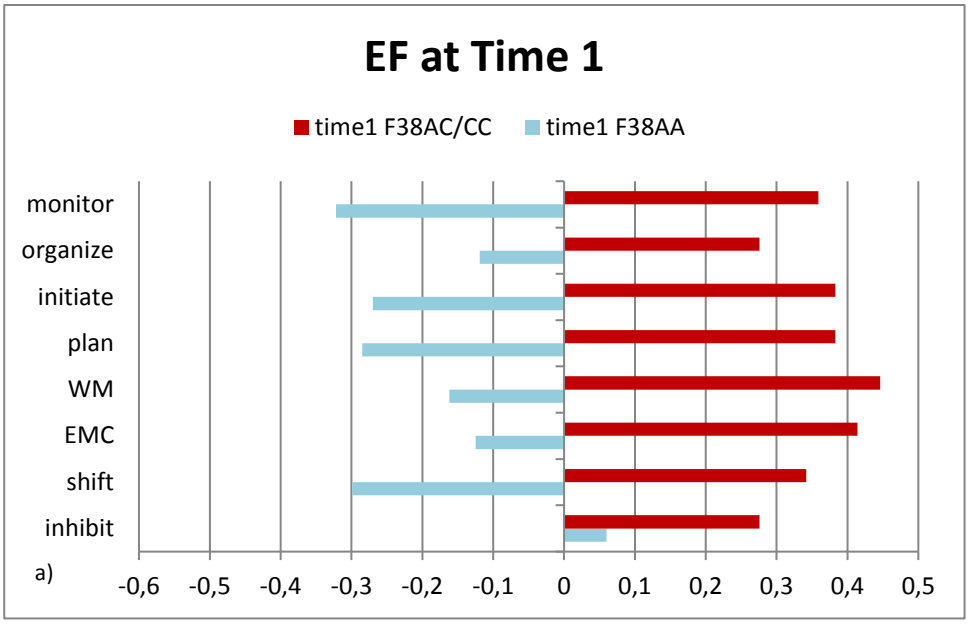


Figure 24 a and b: Change in relation to FKBP5 rs1360780 polymorphism, the risk allele confers more vulnerability over time



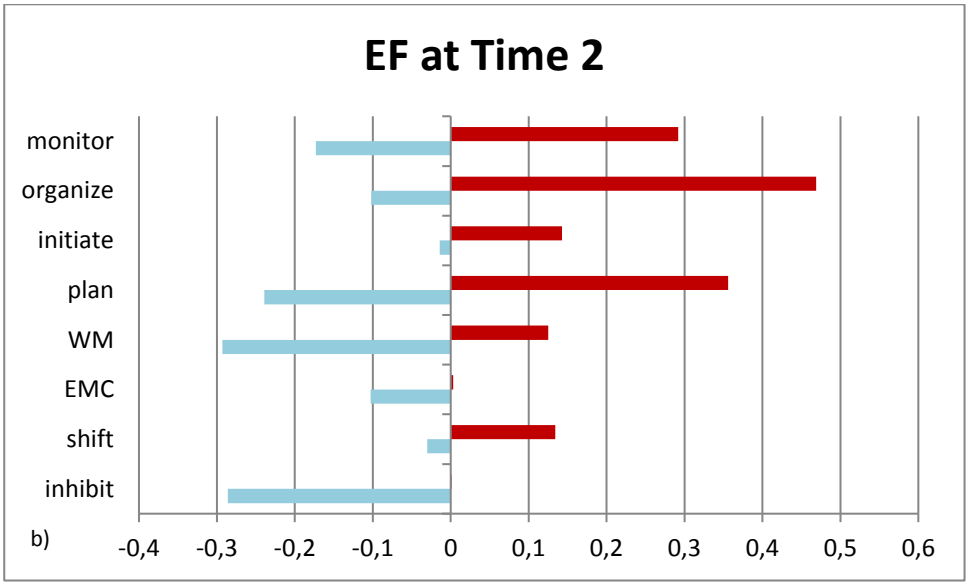


Figure 25 a and b: Change in relation to FKBP5 rs3800373 polymorphism, the risk allele confer more vulnerability over time

4.3 Patients with solid tumors (PWST) and with acute lymphoblastic leukemia (ALL)

OBJECT 1: cumulative prevalence of adverse neurocognitive, emotional/behavioral and psychosocial outcomes by exposure and diagnosis.

The data reported here include those from 3 questionnaires, namely The BRIEF, SDQ and the child related sub domain of the PAT. We included 8 subscales of the BRIEF, measuring behavior problems related to inhibition, shifting, emotional control, initiation, working memory, planning/organizing, organization of materials, and monitoring; 5 subscales from the SDQ as well as the total score, and 6 subscales from the PAT. To comprehensively evaluate cognition and emotion, these tests were administered to 21 patients (regulary followed for ALL and pediatric tumor follow up) which measures reflect performance across the domains of executive function, behavior and emotion and psychosocial function of the family. Test scores were standardized according to age and gender norms.

Executive Function in children with cancer

Here, we considered the executive function during the first year of Follow up in ALL and PWST group compared to normative data, obtained from typical development children provided by the BRIEF test manual. Scores above 50 are higher than the normative mean but do not indicate compromised function. We considered scores as being compromised when T-scores were equal or higher than 55. A score of 65 or above was considered to be in the clinical range. Overall children with solid tumors displayed the same pattern of strengths and need with respect to executive function as children diagnosed with ALL but worse. Especially, mental flexibility (shift), emotional control and the capacity to monitor ones thought and actions were more significantly more compromised in PWST (Figure 26).

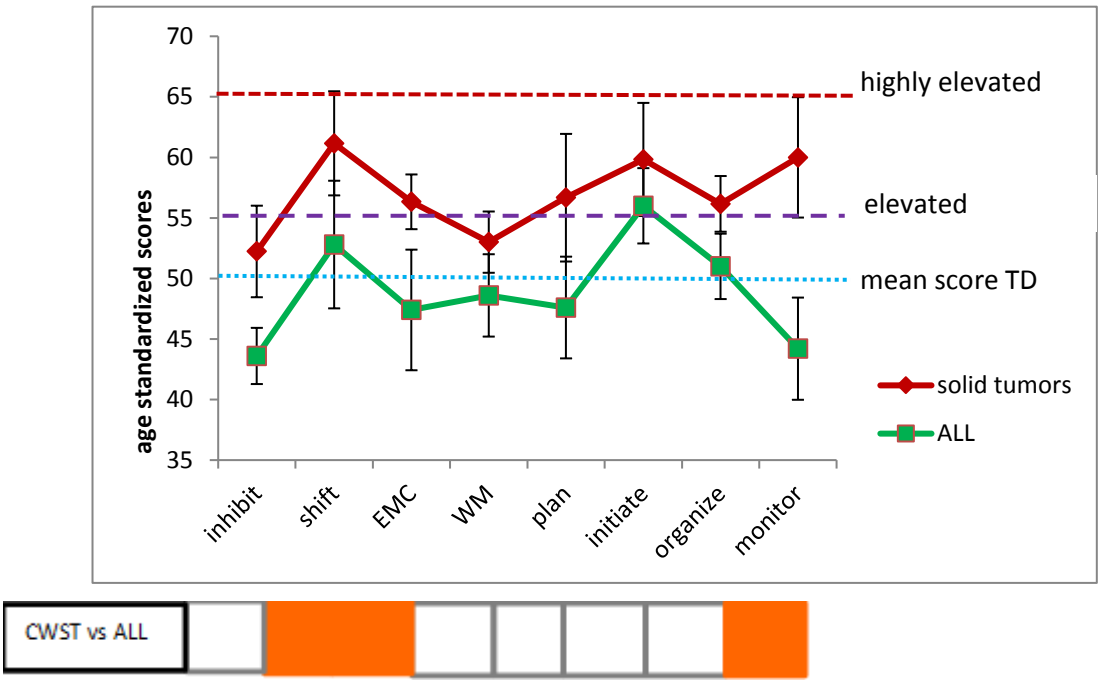


Figure 26: differences in BRIEF test subscale scores in PWST and with ALL

Behavioral and emotional problems in children with cancer

The hypothesis was tested that in children and adolescents' diverse types of tumors belonging to the cluster of compromised executive function, behavioral and emotional domains were compromised as well. PWST, overall displayed less problems overall (<.05). This effect seems to especially be related to differences regarding enhanced emotional problems (p<.01) and peer related problems (p<.01).

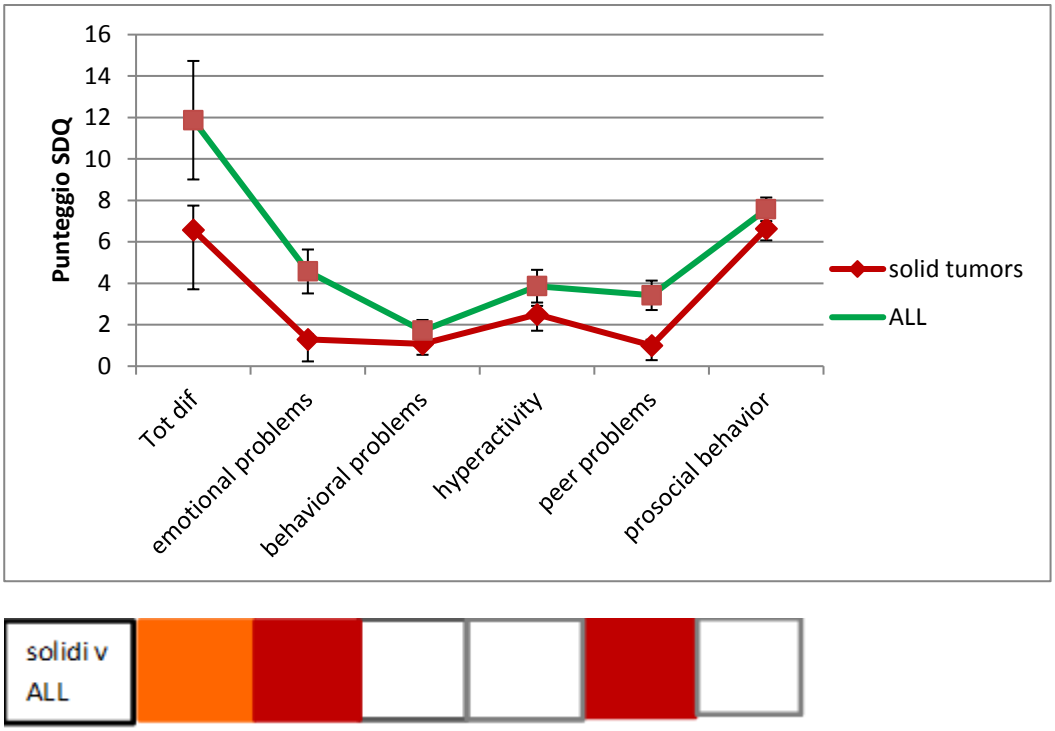


Figure 27: Differences in behavior and emotional domain tested with SDQ questionnaire in PWST and ALL.

4.3.1 PWST group

Executive function in patients diagnosed with PWST: changes over time

The difference from the onset of follow-up (SFU) to one year after the start of follow-up (SFU+1) in PWST and children with ALL in various domains of EF using time as factor. All tests showed main effects for group in which PWST almost always performed worse than age-corrected standard score performance of typical developing children provided by the manual as reference group. Main effects for time (regardless of group) revealed significant improvement for most domains of executive function. None of the tests revealed significant Group x Time interactions.

In general, PWST performed in the compromised range on mental flexibility (shift), emotional control, plan, initiate and monitor (figure 28). Except for shift and monitor these differences were not statistically different. However, this traditional manner of looking at data doesn't put in evidence the dynamic nature of the in executive function early in Follow Up.

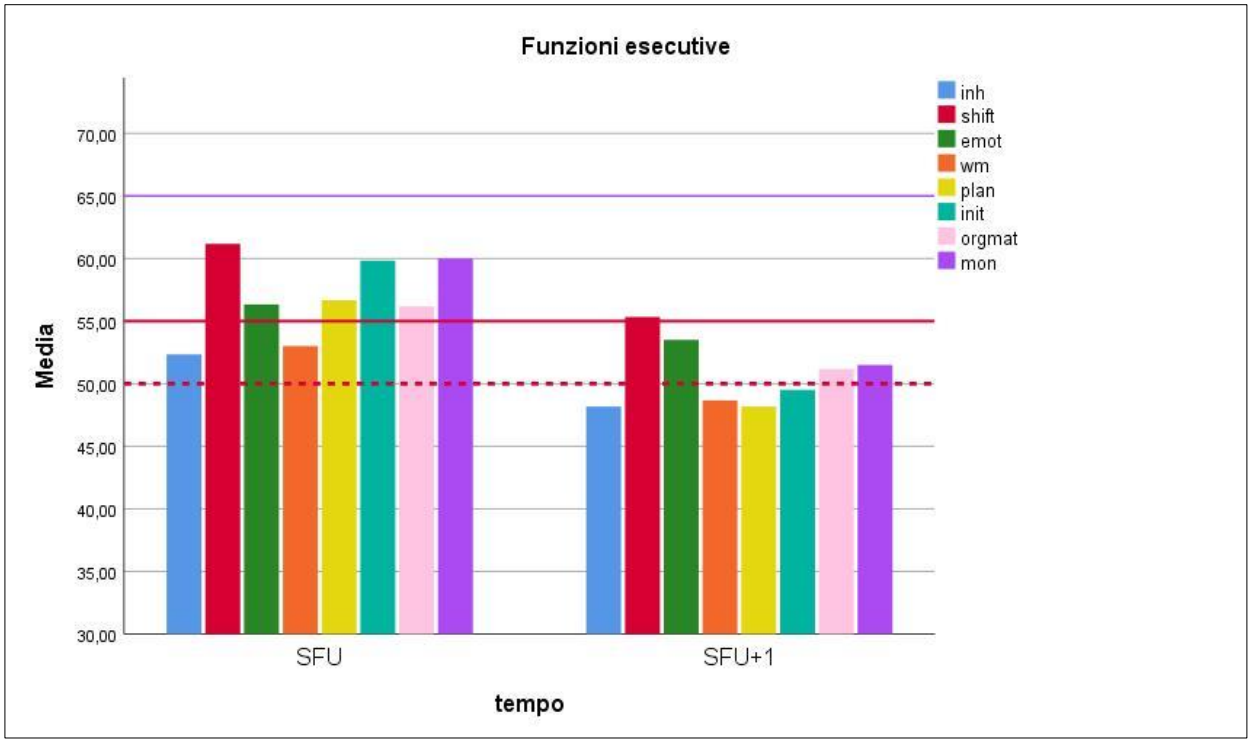


Figure 28. Difference between executive function in PWST at the time point of follow-up (SFU) to one year after (SFU+1). All scores are corrected by gender and age. The base line is 50 as reported in the manual, scores over 65 are considered of clinical relevance, score over 55 are considered of moderate severity. (inh=Inhibit; emot= emotional control; wm= working memory; plan= plan and organize; init= initiate: orgmat= organization of materials; mon= monitor)

When considering the percent of change and not the difference score, patient 4 and 9 stand out for their negative (patient 4) and positive change (patient 9) over time (figure 29). Three patients dramatically worsen over a one year period especially with respect the domains, such as, emotional control, initiate and working memory. Note that, patients have very personal patterns of strengths and needs. Thus, taking these patients as a group would not help identify where to direct our attention most effectively.

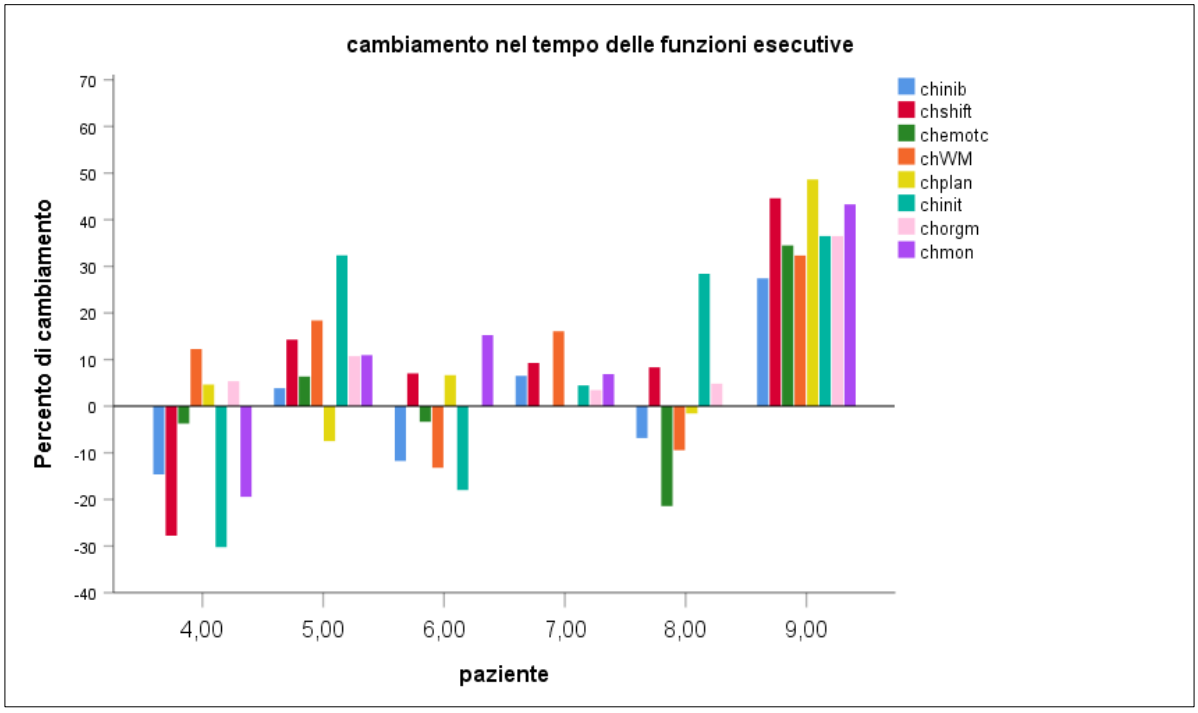


Figure 29: percentage of change in PWST on the Executive Function tested by BRIEF test over time. Patient 1 is excluded because too younger (age < 5 years old) and patient 2 because didn't complete the test.

(chinibit: change in inhibit; chshift= change in shift; chemot= change in emotional control; chwm= change in working memory; chplan = change in plan and organize; chinit= change in initiate; chorgm= change in organization of materials, chmon=change in monitor)

Emotional and Behavior in PWST over time: changes over time

The change in behavioral and emotional strengths and difficulties indicates a different pattern of change, (figure 30) with the area most compromised that of behavioral problems. When looking at individual items on the child problem scale, we found that PWST have more attention related problems and problems related to mood and anxiety (data not shown)

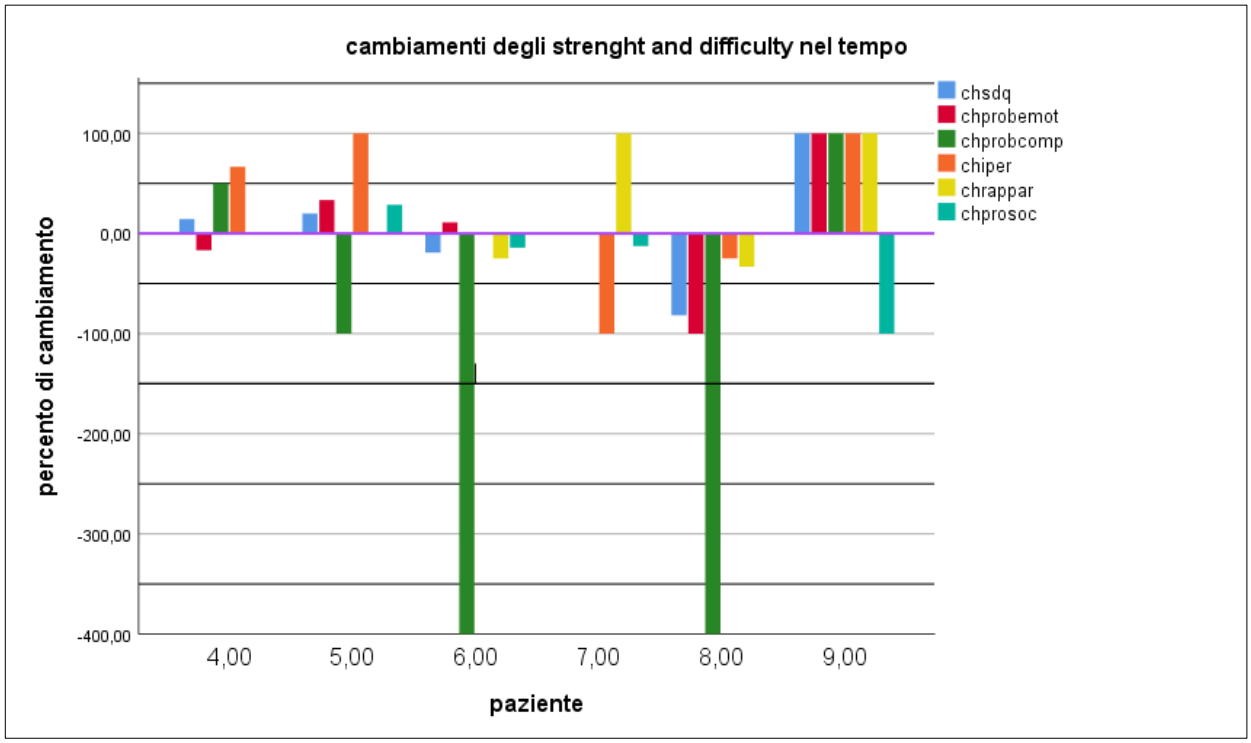


Figure 30: Change in The Strengths and Difficulties Questionnaire (SDQ) completed by parents. All in all, the item more compromised is related to the behaviour. Patient 1 is not reported because too young (age < 4 years old) and patient 2 didin't complete the questionnaire.

(chsdq= change in total score difficulties; chprobemot= change in emotional symptoms; chprocomp= change in conduct problems; chipper= change in hyperactivity/inattention; chrappar= change in peer relationship; chprosoc: change in prosocial behavior)

Psychosocial Risk in PWST over time: changes over time

Patients 6 and patients 8 present negative changes in behavior domain which is confirmed by the problems related to the behavior of the patients reported by the parents on the PAT (figure 31) and the stress caused by the disease. Patients 9 get better in all domains, it may be related to a good psychosocial environment.

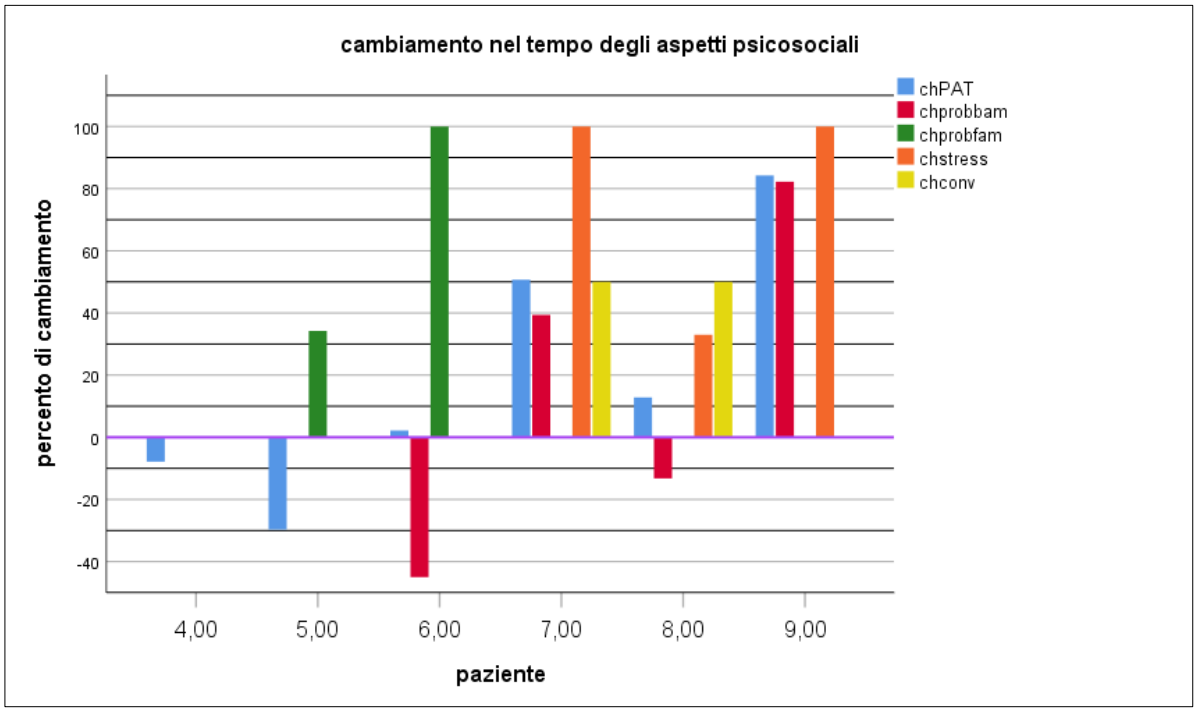


Figure 31: Changes over time in psychosocial domain measured by PAT 2.0.

(chPAT= change in PAT score; chprobbam = change in children related problem; chprobfam = change in family problem; chstress: change in stress of family; chcon= change in family beliefs)

4.3.2 ALL group

Executive function in patients diagnosed with ALL: changes over time

Time plays an important factor in this group of patients. An important worsening was observed between the start of follow-up and one year after the start of follow-up (Figure 32). Particularly compromised were, the capacity to shift, which is related to mental flexibility and emotional control over one thoughts and actions and well as the capacity to monitor own actions. Patients displayed between a 10 % and 18 % deteriorating within a one year period, which represent a substantial loss of function. Only the capacity to plan a thought or action was relatively spared (figure 33).

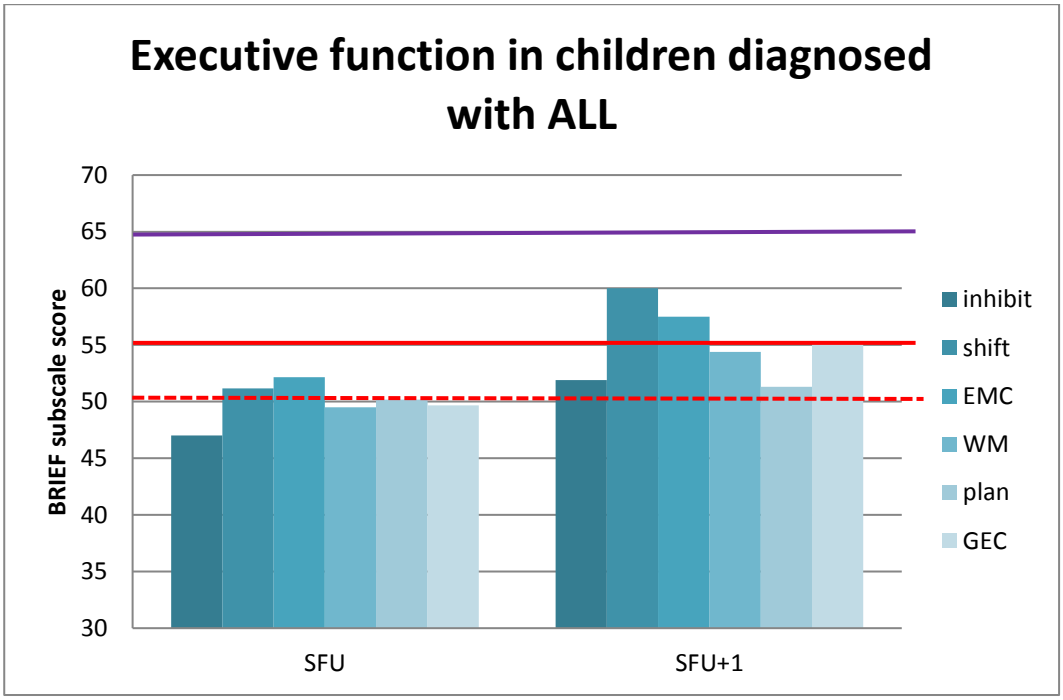


Figure 32: Differences on executive function profile in ALL group tested by BRIEF. All in all, there is a worsening in all executive function domain.

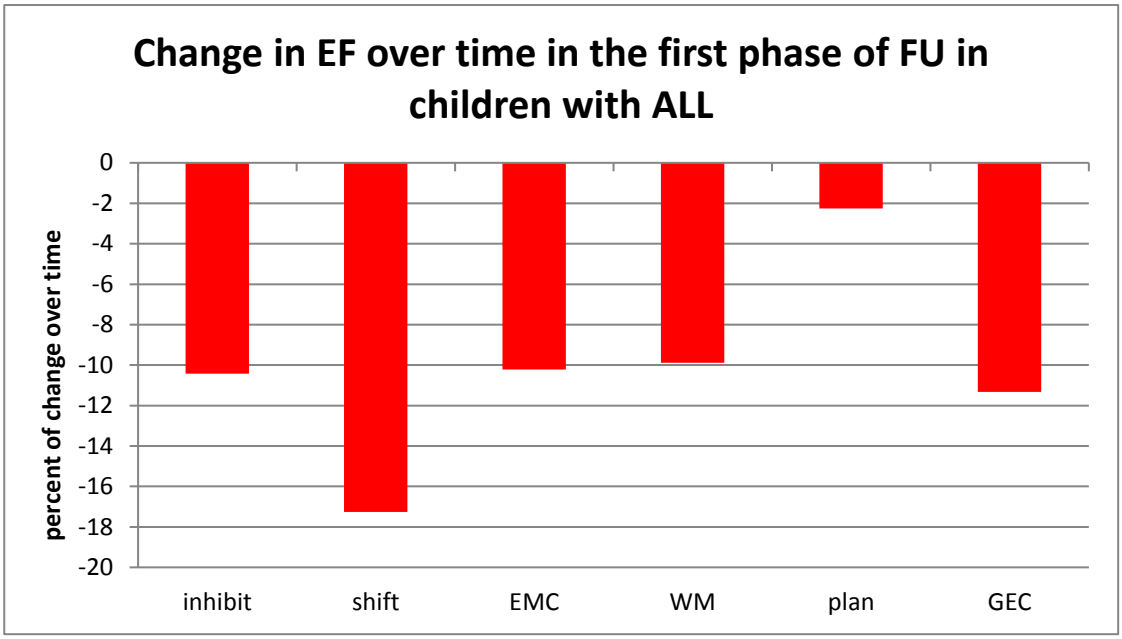


Figure 33: Percentages of changes in ALL group between the two first time point of follow up. As reported before all domains get worse with exclusion of one related to plan and organize ability.

Emotional and Behavior in ALL over time: changes over time

The same pattern of overall worsening was observed in behavioral and emotional domains, where substantial aggravation of 30% was observed with extreme exacerbation regarding emotional problems. The only domain in which some improvement was observed, was the capacity to relate to peers.

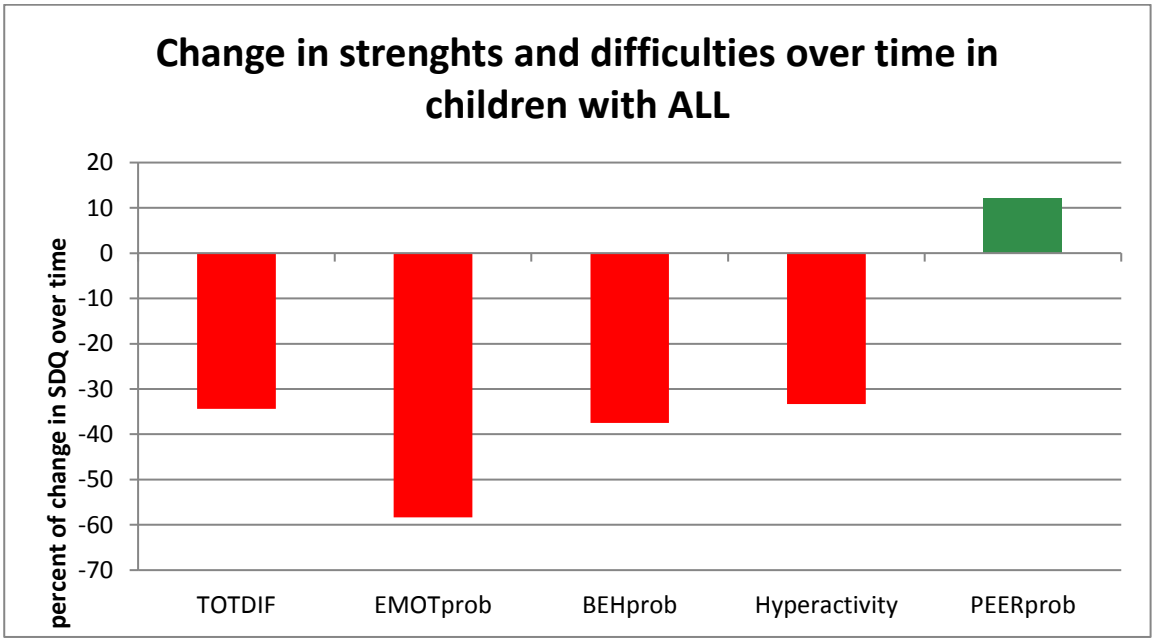


Figure 34: percentages of changes in SDQ scale during fist phases of follow up. All domains of emotional and behavior skills get worse but the relationship with peers remains good.

Psychosocial Risk in ALL over time: changes over time

In the psychosocial realm, an improvement was observed regarding problems related to the sick child as well as siblings and problems related to the family. In contrast, social support was less. Especially the stress reactivity of the family and the patient seems to worsen over time, which may be related to the uncertainty of this new phase of care. The higher stress reactivity is also reflected by the fact that family attitudes worsen which suggest a high burden related to uncertainty with respect to possible future events and sequelae related to the disease.

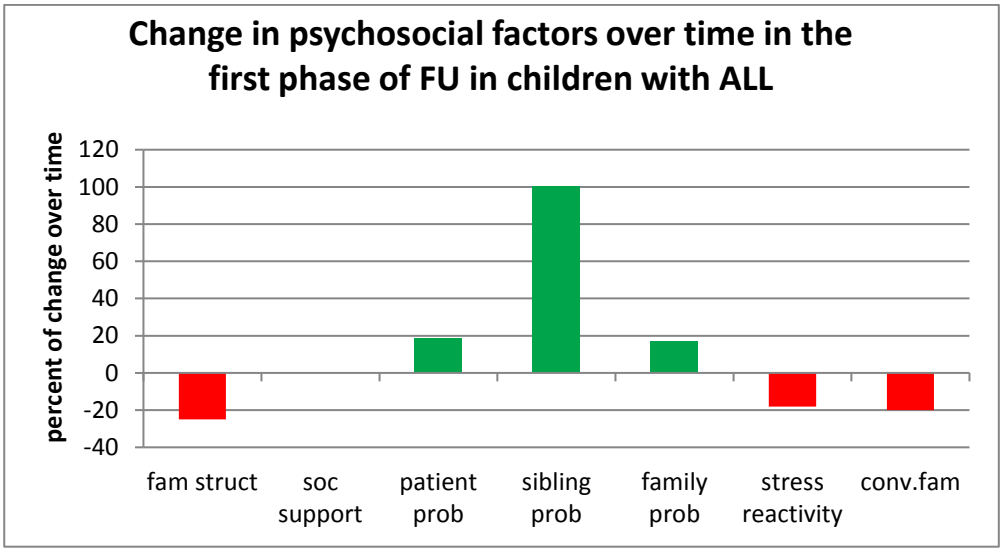


Figure 35: Changes in psychosocial factors over time. In ALL group no modification in social support needs but family structure, stress reactivity of the family and family beliefs get worse.

OBJECT 2: Possible patient related predictive factors of neurocognitive deficits

Age at diagnosis

Only in ALL group age at diagnosis was highly correlated with Executive function (EF). Age correlated negatively with inhibit ($r = -.514$; $p < 0.01$), working memory ($r= -.473$; $p <0.01$) and overall EF: Global Executive Composite (GEC) ($r= -.415$; $p < 0.05$). Thus, the younger the patient, the more compromised these functions are. Therefore, we inserted age at diagnosis as a covariate. This allowed us to control for this variable and concentrate on the changes in EF over time without this confounding factor.

Gender

Gender has an important effect on executive function only in ALL gruop ($F (6,26) = 1,214$ $p=.331$ $\eta2= 219$) which in general indicates that females perform almost 7 times worse than males when we control for the age of onset. Gender, especially affected Working Memory ($F(1,31)=6,368$ $p <0.05$, $\eta2=170$ with and OR of 5.16), plan and organize ($F(1,31)=6,428$ $p <0.05$, $\eta2=172$ with and OR of 5.22),and GEC ($F(1,31)=5,98$ $p <0.05$, $\eta2=162$ with and OR of 4,92). Shift and inhibit were only mildly affected. Females performed better than males on all these subscales.

OBJECT 3: Genes as potential predictors of the severity of deficits in children with cancer during the early phase of follow-up.

A binary logistic regression was performed to ascertain the effect of group (type of diagnosis), time (SFU and SFU+1) and genetic expression of 4 polymorphisms (5HTTLPR, BDNF Val66Met, COMT Val158Met and MTHFR C677T) on the likelihood that patients had clinically compromised scores in various domains of executive function. We used binary logistic regression analysis (figure 36) because, by design, it overcomes many of the restrictive assumptions of linear regressions. For example, linearity, normality and equal variances are not assumed, nor is it assumed that the error term variance is normally distributed. The major assumption is that the outcome variable must be dichotomous. The outcome variable for the executive domains was set at above a T-score of 54 yes or no.

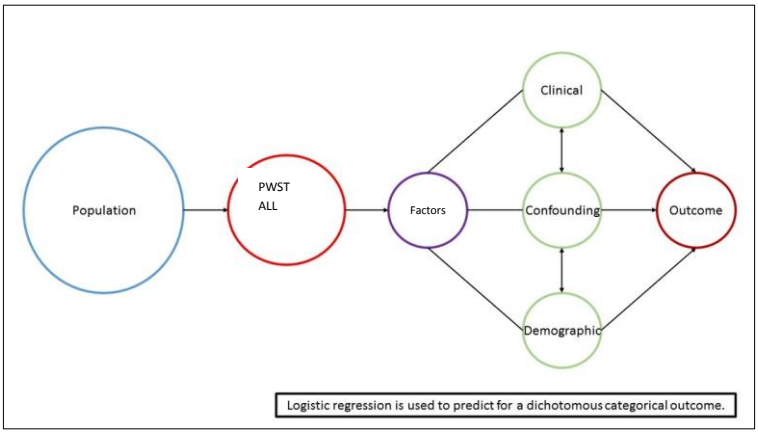


Figure 36: binary logistic regression analysis

Genetic factors as predictors on executive function in children with cancer

1. INHIBIT

The model explained 48,7% (R^2 Nagelkerke) of the variance in deficits in the capacity to inhibit ones thought or action and correctly classified 90% of the cases.

Tabella di classificazione[a]

Observed			Expeted inhcompr normal	compromised	Percentage of correction
phase 1	inhcompr	normal	23	1	95,8
		compromised	2	4	66,7
	Percentuale globale				90,0

a. Il valore di divisione è ,500

Patients with at least one at least one Met allele of the COMT gene were 11 times more likely to have compromised inhibition and MTHFR homozygous CC patients were 14 times more likely to experience problems. This suggests that availability of dopamine may play a role in this domain of executive function. No difference exists between the risk conferred by the type of diagnosis.

Variabili nell'equazione

		B	S.E.	Wald	gl	Sign.	Exp(B)	95% C.I.per EXP(B) inf	sup
phase 1[a]	group(1)	,315	1,245	,064	1	,800	1,370	,119	15,705
	tempo(1)	,619	1,231	,253	1	,615	1,856	,166	20,707
	ser(1)	-22,041	11669,258	,000	1	,998	,000	,000	.
	bdnf(1)	-1,413	1,403	1,014	1	,314	,243	,016	3,809
	comt(1)	2,664	1,752	2,310	1	,129	14,349	,463	445,149
	mthfr(1)	2,503	1,468	2,907	1	,088	12,215	,688	216,939
	Costante	-2,968	2,055	2,087	1	,149	,051		

2. MENTAL FLEXIBILITY

The model, that is group membership as well as change over the first year of follow-up, together with the expression of various polymorphisms explained 41,2 % (R2 Nagelkerke) of the variance in mental flexibility. Patients classified as having problems were identified correctly 76,7% of the cases. Group was not an important predictive factor in indicating who was more likely to have problems related to mental flexibility. However, patients homozygous for the Val allele (Val/Val) were significantly more likely to develop ($p<0.05$) compromised mental flexibility (SHIFT), odds ratio 10.946. Also, SHIFT was 6 times more likely to display deficits at the onset of follow-up; odds ratio5,955.

Tabella di classificazione[a]

	Osservato		Previsto shiftcompr normal	Previsto shiftcompr compromised	Percentuale di correttezza
Fase 1	shiftcompr	normal	14	2	87,5
		compromised	5	9	64,3
	Percentuale globale				76,7

Variabili nell'equazione

		B	S.E.	Wald	gl	Sign.	Exp(B)	95% C.I.per EXP(B) inferiore	superiore
Fase 1[a]	group(1)	-,366	,964	,144	1	,704	,694	,105	4,588
	tempo(1)	1,784	1,026	3,023	1	,082	5,955	,797	44,500
	ser(1)	-,275	1,086	,064	1	,800	,759	,090	6,383
	bdnf(1)	2,393	1,037	5,325	1	,021	10,946	1,434	83,546
	comt(1)	,252	1,143	,049	1	,826	1,286	,137	12,079
	mthfr(1)	-,057	,962	,003	1	,953	,945	,143	6,224
	Costante	-2,398	1,458	2,706	1	,100	,091		

a. Variabili inserite nella fase 1: group, tempo, ser, bdnf, comt, mthfr.

3. EMOTIONAL CONTROL

The model significantly explained (Chi Quadrato=13.746, p<.05) 49% (R^2 Nagelkerke) of the variance observed in emotional control. Patients classified as having problems were identified correctly 83,3% of the cases. Group was not an important predictive factor in indicating who was more likely to have problems related to mental flexibility. Patients, independent of diagnosis, homozygous for the Val allele (Val/Val) were significantly more likely to develop (p<0.05) problems regarding emotional odds ratio 25.529. PWST had a slightly enhanced risk (OR 3,659).

Tabella di classificazione[a]

			Previsto emotcompr		
	Osservato		normal	compromised	Percentuale di correttezza
Fase 1	emotcompr	normal	13	3	81,3
		compromised	2	12	85,7
	Percentuale globale				83,3

a. Il valore di divisione è ,500

Variabili nell'equazione

		B	S.E.	Wald	gl	Sign.	Exp(B)	95% C.I.per EXP(B) Inferiore	Superiore
Fase 1[a]	group(1)	1,297	1,070	1,470	1	,225	3,659	,450	29,782
	tempo(1)	,388	1,025	,144	1	,705	1,475	,198	10,990
	ser(1)	,483	1,322	,134	1	,715	1,621	,122	21,612
	bdnf(1)	3,240	1,163	7,762	1	,005	25,529	2,613	249,376
	comt(1)	,045	1,205	,001	1	,970	1,046	,099	11,105
	mthfr(1)	,665	1,068	,388	1	,533	1,944	,240	15,767
	Costante	-3,285	1,659	3,923	1	,048	,037		

a. Variabili inserite nella fase 1: group, tempo, ser, bdnf, comt, mthfr.

4. WORKING MEMORY

The model explained 23,6% (R^2 Nagelkerke) of the variance in deficits in working memory and correctly classified 76,7 % of the cases. Working memory integrity was non predicted by type of diagnosis nor by any of the polymorphisms. In PWST, Working Memory was not compromised in the early phase of follow-up and the various polymorphisms did not help to predict who would be more vulnerable.

Tabella di classificazione[a]

			Previsto wmcompr		
	Osservato		,00	1,00	Percentuale di correttezza
Fase 1	wmcompr	,00	23	0	100,0
		1,00	7	0	,0
	Percentuale globale				76,7

a. Il valore di divisione è ,500

Variabili nell'equazione

								95% C.I.per EXP(B)	
		B	S.E.	Wald	gl	Sign.	Exp(B)	Inferiore	Superiore
Fase 1[a]	group(1)	,268	,948	,080	1	,778	1,307	,204	8,386
	tempo(1)	,427	1,008	,180	1	,672	1,533	,212	11,064
	ser(1)	-,725	1,281	,320	1	,572	,484	,039	5,969
	bdnf(1)	-,353	,938	,142	1	,707	,703	,112	4,417
	comt(1)	-20,038	15015,255	,000	1	,999	,000	,000	.
	mthfr(1)	-,238	,964	,061	1	,805	,788	,119	5,215
	Costante	-,848	1,294	,429	1	,513	,428	,204	8,386

a. Variabili inserite nella fase 1: group, tempo, ser, bdnf, comt, mthfr.

5. PLAN AND ORGANIZE

The model explained 26,3% (R^2 Nagelkerke) of the variance of possible deficits in the planning of a thought or action and correctly classified 80% of the cases, however, compromised cases were predicted in only 44,4 percent. Deficits in planning were 4 times more likely for PWST. In PWST, PLAN was was not compromised in the early phase of follow-up and the various polymorphisms did not help to predict who would be more vulnerable.

Tabella di classificazione[a]

			Previsto plancompr		
	Osservato		,00	1,00	Percentuale di correttezza
Fase 1	plancompr	,00	20	1	95,2
		1,00	5	4	44,4
	Percentuale globale				80,0

a. Il valore di divisione è ,500

Variabili nell'equazione

								95% C.I.per EXP(B)	
		B	S.E.	Wald	gl	Sign.	Exp(B)	Inferiore	Superiore
Fase 1[a]	group(1)	1,423	,992	2,057	1	,151	4,149	,594	28,997
	tempo(1)	,374	,969	,149	1	,699	1,454	,218	9,710
	ser(1)	,622	1,141	,297	1	,586	1,862	,199	17,413
	bdnf(1)	1,371	,987	1,929	1	,165	3,939	,569	27,264
	comt(1)	-1,328	1,251	1,128	1	,288	,265	,023	3,074
	mthfr(1)	1,299	1,005	1,672	1	,196	3,666	,512	26,273
	Costante	-3,130	1,552	4,070	1	,044	,044		

a. Variabili inserite nella fase 1: group, tempo, ser, bdnf, comt, mthfr.

6. INITIATE

The model explained 37,3% (R^2 Nagelkerke) of the variance of possible deficits in initiating a thought or action and correctly classified 74,1% of the cases, however, compromised cases were predicted in only 44,4 percent. The type of diagnosis (Group) represents a significant predictive factor in indicating who was more likely to have problems related to initiating a thought or action. PWST were almost 17 times more likely to display compromised function in this domain. Also, INITIATE was 6 times more likely to display deficits at the onset of follow-up.

Tabella di classificazione[a]

			Previsto initcompr		
	Osservato		,00	1,00	Percentuale di correttezza
Fase 1	initcompr	,00	16	2	88,9
		1,00	5	4	44,4
	Percentuale globale				74,1

a. Il valore di divisione è ,500

Variabili nell'equazione

								95% C.I.per EXP(B)	
		B	S.E.	Wald	gl	Sign.	Exp(B)	Inferiore	Superiore
Fase 1[a]	group(1)	2,820	1,387	4,132	1	,042	16,776	1,106	254,459
	tempo(1)	1,803	1,138	2,510	1	,113	6,067	,652	56,424
	ser(1)	1,649	1,293	1,625	1	,202	5,201	,412	65,627
	bdnf(1)	1,198	1,180	1,031	1	,310	3,313	,328	33,446
	comt(1)	1,145	1,170	,956	1	,328	3,141	,317	31,149
	mthfr(1)	1,637	1,273	1,654	1	,198	5,138	,424	62,248
	Costante	-5,836	2,500	5,449	1	,020	,003		

7. ORGANIZATION OF MATERIAL

The model significantly explained (Chi Quadrato=19.143, p<.01) 78,4% (R^2 Nagelkerke) of the variance observed in the capacity to organize material. Patients classified as having problems were identified correctly 81,8% of the cases. None of the factors played a determining role in predicting who was more at risk for compromised function. This is most likely related to the fact that this domain of executive functions seems to be one of the least at risk for deficits in both patient groups.

Tabella di classificazione[a]

			Previsto orgcompr		
	Osservato		,00	1,00	Percentuale di correttezza
Fase 1	orgcompr	,00	11	2	84,6
		1,00	2	7	77,8
	Percentuale globale				81,8

a. Il valore di divisione è ,500

8. MONITOR

The model explained 31,3% (R^2 Nagelkerke) of the variance of possible deficits in initiating a thought or action and correctly classified 82,6% of the cases. Monitor was almost 4 times more likely to be compromised in PWST (OR 3,744). None of the other factors represent important predictors

Tabella di classificazione[a]

			Previsto moncompr		
	Osservato		,00	1,00	Percentuale di correttezza
Fase 1	moncompr	,00	12	2	85,7
		1,00	2	7	77,8
	Percentuale globale				82,6

a. Il valore di divisione è ,5

Variabili nell'equazione

		B	S.E.	Wald	gl	Sign.	Exp(B)	95% C.I.per EXP(B) Inferiore	Superiore
Fase 1[a]	group(1)	1,320	1,164	1,286	1	,257	3,744	,382	36,663
	tempo(1)	,919	1,054	,760	1	,383	2,507	,317	19,805
	ser(1)	,398	1,445	,076	1	,783	1,488	,088	25,288
	bdnf(1)	1,094	1,038	1,111	1	,292	2,986	,391	22,826
	comt(1)	-1,651	1,412	1,368	1	,242	,192	,012	3,052
	mthfr(1)	-,147	1,132	,017	1	,896	,863	,094	7,928
	Costante	-2,058	1,644	1,567	1	,211	,128		

a. Variabili inserite nella fase 1: group, tempo, ser, bdnf, comt, mthfr.

Polymorphisms of FK506 did not predict compromised executive function nor did it predict compromised emotional or behavioral problems.

In sum, variation in the expression of BDNF predicted heightened risk for compromised emotional control in PWST: carriers homozygous for BDNF val/Val perform worse (figure 38 a). In PWST 5HTTLPR differential expression did not predict compromised executive function. In contrast, in children with ALL, 5HTTLPR polymorphism was predictive of compromised function in various executive domains (figure 37), while BNDF polymorphic expression only predicted compromised emotional control for children homozygous for Val/Val (Figure 38 b).

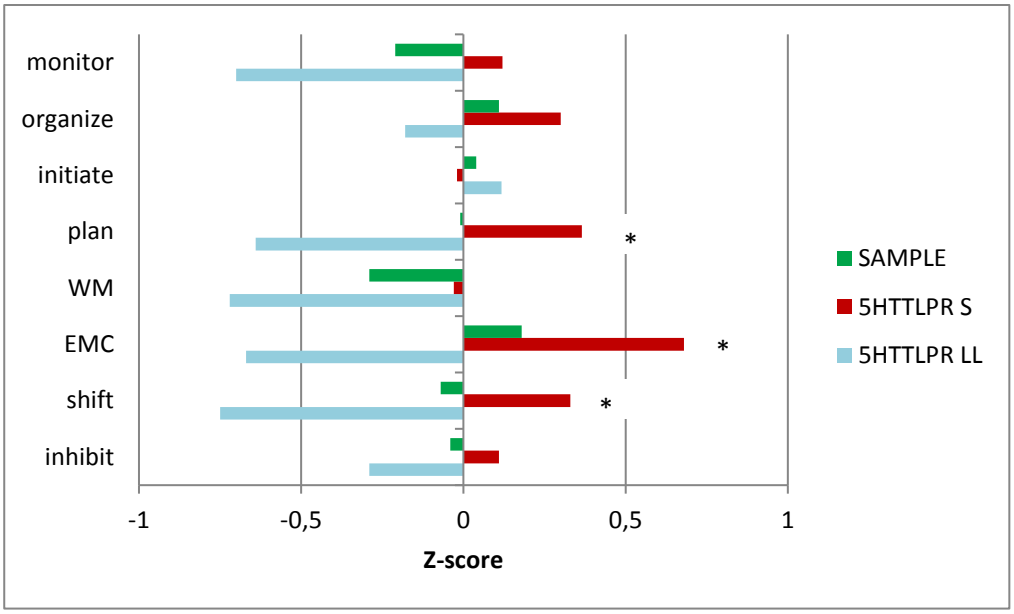


Figure 37: 5HTT LPR polymorphism, is an additive factor on EF in ALL: children with at least on S allele

perform worse.

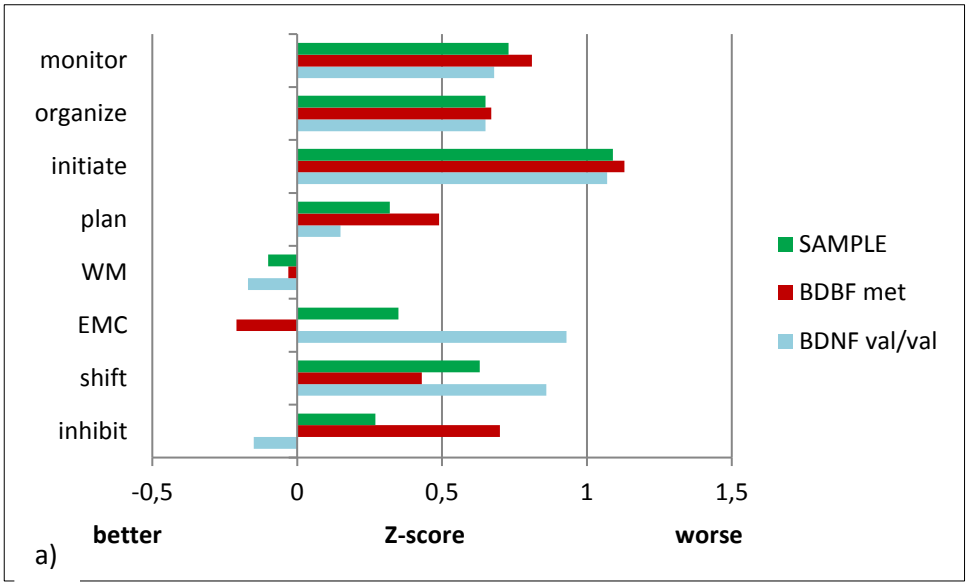


Figure 38 part a: BDNF expression in children with solid tumors and EF, children Val/Val carriers performe worse on emotional control and shift.

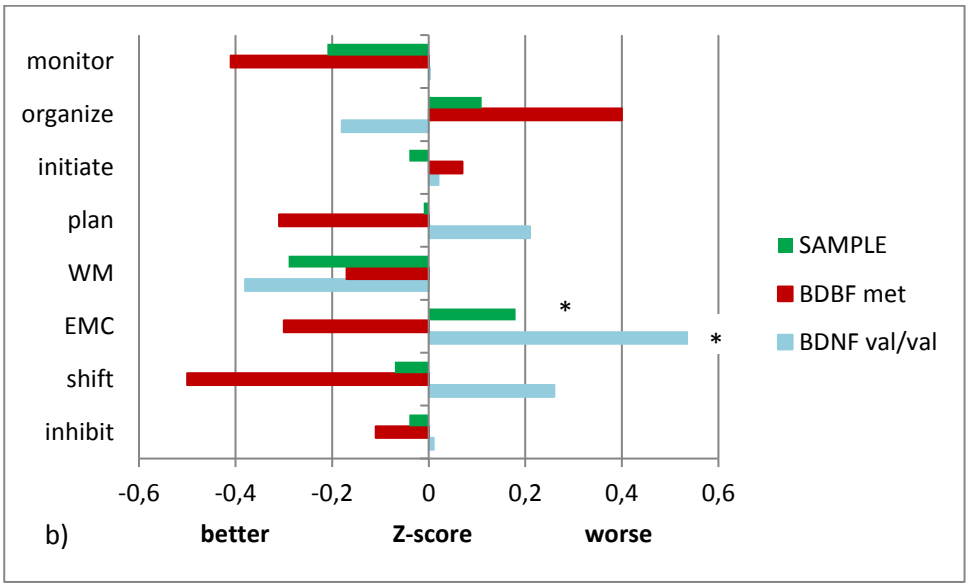


Figure 38 part b: BDNF expression in children with ALL and EF

Our data suggest that in general PWST have more problems related to executive function during the first phase of follow-up but most of them seem to diminish within the first year.

Furthermore, mental flexibility and emotional control seem to be most likely compromised and are often reliably predicted by polymorphisms of BDNF; Val/Val carriers are more likely to

experience deficits in emotional control than Met carriers.

Genetic factors as predictors on behavior and emotional domain in children with cancer

The best predictor of total risk was to what group the child belonged, that is, with what class of tumor had the child or adolescent been diagnosed (B=6,866, t=2,197, p<.05, 95CI 0,205-13,527). PWST had more problems than children with ALL. Altogether, the model containing group as well as all the genes tested, explained 35,4% of the variance observed in the total number of behavioral and emotional problems. However, genes did not contribute in an important way to predicting who is and most likely will be at risk.

Emotional problems

When considering emotional problems alone, group correlated highly (R=.680) and 5-HTT genotype markedly (R=.337) with the heightened presence of emotional problems, and the model explained 54,5 % of the variance. However, only group membership, predicted emotional deficits in a significant way (B =3,456, t=3,552 , p=.003, 95CI 1,369-5,543).

Behavioral problems

Behavioral problems were significantly correlated with 5HTT genotype (R= .387, p< .05), that is, patients with at least one S allele displayed more behavioral problems. The model predicted 27.7 % of the variance related to behavioral problems. However, none of the factors significantly predicted outcome.

Hyperactivity

Hyperactivity was markedly correlated with group membership, with PWST showing a higher level of hyperactivity related behaviors (R=.358, p=.055). The model explained 24,4% of the variance observed in hyperactivity.

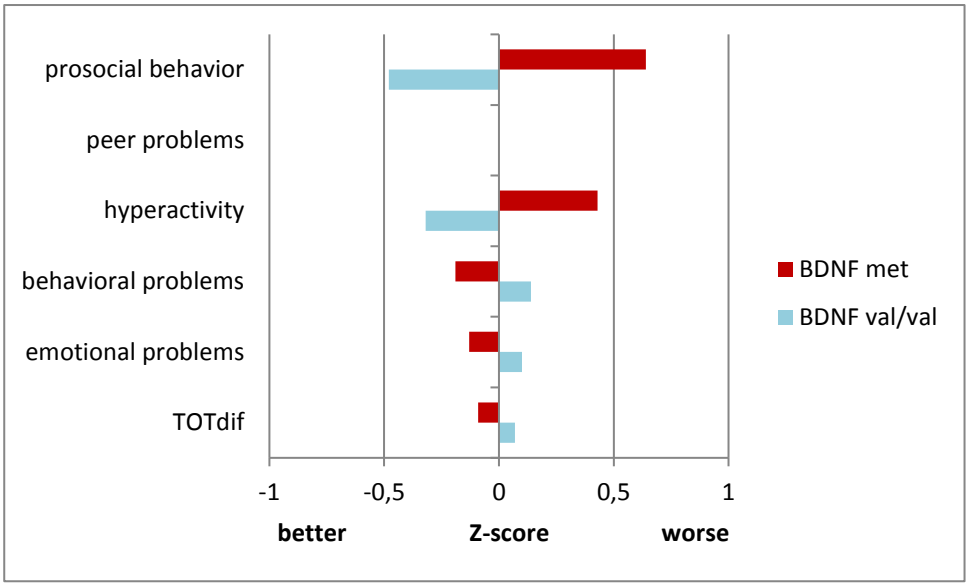
Peer related problems

Peer related problems correlated significantly with group (R=.660, p<.001) and markedly with 5-HTT genotype (R=.356, p=.057). PWST presented more peer related problems than children diagnosed with ALL. While the model explained 55,1 % of the variance, only group membership, predicted peer related problems in a significant way (B =2,611, t=3,552, p=.003, 95CI 1,035-4,187).

In short, PWST were somewhat more at risk. Also , with respect to emotional and behavioral problems, children previously diagnosed and treated for ALL display reduced pro-social capacities as well as enhanced hyperactivity. PWST are significantly more hyperactive in the presence of at least one Met allele, whereas

Val/Val homozygous PWST display more overall behavioral and emotional difficulties, which is mostly linked to worsening of emotional problems (figure 39 a,b).

a) ALL



b) PWST

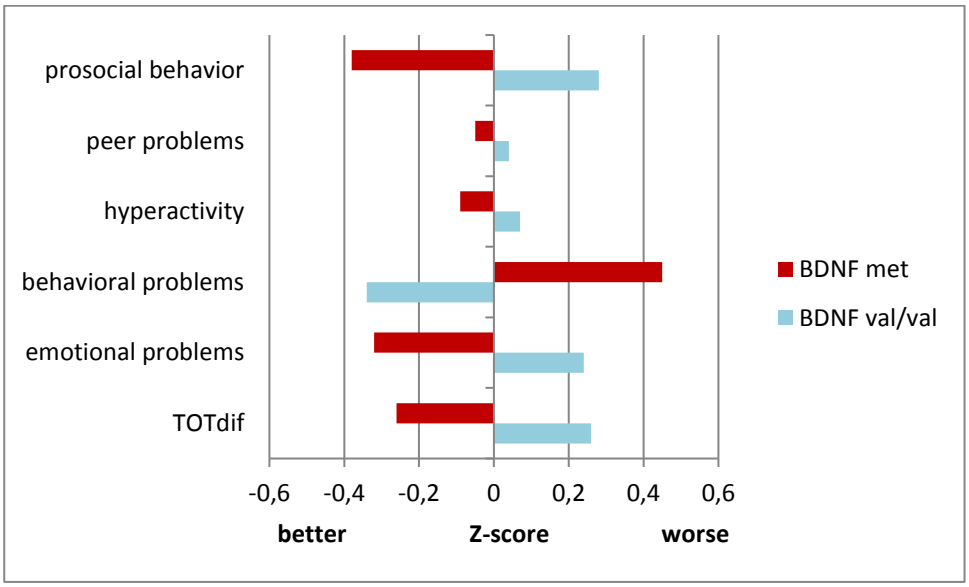


Figure 39 a and b: BDNF polymorphisms predict changes in emotional and behavioral problems

Polymorphisms of the 5HTTLPR predict compromised prosocial capacity only in children homozygous for the L allele (Figure 40).

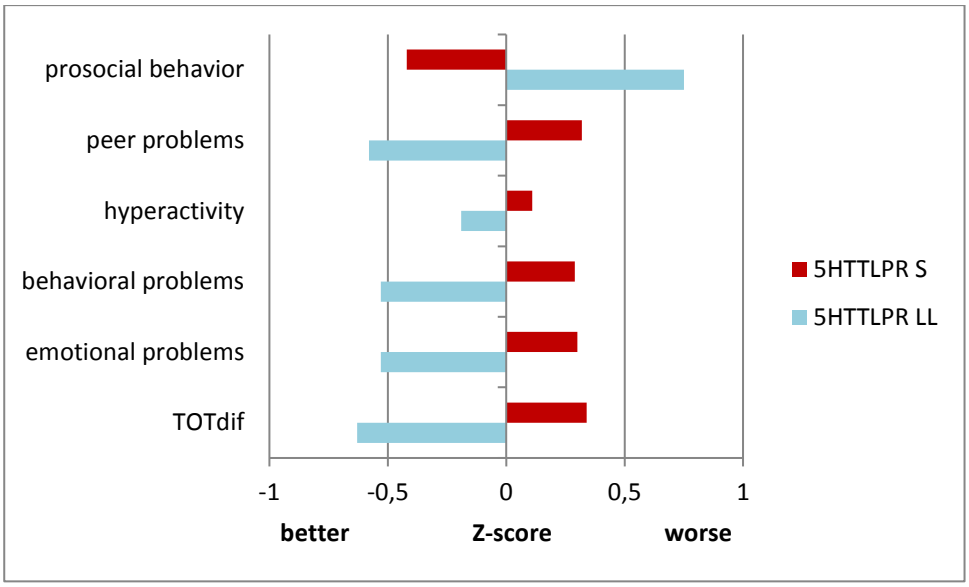


Figure 40: 5HTT and emotional and behavioral problems in children with ALL

4.4.1 Pattern of connectivity among of domains of EF function and emotional and behavioral domains influenced by EF in children with Epilepsy

We explored the applicability of network and graph theory to understand the impact of epilepsy on cognition compared with other severe or chronic disease, such as asthma and pediatric cancer. Consequently, the patterns of cognitive development the sick children and their development would set the stage for prospective comparisons of how disability might develop in these children over time and what variables may help us to predict what problems may arise or get worse. In children with epilepsy and other pathologies. The overall goal is to examine the potential utility of this analytic tool and approach to conceptualize the cognitive comorbidities in epilepsy. Given that the major cognitive domains representing cognitive function are interdependent, the associations between neuropsychological abilities underlying these domains can be referred to as a cognitive network. Therefore, the architecture of this cognitive network can be quantified and assessed using graph theory Methods, rendering a novel approach to the characterization of cognitive status.

When considering the network of executive and behavioral functions in our patients one can observe that the bigger spheres represent the hubs of the network which are those domains that facilitate the interactions between other domains. The change in the importance of these hubs between the first and second year of follow-up in the group of epileptic patients was highly dynamic especially as related to domains, such as, emotional control, WM and the capacity to organize, while other domains were more on the outside of the network.

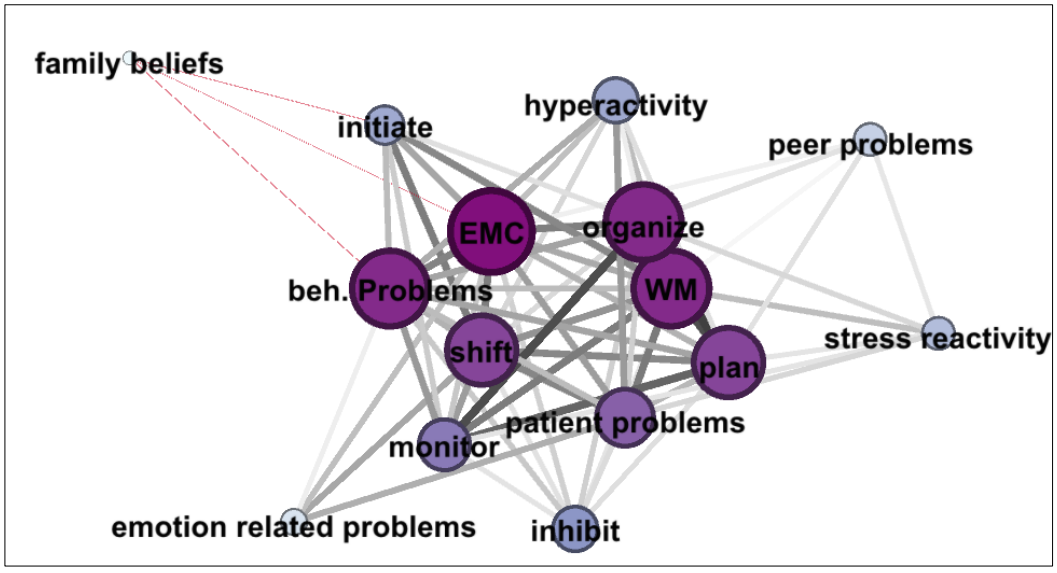


Figure 41: network analysis applied to executive functions, emotional, behavior and psychosocial domains in children with epilepsy.

Domains related to executive functions, such as working memory (WM), plan and organize, shift, together with behavioral problems are those domains more susceptible to change and appear related one to each other. On the other hand, Family believes (psychosocial domain) for example is out of the network.

Next, we considered the importance of two genes in driving the organization of the network. (Figure 42 and figure 43). The network of patients homozygous for Val/Val was a sparsely connected network where change toke place primarily in four domains with little connectivity among them (Figure 42). The network representation of patients carrying at least one Met allele was more connected and change take place in more connected domains. In presence of Val/Val the network is concentrated on organize, working memory and plan a thought or action were central hubs in driving this change (nodes key players). In presence of Met allele network is more diffused involving montitor, initate and organize abilities. Working memory and the capacity to plan and organize similar pattern can be observed for the MTHFR gene (Figure 43). Patients homozygous for the C allele were characterized by a thinly connected network of EF domains, with a complete different set of domains in which major change toke place. Here, change was liked to a network among emotional control, and initiate and inhibit. WM and plan were less connected. In contrast against a different background represented by the presence of at least one T allele, patients displayed diffuse change in EF. In, these patients change seemed to be driven by the capacity to organize a thought or action and less so by emotional control.

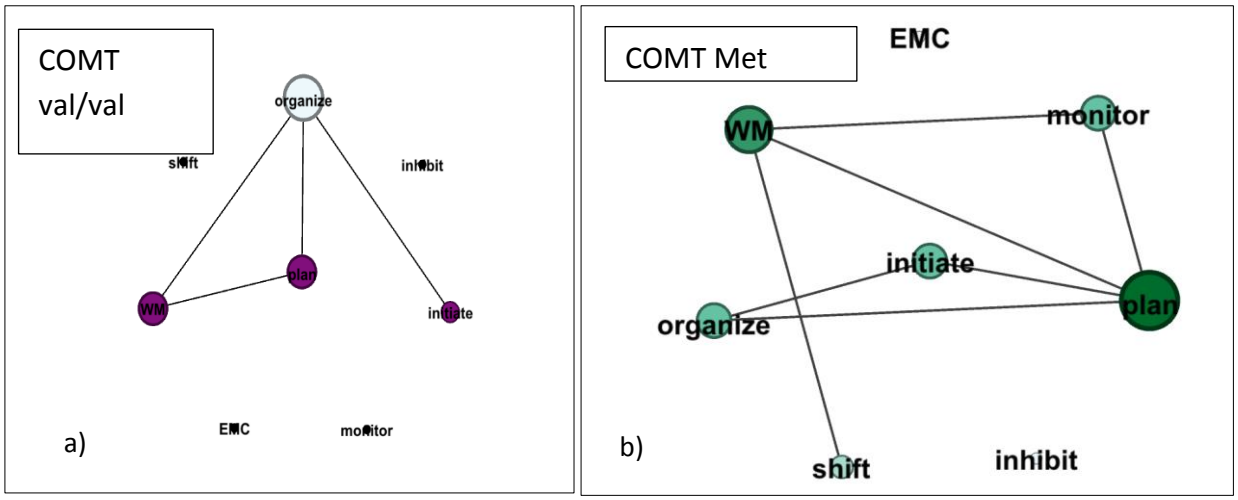


Figure 42 a and b: Comt Val158Met on change in executive functions domains network

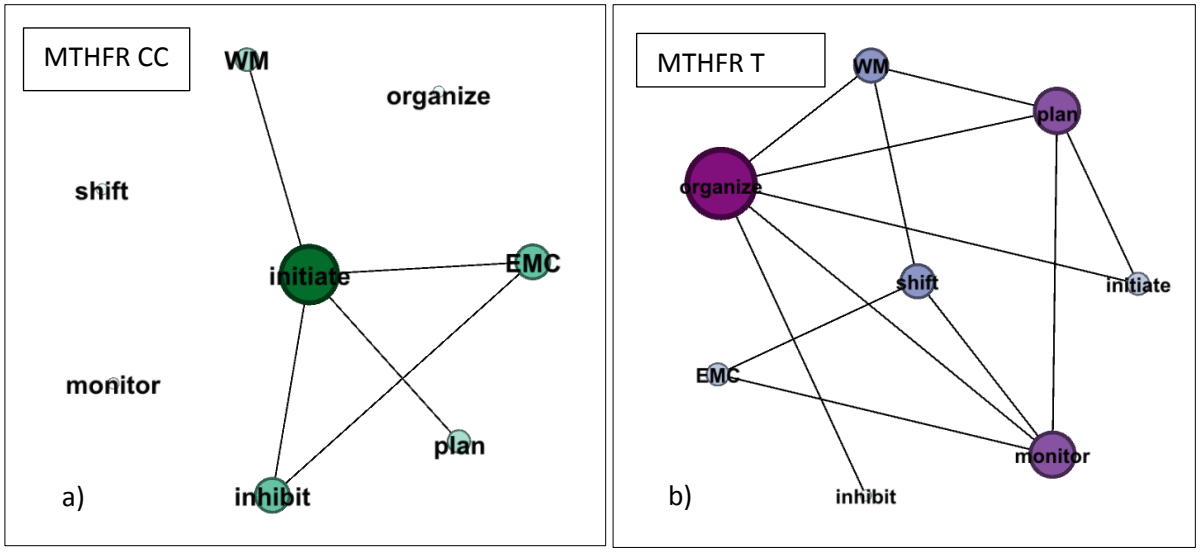


Figure 43 a and b: The effect of MTHFR C677T on change in executive functions domains network

The community structure of male patients and female patients were calculated over a one year period (Figure 44) in order to qualitatively visualize developmental changes and the differences in network formation and structure related to gender because gender has been found to play an important role in defining compromised EF and behavioral and emotional capacities. Differences could be easily discerned between males and females whereby the male patients display diffuse developmental change in many areas of EF, which could be appreciated in the arrangement of nodes. Note that the domain Initiate is not connected and has not been related to change over time. The cognitive networks of male patients were more diffuse than those of female patients where change over a one year period was concentrated in two domain, emotional control and the capacity to shift one's attention. The modularity of these changes reflects such qualitative results in which female patients have a significant increase in network organization while male patients display change in many domains which are not highly connected among each other.

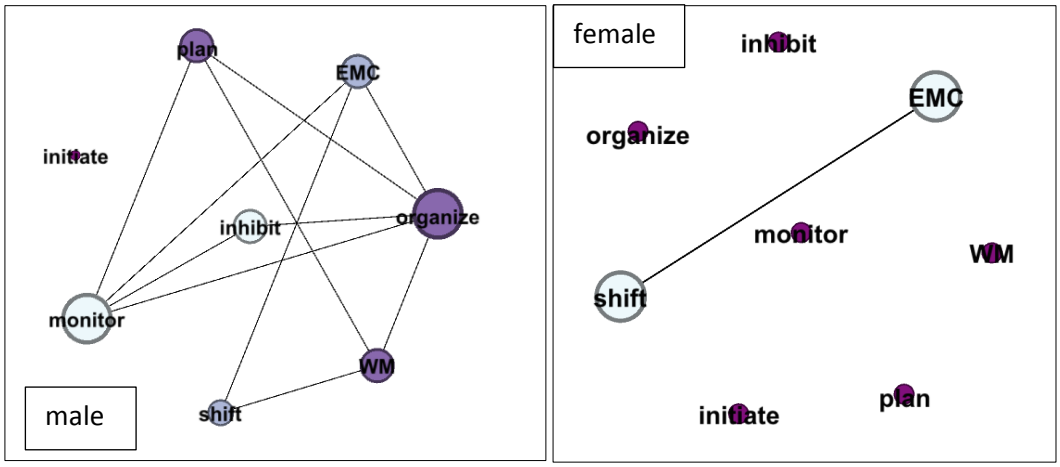


Figure 44: Gender effect on executive function network

The community structure of patients younger than 10 and older than 10 years of age where calculated over a one year period (Figure 45) in order to qualitatively visualize developmental changes and the differences in network formation and structure related to age. Differences could be easily discerned between groups whereby the younger patients seemed to present more diffuse developmental change, which could be appreciated in the arrangement of nodes. The cognitive networks of younger patients were not as organized as those of older patients where major changes were observed in a few connected domains. The modularity of these changes reflects such qualitative results in which older patients have a significant increase in network organization while younger patients display change in many domains which are not highly connected among each other.

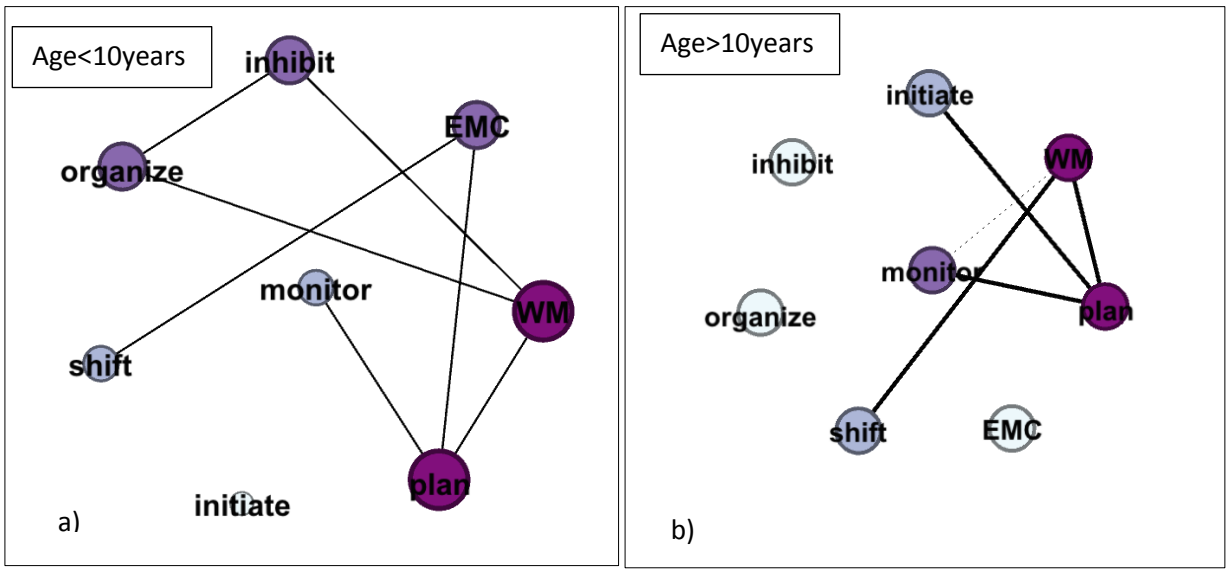


Figure 45 a and b: Age effect on executive functions network

4.4.2 Pattern of connectivity among of domains of EF function and emotional and behavioral domains children with Asthma

Cognitive abilities do not exist in isolation. It is the dynamic interaction among different domains that permit the development of healthy behavioral, emotional and cognitive function. The development of and Interactions between these domains may vary as a function of external demands as well as genetic predisposition. Nonetheless, damage due to a disease or its treatment and cognitive and behavioral functions may result in the less efficient overall functional architecture of cognitive processes. While this is a reasonable inference, there has been little empirical research to support or refute the hypothesis of an altered cognitive network using conventional neuropsychological measures.

Given this, in this thesis a novel approach was explored to characterize the effect of childhood asthma, epilepsy and pediatric cancer on the global landscape of cognition, defined by the interaction of multiple cognitive domains, especially EF. Given that different EF domains are interdependent with each other, the associations between the neuropsychological abilities underlying these domains could be referred to as a cognitive network. Thus, the architecture of the cognitive network can be quantified and assessed using formal methods to determine network conformation, i.e., graph theory. Graph theory, in essence, can provide a measure of the architectural organization of cognitive function, as defined by the network formed by the interrelationships between multiple cognitive abilities and domains. As such, graph theory is an expansion on conventional statistical approaches because it permits the evaluation not only of grouping of cognitive modules but also the participation of cognitive functions/domains within the entire cognitive design. Therefore, cognitive networks may provide novel insights into the cross-sectional status and longitudinal changes in cognitive structure, especially in regard to the abnormal conformation that may be driven by certain types of pathologies. Figure Two-dimensional graph representation illustrating the spatial (and functional) relationship between cognitive and behavioral tests in a sample of children with asthma. The spatial distribution was calculated using the force-atlas graph algorithm, where nodes that demonstrate stronger connections are located closer in space, while nodes with fewer connections tend to lie on the outside. Nodes with a similar color belong to the same cluster/domain. In the overall sample of children diagnosed with asthma (high and low performers together), two main clusters of connected domains could be identified, a cluster centered on executive function with domains, such as, distractibility and hyperactivity connected but to a lesser degree. Second, a cluster centered around emotional problems, such as, sadness associated with peer related problems and attention problems. In the first cluster, the central domains displaying problems are linked to the capacity to monitor, as well as, to plan and WM. No clear community structure could be observed, although the two clusters underlie distinct functions for which specific therapies might be developed. High performing patients with asthma, a hub function can be attributed to the sadness and anxiety of the child, which in turn is strongly related to the attitude of the

family towards the disease and the professionals that manage the care of the patient as well as problems patients experience in relation to their disease.

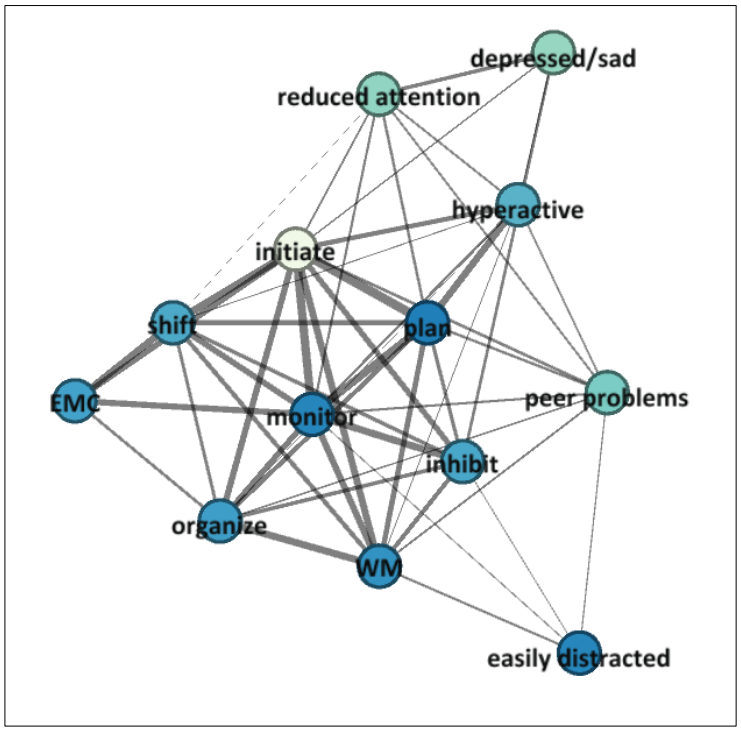


Figure 46: executive function domains networks in all asthma patients

Number of connected edges in patients with asthma. This indicates the number of nodes that have a certain number of connections with other nodes. More simply, how well is a domain of function connected to other domains. The network represents a high degree of connectivity with many nodes highly connected and few nodes with few connections.

Patients with asthma performing in the high range of Executive Function (high performers)

From recent research it has emerged that cognitive function and more specifically EF can be adversely affected in childhood asthma, even among the so-called less severe forms (as our patients). In such groups of patients, intelligence is generally within the norm but with abnormalities in specific areas of cognitive ability including executive function. Prospective investigations tracking children from the time of onset and diagnosis indicate that cognitive differences can be present at or near the time of diagnosis with these differences typically maintained over time, without evidence of progressive decline or significant improvement over the years following. Here we present the data at the onset of a prospective long-term follow up study. In the first part of this research, patterns of EF and behavioral strengths and difficulties have been developed and characterized by analysis of individual test scores, combinations of test scores, or

factor scores at baseline and one year after (represented as change over time). While cognitive differences between participants asthma can be identified and tracked over time with sophisticated test batteries, it is unclear, however, how these diverse cognitive abilities and domains interact with one another in the participants with asthma and other chronic or severe diseases such as cancer, and if the interrelationships are different and, if so, in what ways. Also unclear is how these cognitive interrelationships and networks may change over time with maturation and how certain genes may add risk to the development of these domains or patterns of function. Increasing chronological age is of course associated with the development and specialization of discrete cognitive skills (e.g., executive functions in adolescence) in the context of maturational brain changes; cognitive abilities increase from infancy to adulthood; however, depending on people's skills, they either decline or stay the same. One approach to examine the network of cognitive abilities and their integration is by using graph theory techniques. As noted previously, these analytic approaches have been utilized in neuroscience research, but rarely has cognition been the sole focus of examination.

As our patient group was rather heterogeneous, thus, we performed a cluster analysis and subsequently analyzed the community structure of the two emerging groups, high performers and low performers (see previous analysis).

The community structure of both high performing and low performing patients were calculated (Figure 47 and Figure 48) in order to qualitatively visualize group differences. Differences could be easily discerned between groups whereby the high performing patients seemed to present the most efficient developmental change, which could be appreciated in the modular arrangement of nodes. However, cognitive networks of younger healthy participants did not seem as organized as might be anticipated. The modularity index reflects such qualitative results in which older controls presented a significant increase while younger controls presented similar values of modularity index at each time point

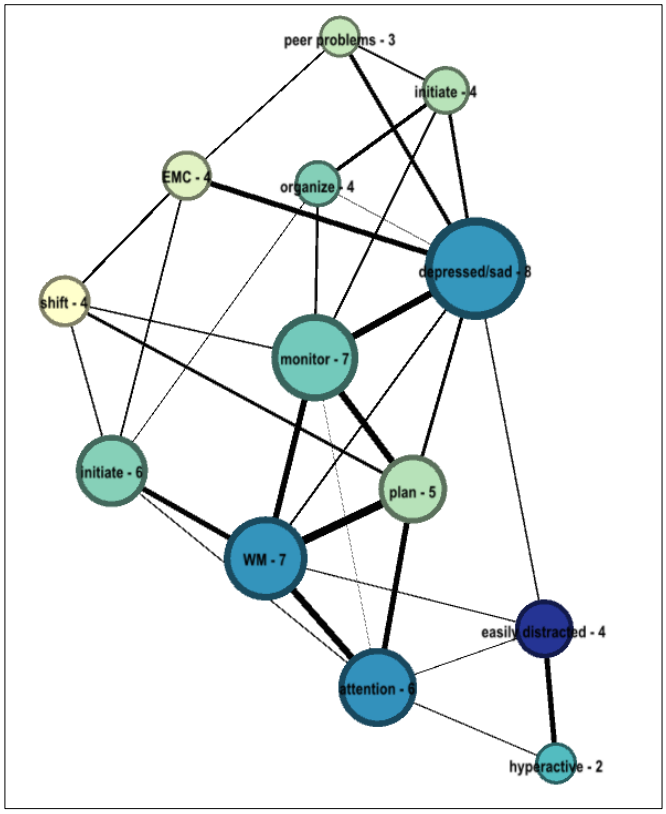


Figure 47: Executive function domains network of asthma “high performers”

Patients with asthma that have substantial difficulties with EFs (low performers)

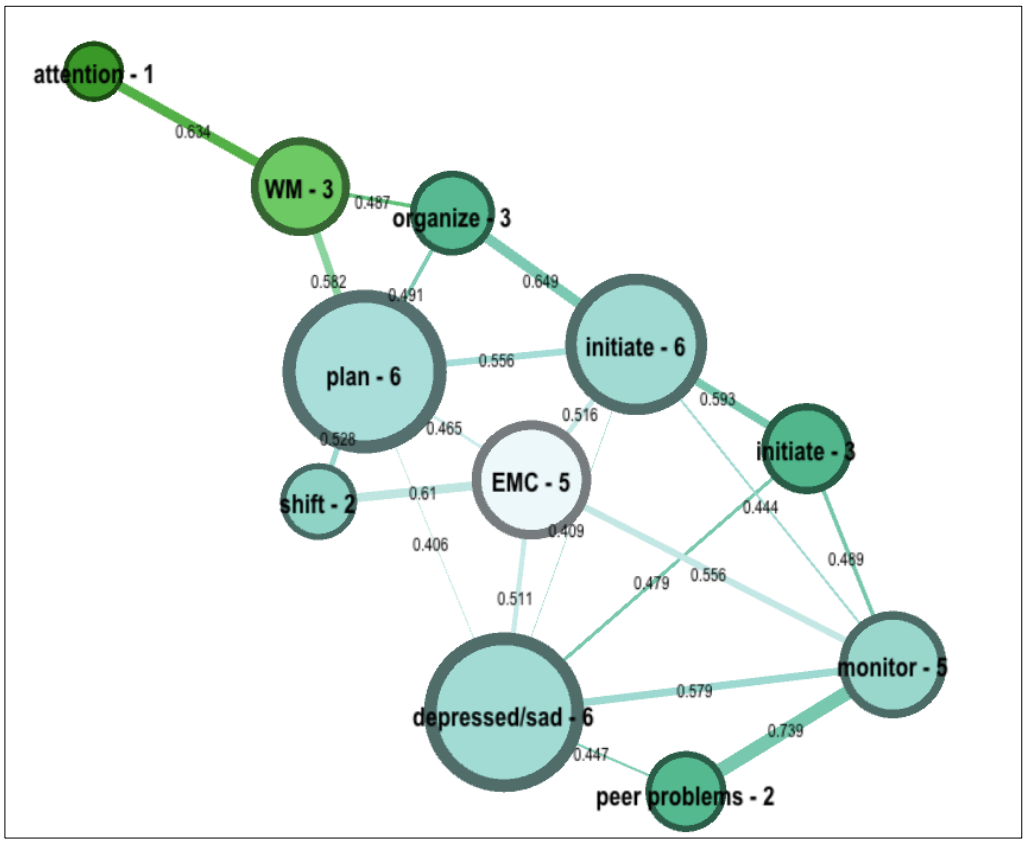


Figure 48: Executive function network in asthma low performer

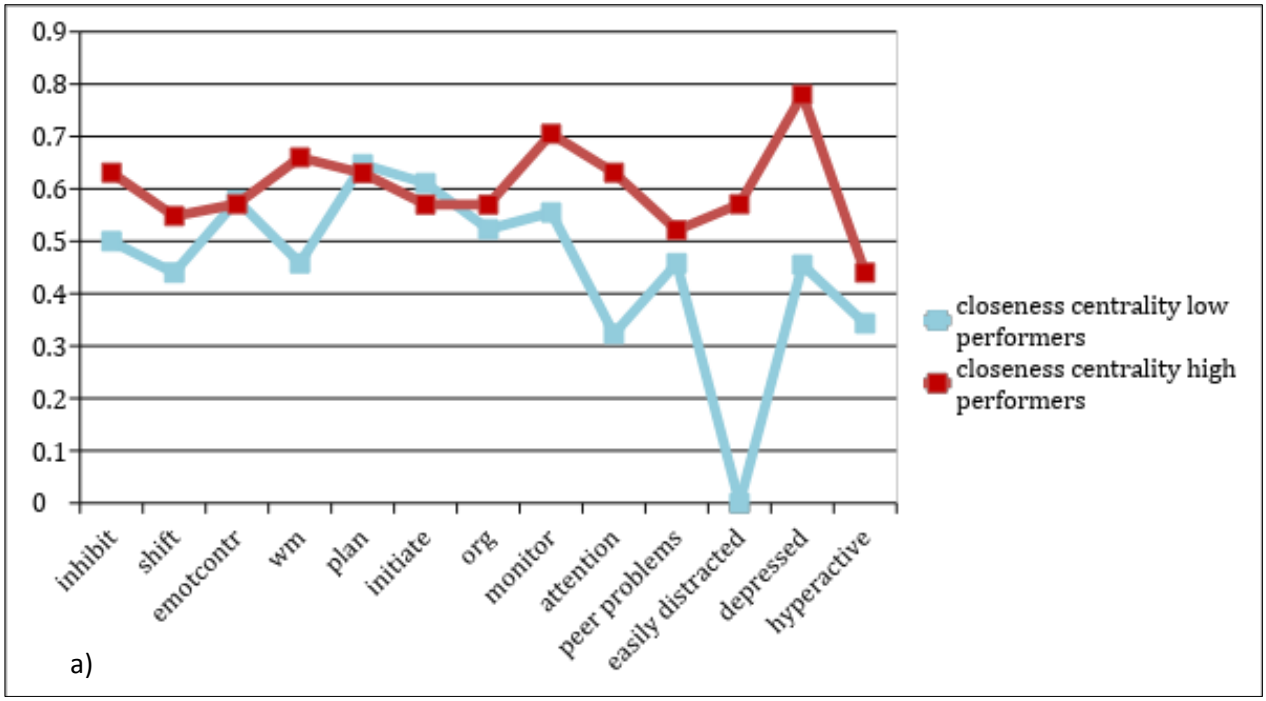
In order to describe the inter-relationship between different cognitive domains, a correlation matrix was computed between the performance in patients with asthma divided in high a low performers by cluster analysis. We evaluated the correlation coefficient between each possible pair of tests, where links represented the correlation coefficient between the tests. Two-dimensional graphs were reconstructed in order to define the overall structure of EF and behvior in each group. The data were exported to the software Gephi and organized using a Force Atlas algorithm (attraction strength =10, repulsion strength =100, gravity=30). To preserve the most important relationships and to improve visualization of the network structure, graphs were reconstructed using only links above the 50% percentile of weight in each group.

Local network metrics

Overall, patients in different clusters displayed different network metrics as demonstrated in Figure 49 a,b. Betweenness centrality showed a peak in low performing patients for WM, and plan and initiate, as opposed to high performers that showed a peak in betweenness in depression. This suggests a suboptimal interconnection of the WM and plan domain in low performers and for high performers in the emotional domain. Increased clustering coefficient and efficiency in patients indicate a more segregated cognitive network. Also, the results suggest a higher influence of specific nodes, i.e., cognitive tests/measures, over the network, as demonstrated by higher efficiency and clustering coefficient higher functioning patients.

Betweeness centrality measures the importance of a node in the communication of a network. Our findings showed that in low performing patients WM and plan play a key role in the communication between other nodes and can be considered a hub of this network. In turn, in high performers the emotional state of the patient has an important role in communicating with other nodes.

Also, being easily distracted in low performers, or suffering from depression or anxiety, interferes with network efficacy.



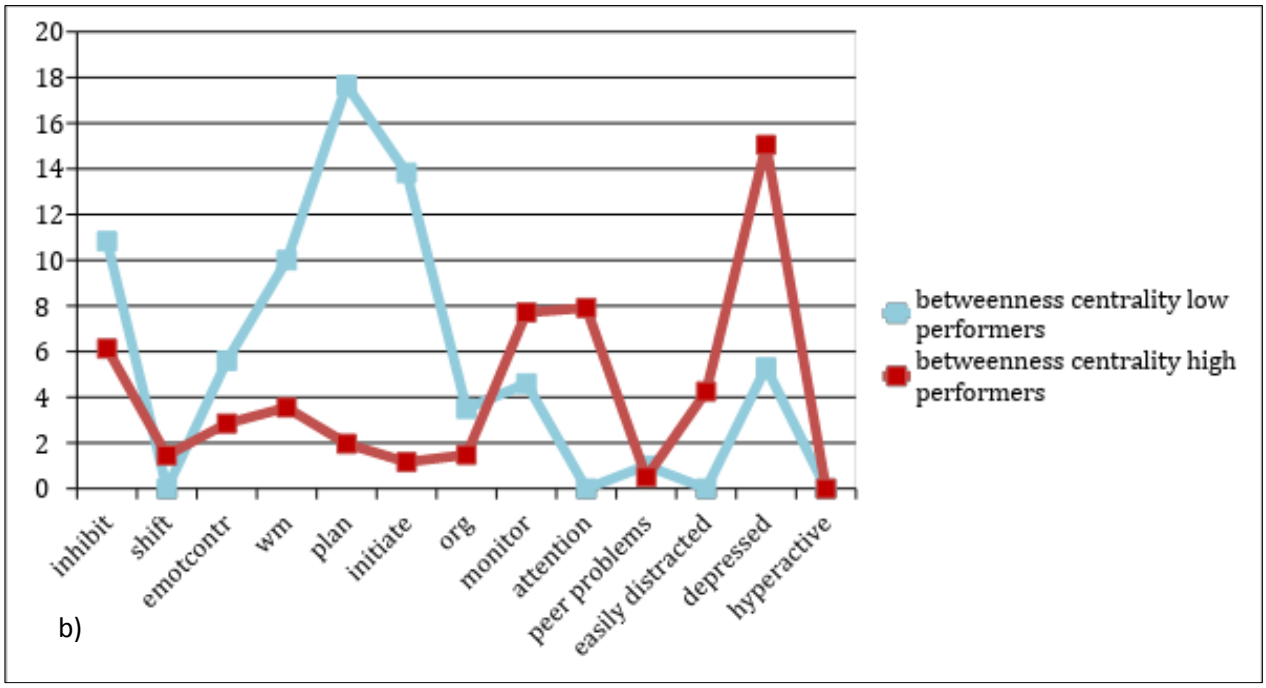


Figure 49 a and b: Distribution plots demonstrating global network measures

4.4.3 Pattern of connectivity among of domains of EF function and emotional and behavioral domains in PWST and ALL

Degree centrality assigns an importance score based purely on the number of links held by each domain o0f function (node). It tells us many direct, 'one hop' connections each domain has to other domain within the network. It helps us to find domains that are highly connected and that play an important role in connecting other domains with each other. Degree centrality is the simplest measure of node connectivity. To domain stands out as being highly connected, the domain of emotional problems and the behavioral problems, both heavily related to child centered problems. Interestingly, family related psychosocial function is much less central to the network and are less connected, while child related secondary, more specialized problems, such as, hyperactivity and problems related to peer interaction are more strongly connected to the two central domains.

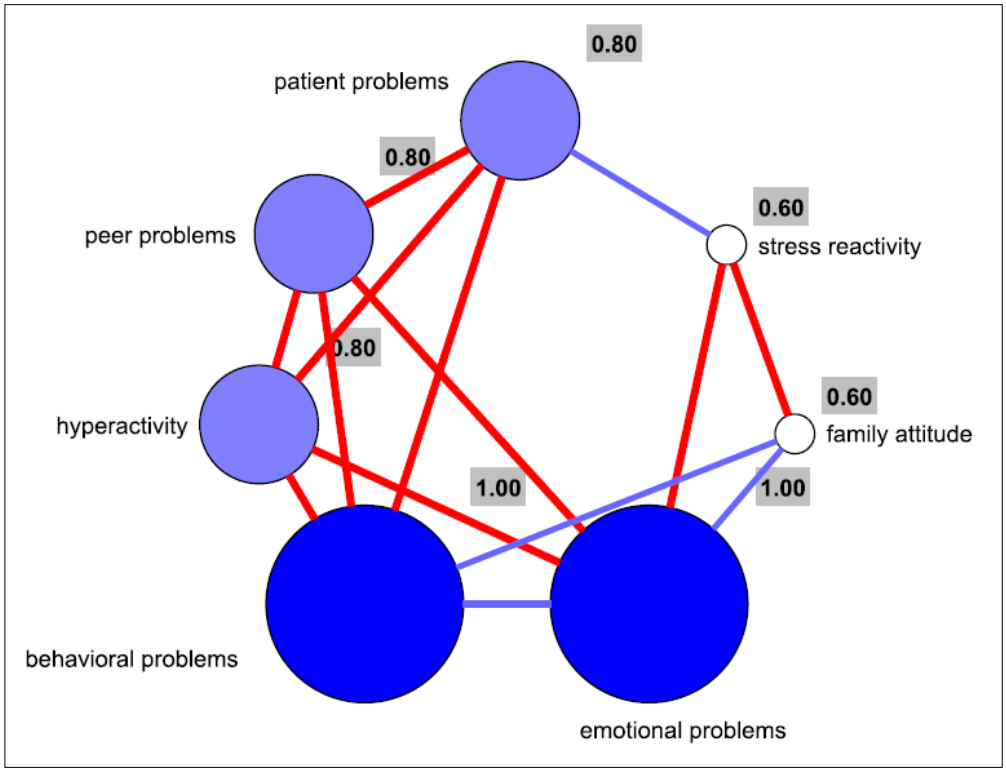


Figure 50: The degree centrality in PWST network between emotional and psychosocial domains. Red lines = p<0.001; Blu line p<0.005

In children diagnosed with ALL, a completely different pattern can be observed. Here, the central domain displaying problems, is related to the stress reactivity of the child and its family as a whole, which in turn is strongly related to family attitude towards the disease and the professionals that manage the care of the patient as well as problems that patients experiences in relation to their disease.

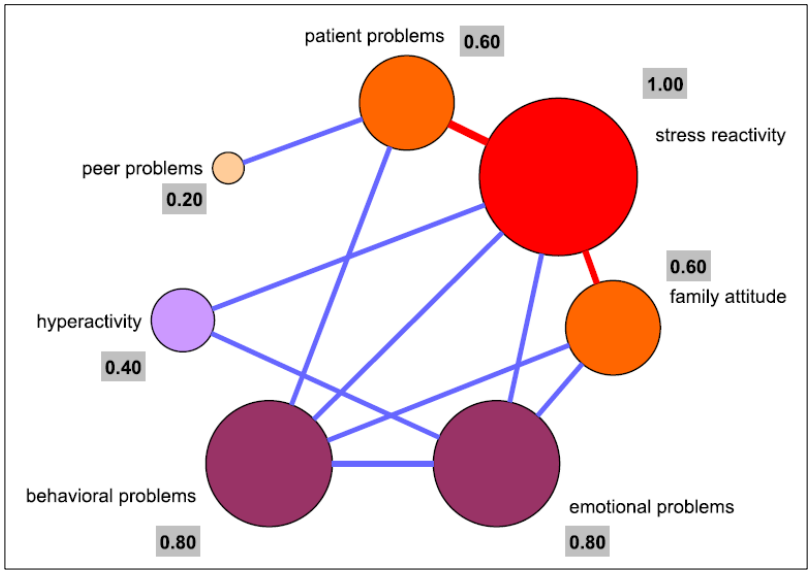


Figure 51: Emotional, psychosocial network degree centrality in ALL, Red lines = p<0.001; Blu line p<0.005

Figure 52 shows which domains act as 'bridges' between other domains in the network. It does this by identifying all the shortest paths and then counting how many times each node falls on one. Here, we used it to determine which domain(s) influence the connectivity among domains which allows us to analyze the dynamics of a network. In children with solid tumors we discovered that emotional problems represent the most "dominant" domain. This together with behavioral problems of the patient and other patient related problems suggest that the therapy should be largely child centered.

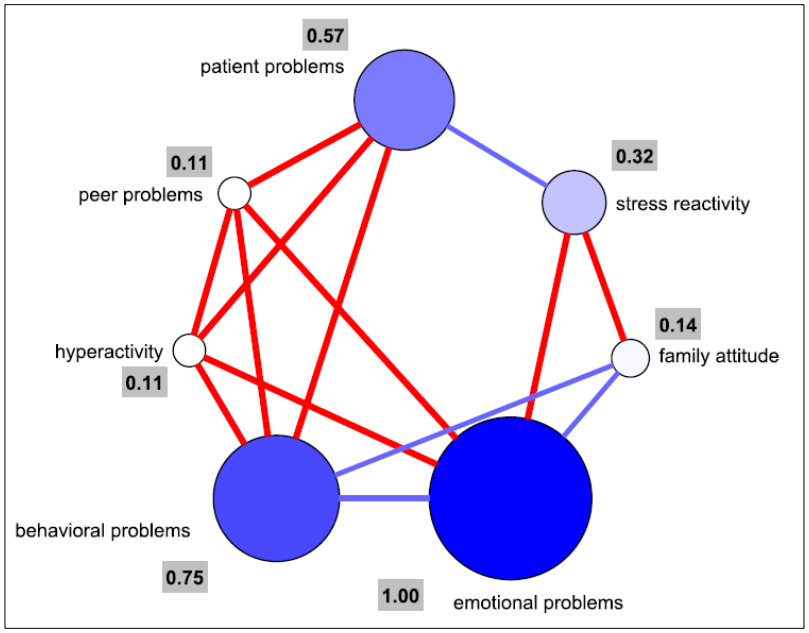


Figure 52: Betweenness centrality in PWST. Red lines = p<0.001; Blu line p<0.005

A complete different pattern of connectivity is represented by the betweenness centrality in the ALL follow-up group Figure 53. Here, two domains prevail and govern the psychosocial and behavioral problems of these patient, namely, stress reactivity of the family and the problems of the patient, often mood related. In this case, therapy should first of all be directed to the family with the purpose of enhancing the resistance to and capacity to deal with the stress surrounding the patient with leukemia, paying attention to the believes of the family regarding the team that takes care of their child. Analysis of the network results in a strong dynamic interplay between these two which if not addressed in time may have negative effects on other domains included in the network. Nonetheless, particular attention should be directed to help the patient deal with disease related problems.

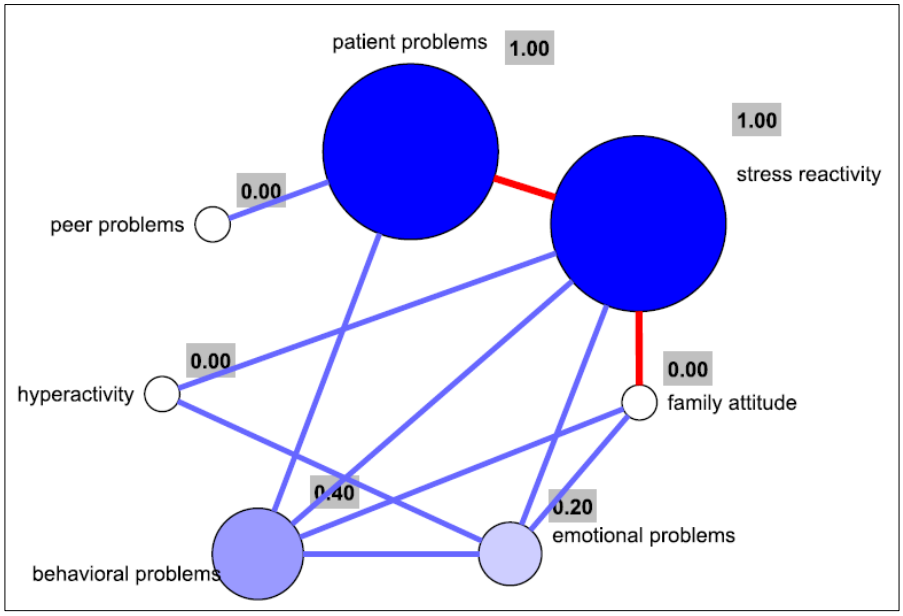


Figure 53: betweenness centrality in ALL. Red lines = p<0.001; Blu line p<0.005

Few domains are highly connected, most domains are linked to two other domains, with monitor being the domain connected to three other domains. This suggests that in PWST, the network of executive function is still highly diffuse, with no domain being central to others. This also indicates that it takes a fair amount of effort to manage difficult time-consuming task because information sort of gets lost in transition. Note that two function, INHIBIT and EMOTIONAL CONTROL, are completely disconnected and do not participate in the network efforts to manage executive function.

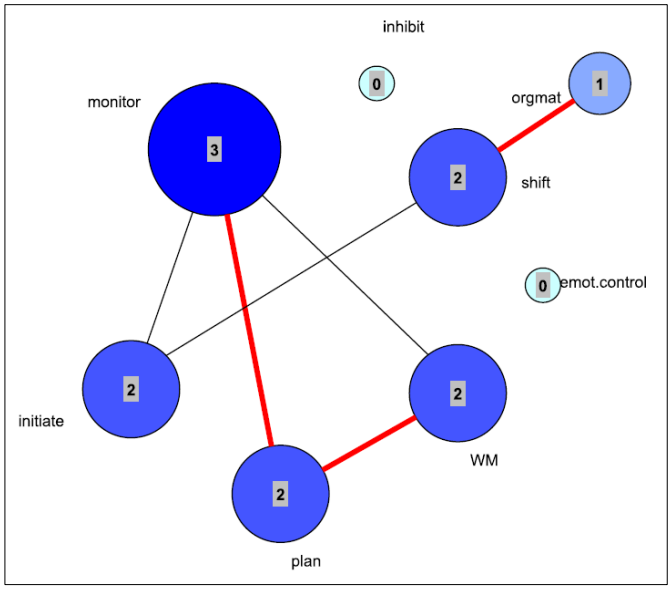


Figure 54: Number of connected edges for each domain of executive function in PWST. Red lines = p<0.001; Blu line p<0.005

In children in follow-up for ALL a different pattern of connectivity and degree of connectivity emerges. Here the domain of mental flexibility (SHIFT) is centrally connected to many other domains and if not connects domains to each other: the network is more connected. However, here also, the domain INHIBIT does not take part in the network.

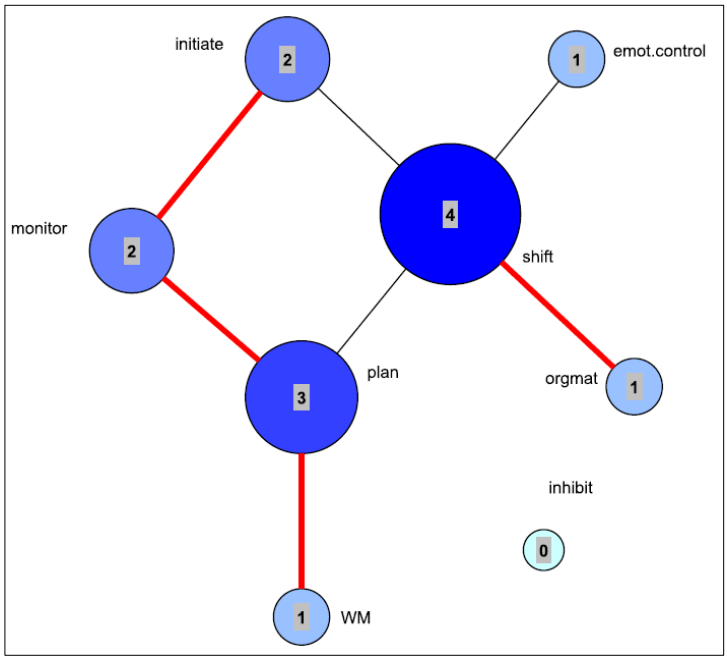


Figure 55: Number of connected edges for each domain of executive function in ALL. Red lines = p<0.001; Blu line p<0.005

CHAPTER 5: Final Discussion

We have considered four of the most important pediatric pathologies for frequency and severity. Altogether, they have an involvement of the central nervous system (CNS) in common although if in a different manner. In epilepsy, the CNS is the seat of the disease thus the CNS is directly involved; in asthma patients the CNS is engaged indirectly, because it depends on the severity of asthma symptoms (ex: intermittent hypoxia, sleep disturbance) and treatment (ex: high corticosteroids doses); finally, in children who are pediatric cancer survivor the CNS is often damaged by treatment. Frequently, it emerges that children with chronic (epilepsy or asthma) and severe disease (as ALL or solid tumors), present difficulties in domains of executive function, despite good cognitive competence (Schillerstrom et al., 2008; Kesler et al., 2018). These difficulties frequently may remain unnoticed or underestimate in routine controls, but they can escalate over time obstructing the correct neurodevelopmental trajectory and the realization of children's life objectives. The question at the heart of our study is why some children are more vulnerable than others although they have the same pathological and treatment profile? While we do not have precise knowledge on what factors cause this variability, we do know that an involvement of genetic factors is probable (Karmiloff-Smith et al., 2014). Given this, the hypothesis was tested that some genes and their polymorphisms (SNPs), selected for their role in the neurocognitive and behavior functions, serve as protective or additive risk factors in our pediatric patients. Moreover, we analyzed which other factors could contribute in the construction of an individualized risk profile for each disease. In this light, we analyzed the characteristics of various pathologies in relation to executive, emotional/behavioral and psychosocial domains, comparing our patients to the norm using traditional statistical analysis and studying their state. However, this type of analysis does not capture the dynamic nature of and interrelationships among the various domains of function, nor does it fully capture the importance of the developmental nature of cognitive and behavioral processes. In order to address this, we introduced network analysis as an innovative way of capturing the dynamic nature of the development of possible deficits (Garcia-Ramos et al., 2015). This type of analysis proved to be an important ally in understanding the developmental trajectory of children. Network analysis evidenced which domains are most involved and most susceptible to change (node or key players), and in turn allay help to direct treatment and therapy in a targeted, effective and personalized way (Pessoa, 2018).

From our results emerge that children with Temporal Lobe Epilepsy (TLE) and Rolandic Epilepsy (RE) displayed difficulties in various domains of executive function (EF). As reported in by others (Helmstaedter et al., 2012), the function most compromised is Working Memory (WM) but in our sample children displayed problems in emotional and behavior domains as well. No main difference was observed with respect to the type of therapy but children who suffered from Temporal Lobe Epilepsy presented more

difficulties especially related to domains of emotional control. Schraegle and collegues (Schraegle et al., 2018) hypothesized that EF deficits may lead to a prolonged focus on negative valence. This, in turn, can maintain or increase negative affect and subsequently maintain or increase emotional control difficulties and increase the risk for depressive symptoms. Measuring the percentage of change, from T0 to T1, a global worsening in all domains was observed with executive functions being most compromised. But what are the factors that make these children more vulnerable? What differentiates them in terms of greater critical problems? Contrary to what one might think, the type of treatment does not affect the severity of the EF difficulties. However, it is fundamental to achieve seizures control: children with poor crisis control score in the elevated or highly elevated range of executive function impairment. Thus, the absence of crisis control is an important additive risk factor. Other additive risk factors are represented by polymorphisms (SNPs) of particular genes. Traditionally, the more studied SNP related to EF is COMT Val158Met on COMT gene enzyme for its role in dopamine (DA) metabolism in the frontal and prefrontal cortex. The COMT gene is defined as pleiotropic (Mier et al.,2010) and its action changes in function of age and cerebral area (Dumonteil et al., 2011). In our sample, Met carriers presents more deficits compared to Val/Val homozygous carriers over time, but the effect is partial (incomplete). In children with epilepsy, the SNP that displays a major effect on domains of executive function and therefore may represent an additive risk factor, is MTHFR C677T: at T0 children who are T carries have more difficulties in all executive function domains tested with the BRIEF, the function most compromised remains Working Memory followed by Shift, Plan and Monitor. In addition, analyzing the percentage of change over time, T carriers remain more compromised. Therefore, it is important to better understand the role of MTHFR on specific domains of executive function. One possibility is interaction on dopamine metabolism? Studying COMT and MTHFR together we see that yes, there is an interaction known the role of MTHFR in methylation processes (Friso et al, 2002; Roffaman et al., 2008; Debost et al., 2014). Overall, T carriers worsen in time while in C carriers the percentage of change is enhanced. Consequently, depending on MTHFR functionality, methylation efficacy may be altered and as a result influence COMT transcription, which in turn influences the availability of dopamine in the frontal and prefrontal regions. In particular, CC carriers produce increased methylation of the COMT promoter gene that thus becomes less available to transcription and results in less circulating enzyme (Roffmann et al., 2008). Hence, Met or Val/Val carriers may contribute to excessive dopamine signaling in patients, similar data reported by Mattay et collegues (Mattay et al., 2003). This enhancement of DA may result as not being functional in relation to executive function because according to the inverted U model, DA level too high or too low situated on either side of the U curve are less efficient. Therefore, the MTHFR T allele may contribute to EF difficulties both for its involvement in the folate pathway as well as for its role in the regulation of homocysteine levels. Alternatively, an epigenetic mechanism may be at work where MTHFR CC and COMT Met carriers result more compromised because they may have too high circulating dopamine levels. Future research must address this issue.

Analyzing the asthma group, patients as a group do not display impairment in domains of executive function. However, data-driven clustering revealed two sub-groups: one composed of children with good executive performances and one with children displaying difficulties in all domains, in particular in the Working Memory, Shift and Monitor domains. Children in this cluster are more compromised in emotional and behavior domains too, where function related to hyperactivity and conduct are the more involved. Asthma is a pathology with a strong connection to the environment, thus, these factors have to be considered as factors of additive risk in children with asthma. Young asthma patients are different from children with epilepsy or leukemia, therefore, we started considering the environment of the child with asthma as factor that may aggravate symptoms and disease. We included exposure to smoke indicated as "smoke", home humidity called "humidity", presence of animals named as "animals" and living in urban areas, as "city". From our study emerged that "smoke", "humidity", and "city", all have an important effect on domains of executive function, in particular on Shift, Inhibit, Emotional Control and Working Memory. These results confirm data from other studies that reported that these environmental factors contribute to a high concentration of aeroallergens that led to increased asthma exacerbation (Bloom et al., 2016; Cipriani et al.,2017). Altogether, smoke exposure results the most important additive risk factor for executive function impairment in children with asthma. Other studies reported similar results (Chamberlin et al., 2012; Heffernan et al., 2014; Rose-Jacobs et al., 2017). We suspect that in children with asthma the effect is twofold, due on the one hand to the direct effect of smoke on EF and on the other by the fact that exposure to smoke aggravates asthma symptoms. Other factors that worsen executive functions are related to the presence of bronchitis in early childhood and the presence of rhino-conjunctivitis. A possible explanation is that all factors that exacerbate asthma symptoms lead to dyspnea, chronic intermittent hypoxia, sleep disturbance, low energy metabolism in the brain, which could modify the structure and function of the brain, such as grey matter, synapse connectivity, and brain plasticity (Sariotomo et al., 2012; Xie et al., 2012; Bian et al., 2018). Treatment had no main effect on executive function and behavioral domains, but treatment with high doses of corticosteroid (>500 mcg/die) do interfere with emotional competence and seems to be predictive of behavioral problems and hyperactivity, confirming what other studies reported (Belanoff et al.,2001). The environmental predictors considered, "smoke", "humidity", "animals", and "city" were not predictive of behavioral and emotional difficulties; except for hyperactivity, where being exposed to smoke in the household significantly predicted enhanced hyperactivity, which is confirmed by other studies (Pagani, 2014; De Alwis et al., 2015; Choi et al., 2016). In sum, in children with asthma, the exposure to factors that potentially exacerbate symptoms have an important role on executive function domains but less on emotional and behavior domains. In contrast, asthma gravity (moderate or severe), spirometry and type of treatment seem to predict emotional and behavior domain impairment.

Next, the role of SNPS as additive risk factors were considered. In asthma patients we observed a particular important role of FKBP5 in its two variants rs3800373(A/C) and rs1360780 (C/T). Children with asthma who

are C or T carriers showed compromised executive function in various domains, especially those related to executive attention, such as, Monitor, Shift, Plan and Organize. Moreover, mutation carriers (C or T) in both SNPs performed worse on tasks that require good emotional control (Emotional Control) as well. With respect to behavior, C and T carriers displayed difficulties in all domains. Surprisingly, children who are SNPs mutation carrier presented poorer change over time. The role of FKBP5 in children with asthma, however, may not be a surprise. FKBP5 is known as an endogenous regulator of the neuroendocrine system that modulates not only glucocorticoid receptor activity in response to stressors but also a multitude of other cellular processes in both the brain and periphery (Zannas et al., 2016). Carriers of the rs1360780 mutation exhibit increased FKBP5 activity leading to a change in Glucocorticoid Receptor (GR) sensitivity even in healthy subjects (Binder et al.,2009), which results in impaired normalization of cortisol levels after exposure to stressful events (Sheuer et al., 2016), and cortisol levels remaining elevated. In asthma, symptoms as well as inhaled corticosteroid can alter hypothalamic–pituitary–adrenal (HPA) axis functionality (Chen and Miller, 2007; Nelson et al., 2002), conferring enhanced stress reactivity to the patient (Matosin et al., 2018). Moreover, the presence of FKBP5 allelic mutation is associated with changes regarding the negative feedback inhibition of the GR, interfering with GR dependent feedback of the HPA axis on systemic levels of cortisol and prolonging the cortisol response (Matosin et al., 2018). Because an asthma attack is a very intense and often repeated stressful event (Vig et al., 2008), and because adequate GR function is necessary to manage asthma symptoms and therapy, the role of FKBP5 polymorphisms in asthmatic children is particularly important. While environmental stress (including symptoms and treatment) can induce HPA axis dysregulation (Zannas et al., 2016) polymorphisms in genes, coding for HPA axis regulation proteins, also generate important individual differences in stress responsivity, and can induce morphological changes in threat-related brain area, such as, the hippocampus. Thus, considering presence of polymorphism in asthmatic children, could serve to monitor and assess future risk and to decide how to manage and moderate the effects of symptoms and treatment, in those who are more vulnerable, by preventing possible known effects on mood, anxiety and EF. In contrast, children with a previous diagnosis of Acute Lymphoblastic Leukemia (ALL) or solid tumor (PWST), one year after the stop of treatment, show different profiles: children with leukemia are less compromised regarding domains of executive function; no function presented a “high elevated” score. The function most compromised was Initiate. Also, mental flexibility (Shift) was slightly compromised, despite the fact that other domains belonging to executive attention, such as, Monitor and Inhibit were in the norm, resulting in children with leukemia that displayed overall adequate control. Children with solid tumors, were more compromised than children with ALL and difficulties were often situated in the “elevated” or “high elevated” range. Shift was the executive function most compromised, followed by Monitor, Initiate and Plan and Organize. Consequently, PWST have reduced mental flexibility with respect to the norm, especially when considering functions that require good attention. One explanation may be that treatment has a particular debilitating

effect on these functions: most children in our sample were treated with Methotrexate and/or Vincristine derivates that have an important effect on white matter integrity causing a loss of flexibility in brain connections (Askins and Moore, 2008; Cheung and Krull, 2015). Comparing children with ALL and PWST with respect to the emotional and behavior, performances differenced as well: the ALL group showed more difficulties regarding emotional control and peer relationships (SDQ questionnaire) displaying these functions to be in the borderline range of impairment. PWST one year after stop treatment did not present difficulties in emotional domains of function. Overall, considering change over time in the PWST group, an improvement in all executive function domains emerged: some functions with "elevated" scores (as Plan, Initiate) one year after the first evaluation returned to scores that fell in the normal range, other functions (as Shift, Emotional Control, Organization of material) improved but remained in the borderline range. The function that remained the most compromised was Shift, which represent the mental flexibility, is highly vulnerable in these patients. Changes in emotional and behavior observed in each patient separately displayed a different pattern: some functions got better, some functions got worse, which reflected the variability in the different type of tumors that characterize this group. On the whole, behavior problems got worse in the majority of patients, in spite of the fact that in psychosocial domains positive changes were observed over time. Two years after the stop of therapy (T1) the perceived family stress, family beliefs, and problems related to their social support seem to have recovered.

Considering SNPs, they have a different role in PWST or in ALL; from the study two different risk profiles clearly emerged. In PWSTs, the SNPs that displayed the most important predictive role of impairment regarding executive function was BDNF Val66Met. Homozygous Val/Val performed worse in particular in the domains, Shift and Emotional Control; not only, PWST Val/Val carriers displayed more overall behavioral and emotional difficulties, which is mostly linked to the worsening of emotional problems. In ALL, the SNP more involved was the 5HTT-LPR S carrier who showed more difficulties on task that require a good control of his/her emotion (Emotional Control), ability to plan (Plan and Organize) and mental flexibility (Shift). Also, with respect to emotional and behavioral problems, children with ALL displayed difficulties in all behavioral and emotional domains tested with the SDQ, also displaying reduced pro-social capacities and markedly enhanced hyperactivity. In PWST, BDNF polymorphisms conferred a differential responsiveness to executive tasks. BDNF is critically involved in neuroplasticity and neurodevelopment and BDNF levels have been found to moderate the association between early adversity and anxiety. Although a number of studies have demonstrated that the Val allele is the protective allele, other researches revealed that the Met allele was protective for neuroticism (Frustaci et al., 2008). Val/Val BDNF alleles may be defined as "plasticity alleles", they may be predictive of enhanced outcomes in positive environments yet of elevated vulnerability in adverse environments (Drury et al., 2012). Because variation in gene expression levels have been demonstrated during early development for most of genes, our current working

hypothesis for children with solid tumors may represent a window of vulnerability that may became opportunity with the correct intervention.

Finally, each pathology emerges with its characteristics, its strengths and weaknesses. However, it is necessary to better understand how to design targeted intervention models that are sensitive to change, and that take into account the dynamic development of the child over time. It is necessary to understand if and how the domains analyzed are linked together, capture those most sensitive to change and thus on which to target therapy effectively.

Introducing a network approach as a component of final analysis allows us to highlight links between domains that otherwise would remain hidden (borsboom and Cramer, 2013; Garcia-Ramos et al., 2015). Children with epilepsy displayed changes in central hubs between the first and second year of follow-up. Especially domains, such as, emotional control, WM and the capacity to organize were found to be highly dynamic and subject to change, while other domains were less central and remained local and peripheral in the network. Also, the network was more cantered on the individual characteristic of the child and family domains such as family believes, or family stress reactivity proved to be marginally involved. Depending on the type of SNPs network dynamics changed and we found that MTHFR had an important effect on different domains of executive function where cognitive control abilities (Organize of materials, Plan and Organize, Monitor) appeared to be the nodes that drive the network. Interestingly, gender and age also result in different network dynamics: the network of male patients and children who were younger than 10 years and six months was more diffuse (Baum et al.,2017). This may be considered as a vulnerability factor because diffuse structures may be more susceptible to damage and altered developing brain trajectories (Garcia-Ramos et al., 2015). The network analysis of children with asthma allowed to better differentiate “high performers” from “low performers”. In the first group, nodes had more equal weighed, while in the second, some functions (nodes) prevail on others, in particular, those related to sustained attention with possible impact not only on school task but also on the administration of treatment (Sonney and Insel, 2018). Also, our results suggest a higher influence of specific nodes within the network, i.e., cognitive tests/measures, as demonstrated by higher efficiency and clustering coefficients in higher functioning patients. Betweenness centrality measures the importance of a node in the communication of a network (Borsboom and Cramer, 2013). Our findings showed that in low performing patients WM and Plan ability play a key role in the communication between other nodes and can be considered as hubs in this network. In turn, the emotional state of the patient has an important role in communicating with other nodes. Network analysis also helped to emphasize the dynamic differences in children who survived a solid tumor and those who survived ALL. Superficially, it may seem that children and families of both malignant pathologies experienced similar experiences, but this proved not to be correct. Important difference was observed in the network of PWST as compared to ALL, in the first group EF and behavioral networks were

more diffuse, while children diagnosed with ALL are more defined and less diffuse. This may be explained by the type of treatment and its effects on brain circuits. However, more interesting was the difference in the weight in the stress perceived by the family and the family beliefs we observed. In PWST the effects that the disease left on the family played a lesser role, while patient problems were perceived as most central and severe. In families of children with ALL, the family reaction to stress was central. Consequently, our current working hypothesis is that the cancer experiences modified beliefs and perceptions of the family. This is important because in terms of follow up this suggest us where to direct our resources and how to better define our goals.

CHAPTER 6: Conclusion

As reported by Karmiloff-Smith (Karmiloff-Smith et al., 2014), timing turns out to be one of the most crucial factors involved in both typical and atypical developmental change. This study underlines that there is not a single time window in the developmental trajectory during which intervention is most successful. All depends on the state of the developing brain, the underling disease, the type of domain considered, the differences in the organism's reaction to environmental inputs, and the additive risk posed by certain SNPs (Figure 56). It has become increasingly clear that emergent phenotypes are not predetermined by genes or disease and that there is a very complex interplay between each domain: genes-disease-neurocognition-behavior-environment.

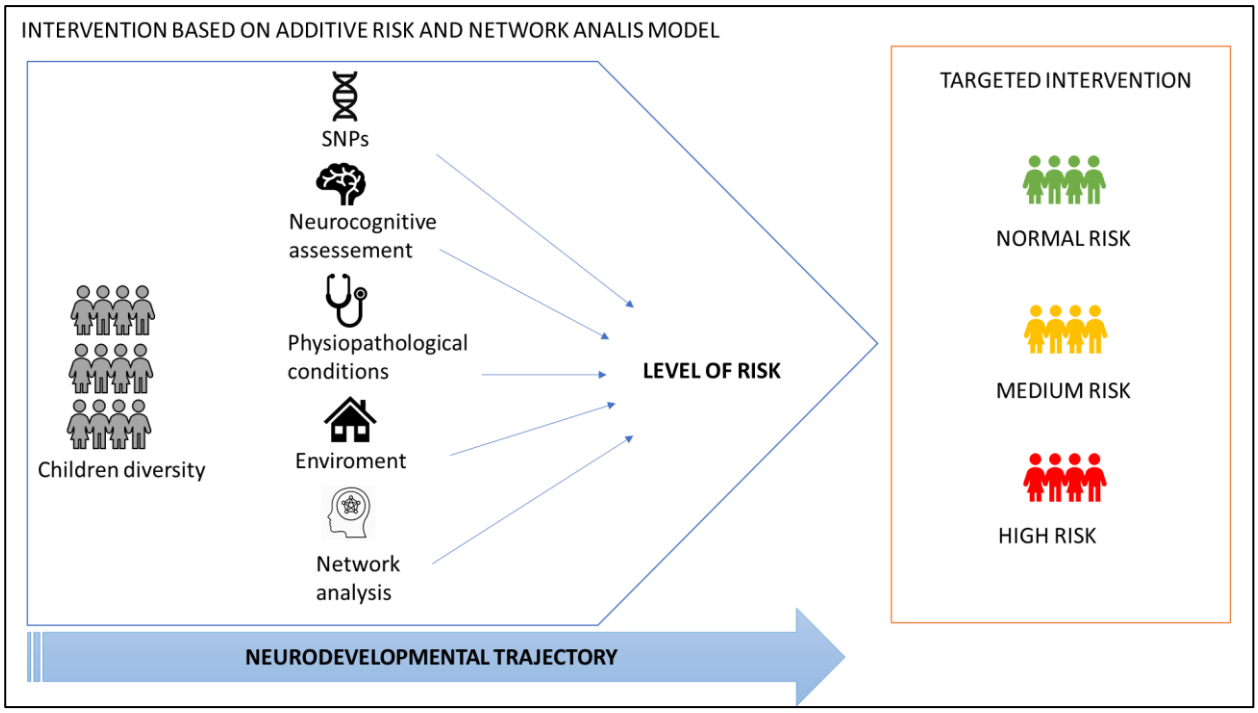


Figure 56: Model of intervention based on additive risk and network analysis who consider the neurodevelopmental child trajectory.

Risk factors identified weave together during the course of developmental, resulting in a final picture that is the sum of various parts coming from the environment, treatment, symptoms and alleles that interact in an additive way that our model considers windows in development. Neuroscience will make significant future progress focusing research on individual differences at multiple levels ranging from genetic- behavioral-cognitive and environmental rather than on data stemming from a single level of analysis. Such a multilevel, multidisciplinary approach should aid the planning of successful dynamic intervention.

The research presented here combined genetic, cognitive and behavioral data as well as the psychosocial background to elucidate some of the intrinsic risk factors that may contribute to current compromised function to help predict future problems. Our approach tried to relate variation in specific genes to variation in cognitive and behavioral phenotypes. While still a developing field, genetic data have proved useful in indicating key questions with respect to neurocognitive and behavioral vulnerability. The aim to integrate cognitive, behavioral and genetic data will ultimately lead to a more detailed understanding of psychological phenotypes and prevent future complications. The first step, however, is to observe and measure cognitive and behavioral strengths and needs as reliable and rigorous as possible. This poses important constraints on the type of approach that should be considered in conducting research.

When evaluating the influence of interactions among behavioral, psychosocial, and genetic factors on the health of severe or chronically ill children, it is fundamental to have a clear concept of interaction both from the biological as well as from a statistical perspective. Not all models or approaches are equally relevant. Therefore, when evaluating the combined effect of multiple factors on the health of children and adolescents, we should try and move beyond the scales and tests used to evaluate and explain the effects of different factors on the present, but more importantly, the future health of these children.

Often, scientists that study the same underlying biological processes may arrive at different or even opposite conclusions using different scales and, consequently, make different treatment recommendations. Moreover, the same data may be forced to fit more than one statistical model, making it very difficult to interpret the results. Therefore, if the goal is to comprehend underlying (neuro)biology, a different way to conceptualize interaction that does not dependent on statistics, should be considered. Given this, we tried to conceptualize the research presented in this thesis using an alternative framework for interaction that is not solely based on statistical models (Rothman and Greenland, 1998; Rothman and Greenland, 2005) but introduces the notion of additive instead of multiplicative risk factors. The importance of the underlying model against which gene-environment interactions are tested (i.e., additive versus multiplicative) should consider that from a statistical standpoint, interaction can be defined as a state in which the effect of one factor (psychosocial, cognitive, behavioral, or genetic) on the development of deficits is the same within levels defined by another factor (Figure 56). This suggests that an interaction is present if the effect of a social or behavioral factor on the risk for compromised outcome varies among individuals with different genotypes, or if the effect of a genotype on compromised outcome varies among individuals with different levels of a social or behavioral factor. Measures such as odds ratios (ORs) evaluate the effects of risk factors on a multiplicative scale, because they imply that the odds (for OR) are multiplied in individuals with the risk factor compared to those without. In contrast, risk differences (RD) evaluate the effects of risk factors on an additive scale, because they reflect how much risk for disability is added in children who have the risk factor, compared with those who do not. Having in mind that the ultimate scope is how to implement

therapy based on the impact of all the factors, and thus, how to build a framework more carefully linked to biology, we should start by observing what occurs at the individual level rather than at the population level. Much of this thinking is based on the counterfactual model, which defines a "cause" of disease, in an individual, as any factor without which the disease would not have occurred (Maldonado and Greenland, 2002; Rothman and Greenland, 2005), and assumes that multiple causal pathways ("sufficient causes") can lead to the same disease, and within each such pathway, various factors can work together (multiple "component causes" within a sufficient cause) to cause the disease. An interaction, then, is defined as the co-participation of two component causes within one sufficient cause, so that both factors are necessary for the sufficient cause to occur: an approach especially useful in the assessment of gene-environment interactions. The research presented in this thesis, considering various types of serious pediatric conditions, epilepsy, asthma, or pediatric cancer, their psychosocial and treatment aspects and, ultimately, their genetic background, found that all may influence the risk for cognitive and behavioral problems either alone or as combined effects which can be additive or non-additive. Furthermore, psychosocial factors, behavioral factors and genes could also be considered as having protective effects. For example, a protective effect was observed only in persons with a particular genotype. Taken together, the complexity of evaluating interactions and the impact of factors is substantial, even for a single genotype and a single psychosocial or behavioral factor. Using different approaches and ultimately, considering how domains of function are related among each other starting from the individual needs and strengths of each patients may offer the most comprehensive approach as it is based on interaction and the development of domains of function over time in the co-presence of various risk or protective factors.

Other studies are necessary to better investigate the complex role of SNPs in chronic and severe pediatric diseases, increasing the number of patients and time-points. It will be important to introduce other procedures and steps to improve our understanding of the mechanisms at the basis of the interaction of these polymorphisms and pediatric pathologies such as epilepsy, asthma and pediatric cancer survivors, for example introducing cortisol analysis in asthma patients or folate integration in epilepsy. Much has to be studied regarding the involvement of these polymorphisms on neurodevelopmental trajectories, but we think that the correct way is put the biopsychosocial wellbeing of children in the center by using a long-term perspective which keeps in mind and valorizes the differences between individuals.

References

- Albert PR. What is a functional genetic polymorphism? Defining classes of functionality. J Psychiatry Neurosci JPN. Novembre 2011; 36(6):363–5.
- Alfstad, K. A., Torgersen, H., Van Roy, B., Hessen, E., Hansen, B. H., Henning, O., et al. Psychiatric comorbidity in children and youth with epilepsy: an association with executive dysfunction? Epilepsy Behav. 2016. 56, 88-94
- Allen DB. Inhaled steroids for children: effects on growth, bone, and adrenal function. Endocrinol Metab Clin North Am. 2005; 34:555–564.
- Anderson FS, Kunin-Batson AS. Neurocognitive late effects of chemotherapy in children: the past 10 years of research on brain structure and function. Pediatr Blood Cancer. Febbraio 2009; 52(2):159–64.
- Annett RD, Aylward EH, Lapidus J, Bender B G., DuHamel T. Neurocognitive functioning in children with mild and moderate asthma in the Childhood Asthma Management Program. Journal of Allergy and Clinical Immunology 2000. Volume 105, Issue 4, 717 - 724
- Arif, A. The association between symptomatic asthma and neurobehavioral comorbidities among children. Journal of Asthma, 2010. 47 , 7, 792-796
- Asher I and Pearce N. Global burden of asthma among children. I J Tuberc Lung Dis. 2014.Nov;18(11):1269-78. doi: 10.5588/ijtld.14.0170.
- Askins MA, Moore III BD. Preventing neurocognitive late effects in childhood cancer survivors. Journal of child neurology. 2008 Oct;23(10):1160-71.
- Bacharier LB., Boner A, Carlsen K, Eigenmann PA, Frischer T, Götz M, Helms PJ, et al. Diagnosis and treatment of asthma in childhood: a PRACTALL consensus report. Allergy 2008. 63: 5-34. doi:10.1111/j.1398-9995.2007.01586.x
- Baum, G. L., Ciric, R., Roalf, D. R., Betzel, R. F., Moore, T. M., Shinohara, R. T., ... & Cook, P. A. (2017). Modular segregation of structural brain networks supports the development of executive function in youth. Current Biology, 27(11), 1561-1572
- Belanoff, J. K., Gross, K., Yager, A., & Schatzberg, A. F. (2001). Corticosteroids and cognition. Journal of psychiatric research, 35(3), 127-145.
- Bell B, Lin JJ, Seidenberg M, Hermann B. The neurobiology of cognitive disorders in temporal lobe epilepsy. Nat Rev Neurol. 2011;7(3):154-64.
- Belsky J, Jonassaint C, Pluess M, Stanton M, Brummett B, Williams R. Vulnerability genes or plasticity genes? Mol Psychiatry. 2009 Aug;14(8):746-54. doi: 10.1038/mp.2009.44. Epub 2009 May 19.
- Bhatia P, Singh N. Homocysteine excess: delineating the possible mechanism of neurotoxicity and depression. Fundam Clin Pharmacol. 2015 Dec;29(6):522-8. doi: 10.1111/fcp.12145. Epub 2015 Sep 17. Review. PubMed PMID: 26376956.
- Bian, R., Zhang, Y., Yang, Y. , Yin, Y. , Zhao, X. , Chen, H. and Yuan, Y. (2018), White Matter Integrity Disruptions Correlate With Cognitive Impairments in Asthma. J. Magn. Reson. Imaging, 48: 748-756. doi:10.1002/jmri.25946
- Binder EB, Bradley RG, Liu W, Epstein MP, Deveau TC, Mercer KBet al. Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. JAMA 2008; 299: 1291–1305
- Binder EB. The role of FKBP5, a co-chaperone of the glucocorticoid receptor in the pathogenesis and therapy of affective and anxiety disorders. Psychoneuroendocrinology. 2009 Dec;34 Suppl 1:S186-195
- Blair RDG. Temporal Lobe Epilepsy Semiology. Epilepsy Research and Treatment. 2012; 2012:751510. doi:10.1155/2012/751510.

- Bloom, C. I., Feary, J., Jarvis, D., Saglani, S., & Quint, J. K. (2018). S103 Changing prevalence of current asthma and inhaled corticosteroid treatment in the UK: population based cohort 2006 to 2016.
- Borsboom D and Cramer AO. Network analysis: an integrative approach to the structure of psychopathology. Annu Rev Clin Psychol. 2013;9:91-121. doi: 10.1146/annurev-clinpsy-050212-185608.
- Boscarino JA, Erlich PM, Hoffman SN, Zhang X. Higher FKBP5, COMT, CHRNA5, and CRHR1 allele burdens are associated with PTSD and interact with trauma exposure: Implications for neuropsychiatric research and treatment. Neuropsychiatr Dis Treat 2012; 8: 131–139.
- Botto LD, Yang Q. 5,10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: a HuGE review. Am J Epidemiol. 2000 May 1;151(9):862-77. Review. PubMed PMID: 10791559.
- British Thoracic Society (BTS), Scottish Intercollegiate Guidelines Network (SIGN). *British Guideline on the Management of Asthma. A national clinical guideline*. BTS, SIGN, Edinburgh, 2012. Available from: https://www.brit-thoracic.org.uk/guidelines-and-quality-standards/asthma-guideline/
- Caldwell W, McInnis OA, McQuaid RJ, Liu G, Stead JD, Anisman H, Hayley S. The Role of the Val66Met Polymorphism of the Brain Derived Neurotrophic Factor Gene in Coping Strategies Relevant to Depressive Symptoms. PLoS One. 2013 Jun 18;8(6):e65547. doi: 10.1371/journal.pone.0065547. Print 2013. PubMed PMID: 23824678; PubMed Central PMCID: PMC3688808.
- Casey BJ, Tottenham N, Liston C, Duston S. Imaging the developing brain: what have we learned about cognitive development? Trend in Cogn Sci. Marzo 2005; 9(3):104-10.
- Caspi A, Hariri AR, Holmes A, Uher R, Moffitt TE. Genetic sensitivity to the environment: the case of the serotonin transporter gene and its implications for studying complex diseases and traits. Am J Psychiatry. 2010 May;167(5):509-27. doi: 10.1176/appi.ajp.2010.09101452. Epub 2010 Mar 15. Review. PubMed PMID: 20231323; PubMed Central PMCID: PMC2943341
- Caspi A, Moffitt T. Gene–environment interactions in psychiatry: joining forces with neuroscience. Nat Rev Neurosci. Luglio 2006; 7(7):583-90.
- Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, McClay J, Mill J, Martin J, Braithwaite A, Poulton R. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science. 2003 Jul 18;301(5631):386-9. PubMed PMID: 12869766.
- Castro R, Rivera I, Ravasco P, Camilo ME, Jakobs C, et al. (2004) 5,10-methylenetetrahydrofolate reductase (MTHFR) 677C→T and 1298A→C mutations are associated with DNA hypomethylation. J Med Genet 41: 454–458.
- Cellerino R, Cetto G, Piga A. Oncologia Clinica - Principi e Pratica. Edizione Selecta Medica; 2011.
- Chamberlain SR, Odlaug BL, Schreiber LR, Grant JE. Association between tobacco smoking and cognitive functioning in young adults. Am J Addict. 2012 Nov;21 Suppl 1:S14-9. doi: 10.1111/j.1521-0391.2012.00290.x.
- Chen E, Gregory E. Miller, Stress and inflammation in exacerbations of asthma. Brain, Behavior, and Immunity, Volume 21, Issue 8, 2007, Pages 993-999, ISSN 0889-1591, https://doi.org/10.1016/j.bbi.2007.03.009.
- Chen MH, Su TP, Chen YS, Hsu JW, Huang KL, Chang WH, et al. Comorbidity of allergic and autoimmune diseases among patients with ADHD: a nationwide population-based study. *J. Atten. Disord.* 2013. 21 219–227. 10.1177/1087054712474686
- Cheung YT, Krull KR. Neurocognitive outcomes in long-term survivors of childhood acute lymphoblastic leukemia treated on contemporary treatment protocols: A systematic review. Neurosci Biobehav Rev. 2015 Jun; 53:108-20. doi: 10.1016/j.neubiorev.2015.03.016.
- Cheung YT, Sabin ND, Reddick WE, Bhojwani D, Liu W, Brinkman TM, Glass JO, Hwang SN, Srivastava D, Pui CH, Robison LL, Hudson MM, Krull KR. Leukoencephalopathy and long-term neurobehavioural, neurocognitive, and brain imaging outcomes in survivors of childhood acute lymphoblastic leukaemia treated with chemotherapy: a longitudinal analysis. Lancet Haematol. 2016 Oct;3(10):e456-e466. doi: 10.1016/S2352-3026(16)30110-7.

- Choi JW, Kim JW. Environmental Lead and Tobacco Smoke Exposure and Neurocognitive Performance in Attention-Deficit/Hyperactivity Disorder. Int J Neuropsychopharmacol. 2016;19(Suppl 1):6. Published 2016 May 27. doi:10.1093/ijnp/pyw044.558
- Cipriani, F., Calamelli, E., & Ricci, G. (2017). Allergen avoidance in allergic asthma. Frontiers in pediatrics, 5, 103.
- Cools R. and D'Esposito M. Inverted-U shaped dopamine actions on human working memory and cognitive control. Biological Psychiatry 2011. 69, e113–e125. doi:10.1016/j.biopsych.2011.03.028
- Cooper SL, Brown PA. Treatment of pediatric acute lymphoblastic leukemia. Pediatr Clin North Am. 2015 Feb;62(1):61-73. doi: 10.1016/j.pcl.2014.09.006. Epub 2014 Oct 18. Review. PubMed PMID: 25435112; PubMed Central PMCID: PMC4366417
- Costello EJ, Egger H, Angold A. 10-year research update review: the epidemiology of child and adolescent psychiatric disorders: I. Methods and public health burden. J Am Acad Child Adolesc Psychiatry. 2005;44(10):972–86. doi: 10.1097/01.chi.0000172552.41596.6f
- Covar RA, Szefler SJ, Martin RJ, Sundstrom DA, Silkoff PE, Murphy J et al. Relations between exhaled nitric oxide and measures of disease activity among children with mild-to-moderate asthma. J Pediatr 2003; 142:469–475.
- Daskalakis NP, Binder EB. Schizophrenia in the spectrum of gene-stress interactions: the FKBP5 example. Schizophr Bull. 2015;41: 323–329. pmid:25592294
- De Alwis D, Tandon M, Tillman R, Luby J. Nonverbal Reasoning in Preschool Children: Investigating the Putative Risk of Secondhand Smoke Exposure and Attention-Deficit/Hyperactivity Disorder as a Mediator. Scand J Child Adolesc Psychiatr Psychol. 2015;3(2):115-125.
- De la Cruz-Cano E, Association between FKBP5 and CRHR1 genes with suicidal behavior: A systematic review, Behavioural Brain Research, Volume 317, 2017, Pages 46-61, ISSN 0166-4328, https://doi.org/10.1016/j.bbr.2016.09.032
- De Vries TW, Van Roon EN., Duiverman EJ. Inhaled corticosteroids do not affect behavior. *Acta Paediatr.* 2008. 97 786–789. 10.1111/j.1651-2227.2008.00770.
- Devlin AM, Brain U, J Austin, TF Oberlander Prenatal Exposure to Maternal Depressed Mood and the MTHFR C677T Variant Affect SLC6A4 Methylation in Infants at Birth- PloS one, 2010 - journals.plos.org
- Di Rosa G., Lenzo, P., Parisi E., Neri, M., Guerrera, S., Nicotera, A., et al. Role of plasma homocysteine levels and MTHFR polymorphisms on IQ scores in children and young adults with epilepsy treated with antiepileptic drugs. Epilepsy Behav. 201329, 548-551. doi: 10.1016/j.yebeh.2013.09.034
- Donati F, Gobbi G, Campistol JM, Rapatz G, Aldenkamp AP et al. Effects of oxcarbazepine on cognitive function in children and adolescents with partial seizures. Neurology 2006. 67(4):679-82 DOI: 10.1212/01.wnl.0000230138.46508.5b
- Dr. Harold S. Nelson MD Dr. Wes Stricker MD Dr. Thomas B. Casale MD Dr. Hershel Raff PhD Dr. Jon A. Fourré MS Dr. David C. Aron MD, MS Dr. Kenneth B. Newman MD . A Comparison of Methods for Assessing Hypothalamic-Pituitary-Adrenal (HPA)Axis Activity in Asthma Patients Treated with Inhaled Corticosteroids. Journal of Clinical Pharmacology, 2002;42:319-326
- Drozdowicz LB, Bostwick JM. Psychiatric adverse effects of pediatric corticosteroid use. Mayo Clin Proc. 2014 Jun;89(6):817-34. doi: 10.1016/j.mayocp.2014.01.010.
- Drury, S.S., Gleason, M.M., Theall, K.P., Smyke, A.T., Nelson, C.A., Fox, N.A. and Zeanah, C.H., 2012. Genetic sensitivity to the caregiving context: The influence of 5httlpr and BDNF val66met on indiscriminate social behavior. Physiology & behavior, 106(5), pp.728-735.
- Dumontheil I, Roggeman C, Ziermans T, Peyrard-Janvid M, Matsson H, Kere J, Klingberg T. Influence of the COMT genotype on working memory and brain activity changes during development. Biol Psychiatry. 2011 Aug 1;70(3):222-9. doi: 10.1016/j.biopsych.2011.02.027.
- Dunlop KA, Carson DJ, Steen HJ, McGovern V, McNaboe J, Shields MD. Monitoring growth in asthmatic children treated with high dose inhaled glucocorticoids does not predict adrenal suppression. Arch Dis Child 2004; 89:713–716.

- Edelmann MN, Ogg RJ, Scoggins MA, Brinkman TM, Sabin ND, Pui CH, Srivastava DK, Robison LL, Hudson MM, Krull KR. Dexamethasone exposure and memory function in adult survivors of childhood acute lymphoblastic leukemia: A report from the SJLIFE cohort. Pediatr Blood Cancer. 2013 Nov;60(11):1778-84. doi: 10.1002/pbc.24644. Epub 2013 Jun 18. PubMed PMID: 23775832; PubMed Central PMCID: PMC3928631.
- Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B, Weinberger DR. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell. 2003 Jan 24;112(2):257-69. PubMed PMID: 12553913.
- Ellis BJ, Boyce WT, Belsky J, Bakermans-Kranenburg MJ, Van Ljzendoorn MH. Differential susceptibility to the environment: An evolutionary–neurodevelopmental theory. Dev Psychopathol. Febbraio 2011; 23(01):7–28.
- Eriksson J, Vogel EK, Lansner A, Bergström F, Nyberg L. Neurocognitive architecture of working memory. Neuron 2015. 88, 33–46. doi:10.1016/j.neuron.2015.09.020
- Fasmer OB, Riise T, Eagan TM, Lund A, Dilsaver SC, Hundal Ø. et al. Comorbidity of asthma with ADHD. *J. Atten. Disord.* 2011. 15 564–571. 10.1177/1087054710372493
- Fastenau, PS., Johnson, CS., Perkins, S. M., Byars, A. W., deGrauw, T. J., Austin, J. K., et al. Neuropsychological status at seizure onset in children: risk factors for early cognitive deficits. Neurology 2009. 73, 526–534. doi: 10.1212/WNL.0b013e3181b23551
- Fayed N, Davis AM, Streiner DL, Rosenbaum PL, Cunningham CE, Lach LM., Boyle MH., Ronen, GM Children's perspective of quality of life in epilepsy. Neurology 2015. 84, 1830-7. doi: 10.1212/WNL.0000000000001536.
- Ferrante G., and La Grutta S. The Burden of Pediatric Asthma. Frontiers in Pediatrics 2018, 6, 186. http://doi.org/10.3389/fped.2018.00186
- Fisher RS, Acevedo C, Arzimanoglou A, et al. ILAE official report: a practical clinical definition of epilepsy. Epilepsia. 2014;55(4):475–482
- Fitzpatrick AM, Teague WG, Meyers DA, Peters SP, Li X, Li H, Wenzel SE, et al. Heterogeneity of severe asthma in childhood: Confirmation by cluster analysis of children in the National Institutes of Health/National Heart, Lung, and Blood Institute Severe Asthma Research Program. Journal of Allergy and Clinical Immunology 2011. 127, Issue 2, 382-389.e13, https://doi.org/10.1016/j.jaci.2010.11.015.
- Fried EI, van Borkulo C, Cramer AOJ, Boschloo L, Schoevers RA, Borsboom D. Mental disorders as networks of problems: a review of recent insights. Social Psychiatry and Psychiatric Epidemiology 2017, Volume 52, Issue 1, pp 1–10
- Friedman T. Diagnosing Asthma in Young Children: Current Research & Recommendations
- Frustaci A., G. Pozzi, F. Gianfagna, L. Manzoli, S. Boccia. Meta-analysis of the brain-derived neurotrophic factor gene (BDNF) Val66Met polymorphism in anxiety disorders and anxiety-related personality traits. Neuropsychobiology, 58 (2008), pp. 163-170
- Fujii T, Ota M, Hori H, Sasayama D, Hattori K, Teraishi T, et al. Association between the functional polymorphism (C3435T) of the gene encoding P-glycoprotein (ABCB1) and major depressive disorder in the Japanese population. J Psychiatr Res 2012 46:555e9
- Fuster JM. Frontal lobe and cognitive development. J Neurocytol. 2002 Mar-Jun;31(3-5):373-85.
- Garcia-Ramos C, Jackson DC, Lin JJ, Dabbs K, Jones JE, Hsu DA, Stafstrom CE, et al. Cognition and brain development in children with benign epilepsy with centrotemporal spikes. Epilepsia. 2015 Oct;56(10):1615-22. doi: 10.1111/epi.13125. Epub 2015 Sep
- Garcia-Ramos, C., Lin, J. J., Prabhakaran, V., & Hermann, B. P. (2015). Developmental reorganization of the cognitive network in pediatric epilepsy. PloS one, 10(10), e0141186.
- Gelernter J, Kranzler HR, Sherva R, Almasy L, Koesterer R, Smith AH, Anton R, Preuss UW, Ridinger M, Rujescu D, Wodarz N, Zill P, Zhao H, Farrer LA. Genome-wide association study of alcohol dependence: significant findings in African- and European-Americans including novel risk loci. Mol Psychiatry. 2014 Jan;19(1):41-9. doi: 10.1038/mp.2013.145. Epub 2013 Oct 29.

- Gelernter J. SLC6A4 polymorphism, population genetics, and psychiatric traits. Hum Genet. 2014 Apr;133(4):459-61. doi: 10.1007/s00439-013-1412-2. Epub 2014 Jan 3. Review. PubMed PMID: 24385047; PubMed Central PMCID: PMC3992709
- Gelfand EW. Pediatric Asthma: A Different Disease. Proceedings of the American Thoracic Society. 2009;6(3):278-282. doi:10.1513/pats.200808-090RM
- Gillaspy SR, Hoff AL, Mullins LL, Van Pelt JC, Chaney JM. Psychological Distress in High-Risk Youth with Asthma. Journal of Pediatric Psychology, 2002. Volume 27, Issue 4, Pages 363–371, https://doi.org/10.1093/jpepsy/27.4.363
- Gioia GA, Espy KA, Isquith PK. Behavior Rating Inventory of Executive Function – Preschool Version (BRIEF-P). Casa Editrice PAR; 2014.
- Gioia GA, Isquith PK, Guy SC, Kenworthy L. Behavior Rating Inventory of Executive Function (BRIEF). Casa Editrice Par; 2013.
- Global Initiative for Asthma Global Strategy for Asthma Management and Prevention (2018). Available online at: www.ginasthma.org
- Goodman A, Goodman R. Strengths and difficulties questionnaire as a dimensional measure of child mental health. J Am Acad Child Adolesc Psychiatry. 2009 Apr;48(4):400-3. doi: 10.1097/CHI.0b013e3181985068.
- Goodman A, Lamping DL, Ploubidis GB. When to use broader internalising and externalising subscales instead of the hypothesised five subscales on the Strengths and Difficulties Questionnaire (SDQ): data from British parents, teachers and children. Journal of Abnormal Child Psychology. 2010; 38(8): 1179–91.
- Goodman R, Meltzer H, Bailey V. The strengths and difficulties questionnaire: A pilot study on the validity of the self-report version. European Child & Adolescent Psychiatry. 1998; 7(3):125–130.
- Goodman R. The extended version of the Strengths and Difficulties Questionnaire as a guide to child psychiatric caseness and consequent burden. J Child Psychol Psychiatry. 1999 Jul;40(5):791-9. PubMed PMID: 10433412.
- Goodman R. The Strengths and Difficulties Questionnaire: a research note. J Child Psychol Psychiatry. 1997 Jul;38(5):581-6. PubMed PMID: 9255702.
- Goodwin RD. Environmental tobacco smoke and the epidemic of asthma in children: the role of cigarette use. Ann. Allergy Asthma Immunol. 2000 . 98 447–454. 10.1016/S1081-1206(10)60759-4
- Guilbert TW, Mauger DT, Lemanske RF Jr. Childhood asthma-predictive phenotype. J Allergy Clin Immunol Pract. 2014 Nov-Dec;2(6):664-70. doi:10.1016/j.jaip.2014.09.010.
- Hajek CA, Yeates KO, Anderson V, Mackay M, Greenham M, Gomes A. Low Cognitive outcomes following arterial ischemic stroke in infants and children. J Child Neurol. 2014; 29(7):887–94. doi:10.1177/0883073813491828
- Hankin BL, Young JF, Abela JR, et al. Depression from childhood into late adolescence: Influence of gender, development, genetic susceptibility, and peer stress. J Abnorm Psychol. 2015;124(4):803-16.
- Harila MJ, Winqvist S, Lanning M, Bloigu R, Harila-Saari AH. Progressive neurocognitive impairment in young adult survivors of childhood acute lymphoblastic leukemia. Pediatr Blood Cancer. 2009 Aug;53(2):156-61. doi: 10.1002/pbc.21992. PubMed PMID: 19405135.
- Harris SE, Fox H, Wright AF, Hayward C, Starr JM, Whalley LJ, Deary IJ. The brain-derived neurotrophic factor Val66Met polymorphism is associated with age-related change in reasoning skills. Mol Psychiatry. 2006 May;11(5):505-13. PubMed PMID: 16446742
- Heffernan TM, Carling A, O'Neill TS, Hamilton C. (Smoking impedes executive function and related prospective memory. Irish Journal of Psychological Medicine , 2014. 31 , (3) , pp. 159-165.
- Hermann B, Seidenberg M, Bell B, Rutecki P, Sheth R, Ruggles K, Wendt G, O'Leary D, Magnotta V. The neurodevelopmental impact of childhood-onset temporal lobe epilepsy on brain structure and function.2002. Epilepsia. Sep;43(9):1062-71
- Hermann BP, Zhao Q, Jackson, DC, Jones JE., Dabbs, K., Almane, D., et al. Cognitive phenotypes in childhood idiopathic epilepsies. Epilepsy Beahv. 2016. 61, 269-274

- Hochberg Z, Feil R, Constancia M, Fraga M, Junien C, Carel J-C, et al. Child health, developmental plasticity, and epigenetic programming. Endocr Rev. Aprile 2011; 32(2):159–224.
- Horak, F., Doberer, D., Eber, E., Horak, E., Pohl, W., Riedler, J., et al. (). Diagnosis and management of asthma – Statement on the 2015 GINA Guidelines. Wiener Klinische Wochenschrift, 2016. 128(15), 541–554. http://doi.org/10.1007/s00508-016-1019-4
- Hossny E, Rosario N, Lee BW, et al. The use of inhaled corticosteroids in pediatric asthma: update. The World Allergy Organization Journal. 2016; 9:26. doi:10.1186/s40413-016-0117-0
- Huffaker M, Phipatanakul, W. Pediatric Asthma: Guidelines-Based Care, Omalizumab, and Other Potential Biologic Agents. Immunology and Allergy Clinics of North America. 2015. Volume 35, Issue 1, Pages 129-144, https://doi.org/10.1016/j.iac.2014.09.005
- Ian C. G. Weaver, Austin C. Korgan , Kristen Lee , Ryan V. Wheeler , Amos S. Hundert and Donna Goguen. Stress and the Emerging Roles of Chromatin Remodeling in Signal Integration and Stable Transmission of Reversible Phenotypes. Front Behav Neurosci. Marzo 2017; 11:41.
- Insel T, Cuthbert B., Garvey M, Heinssen R, Pine DS, Quinn K, et al. Research domain criteria (RDoc): Toward a new classification framework for research on mental disorders. Am J Psychiatry 2010, 167, pp. 748-751
- Irani F, Barbone JM, Beausoleil J, Gerald L. Is asthma associated with cognitive impairments? A meta-analytic review. J Clin Exp Neuropsychol. 2017 Dec;39(10):965-978. doi: 10.1080/13803395.2017.1288802. Epub 2017 Mar 21.
- Ismail FY, Fatemi A, Johnston MV. Cerebral plasticity: Windows of opportunity in the developing brain. Eur J Paediatr Neurol EJPN Off J Eur Paediatr Neurol Soc. Gennaio 2017; 21(1):23–48.
- Jankovic M, Locatelli F, Maule M, Mosso ML, Pastore G, Pession A, et al. AIRTUM Working Group and AIEOP Working Group. Epidemiol Prev. 2012; 225.
- Janszky J, Schulz R, Janszky I, Ebner A. Medial temporal lobe epilepsy: gender differences. J Neurol Neurosurg Psychiatry. 2004 May;75(5):773-5.
- Kamdar KY, Krull KR, El-Zein RA, Brouwers P, Potter BS, Harris LL, Holm S, Dreyer Z, Scaglia F, Etzel CJ, Bondy M, Okcu MF. Folate pathway polymorphisms predict deficits in attention and processing speed after childhood leukemia therapy. Pediatr Blood Cancer. 2011 Sep;57(3):454-60. doi: 10.1002/pbc.23162.
- Karmiloff-Smith A, Casey BJ, Massand E, Tomalski PT. Environmental and genetic influences on neurocognitive development: The importance of multiple methodologies and time-dependent intervention. Clin Psychol Sci. 2014. doi:10.1177/2167702614521188
- Katon WJ, Richardson L, Lozano P; McCauley E. The Relationship of Asthma and Anxiety Disorders. Psychosomatic Medicine: May-June 2004 - Volume 66 - Issue 3 - p 349-355
- Kavanaugh BC, Scarborough VR., and Salorio CF. Parent-rated emotional–behavioral and executive functioning in childhood epilepsy. Epilepsy Behav. 2015. 42, 22–8.
- Kazak A. Pediatric Psychosocial Preventative Health Model (PPPHM): Research, practice and collaboration in pediatric family systems medicine. Families, Systems and Health, 24, 381-395. 2006; 24, 382-395.
- Kazak AE, Brier M, Alderfer MA, et al. Screening for psychosocial risk in pediatric cancer. Pediatr Blood Cancer. 2012;59(5):822-7.
- Kazak AE, Barakat LP, Ditaranto S, Biros D, Hwang WT, Beele D, Kersun L, Alderfer MA, Mougianis I, Hocking MC, Reilly A. Screening for psychosocial risk at pediatric cancer diagnosis: the psychosocial assessment tool. J Pediatr Hematol Oncol. 2011 May;33(4):289-94. doi: 10.1097/MPH.0b013e31820c3b52.
- Kellerman TS, Bonilha L, Lin JJ and Hermann PB. Mapping the landscape of cognitive development in children with epilepsy. Cortex 2015. 66, 1-8
- Kenna GA, Roder-Hanna N, Leggio L, Zywiak WH, Clifford J, Edwards S, Kenna JA, Shoaff J, Swift RM. Association of the 5-HTT gene-linked promoter region (5-HTTLPR) polymorphism with psychiatric disorders: review of psychopathology and pharmacotherapy. Pharmgenomics Pers Med. 2012;5:19-

35. doi: 10.2147/PGPM.S23462. Epub 2012 Jan 13. PubMed PMID: 23226060; PubMed Central PMCID: PMC3513226.
- Kernan CL, Asarnow R, Siddarth P, Gurbani S, Lanphier EK, Sankar R. and Caplan R. Neurocognitive profiles in children with epilepsy. Epilepsia 2012. 53, 2156–2163. doi:10.1111/j.1528-1167.2012.03706.x
- Kesler SR, Ogg R, Reddick WE, Phillips N, Scoggins M, Glass JO, Cheung YT, Pui CH, Robison LL, Hudson MM, Krull KR. Brain network connectivity and executive function in long-term survivors of childhood acute lymphoblastic leukemia. Brain connectivity. 2018 Aug 1;8(6):333-42.
- Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE. Lifetime prevalence and age-of-onset distributions of dsm-iv disorders in the national comorbidity survey replication. Archives of General Psychiatry. 2005;62(6):593–602. doi: 10.1001/archpsyc.62.6.593
- Kinnert MD, Kaugars AS, Strand M. and Silveira l. Family Psychological Factors in Relation to Children's Asthma Status and Behavioral Adjustment at Age 4. Family Process,2008. 47: 41-61. doi:10.1111/j.1545-5300.2008.00238.x
- Kirke PN, Mills JL, Molloy AM, Brody LC, O'Leary VB, Daly L, Murray S, Conley M, Mayne PD, Smith O, Scott JM. Impact of the MTHFR C677T polymorphism on risk of neural tube defects: case-control study. BMJ. 2004 Jun 26;328(7455):1535-6. Epub 2004 May 21. PubMed PMID: 15155469; PubMed Central PMCID: PMC437144.
- Klein M, Van Donkelaar M, Verhoef E, Franke B. Imaging genetics in neurodevelopmental psychopathology. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics. 2017 Jul;174(5):485-537.
- Kliegman RM, Bonita MD, Stanton, MD, Joseph St. Geme, MD and Nina F Schor, MD, PhD, Nelson Textbook of Pediatrics, 20th Edition, 2016
- Kliegman RM, Stanton BF, Schor NF, Behrman RE. Pediatria di Nelson. 19° edizione. Volume 2. 2012.
- Koinis-Mitchell D, McQuaid E, Seifer R, Kopel S, Nassau J, Klein R, Feldman J, Wamboldt M, Fritz G. Symptom perception in children with asthma: cognitive and psychological factors. Health Psychology. 2009;28:226–237
- Kolb B, and Gibb R. Searching for the principles of brain plasticity and behavior. Cortex 2014. http://doi.org/10.1016/j.cortex.2013.11.012
- Kozyrskyj AL, Mai XM, McGrath P, Hayglass KT, Becker AB, Macneil B. Continued exposure to maternal distress in early life is associated with an increased risk of childhood asthma. Am J Respir Crit Care Med 2008; 177: 142–147.
- Krasny-Pacini A, Evans J. Single-case experimental designs to assess intervention effectiveness in rehabilitation: A practical guide. Annals of Physical and Rehabilitation Medicine, Volume 61, Issue 3, May 2018, Pages 164-179
- Krull KR, Bhojwani D, Conklin HM, Pei D, Cheng C, Reddick WE, Sandlund JT, Pui CH. Genetic mediators of neurocognitive outcomes in survivors of childhood acute lymphoblastic leukemia. J Clin Oncol. 2013 Jun 10;31(17):2182-8. doi: 10.1200/JCO.2012.46.7944. Epub 2013 May 6. PubMed PMID: 23650422; PubMed Central PMCID: PMC3731978.
- Krull KR, Brinkman TM, Li C, Armstrong GT, Ness KK, Srivastava DK, Gurney JG, Kimberg C, Krasin MJ, Pui CH, Robison LL, Hudson MM. Neurocognitive outcomes decades after treatment for childhood acute lymphoblastic leukemia: a report from the St Jude lifetime cohort study. J Clin Oncol. 2013 Dec 10;31(35):4407-15. doi: 10.1200/JCO.2012.48.2315.
- Kwan P, Brodie MJ. Effectiveness of first antiepileptic drug. Epilepsia. 2001 Oct;42(10):1255-60.
- La Vecchia C, Bosetti C, Lucchini F, Bertuccio P, Negri E, Boyle P, et al. Cancer mortality in Europe, 2000-2004, and an overview of trends since 1975. Ann Oncol Off J Eur Soc Med Oncol. Giugno 2010;21(6):1323–60.
- Lee LO, Prescott CA. Association of the catechol-O-methyltransferase val158met polymorphism and anxiety-related traits: a meta-analysis. Psychiatr Genet. 2014 Apr;24(2):52-69. doi: 10.1097/YPG.0000000000000018. PubMed PMID: 24300663; PubMed Central PMCID:

- Lenroot RK, Giedd JN. Brain development in children and adolescents: insights from anatomical magnetic resonance imaging. Neurosci Biobehav Rev. 2006;30(6):718-29. Epub 2006 Aug 2. Review. PubMed PMID: 16887188
- Lesca G, Rudolf G, Bruneau N, Lozovaya N, Labalme A, Boutry-Kryza N, Salmi M, et al. GRIN2A mutations in acquired epileptic aphasia and related childhood focal epilepsies and encephalopathies with speech and language dysfunction. Nat Genet. 2013 Sep;45(9):1061-6.
- Linda S. Pagani, Environmental tobacco smoke exposure and brain development: The case of attention deficit/hyperactivity disorder, Neuroscience & Biobehavioral Reviews, Volume 44, 2014, Pages 195-205,ISSN 0149-7634, https://doi.org/10.1016/j.neubiorev.2013.03.008.
- Longo CA, Kerr EN and Smith ML. Executive functioning in children with intractable frontal lobe or temporal lobe epilepsy. Epilepsy Behav. 2013. 26, 102-108
- MacAllister WS, Vasserman M, Coulehan K, Hall AF, Bender HA.Cognitive estimations as a measure of executive dysfunction in childhood epilepsy. Child Neuropsychology 2016. Vol. 22, 65-80. doi:10.1080/09297049.2014.967670
- MacAllister, W. S., Bender, H. A., Whitman, L., Welsh, A., Keller, S., Granader, Y., et al. Assessment of executive functioning in childhood epilepsy: The Tower of London and BRIEF. Child Neuropsychol. 2012 18, 404-15. doi: 10.1080/09297049.2011.613812
- Maiman, M., Salinas C. M., Gindlesperger, M. F., Westerveld, M., Marsha Vasserman, MacAllister W. S. Utility of the Behavior Rating Inventory of Executive Function – Preschool version (BRIEF-P) in young children with epilepsy. Child Neuropsychol. 2012 29, 1-11. doi: 10.1080/09297049.2017.1365829
- Maldonado, G., & Greenland, S. (2002). Estimating causal effects. International journal of epidemiology, 31(2), 422-429
- Markant J, Cicchetti D, Hetzel S, Thomas KM. Contributions of COMT Val^{158} Met to cognitive stability and flexibility in infancy. Dev Sci. 2014 May;17(3):396-411. doi: 10.1111/desc.12128. Epub 2014 Jan 11. PubMed PMID: 24410746; PubMed Central PMCID: PMC4034686.
- Marusak HA, Kuruvadi N, Vila AM, Shattuck DW, Joshi SH, Joshi AA, Jella PK, Thomason ME. Interactive effects of BDNF Val66Met genotype and trauma on limbic brain anatomy in childhood. Eur Child Adolesc Psychiatry. 2016 May;25(5):509-18. doi: 10.1007/s00787-015-0759-4. Epub 2015 Aug 19. PubMed PMID: 26286685; PubMed Central PMCID: PMC4760899.
- Matosin N, Halldorsdottir T, Binder E.B. Understanding the Molecular Mechanisms Underpinning Gene by Environment Interactions in Psychiatric Disorders: The FKBP5 Model. 2018. Biological Psychiatry, Volume 83, Issue 10, Pages 821-830, https://doi.org/10.1016/j.biopsych.2018.01.021.
- McQuaid, EL, Kopel, SJ, & Nassau, JH. Behavioral adjustment in children with asthma: a meta-analysis. Journal of Developmental & Behavioral Pediatrics, 2001. 22(6), 430-439.
- Merikangas KR, He J-p, Burstein M, Swanson SA, Avenevoli S, Cui L, et al. Lifetime Prevalence of Mental Disorders in U.S. Adolescents: Results from the National Comorbidity Survey Replication–Adolescent Supplement (NCS-A). Journal of the American Academy of Child and Adolescent Psychiatry. 2010;49(10):980–9. doi: 10.1016/j.jaac.2010.05.017
- Meyer-Lindenberg A, Weinberger DR. Intermediate phenotypes and genetic mechanisms of psychiatric disorders. Nat Rev Neurosci. 2006 Oct;7(10):818-27. Review. PubMed PMID: 16988657.
- Mier D, Kirsch P, Meyer-Lindenberg A. Neural substrates of pleiotropic action of genetic variation in COMT: a meta-analysis. Molecular psychiatry. 2010 Sep;15(9):918.
- Moricke A, Zimmermann M, Valsecchi MG, Stanulla M, Biondi A, Mann G, Locatelli F, Cazzaniga G, Niggli F, Arico M, Bartram CR, Attarbaschi A, Silvestri D, Beier R, Basso G, Ratei R, Kulozik AE, Lo Nigro L, Kremens B, Greiner J, Parasole R, Harbott J, Caruso R, von Stackelberg A, Barisone E, Rossig C, Conter V, Schrappe M. Dexamethasone vs prednisone in induction treatment of pediatric ALL: results of the randomized trial AIEOP-BFM ALL 2000. Blood. 2016 Apr 28;127(17):2101-12. doi: 10.1182/blood-2015-09-670729. Epub 2016 Feb 17. PubMed PMID: 26888258
- Morrison KM, Goli A, Van Wagoner J, Brown ES, Khan DA. Depressive Symptoms in Inner-City Children With Asthma. Primary Care Companion to The Journal of Clinical Psychiatry. 2002;4(5):174-177.

- Nederhof, E.Email Author, Bouma, E.M.C., Riese, H., Laceulle, O.M., Ormel, J., Oldehinkel, A.J. Evidence for plasticity genotypes in a gene-gene-environment interaction: The TRAILS study. Genes, Brain and Behavior Volume 9, Issue 8, November 2010, Pages 968-973
- Nelson AC. Neural plasticity and human development: the role of early experience in sculpting memory systems. Developmental Science. Maggio 2000; 3(2): 115-136.
- Newton HB. Neurological complications of chemotherapy to the central nervous system. Handb Clin Neurol. 2012; 105:903–16.
- Nickels KC, Zaccariello MJ, Hamiwka LD, Wirrell EC. Cognitive and neurodevelopmental comorbidities in paediatric epilepsy. Nature Reviews Neurology 2016. 12, 465–476. doi:10.1038/nrneurol.2016.98
- Nordquist N, Oreland L. Serotonin, genetic variability, behaviour, and psychiatric disorders--a review. Ups J Med Sci. 2010 Feb;115(1):2-10. doi: 10.3109/03009730903573246. Review. PubMed PMID: 20187845; PubMed Central PMCID: PMC2853349.
- Notaras M, Hill R, van den Buuse M, The BDNF gene Val66Met polymorphism as a modifier of psychiatric disorder susceptibility: progress and controversy. Molecular Psychiatry. 2015 2015/03/31/online
- O'Brien LM, Gozal D. Behavioural and neurocognitive implications of snoring and obstructive sleep apnoea in children: facts and theory. Paediatr Respir Rev. 2002
- Ortega AN, Gergen PJ, Paltiel AD, Bauchner H, Belanger KD, and Leaderer BP. Impact of site of care, race, and Hispanic ethnicity on medication use for childhood asthma. Pediatrics, 2002. 109(1), e1-e1.
- Ortega AN, Goodwin RD, McQuaid EL, Canino G.Parental Mental Health, Childhood Psychiatric Disorders, and Asthma Attacks in Island Puerto Rican Youth. Ambulatory Pediatrics, 2004. Volume 4, Issue 4, Pages 308-315, ISSN 1530-1567, https://doi.org/10.1367/A03-169R1.1
- Pai AL, Patiño-Fernández AM, McSherry M, Beele D, Alderfer MA, Reilly AT, Hwang WT, Kazak AE. The Psychosocial Assessment Tool (PAT2.0): psychometric properties of a screener for psychosocial distress in families of children newly diagnosed with cancer. J Pediatr Psychol. 2008 Jan-Feb;33(1):50-62. Epub 2007 Jul 3. PubMed PMID: 17609228; PubMed Central PMCID: PMC2819976
- Pai AL, Tackett A, Hente EA, Ernst MM, Denson LA, Hommel KA. Assessing psychosocial risk in pediatric inflammatory bowel disease: validation of the Psychosocial Assessment Tool 2.0_General. J Pediatr Gastroenterol Nutr. 2014;58(1):51-6.
- Pai ALH, Tackett A, Ittenbach RF, Goebel J. Psychosocial Assessment Tool 2.0 _General: Validity of a psychosocial risk screener in a pediatric kidney transplant sample. Pediatric transplantation. 2011;16(1):92–98
- Pan CC, McQuoid DR, Taylor WD, Payne ME, Ashley-Koch A, Steffens DC. Association analysis of the COMT/MTHFR genes and geriatric depression: an MRI study of the putamen. Int J Geriatr Psychiatry. 2009;24(8):847-55.
- Park SP, Kwon SH. Cognitive effects of antiepileptic drugs. J Clin Neurol. 2008 Sep;4(3):99-106. doi: 10.3988/jcn.2008.4.3.99
- Passion A, Rondelli R. I tumori dei bambini e adolescenti in Italia. Oncologia Pediatrica. Dicembre 2013; 43 (172):226-232.
- PAT2.0 Scoring Manual. 2008
- Pearl PL. Epilepsy Syndromes in Childhood. Child Neurology. 2018 Feb;24(1):186-209. doi: 10.1212/CON.0000000000000568.
- Pedersen, S. E., Hurd, S. S., Lemanske, R. F., Becker, A., Zar, H. J., Sly, P. D., Soto-Quiroz, M, Wong, G. and Bateman, E. D.Global strategy for the diagnosis and management of asthma in children 5 years and younger. Pediatr. Pulmonol. 2011, 46: 1-17. doi:10.1002/ppul.21321
- Pérez-Pérez B, Cristóbal-Narváez P, Sheinbaum T, Kwapil TR, Ballespí S, et al. Interaction between FKBP5 variability and recent life events in the anxiety spectrum: Evidence for the differential susceptibility model. PLOS ONE 2018 13(2): e0193044. https://doi.org/10.1371/journal.pone.0193044

- Perucca P, Dubeau F, Gotman J. Intracranial electroencephalographic seizure-onset patterns: effect of underlying pathology. Brain. 2014 Jan;137(Pt 1):183-96. doi: 10.1093/brain/awt299.
- Pessoa L. Understanding emotion with brain networks. Current opinion in behavioural science 2018, 19:19-25
- Pezawas L, Verchinski BA, Mattay VS, Callicott JH, Kolachana BS, Straub RE, Egan MF, Meyer-Lindenberg A, Weinberger DR. The brain-derived neurotrophic factor val66met polymorphism and variation in human cortical morphology. J Neurosci. 2004 Nov 10;24(45):10099-102. PubMed PMID: 15537879.
- Polanczyk GV, Salum GA, Sugaya LS, Caye A, Rohde LA. Annual research review: A meta-analysis of the worldwide prevalence of mental disorders in children and adolescents. J Child Psychol Psychiatry. 2015;56(3):345–65. doi: 10.1111/jcpp.12381
- Priftis KN, Papadimitriou A, Gatsopoulou E, Yiallouros PK, Fretzayas A, Nicolaidou P. The effect of inhaled budesonide on adrenal and growth suppression in asthmatic children. Eur Respir J 2006; 27:316–320.
- Protocol AIEOP BFM 2009
- Protocol AIEOP BFM-LLA 2009
- Protocol AIEOP LLA 2000
- Ralhan R, Kaur J. Alkylating agents and cancer therapy. Expert Opin Ther Patents. 2007; 17(9):1061-75.
- Raspall-Chaure M, Neville BG, Scott RC. The medical management of the epilepsies in children: conceptual and practical considerations. Lancet Neurol. 2008 Jan;7(1):57-69.
- Reddick WE, Taghipour DJ, Glass JO, Ashford J, Xiong X, Wu S, et al. Prognostic factors that increase the risk for reduced white matter volumes and deficits in attention and learning for survivors of childhood cancers. Pediatr Blood Cancer. Giugno 2014; 61(6):1074–9.
- Reilly, C., Atkinson, P., Das, K. B., Chin, R. F. M. C., Aylett, S. E., Burch,V., et al. Neurobehavioral Comorbidities in Children With Active Epilepsy: A Population-Based Study. Pediatrics 2014. 133, e1586-e1593. doi: 10.1542/peds.2013-3787
- Rein T. FK506 binding protein 51 integrates pathways of adaptation: FKBP51 shapes the reactivity to environmental change Bioessays, 38 (2016), pp. 894-902
- Replication–Adolescent Supplement (NCS-A). Journal of the American Academy of Child and Adolescent Psychiatry. 2010;49(10):980–9. doi: 10.1016/j.jaac.2010.05.017
- Richardson J, Steiger H, Schmitz N, Joober R, Bruce KR, Israel M, Gauvin L, Anestin AS, Dandurand C, Howard H, de Guzman R. Relevance of the 5-HTTLPR polymorphism and childhood abuse to increased psychiatric comorbidity in women with bulimia-spectrum disorders. J Clin Psychiatry. 2008 Jun;69(6):981-90. PubMed PMID: 18505306.Scheuer et al., 2015
- Robison LL, Hudson MM. Survivors of childhood and adolescent cancer: life-long risks and responsibilities. Nat Rev Cancer. 2014 Jan;14(1):61-70. doi: 10.1038/nrc3634. Epub 2013 Dec 5. Review. PubMed PMID: 24304873
- Roffman JL, Gollub RL, Calhoun VD, et al. MTHFR 677C --> T genotype disrupts prefrontal function in schizophrenia through an interaction with COMT 158Val --> Met. Proc Natl Acad Sci U S A. 2008;105(45):17573-8.
- Rosenbaum DH, Siegel M, Barr B, Rowan AJ. Epileptic aphasia Neurology. 1986. 36 (6) 822; DOI: 10.1212/WNL.36.6.822
- Rothman, K. J., & Greenland, S. (2005). Causation and causal inference in epidemiology. American journal of public health, 95(S1), S144-S150
- Rothman, K. J., Greenland, S., & Lash, T. L. (1998). Types of epidemiologic studies. Modern epidemiology, 3, 95-7
- Ruth Rose-Jacobs, Mark A. Richardson, Kathryn Buchanan-Howland, Clara A. Chen, Howard Cabral, Timothy C. Heeren, Jane Liebschutz, Leah Forman, Deborah A. Frank. Intrauterine exposure to tobacco and executive functioning in high school. Drug and Alcohol Dependence, 2017; 176: 169 DOI: 10.1016/j.drugalcdep.2017.02.022

- Satriotomo I, Dale EA, Dahlberg JM, et al. Repetitive acute intermittent hypoxia increases expression of proteins associated with plasticity in the phrenic motor nucleus. Exp Neurol 2012; 237:103–115.
- Savitz J, Solms M, Ramesar R. The molecular genetics of cognition: dopamine, COMT and BDNF. Genes Brain Behav. 2006 Jun;5(4):311-28. Review. PubMed PMID: 16716201.
- Scheffer IE. Autosomal dominant rolandic epilepsy with speech dyspraxia.2000. Epileptic Disord. 2 Suppl 1: S19-22.
- Scheuer S, Ising M, Uhr M, Otto Y, von Klitzing K, Klein AM. FKBP5 polymorphisms moderate the influence of adverse life events on the risk of anxiety and depressive disorders in preschool children. Journal of Psychiatric Research. 2016 Jan 1;72:30–6
- Schillerstrom JE, Horton MS, Royall DR. The impact of medical illness on executive function. Psychosomatics. 2005 Nov 1;46(6):508-16.
- Schraegle, WA and Titus JB. Executive function and health-related quality of life in pediatric epilepsy. Epilepsy Behav. 2016. 62, 20-26, doi: 10.1016/j.yebeh.2016.06.006.
- Schraegle, William A., Nancy L. Nussbaum, and Jeffrey B. Titus. "Executive Dysfunction and Depression in Pediatric Temporal Lobe Epilepsy: The Contribution of Hippocampal Sclerosis and Psychosocial Factors." Journal of the International Neuropsychological Society (2018): 1-11.
- Shibuya N, Suzuki A, Sadahiro R, Kamata M, Matsumoto Y, Goto K, et al. Association study between a functional polymorphism of FK506-binding protein 51 (FKBP5) gene and personality traits in healthy subjects. Neurosci Lett. 2010;485: 194–197. pmid:20849924
- Silverman LB. Balancing cure and long-term risks in acute lymphoblastic leukemia. Hematology Am Soc Hematol Educ Program. 2014 Dec 5;2014(1):190-7. doi: 10.1182/asheducation- 2014.1.190. Epub 2014 Nov 18. Review. PubMed PMID: 25696854
- Soleimani E, Saliminejad K, Akbari MT, Kamali K, Ahani A. Association study of the common polymorphisms in the folate-methionine pathway with retinoblastoma. Ophthalmic Genet. 2016 Dec;37(4):384-387
- Sonney J, Insel KC. Exploring the intersection of executive function and medication adherence in school-age children with asthma. J Asthma. 2018 Mar 7:1-11. doi: 10.1080/02770903.2018.1441870
- Soriano JB, Abajobir AA, Abate KH, Abera SF, Agrawal A, Ahmed MB, et al. Global, regional, and national deaths, prevalence, disability-adjusted life years, and years lived with disability for chronic obstructive pulmonary disease and asthma, 1990–2015: a systematic analysis for the Global Burden of Disease Study. 2015. Lancet Respir Med. 2017. 5:691–706. 10.1016/S2213-2600(17)30293-X
- Steliarova-Foucher E, Stiller C, Lacour B, Kaatsch P. International Classification of Childhood Cancer, third edition. Cancer. 1 aprile 2005; 103(7):1457–67.
- Stone LL, Janssens JMAM, Vermulst, AA, Van Der Maten, M, Engels, RCME, Otten, R. The Strengths and Difficulties Questionnaire: psychometric properties of the parent and teacher version in children aged 4–7. BMC Psychology, 2015, 3(1), 4. http://doi.org/10.1186/s40359-015-0061-8
- Strachan DP. The role of environmental factors in asthma. *Br. Med. Bull.* 2000. 56 865–882. 10.1258/0007142001903562
- Taha H. Poor Executive Functions among Children with Moderate-into-Severe Asthma: Evidence from WCST Performance. Frontiers in Psychology. 2017;8:793. doi:10.3389/fpsyg.2017.00793.
- Tahara T, Shibata T, Arisawa T, Nakamura M, Yamashita H, Yoshioka D, Okubo M, Maruyama N, Kamano T, Kamiya Y, Fujita H, Nagasaka M, Iwata M, Takahama K, Watanabe M, Hirata I. Impact of catechol-O-methyltransferase (COMT) gene polymorphism on promoter methylation status in gastric mucosa. Anticancer Res. 2009 Jul;29(7):2857-61. PubMed PMID: 19596974.
- Triplett, R. L., Asato M. S., and Asato M. R. Brief Cognitive and behavioral screening in children with new-onset epilepsy: a pilot feasibility trial. Pediatr Neurol. 2015 52, 49-55
- Tsai MH, Vears DF, Turner SJ, Smith RL, Berkovic SF, Sadleir LG, Scheffer IE. Clinical genetic study of the epilepsy-aphasia spectrum. Epilepsia. 2013 Feb;54(2):280-7. doi: 10.1111/epi.12065.

- Van Rooij SJ, Stevens JS, Ely TD, Fani N, Smith AK, Kerley KA, Lori A, Ressler KJ, Jovanovic T. Childhood Trauma and COMT Genotype Interact to Increase Hippocampal Activation in Resilient Individuals. Front Psychiatry. 2016 Sep 14;7:156. doi: 10.3389/fpsyt.2016.00156. eCollection 2016. PubMed PMID: 27683563; PubMed Central PMCID: PMC5021680.
- Vig RS, Forsythe P, Vliagoftis H. The Role of Stress in Asthma. Annals of the New York Academy of Sciences. 2006;1088(1):65–77
- Walters EH, Walters J. Inhaled short acting beta2-agonist use in chronic asthma: regular versus as needed treatment. Cochrane Database Syst Rev. 2003; CD001285.
- Warris LT, van den Heuvel-Eibrink MM, den Hoed MA, Aarsen FK, Pieters R, van den Akker EL. Does dexamethasone induce more neuropsychological side effects than prednisone in pediatric acute lymphoblastic leukemia? A systematic review. Pediatr Blood Cancer. 2014 Jul;61(7):1313-8. doi: 10.1002/pbc.24988. Epub 2014 Feb 16. Review. PubMed PMID: 24532490.
- White MG, Bogdan R, Fisher PM, Muñoz KE, Williamson DE, Hariri AR. FKBP5 and emotional neglect interact to predict individual differences in amygdala reactivity. Genes Brain Behav. 2012;11: 869–878. pmid:22979952
- Wingrove, B. An Overview of Pediatric Asthma. Physician Assistant Clinics, 2016Volume 1, Issue 4, Pages 563-582, https://doi.org/10.1016/j.cpha.2016.05.005.
- Wolthers OD, Heuck C. Assessment of the relation between short and intermediate term growth in children with asthma treated with inhaled glucocorticoids. Allergy 2004; 59:1193–1197.
- Wright RJ, Cohen RT, Cohen S. The impact of stress on the development and expression of atopy. Curr Opin Allergy Clin Immunol 2005; 5: 23–29.
- Xie H, Yung WH. Chronic intermittent hypoxia-induced deficits in synaptic plasticity and neurocognitive functions: a role for brain-derived neurotrophic factor. Acta Pharmacol Sin 2012; 33:5–10.
- Xie P, Kranzler HR, Poling J, Stein MB, Anton RF, Farrer LA et al. Interaction of FKBP5 with childhood adversity on risk for post-traumatic stress disorder. Neuropsychopharmacology 2010; 35: 1684–1692.
- Yuen, A. W. C., Keezer, M. R., Sander, J. W. Epilepsy is a neurological and systemic disorder. Epilpesy Behav. 2018. 78, 57-61
- Zannas AS, Binder EB. Gene-environment interactions at the FKBP5 locus: Sensitive periods, mechanisms and pleiotropism. Genes Brain Behav. 2014;13: 25–37. pmid:24219237
- Zannas AS, Wiechmann T, Gassen NC & Binder EB. Gene–Stress–Epigenetic Regulation of FKBP5: Clinical and Translational Implications. neuropsychopharmacology volume 41, pages 261–274 (2016)
- Zimmermann P, Bruckl T, Nocon A, Pfister H, Binder EB, Uhr M et al. Interaction of FKBP5 gene variants and adverse life events in predicting depression onset: results from a 10-year prospective community study. Am J Psychiatry 2011; 168: 1107–1116