Università degli Studi di Cagliari

PHD DEGREE

Neuroscience

Cycle XXXIII

Attention-Deficit/Hyperactivity Disorder (ADHD) and Autism Spectrum Disorder (ASD) prenatal and postnatal risk factors and polygenetic risk scores.

Scientific Disciplinary Sector

MED-39

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Final exam. Academic Year 2019 – 2020
Thesis defense session: January 2021.

Acknowledgements

I am grateful to the University of Cagliari for the opportunity to undertake this PhD.
I would like to thank my supervisor Professor Alessandro Zuddas for his guidance and help over these years of PhD studies. Also, I would like to thank Professor Antonella Gagliano for her advice and help.

I would like to thank Professor Pieter Hoekstra for the opportunity to carry out my research abroad period at the University of Groningen and contribute to my career development.
I would especially like to thank all parents and children who took part in the study, without their help it would not have been possible to conduct this research.

Also, I would like to thank my colleagues at the Department of Child and Adolescent Neuropsychiatric at "Antonio Cao" Paediatric Hospital, Cagliari, and my colleagues at the Department of Child and Adolescent Psychiatry at ACCARE Groningen, for their help and collaboration.

Finally, I would like to thank my family and friends for their support.

Thesis summary

Attention-Deficit/Hyperactivity Disorder (ADHD) and Autism Spectrum Disorder (ASD) are neurodevelopmental disorders with onset in the developmental period. The etiology of ADHD and ASD involves both genetic and environmental factors. Most of the environmental risk factors studies have been conducted separately for each disorder, and still more genetic studies should be done to investigate the relation with the environmental factors and these disorders.

The aim of this thesis is to explore environmental risk factors for ADHD and ASD and identify common risk factors between the groups. Additionally, it aims to investigate the interplay between environmental risk factors and polygenetic risk scores. For this purpose, two studies have been carried out. To investigate ADHD and ASD exposure to environmental factors a case control study was developed at the University of Cagliari, Italy, at the Unit of Child and Adolescent Neuropsychiatry at "A.Cao" Paediatric Hospital and "S. Michele" Hospital, Hospital Trust, Cagliari. The other study was carried out at the University of Groningen, The Netherlands, at the Child and Psychiatric Unit, University Medical Center Groningen, using a prospective birth cohort study "The Avon Longitudinal Study of Parents and Children (ALSPAC)" to investigate the interplay between maltreatment and different forms of maltreatment and ADHD traits.

Environmental factors, such as cesarean delivery, formula feeding, high sugar consumption, family adverse situations, psychological aggression, bullying, inconsistent discipline and hostility of the mother were associated with an increased risk of ADHD. Regarding the interplay between environmental factors and polygenetic risk scores in relation to ADHD traits, a gene-environment correlation predicted maltreatment in children and a genetic liability of the parents may be involved. Gene-environment correlation (rGE) plays an important causal role in psychiatric disorders, and the identification of rGE may suggest targets for environmental intervention.

These findings showed the important role of the genetic and environmental factors in neurodevelopmental disorders such as ADHD. An emphasis on environmental factors should be taken into consideration as a clinical intervention of ADHD.

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Chapter 1

1. Introduction

1.1 Neurodevelopmental Disorders.

The section "Disorders usually first diagnosed in infancy, childhood, or adolescence" of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) was replaced in the most recent 5th Edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) by the section "Neurodevelopmental disorders" (Harris, 2014).

According to the DSM-5, Neurodevelopmental disorders are a group of conditions typically manifested early in development. The onset of these conditions often occurs before entry into school and they are characterized by developmental deficits with impairments in personal, social, academic or occupational functioning (American Psychiatric Association, 2013). The multifaceted conditions of the disorders include impairments in cognition, communication, behavior and/or motor skills (Mullin et al., 2013). The deficits caused by disorders vary from specific limitations such as learning or control of executive functions to global impairments such as social skills or intelligence (American Psychiatric Association, 2013).

DSM-5 classifies Attention-Deficit/Hyperactivity Disorder (ADHD) and Autism Spectrum Disorder (ASD) as neurodevelopmental disorders (Du Rietz et al., 2020; Visser et al., 2016). This classification is supported by the similarity in the characteristics and the comorbidity between the neurodevelopmental disorders (Du Rietz et al., 2020). Neurodevelopmental disorders frequently co-occur, and ADHD and ASD are part of the most common co-occurring neurodevelopmental disorders (American Psychiatric Association, 2013). A comorbidity between ADHD and ASD is also frequent, and both disorders are highly heritable (Demontis et al., 2019; Grove et al., 2019; Waddington et al., 2020) and they also share genetic influences (Stergiakouli et al., 2017). These disorders are more commonly diagnosed in men than in women (Pinares-Garcia et al., 2018; Sayal et al., 2018).

1.1.1 Attention-Deficit/Hyperactivity Disorder (ADHD).

According to the DSM-5, the essential feature of attention-deficit/hyperactivity disorder (ADHD) is a persistent pattern of inattention and/or hyperactivity-impulsivity at levels that are inconsistent with age and developmental level, and which interferes with functioning or development and negatively impacts on social and academic/occupational activities (American Psychiatric Association, 2013).

In DSM-IV-TR, ADHD was classified as a disruptive behavior disorder, referred to as "externalizing disorder", while is currently classified as a neurodevelopmental disorder in the DSM-5 (Du Rietz et al., 2020). ADHD commonly occurs in childhood and in most of the cases persists into adulthood. (American Psychiatric Association, 2013; Sayal et al., 2018).

Specific ADHD diagnostic criteria have changed from the previous DSM version. Currently, symptoms onset is before 12 years, instead of 7 year, as was the case previously. Diagnostic criteria for adults have also changed, and five symptoms are required instead of the six required for a diagnosis in young people (Du Rietz et al., 2020). For an ADHD diagnosis based on DSM-5 criteria, symptoms must be present for at least six months, and several symptoms are present before the age of 12 in two or more different settings, such as home, school, with friends, or in other activities.

A total of six or more symptoms of criterion inattention or hyperactivity-impulsivity are required for a diagnosis of ADHD in children and adolescents the age below of 17 years. For adults or adolescents from their 17th year, a minimum of 5 symptoms are required for a diagnosis.

A specification is also provided for the disorder, a combined presentation is given if both the criteria inattention and hyperactivity-impulsivity are present, a predominantly inattentive presentation is specified if only the inattention criterion is met, and predominantly hyperactive/impulsive presentation is given if only the hyperactivity-impulsivity criterion is met during the last six months (American Psychiatric Association, 2013). In childhood, some inattention and disorganization symptoms involve difficulties to stay on a task, losing materials, and appearing not to listen, and hyperactivity symptoms include overactivity, inability to wait

or to stay seated, and intruding into other people's activities. ADHD persisting in adulthood is characterized by impairments of social, academic and occupational functioning (American Psychiatric Association, 2013).

Prevalence

The worldwide prevalence of ADHD in children is estimated at approximately 5% (American Psychiatric Association, 2013; Polanczyk et al., 2007; Sayal et al., 2018), and around 2.5% in adults (American Psychiatric Association, 2013).

In the general population, ADHD is more frequently in males than in females, with a ratio 2:1 in children, and 1.6:1 in adults (American Psychiatric Association, 2013)

Comorbidity

ADHD is frequently comorbid with Oppositional Defiant Disorder (ODD) and Conduct Disorder (CD). Approximately half of the children with a diagnosis of combined ADHD and a quarter of the predominantly inattentive share a diagnosis of ODD. Combined presentation also co-occurs with CD in around a quarter. Specific Learning Disorder is another disorder that is usually comorbid with ADHD. Obsessive-Compulsive Disorder, Tic Disorder, and Autism Spectrum Disorder may also co-occur with ADHD (American Psychiatric Association, 2013).

Etiology

Genetic and environmental factors are involved in the ADHD etiology (American Psychiatric Association, 2013). In recent studies, the heritability of ADHD has been estimated at 74% (Faraone & Larsson, 2019).

A wide variety of environmental factors have been associated with an increased risk of ADHD in offspring. Child exposure in utero to alcohol, smoking, antibiotics, paracetamol, antidepressants, diabetes, depression, and stressful life events, have all been linked to ADHD. Perinatal factors such as cesarean delivery, prematurity, and low birth weight have been suggested as a risk factors of ADHD. Postnatal factors such as physical and psychological

aggression, bullying, and parenting style, have been associated with an increased risk of ADHD in children (Millichap, 2008; Sciberras et al., 2011; Sellers et al., 2020).

ADHD is also elevated in first-degree relatives (American Psychiatric Association, 2013), and parents with ADHD have been associated with offspring with an ADHD diagnosis (Millichap, 2008).

Candidate genes have also been linked to ADHD; a meta-analytic review was conducted to determine which candidate genes were associated with ADHD in children. A significant association was found between ADHD and several candidate genes linked to dopaminergic and serotonergic systems such as DAT1, DRD4, DRD5, 5HTT, HTR1B. ADHD in childhood was associated well with SNAP25 (Gizer et al., 2009).

Interplay between genetic and environmental factors has also been studied. Gene-environment interplay includes different mechanisms, such as gene-environment correlation (rGE), the genetic liability for a disorder is related to environmental factors linked to the disorder (genetic influences on environmental exposure), and gene-environment interaction (GxE), genetic factors may moderate the susceptibility to environmental factors (Jaffee & Price, 2007). For ADHD, rGE studies have been conducted with polygenic risk scores (PRS) and environmental factors. Prenatal exposure to smoking, paracetamol use, and life events during pregnancy have been correlated with mother's polygenetic scores (Leppert et al., 2019).

Another ADHD study used a twin sample and a longitudinal sample of adolescents divided by gender to assess whether parental hostility has a risk effect on ADHD symptoms using a genetic sensitive and longitudinal analysis. The results suggested a genetic and environmental contribution between mother and son hostility and ADHD symptoms, and genetic contribution explained the association between father and son hostility and ADHD symptoms. In females, the association between ADHD symptoms and mothers and father's hostility was explained only by genetic factors. The longitudinal study suggested evidence in males ADHD symptoms have an impact on mother and son hostility and not in the opposite way. In this study, a causal effect of family relations influencing ADHD symptoms in offspring was not supported (Lifford et al., 2009).

1.1.2 Autism Spectrum Disorder (ASD).

According to the DSM-5, the essential features of Autism Spectrum Disorder (ASD) are persistent impairment in reciprocal social communication and social interaction, as well as restricted, repetitive patterns of behavior, interests, or activities. These symptoms are present from early childhood and limit or impair everyday functioning (American Psychiatric Association, 2013).

DSM-5 created a new category in the Neurodevelopmental Disorders section, Autism Spectrum Disorder, and the category in DSM-IV, Pervasive Developmental Disorder and subgroups (autistic disorder, Asperger's disorder, childhood disintegrative disorder, Rett's disorder and pervasive developmental disorder not otherwise specified) was eliminated (Du Rietz et al., 2020). According to the DSM-5, the consolidation of Autistic disorder, Asperger's disorders, and Pervasive developmental disorders into a new category "Autism Spectrum Disorder" is due to these disorders, rather than being different, involve impairments in domains of social communication and restrictive repetitive behaviors/interest (American Psychiatric Association, 2013).

Another difference between DSM-5 and DSM-IV is the requirement of deficits in two core domains instead of three (social communication and social interaction, and restricted repetitive patterns of behavior, interests and activities). Additionally, for cases of verbal and nonverbal deficit that do not meet the criteria for restrictive repetitive behaviors/interest required for an ASD diagnosis, the category Social (Pragmatic) Communication Disorder was created in the section "Communications Disorders" (Du Rietz et al., 2020).

The clinical ASD presentation based in DSM-5 include symptoms of excess, deficits and delays, and ASD is diagnosed only when the deficits of social communication are accompanied by the presence of excessively repetitive behaviors, restricted interests, and insistence on sameness, as described below:

- Social communication and social interaction: the deficits in social communication and social interaction persist across multiple contexts and include:

    - Deficits in social reciprocity (abnormal social approach, failure of normal conversation or to initiate/respond to social interactions, reduced interests or emotions).

    - Deficits in nonverbal communication used for social interaction varies depending on age, intellectual level, and ability language, as well as treatment history and current support. The symptoms are manifest by: impairments to integrate verbal and nonverbal communication; absent, reduced, or atypical eye contact (considering cultural norms); impairments in body language; in body orientation; in use speech intonation; to understand and use gestures; in facial expression; also a total lack of facial expressions and nonverbal communication are symptoms of ASD.

      A characteristic in early age is impairment in joint attention (lack of pointing; showing or bringing objects to share interest with others; or failure to follow someone's pointing or eye gaze).

      Adults with fluent language, but with difficulty to coordinate nonverbal communication with speech may show an odd, wooden, or exaggerated body language. Individuals may have relatively good eye contact, but poor integration with gesture; body posture; prosody; and facial expression; or may learn a few functional gestures, but with a small repertoire and often fail using expressive gestures spontaneously.

    - Skills impairments to develop, maintain, and understand relationships (impairments to adjust behavior to different social contexts; share imaginative play or make friends; or lack of interest in peers). (American Psychiatric Association, 2013).

- Restricted, repetitive patterns of behavior, interests, or activities: manifestations of these symptoms are according to age and ability, intervention, and current supports. The restricted and repetitive patterns of behavior, interests, and activities are present during early development, and an ASD diagnosis is also possible, even though these symptoms are not currently present but were present in the past. According to DSM-5, two or more of these characteristics must be present for a diagnosis:

    - Stereotyped or repetitive behaviors include: simple motor stereotypes (hand flapping, finger flicking), repetitive use of objects (spinning coins, lining up toys or flipping objects), and repetitive speech (echolalia, the delayed or immediate parroting of heard words, use of "you" when referring to self, stereotyped use of words, phrases, or prosodic patterns).

    - Excessive adherence to routines and restricted patterns of behavior manifested in resistance to change (extreme distress at small changes, insistence on sameness, insistence on adherence to rules, inflexibility with routines, rigidity of thinking) or verbal and nonverbal ritualized behavior (greeting rituals, no changes in what is eaten every day or routes taken, repetitive questioning).

    - Highly restricted, fixated interests that are abnormal in intensity or focus: strong attachment to or preoccupation with unusual objects (a child strongly attached to a pan or worried about vacuum cleaners, or an adult spending hours writing a schedule), hyper-or hyporeactivity to sensory input (apparent indifference to pain, or to heat/cold temperature, extreme responses to specific sounds or textures) or unusual interest in sensory aspects of the environment (excessive smelling or touching of objects, textures, visual fascination with lights or spinning objects). (American Psychiatric Association, 2013)

Language deficits is a common symptom in ASD and varies from a complete lack of speech through language delays, poor comprehension of speech, echoed speech, or stilted and overly literal language. Even though individuals with ASD have good formal language skills such as vocabulary and grammar, the language for reciprocal social communication is impaired.

Extreme reaction or rituals involving taste, smell, texture, or appearance or excessive food restrictions are also common in ASD.

Symptoms must be present during early development and may fully manifest when social demands exceed the capacities or may be masked by learned strategies in later life. A loss of established skills may also manifest. Symptoms cause clinically significant impairment in social, occupational, or other important areas of current functioning (American Psychiatric Association, 2013)

Prevalence

The worldwide prevalence of ASD in child and in adults is estimates at around 1% (American Psychiatric Association, 2013; Lord et al., 2020; Ornoy et al., 2015).
ASD is more frequently found in males than in females, males are diagnosed four times more in comparison to females. ASD diagnosis in females may be unrecognized, due to lack of intellectual disability or language delay, and a subtler manifestation of social and communication difficulties (American Psychiatric Association, 2013).

Comorbidity

Autism spectrum disorder is commonly comorbid with intellectual disability and structural language disorder. ASD is also frequently comorbid with mental disorders, around 70% of people with a diagnosis of ASD may have a mental disorder, and about 40% may have two or more of these disorders. ADHD may also co-occur with ASD, and both diagnoses are given when criteria for both are met. Specific learning difficulties are also common, as well as developmental coordination disorder.

Medical conditions commonly associated with autism spectrum include: epilepsy, sleep problems, and constipation. Avoidant-restrictive food intake disorder is also frequently present (American Psychiatric Association, 2013).

Etiology

ASD is a multifaceted disorder and the etiology involves both genetic and environmental factors (Ornoy et al., 2015; Waltes et al., 2019). Based on twin studies genetic and environmental contribution to ASD have been estimated (Huguet et al., 2016).

Family studies have showed that the recurrence of having a child with ASD increases with the proportion of the genome that the individual shares with one affected sibling or parent. Epidemiological studies provide information about ASD heritability, however, genetic information is missing (Huguet et al., 2016).

In a recent study, the ASD heritability has been estimated at around 83% (Sandin et al., 2017). Prenatal and postnatal environmental risk factors associated with ASD in offspring have been studied. Some environmental factors linked to ASD are: maternal paracetamol use, stressful life events, infections and antibiotic use during pregnancy (Jiang et al., 2016; Lee et al., 2019; Manzari et al., 2019; Masarwa et al., 2018).

Gene-environment interplay studies, such as gene-environment correlation (rGE) and gene-environment interaction (GxE) have been conducted using polygenetic risk scores (PRS) and environmental factors (Jaffee & Price, 2007). A rGE between mother's PRS and stressful life events during pregnancy has been reported (Leppert et al., 2019).

Genes have been linked to ASD, some of them have been involved in the dopaminergic (DRD2) and serotonergic (HTR2A) pathway, and regulation of the hippo signaling pathway (implicating neural development and maintenance). Chromatin modifications were also identified in ASD, and have been characterized to influence the genomic locations of de novo mutations. Some factors including gender, environmental toxins, nutrition, and immune response have been involved in influence chromatin (O'Connell et al., 2018). Another candidate gene study suggested an association between DRD3 gene and ASD related to stereotype behavior (De Krom et al., 2009).

The genetic architecture of autism spectrum disorder involves the interplay of common and rare variation. Chromatic remodeling, related with typical development, are damaged by risk variation affecting the neural connections, as well as synaptic function, which is important for neural physiology, and could be involved in neuropsychiatric disorders. In ASD, de novo mutation cluster to synaptic genes, and synaptic defects have been reported (De Rubeis et al., 2014).

Based on epidemiological and molecular studies, it is now accepted that the genetic susceptibility to ASD can be different from one individual to another. Most of the ASD heritable seems to be due to common variants observed in the general population, with a small contribution of rare variants. De novo mutations are genetic causes of ASD but do not contribute to the heritability since there are only present in the patient (Huguet et al., 2016).

1.2 ADHD and ASD environmental risk factors.

A variety of environmental risk factors have been linked to neurodevelopmental disorders such as ADHD and ASD. Some prenatal risk factors associated with ADHD and ASD are, maternal alcohol consumption, smoking or exposure to smoke during pregnancy, medication use, diseases and mental disorders during pregnancy. Some perinatal and postnatal risk factors associated with both disorders are, type of delivery, child physical and psychological aggression, bullying, and parenting. However, there are still inconsistencies in some findings. An explanation of ADHD and ASD prenatal, perinatal, and postnatal environmental risk factor is provided below.

1.2.1 Prenatal factors.

1.2.1.1 Mother's diseases and mental disorders.

- **Gestational diabetes:**

Prenatal risk factors studies have related mother's diseases and mental disorders during pregnancy with ASD and ADHD in offspring. A systematic review and meta-analysis suggested an association between offspring exposed in utero to pre-existing maternal diabetes and neurodevelopmental disorder including ADHD and ASD (Yamamoto et al., 2019), and another review suggested and association between pre-existing maternal diabetes and ASD, but there was no association with ADHD (Ornoy et al., 2015).

Despite these findings, studies about the association between gestational diabetes and ADHD and ASD are still contradictory. Some studies have found an association between gestational diabetes and ASD (Alshaban et al., 2019; Ornoy et al., 2015), as well as an association with ADHD (Roigé et al., 2020).

Other studies have found that gestational diabetes was not associated as a whole with ADHD or ASD, but, when adjusted by medication for diabetes, it was significantly associated with ADHD (Xiang et al., 2018), and when combined with pre-pregnancy obesity was associated with both disorders (Kong et al., 2020; Li et al., 2016).

- **Psychiatric disorders:**

Prenatal psychiatric disorders have also been linked with an increased risk of ADHD and ASD. Depression in pregnancy has been associated with developmental and behavioral problems in children, such as ADHD (Bleker et al., 2018). In a two cohort study, prenatal symptoms of depression and anxiety in mothers in both cohorts, and depression symptoms in fathers in one cohort were associated with a higher risk of attention problems in children (Van Batenburg-Eddes et al., 2013).

- **Infections:**

Infections during pregnancy have been associated with ASD in offspring in a systematic review and meta-analysis, suggesting that infectious agent, time and site of infection may modulate the risk (Jiang et al., 2016).

During pregnancy, maternal infection and subsequent immunological activation may be associated with and increased risk of ASD in offspring, as well as the presence of maternally derived anti-brain autoantibodies found in approximately 20% of mothers whose children are at risk of developing ASD has defined an additional subphenotype of ASD (Meltzer & Van de Water, 2017).

Maternal immune dysregulation during pregnancy has been linked as a risk factor for ASD. Infection in pregnancy, such as by rubella or influenza virus, can create an inflammatory immune environment and in consequence the production of maternal cytokines, which can affect directly the placenta and, to a limited degree may cross the placenta and affect the fetus development (Meltzer & Van de Water, 2017).

1.2.1.2 Family history of diseases and neurodevelopmental disorders

- **Autoimmune diseases**

Family history of diseases is an important risk factor for ADHD and ASD in children. In autoimmune disease, the immune system activates against self-antigens. Family history of autoimmune diseases first and second-degree relatives have been associated with ADHD and ASD in offspring (Li et al., 2019; Scott et al., 2017).

In a study about family history of autoimmune diseases (first-degree relatives), some autoimmune diseases were associated with ADHD such as: celiac disease, diabetes mellitus type 1, multiple sclerosis, psoriasis, and rheumatoid arthritis (Li et al., 2019).

A study using a sample of ADHD children and association with autoimmune diseases, has found that individuals with a personal history of autoimmune diseases, as well as maternal history of autoimmune diseases were associated with an increased risk of ADHD, but no significant association was found in paternal history of the diseases. In this study, an increased risk of ADHD was associated to individuals with a family history of some autoimmune diseases, such as type 1 diabetes, autoimmune hepatitis, and psoriasis (Nielsen et al., 2017).

In a study with an ASD population, family history of autoimmune disease such as type 1 diabetes and autoimmune thyroiditis in first and second-degree relatives were common in children with ASD (Scott et al., 2017).

Women may produce anti-brain autoantibodies during pregnancy that can access the developing fetal brain and link to fetal proteins altering the course of neurodevelopment. Activation of the maternal immune system during fetal development is an important factor in the etiology of ASD, and may lead to changes in neurodevelopment (Meltzer & Van de Water, 2017).

1.2.1.3 Medication use in pregnancy

- **Paracetamol:**

Paracetamol is widely used during pregnancy and is categorized as a safe drug by regulatory authorities and is also considered as a first-choice medication for fever and pain in pregnant women. Paracetamol crosses the placental barrier, and meta-analyses have linked paracetamol use during pregnancy with an increased risk of ADHD and ASD (Gou et al., 2019; Masarwa et al., 2018).

Children exposed to acetaminophen in pregnancy have been associated with at increased risk of multiple behavioral difficulties. Acetaminophen use at 18 and 32 weeks of pregnancy was associated with higher risk of conduct problems and hyperactivity in offspring (Stergiakouli et al., 2016).

- **Antidepressants**

A systematic review and meta-analysis showed a significant association between maternal antidepressant use during pregnancy and ASD, and a significant association when assessed by trimester of pregnancy exposure.

A significant association was also found between maternal antidepressant use before pregnancy and ASD. The increased risk of ASD in children could also be related to maternal psychiatric disorders rather than to antidepressant medications (Mezzacappa et al., 2017). A review of meta-analyses concluded that maternal antidepressant use in pregnancy was associated with ASD in children, but the association was not significant after correcting for maternal mental illness. Antidepressant use before pregnancy was also associated with an increased risk of ASD. Maternal mental illness was suggested as an important determinant of ASD risk when associated with maternal antidepressant in pregnancy (Andrade, 2017).

A meta-analysis has associated maternal antidepressant prenatal use of with an increased risk of ADHD in offspring. However, when considering mothers with psychiatric disorders but without treatment during pregnancy, the association was not significant. Additionally, antidepressant exposure before pregnancy was also related with an increased risk of ADHD (Jiang et al., 2018).

1.2.1.4 Smoking

Some studies and a meta-analysis have linked prenatal maternal smoking with ADHD in offspring (Huang et al., 2018; Sciberras et al., 2011), but there was no association between smoking and ASD in other studies including a meta-analysis (Caramaschi et al., 2018; Tang et al., 2015).

1.2.1.5 Stressful life events

Stressful life events during pregnancy have been associated with an increased risk of ADHD and ASD in a variety of studies (MacKinnon et al., 2018; Manzari et al., 2019; Rosenqvist et al., 2019).

Exposure to prenatal stressful events has been correlated with symptoms of ADHD in children (MacKinnon et al., 2018). Prenatal stress has been suggested as a factor that influences the child's neurodevelopment, and has been also linked to a later risk of developing ADHD due to exposure to high levels of maternal glucocorticoids (stress hormones) (Rosenqvist et al., 2019).

1.2.2 Perinatal factors.

Perinatal studies have shown an association between environmental factors such as cesarean section and an increased risk of ADHD and ASD (Curran et al., 2015; Zhang et al., 2019).

A meta-analysis has found that very preterm and very low birth weight in newborns are associated with ADHD, and these findings were stronger in offspring with extremely preterm and extremely low birth weight (Franz et al., 2018). A low birth weight has also been associated with ASD in a meta-analysis (Gardener et al., 2011).

1.2.3 Postnatal factors.

1.2.3.1 Child feeding

Postnatal studies have showed an association between breastfeeding and ADHD or ASD, and breastfeeding has been associated as a protector factor for both disorders (Tseng et al., 2019; Tseng, Yen et al., 2019).

Child high consumption of sugar and/or artificial colors has been also associated as a risk factor for ADHD (Del-ponte et al., 2019).

1.2.3.2 Physical and psychological aggression against child

- **Bullying at school:**

Childhood exposure to bullying directly contributes to multiple mental health domains (Singham et al., 2017). Being bullied is an adverse and stressful experience for children and adolescents that affect well-being and development (Arseneault, 2018).

Exposure to bullying in childhood was related with distress and mental problems. (Arseneault, 2018; Singham et al., 2017).
Exposure to bulling at 11 years, was identified as a causal contribution to anxiety, depression, hyperactivity and impulsivity, inattention, and conduct problems (Singham et al., 2017).

- **Child maltreatment:**

Child maltreatment, including physical, emotional, sexual abuse and neglect, was associated as a risk factor for psychiatric disorders, as well as neurodevelopmental disorders such as ADHD and ASD (González et al., 2019; Okazaki et al., 2020).

Emotional and physical abuse in children and adolescents in foster placements were associated with ADHD in a study, but physical abuse after adjusting for demographics, parental psychopathology and ADHD medication was not associated with ADHD; however, emotional abuse remained associated after adjustments. After analysis by gender, only emotional abuse was related to ADHD in males, and only physical abuse with ADHD in females (González et al., 2019).

Another study conducted in a sample of ASD adults to evaluate the relationship between childhood experiences and brain function using the event-related potential (ERP) components. This study has found that sexual abuse was related with P300 amplitudes, and there was no relation between neglect/negative home atmosphere, punishment and emotional abuse (Okazaki et al., 2020).

Another study carried out using a sample of discordant monozygotic twins suggested that child maltreatment was not associated with an increased risk of neurodevelopmental disorders when adjusted by genetic and shared environmental factors. Child maltreatment was associated also with a small increase in ADHD and ASD symptoms, although most of the covariance of child maltreatment with neurodevelopmental symptoms was explained by common genetic effects (Dinkler et al., 2017).

Another study using a sample of mothers and adopted children suggested that hostile parenting was a factor in the developmental course of ADHD. Mother hostility was associated with future child ADHD symptoms and aggression (Sellers et al., 2020).

1.2.3.3 Parenting

Parents of children with ADHD were associated in a study with a lower self-confidence and less warmth and involvement with their children and were associated as well with more use of corporal punishment in comparison to parents of children without ADHD. The study suggested that children with ADHD are at considerable risk of abuse by their parents, and treatment may also need to address the parent's functioning (Alizadeh et al., 2007).

1.3 ADHD and ASD polygenetic risk scores

Most of the genetic studies in epidemiology are based on genotypes, and DNA samples are required in each participant for the analysis. In the basic structure in genetics, a gene is the physical entity transmitted from parents to offspring and contains a sequence of nucleotides, Adenine (A), Guanine, (G) Thymine (T), and Cytosine (C), to create proteins with specific functions. A chromosome contains several thousands of genes. The human genome is composed of 22 unique chromosomes, autosomes, and a sex chromosome, X or Y, and individuals have two copies, one inherited from the mother and the other one from the father, resulting in a genome make up of 46 chromosomes (44 autosomes, and two sex chromosomes) (Fallin & Kao, 2009).

A locus corresponds to a location and despite individuals carry almost entirely identical sequences in the entire human genome, there is still some locus with polymorphic sequences, and this locus is defined as a polymorphism. An allele is the sequence that defines different forms, and any person carries two, one from the mother and one from the father, at any locus. An individual's genotype is the combination of two alleles at any locus in the chromosome. If a person's genotype contains two identical alleles, that person is homozygous at that locus, but if the two alleles are different, then the person is heterozygous at the locus (Fallin & Kao, 2009).

One type of polymorphism is the genetic polymorphism, and one of the three kinds of genetic polymorphisms is the single nucleotide polymorphism (SNPs). In a single nucleotide polymorphism, one nucleotide is replaced with another. SNPs are common, but are minute, and alterations occur at a frequency of around one every 1,000 bases. Many SNPs have no effect on cellular function, but others may directly predispose individuals to disease or influence their response for example to a drug. SNPs are abundant, stable, and are widely distributed across the genome (Fallin & Kao, 2009).

According to Genome-wide association studies (GWAS), part of the genetic liability to neurodevelopmental disorders is due to a large number of common single-nucleotide polymorphisms (SNPs), that are present in >1% of the population (Leppert et al., 2019). The SNPs can be used to calculate polygenic risk scores (PRSs) (Euesden et al., 2015).

Polygenetic risk scores are typically calculated as a weighted sum of the risk alleles of single nucleotide polymorphisms (SNPSs) across multiple genetic loci using effect sizes from genome-wide association studies as their weights (Euesden et al., 2015; Torkamani, 2018). The polygenic model of human phenotypes reveals that much of the genetic basis for most complex traits comprises small effects of hundreds or even thousands of variants. This polygenic effect can be considered a genetic liability to disease risk, while prediction of phenotype from an individual's genetic profile is compromised by this polygenicity (Euesden et al., 2015).

The construction of PRSs has been improved, a more appropriate selection of independent single-nucleotide polymorphisms (SNPs) was carried out, as well as an optimized estimation of their weights. The latest genome-wide association studies (GWAS), using a sample of 20.183 individuals with a diagnosis of ADHD and 35.191 controls, has identified variants in 12 independent loci (Demontis et al., 2019), and using a sample of 18.381 individuals with a diagnosis of ASD and 27.969 controls has identified five genome-wide-significant loci (Grove et al., 2019).

GWAS has also estimated a single nucleotide polymorphism (SNP) based heritability for ADHD at around 22% (Demontis et al., 2019), and for ASD the SNP-based heritability has been estimated at approximately 12% (Grove et al., 2019). The interplay between genetic and environmental factors has been linked with ADHD and ASD. Gene-environment interplay includes different mechanisms, such as gene-environment correlation (rGE), and gene-environment interaction (GxE) (Jaffee & Price, 2007).

Chapter 2

2. Research at University of Cagliari: "ADHD and ASD prenatal and postnatal risk factor".

This chapter describes in detail the study carried out at the University of Cagliari, Italy, a retrospective case control study using a clinical sample of ADHD and ASD patients of the Unit of Child and Adolescent Neuropsychiatry, "*A.Cao*" Paediatric Hospital, and a control group of patients of "S. Michele" Hospital, Hospital Trust, Cagliari.

Rational of the study

The aim of this study was to investigate prenatal and postnatal exposure to environmental risk factors in children and adolescents with a diagnosis of ADHD and ASD and to identify common risk factors exposure between the groups. Several environmental risk factors studies have been conducted for ADHD and ASD, and comparison between the groups and exposure is still missing, and there are also inconsistencies between the studies. For this purpose, a questionnaire about prenatal and postnatal risk factors was developed and applied to parents of children, mainly through an interview, as well as by using scales to measure psychiatric disorders and parenting. A comparison between clinical groups and non-clinical group exposure to the risk factors was analyzed.

2.1 Methods:

2.1.1 Objectives

General objective:

To investigate ADHD and ASD prenatal and postnatal risk factors in children and adolescents between the ages of 6 to 18 years old, patients of the Unit of Child and Adolescent Neuropsychiatry, "*A.Cao*" Paediatric Hospital.

Specific objectives:

- To develop a questionnaire for ADHD and ASD prenatal and postnatal risk factors to find out the exposure to environmental risk factors and other associated factors.
- To explore psychopathological and environmental family risk factors through scales.
- To identify prenatal and postnatal risk factor in each clinical group and identify common risk factors between the groups.

2.1.2 Type of study

This is an observational case-control retrospective study. In a case-control study, cases are already cases and exposure has already occurred. In a retrospective study, data is collected looking into the past to explore an exposure. A case-control compares two identical groups that differ only in the characteristic of having or not having a disease (Bruce, Pope, & Stanistreet, 2017). Comparison is between a clinical group (cases) and a group without the disease (control group), and comparability is about prior exposure of the groups. The differences between exposed or unexposed is calculated through the Odds ratio. (Bruce et al., 2017; Fallin & Kao, 2009; Susser et al., 2009).

In this study, data was collected retrospectively to find out the exposure or non-exposure to environmental factor between the groups, a clinical group ADHD or ASD compared with a control group without the disorders.

2.1.3 Sample

The total sample consisted of N=97 participants, age range 6-18 years, divided into three groups, two clinical (ADHD and ASD) and one control, as follows:

- ADHD sample: n=42.
- ASD samples: n=21.
- Control sample: n=34.

The total sample consisted of patients of pediatric Units of the "G Brotzu" Hospital Trust in Cagliari: ADHD and ASD were patients at the Child and Adolescent Neuropsychiatry Unit, "*A.Cao*" Paediatric Hospital, and controls at the General Pediatrics (including emergencies) Outpatient Unit "S. Michele" Hospital.

2.1.4 Measures

Diagnostic assessments were carried out on parents of children in order to evaluate the risk factors related to mental disorders, ADHD and ASD traits in parents of children, as well as parenting. A description of the scales applied to parents of children is provided below:

Adult ADHD Self-Report Scale (ASRS-V1.1) Symptom Checklist:

The ASRS-V1.1 is a validated screening scale to assess ADHD in adults. A self-report 18-item instrument based on the ADHD 18 criteria of the DSM-IV-TR. The instrument is divided into two parts, part A (items 1-6) and part B (items 7-18). Adults were asked how they felt and how they conducted themselves in the last 6 months. Five possible answers were provided "Never", "Rarely", "Sometimes", "Often", "Very Often". Part A is the most predictive for ADHD symptoms in adults. A total of 4 or more marks in the darkly shaded boxes in Part A is highly consistent with ADHD. Part B is used as a complement if Part A total score is ≥4 (Corbisiero et al., 2017; Kessler, 2003; Lennard et al., 2017). A total score <4 in Part A was codified as "no ADHD traits" and a score ≥4 as "ADHD traits".

Autism-Spectrum Quotient (AQ):

The AQ is a validated 50-items self-report instrument originally used to assess ASD traits in adults (subjects aged ≥16 years). AQ measures ASD traits across five domains: "communication", "social skills", "attention switching", "imagination", and "attention to detail". (Baron-Cohen et al., 2001; Ruta et al., 2012; Wheelwright et al., 2010). Adults were asked to rate how strongly they agreed or disagreed with a list of statements through four possible answers "Definitely agree", "Slightly agree", "Slightly disagree", "Definitely disagree". Items scored as 1 for autistic traits and 0 for non-autistic traits. The total score was obtained by the sum of all items, and the scores ranges from 0 to 50. (Ruta et al., 2012; Wheelwright et al., 2010). A total score of ≥32 in the original AQ version and a total score of ≥33 in the Italian version show clinical significance for ASD traits (Baron-Cohen et al., 2001; Ruta et al., 2012).

The Italian version was applied in this study (Ruta, 2012) with the original version cutoff. A total score of <32 was recoded as "no autistic traits" and a total score of ≥32 was recoded as "autistic traits".

Symptom Check-list-90-R (SCL-90-R):

The SCL-90-R is a validated 90-items self-report instrument to measure general psychological distress and psychiatric symptoms in subjects aged ≥13 years. Subjects responded on the intensity of the symptoms in the last 7 days in a 5-step Likert scale (0-4), 0 was "Not at all" and 5 "Extremely. (Derogatis, 1994; Preti et al., 2018; E. Preti et al., 2011; Prunas et al. 2012). The Italian version is a 5-step Likert scale (1-5) and scoring is codified as the original version (0-4), in this way answer values are codified as: 1=0, 2=1, 3=2, 4=3, 5=4. (E. Preti et al., 2011). A raw score is calculated for 3 global indices and 9 symptomatic dimensions. Global indices reflect the degree of symptomatology and are divided in: "Global Severity Index (GSI)" (an average of depth distress, the best global measure of psychological distress), "Positive Symptom Total (PST)" (the total number of symptoms reported), and "Positive Symptom Distress Index (PSDI)" (the level of symptoms adjusted by the number of symptoms). The 9 symptomatic dimensions are divided in: "Somatization", "Obsessive-compulsive", "Interpersonal sensitivity", "Depression", "Anxiety", "Hostility", "Phobic anxiety", "Paranoid

ideation", and "Psychoticism". (Preti et al., 2018; E. Preti et al., 2011; Prunas et al., 2012). Raw scores are converted in T scores using the appropriate norm group. Criteria for index and dimension interpretation: T<45= General level of distress is not noteworthy, 45≤T<55= General level of distress is normal, 55≤T<65: General level of distress is moderate to high in intensity, 65≤T≤75= General level of distress beyond the threshold of clinical attention. (E. Preti et al., 2011).

Alabama Parenting Questionnaire (APQ):

The Alabama Parenting Questionnaire (APQ) is a validated 42 items instrument to assess parenting through five dimensions: "Involvement", "Positive parenting", "Poor monitoring/supervision", "Inconsistent discipline", "Corporal punishment". APQ is applied to parents of children and adolescents aged 6-18 years. Parents were asked to rate each item according to the relation with the child or adolescent. A 5-step Likert scale was used for rating (1-5): "Never", "Almost never", "Sometimes", "Often", "Always". (Benedetto & Ingrassia, 2012; Frick, 1991).

Scales in the control group were completed mainly by mothers, and in clinical groups they were completed mostly by both parents during their child's clinical visit. The data not collected in the ADHD group is due to non-application of scales initially in the study or due to the impossibility of parents to complete them by telephone or send them by email during 2020, as well as in the case of ASD group. Scales applied by telephone to ASD and ADHD groups were completed only by mothers.

2.1.5 Development and description of the questionnaire.

A prenatal and postnatal risk factor questionnaire was developed to explore the exposure of the groups to the environmental factor. The questionnaire was administered to parents of children through an interview. In case it was not possible to conduct an interview, the questionnaire was given to the parents or sent by email to be completed by them and to be returned during the next clinical visit or by email. Some interviews were carried out by telephone during 2020.

The questionnaire was composed of 107 questions and explores topics related to demographics, socio-economical, and prenatal and postnatal risk factors. The questionnaire was divided into three sections: General information, Information during pregnancy, and Postnatal information.

A description of the sections and topic is provided below:

- **General information section**: Includes general information about the child (name, age, data of birth, gender, diagnosis, medical record number), and parents (interviewee name, telephone number, relation with the child, province and locality of residence).

- **Information during pregnancy:** This section begins with demographic and economic information about pregnancy period (social class during pregnancy; monthly income; marital status; mother's employment; educational level of parents; parents age).

  Then follow questions in topics related to risk factors for the disorders during pregnancy including: mother's weight before and during pregnancy; use of assisted reproductive techniques; mother's psychiatric disorder during pregnancy; diabetes in pregnancy and treatment; thyroid disease in pregnancy; medications use and type, pregnancy week of consumption, time of consumption; infection and type, infectious agent, pregnancy week and duration of the infection; vaginal bleeding, and pregnancy week, intervention; preeclampsia, pregnancy week, intervention; previous abortions; threatened abortion during pregnancy, treatment; exposure to chemicals or environmental pollutants in pregnancy, pregnancy week of exposure; number of ultrasounds, and number by trimester; family history of the disorder; family history of other pathologies or psychiatric disorders; sleep problems during pregnancy; number of sleep hours; folic acid taken before or during pregnancy, period or pregnancy trimester; X-rays exposure; alcohol consumption; smoking during pregnancy or smoke exposure; drugs consumption; physical and/or psychological stress; prenatal medical check-ups; restricted or poor diet during pregnancy.

- **Postnatal information:** A number of perinatal and postnatal questions were asked in this section, including: delivery type; complication during delivery, type of complication; child medical problems after birth; prematurity child; pregnancy week of delivery; child birth weight; child in intensive care unit, and time; type of feeding, time of breastfeeding; postpartum depression; child traumatic experience during childhood; child diet; child medical prescriptions for dietary supplements; other pathologies or psychiatric disorders of the child; father's child psychiatric disorders; father's child antisocial behavior; family life adverse situation; child exposed to chemicals or environmental pollutants; social class after pregnancy; alcohol consumption; drugs consumption; poor diet after pregnancy; child exposed to smoking; hostility/aggression between child's parents; physical violence between child's parents; verbal and psychological violence between child's parents; family environment hostile/aggressive; hostility/aggression between parents and child; child verbally or psychologically assaulted in the family; physical aggression against child in the family; bullying at school; teacher aggression against child.

2.1.6 Inclusion and exclusion criteria

Patients and controls were recruited at two different pediatric Units of the " G Brotzu" Hospital Trust in Cagliari: ADHD and ASD patients at the Child and Adolescent Neuropsychiatry Unit, "*A.Cao*" Paediatric Hospital, controls at the General Pediatrics (including emergencies) Outpatient Unit "S. Michele" Hospital, respectively.

Information about the study was provided to parents during their child's clinical visit. Prenatal and postnatal risk factor questionnaire and scales were applied to parents of children once they agreed to participate in the study. Written informed consent from parents was obtained to participate in the study.

Inclusion criteria:

ADHD group:

- Males and females diagnosed with Attention-Deficit/Hyperactivity Disorder.
- Age between 6 and 18 years.
- Patients of the Unit of Child and Adolescent Neuropsychiatry, "*A.Cao*" Paediatric Hospital. ADHD diagnosis based on DSM-5 criteria, and measures through Conners' Parent Rating Scale-Revised (CPRS-RS) and K-SADS-Present and Lifetime Version (KSADS-PL).

ASD group:

- Males and females diagnosed with Autism Spectrum Disorder.
- Age between 6 and 18 years.
- Patients of the Unit of Child and Adolescent Neuropsychiatry, "*A.Cao*" Paediatric Hospital. ASD diagnosis based on DSM-5 criteria, and measures through Autism Diagnostic Observational Scale (ADOS-2) and Autism Diagnostic Interview-Revised (ADI-R).

Control group:

- Males and females without diagnosis of Attention-Deficit/Hyperactivity Disorder, Autism Spectrum Disorder or other psychiatric disorders.
- Age between 6 and 18 years.
- Patients of the General Pediatrics (including emergencies) Outpatient Unit "S. Michele" Hospital, "*G.Brotzu*" Hospital Trust. Patients were included based on medical records and parent confirmation of absence of ADHD and ASD diagnosis.

Exclusion criteria

ADHD group:

- Males and females diagnosed with Autism Spectrum Disorder.
- Males and females aged under 5 and over 18 years.

ASD group:

- Males and females diagnosed with Attention-Deficit/Hyperactivity Disorder.
- Males and females aged under 5 and over 18 years.

Control group:

- Males and females with diagnosis of psychiatric disorders, Attention-Deficit/Hyperactivity Disorder or Autism Spectrum Disorder.
- Males and females aged under 5 and over 18 years.

2.1.7 Recruitment and procedures

The recruitment process for the ADHD and ASD clinical groups and for the control group was carried out at the pediatric Units of "G. Brotzu" Hospital Trust. An explanation of the process is provided in the next sections:

Clinical groups:

A clinical sample of children and adolescents with an ADHD or ASD diagnosis and their parents was recruited at the Unit of Child and Adolescent Neuropsychiatry, "*A.Cao*" Paediatric Hospital. Parents of children in their child's regular visit were provided of information about the study and they were asked about the interest in participating in the research. Written information about the study and the processes was provided.

In case the parents of children agreed to participate in the study, the questionnaire of prenatal and postnatal risk factors was applied through an interview. Explanation about the scales was given in order to be completed by parents. One or more visits were required to complete the questionnaires and the scales. Some questionnaires and scales were given to parents to be completed on their own and to be returned during the next clinical visit. In 2020, some parents of children were contacted by telephone to enroll in the study, explanation about the research was given, and they were asked about their interest in participating in the study. Once parents confirmed their participation, the interview and scales were applied by telephone or sent by email according to their preferences.

Control group:

A sample of children and their parents were invited to participate in the study during their visit to "*G.Brotzu*" Hospital Trust, Cagliari. Information about the research and the process was provided. Once parents agreed to participate in the study the questionnaire was administered through an interview and explanation about the scales were provided to parents to be completed. Written informed consent to participate was obtained from parents.

2.1.8 Statistical analyses

A multivariate logistic regression analysis was carried out as statistical measure for ASD and ADHD groups and exposure to environmental factors. The analyses were adjusted by sex of the child, mother's academic level and social economic status.

The IBM Statistical Package for the Social Science SPSS version 22 (IBM SPSS, 2014) was used to create the database and to calculate the logistic regression analysis.

Binary variables were created as exposed or not exposed to the environmental factors. Binary variables were recoded as: Type of delivery: 0=Natural, 1= Cesarean. The reference variable was natural childbirth. In some reviews and meta-analysis vaginal delivery is compared to cesarean delivery in psychiatric disorders, being cesarean delivery a risk for ADHD and ASD disorders (Curran et al., 2015; Zhang et al., 2019). Smoking or exposure to smoke during pregnancy: 0=No, 1=Yes (Huang et al., 2018). Child feeding: 0=Breastfeeding, 1=Formula feeding. Breastfeeding was the reference variable. In some studies, breastfeeding is associated with a lower risk of ADHD and it is associated as a protective factor for ASD (Tseng et al., 2019; Tseng, Yen, et al., 2019). Gestational diabetes was recoded as: 0=No, 1=Yes (Alshaban et al., 2019; Ornoy et al., 2015; Roigé et al., 2020). Child high consumption of sugar and/or artificial colors was recoded: 0=No, 1=Yes (Del-ponte et al., 2019). Postnatal adverse situations in family was recoded as: 0=No, 1=Yes (Lost of job, Economic problems, Parents separation, Death of a loved one) based on stressful life events during pregnancy (MacKinnon et al., 2018; Rosenqvist et al., 2019). Child verbally or psychological assaulted in the family: 0=No, 1=Yes (hostility/aggression between parents and child, child has been verbally or psychologically assaulted in the family) (González et al., 2019; Okazaki et al., 2020); Bullying at school was part of a 5-step Likert scale with the next possible options: 1=Never, 2=Almost Never, 3=Sometimes, 4=Often, 5=Always. Binary variable for bullying was recoded: 0=No ("Never"), 1=Yes ("Almost Never", "Sometimes", "Often", "Always") (Arseneault, 2018; Singham et al., 2017); Immune mediated pathologies in family (first and second degree of consanguinity) was coded as: 0=Absent, 1=Present (Li et al., 2019; Scott et al., 2017).

All models were adjusted by sex of the child, social status and educational level of the mother. Covariates were recoded as: Sex of the child: 0=Female, 1=Male; Social status: 0=Middle or High, 1=Low; Educational level of the mother: 0=University, 1=Primary school or High school.

These variables were selected as covariates according to other researches in the file. For all binary variable models, the reference variable was 0.

Scales were codified as having or not having symptoms or traits and were codified as follows: Adult ADHD self-report scale (ASRS-V1.1): 0=Non ADHD symptoms (score <5) vs 1=ADHD symptoms (score ≥5); Autism-Spectrum Quotient (AQ): 0=Non ASD traits (score <32) vs 1=ASD traits (score ≥32); Symptom Check-list-90-R (SCL-90-R) and Alabama Parenting Questionnaire (APQ) scores were used as continuous variable.

2.2 Results

The total sample of the study consisted of N=97 participants, age range 6-18 years (mean age=11 years, SD=3.17). Divided by gender in 70 males (72%) and 27 females (28%).

The clinical ADHD and ASD samples were patients of the Unit of Child and Adolescent Neuropsychiatry, "*A.Cao*" Paediatric Hospital. ADHD sample n=42, 36 (86%) males and 6 (14%) females, age range 6-18 years (mean age=12, SD=3.2). ASD clinical sample n=21, 17 (81%) males and 4 (19%) females, age range 6-18 (mean age=11, SD=3.57). The control sample n=34, 17 (50%) males and 17 (50%) females, age range 6-16 (mean age=10, SD=2.34), were patients of General Pediatrics (including emergencies) Outpatient Unit "S. Michele" Hospital. Characteristics of the sample are provided in Table 2.1.

Table 2.1 Characteristics of the sample in the ADHD and ASD prenatal and postnatal risk factors study by group

	ADHD (n=42)	**ASD (n=21)**	**Control (n=34)**	**Total sample (N=97)**
Participants, n (%)	42 (43%)	21 (22%)	34 (35%)	97 (100%)
Age, mean in years ± SD (range)	12±3.2 (6-18)	11±3.57 (6-18)	10±2.34 (6-16)	11±3.17 (6-18)
Gender, n (%)				
Males	36 (86%)	17 (81%)	17 (50%)	70 (72%)
Females	6 (14%)	4 (19%)	17 (50%)	27 (28%)

Regarding the questionnaires and scales applied to parents of children, a total of 97 questionnaires were collected, 89 (92%) applied through an interview and 8 (8%) completed directly by parents of children, and 310 scales. A total of 42 (43%) questionnaires and 124 (40%) scales were collected for ADHD group, 21 (22%) questionnaires and 67 (22%) scales for ASD group, and 34 (35%) questionnaires and 119 (38%) scales for the control group (Tables 2.2 and 2.3).

Table 2.2 Summary of the questionnaires and scales applied in the ADHD and ASD prenatal and postnatal risk factors study divided by sample

Scales	ADHD (%)	ASD (%)	Control (%)	Total sample (%)
Questionnaire	42 (43%)	21 (22%)	34 (35%)	**97 (100%)**
Adult ADHD Self-Report Scale (ASRS-V1.1)	34 (40%)	15 (18%)	36 (42%)	**85 (100%)**
Autism-Spectrum Quotient (AQ)	32 (40%)	17 (22%)	30 (38%)	**79 (100%)**
Symptom Check-list-90-R (SCL-90-R)	28 (39%)	16 (22%)	28 (39%)	**72 (100%)**
Alabama Parenting Questionnaire (APQ)	30 (40)	19 (26%)	25 (34%)	**74 (100%)**

Scales were applied to measure psychiatric symptoms, and ADHD and ASD traits in parents of children, as well as parenting. A total sample of 310 scales was collected, divided in: ADHD group 124 (40%), 68 (55%) completed by mothers, and 56 (45%) by fathers; ASD group 67 (22%), 40 (60%) completed by mothers, and 27 (40%) by fathers; Control group 119 (38%), 90 (76%) completed by mothers, and 29 (24%) by fathers (Table 2.3).

A total of 85 Adult ADHD Self-Report Scale (ASRS-V1.1) were collected, divided by group: ADHD group: 34 (40%), 18 (21%) completes by mothers, and 16 (19%) by fathers; ASD group: 15 (18%), 9 (11%) completed by mothers, and 6 by fathers (7%); Control group: 36 (42%), 28 (33%) completed by mothers, and 8 (9%) by fathers.

For Autism-Spectrum Quotient (AQ), a total of 79 scales were collected, divided in: ADHD group: 32 (40%), 17 (21%) by mothers, and 15 (19%) by fathers; ASD group: 17 (22%), 10 (13%) by mothers, and 7 (9%) by fathers; Control group: 30 (38%), 23 (29%) by mothers, and 7 (9%) by fathers.

A sample of 72 Symptom Check-list-90-R (SCL-90-R) were collected, divided in: ADHD group: 28 (39%), 16 (22%) by mothers, and 12 (17%) by fathers; ASD group: 16 (22%), 10 (14%) by mothers, and 6 (8%) by fathers; Control group: 28 (39%), 21 (29%) by mothers, and 7 (10%) by fathers.

For Alabama Parenting Questionnaire (APQ), a total sample of 74 was collected, divided in: ADHD group: 30 (40%), 17 (23%) by mother, and 13 (17%) by fathers; ASD group: 19 (26%), 11 (15%) by mothers, and 8 (11%) by fathers; Control group: 25 (34%), 18 (24%) by mother, and 7 (9%) by fathers.

A summary of the scales is provided in Table 2.3.

Table 2.3 Summary of scales applied in the ADHD and ASD prenatal and postnatal risk factors study divided by group and parents of the child

Scales	ADHD group (%)	ASD group (%)	Control group (%)	Total sample (%)
Adult ADHD Self-Report Scale (ASRS-V1.1)				
Mother	18 (21%)	9 (11%)	28 (33%)	**55 (65%)**
Father	16 (19%)	6 (7%)	8 (9%)	**30 (35%)**
Autism-Spectrum Quotient (AQ)				
Mother	17 (21%)	10 (13%)	23 (29%)	**50 (63%)**
Father	15 (19%)	7 (9%)	7 (9%)	**29 (37%)**
Symptom Check-list-90-R (SCL-90-R)				
Mother	16 (22%)	10 (14%)	21 (29%)	**47 (65%)**
Father	12 (17%)	6 (8%)	7 (10%)	**25 (35%)**
Alabama Parenting Questionnaire (APQ)				
Mother	17 (23%)	11 (15%)	18 (24%)	**46 (62%)**
Father	13 (17%)	8 (11%)	7 (9%)	**28 (38%)**
Total	**124 (40%)**	**67 (22%)**	**119 (38%)**	**310 (100%)**

Logistic regression analyses were carried out for the variables of the questionnaire. The analyses indicated for ADHD postnatal risk factors that Formula feeding (OR=5.19; 95% CI, 1.03-26.11), High consumption of sugar and/or artificial colors (OR=4.89; 95% CI, 1.01-23.64), Family adverse situations (OR=3.05; 95% CI, 1.04-8.95), Child verbally or psychologically assaulted (OR=9.00; 95% CI, 1.59-50.81), and Bullying at school (OR=8.08; 95% CI, 1.97-33.11), were associated with an increased risk of ADHD.

A prenatal factor such as Cesarean delivery was associated with an increased risk of ADHD (OR=4.29; 95% CI, 1.17-15.78). Immune mediated pathologies in the family (first and second-degree relatives) was another factor associated with an increased risk of ADHD (OR=9.17; 95% CI, 2.07-40.66), as well as for ASD (OR=5.43; 95% CI, 1.10-26.85).

The analyses were associated mainly as risk factors for ADHD. Other risk factors also explored through the questionnaire were not associated with an increased risk of ADHD or ASD in children, such as gestational diabetes, and smoking or exposure to smoke during pregnancy (Table 2.4).

Table 2.4 Binary logistic regression analysis for ADHD and ASD prenatal and postnatal risk factors

	ADHD		**ASD**	
	OR (95%CI)	p	OR (95%CI)	p
Prenatal risk factor				
Immune mediated pathologies in family*	9.17 (2.07-40.66)	**0.00**	5.43 (1.10-26.85)	**0.04**
Smoking or exposure to smoke	2.16 (0.71-6.60)	0.17	1.556 (0.39-6.23)	0.53
Gestational diabetes	0.28 (0.07-1.24)	0.09	0.39 (0.07-2.07)	0.27
Cesarean delivery	4.29 (1.17-15.78)	**0.03**	5.57 (0.96-32.18)	0.05
Postnatal risk factor				
Formula feeding	5.19 (1.03-26.11)	**0.04**	0.00	0.10
High consumption of sugar and/or artificial colors	4.89 (1.01-23.64)	**0.04**	2.31 (0.39-13.83)	0.36
Family adverse situations	3.05 (1.04-8.95)	**0.04**	0.63 (0.17-2.34)	0.49
Child verbally or psychologically assaulted	9.00 (1.59-50.81)	**0.01**	3.55 (0.51-24.70)	0.20
Bullying at school	8.08 (1.97-33.11)	**0.00**	3.83 (0.73-19.99)	0.11

Models adjusted by sex of the child, mother's educational level and social class. $p < 0.05$.
First and second-degree relatives*

Scales were applied to parents of children to assess ADHD and ASD traits, as well as psychological distress and psychiatric symptoms, and parenting.

ADHD traits in mother and fathers were assessed using the Adult ADHD Self-Report Scale (ASRS-V1.1). ADHD traits in parents were not associated with an increased risk of ADHD or ASD in children. (Table 2.5).

Table 2.5 Binary logistic regression analysis for ADHD traits in parents divided by clinical group

	ADHD group		**ASD group**	
	OR (95%CI)	p	OR (95%CI)	p
ADHD traits mothers*	2.10 (0.10-43.86)	0.63	0.00	0.10
ADHD traits fathers*	0.00		0.00	

Adjusted by sex of the child, mother's educational level and social class. p <0.05.
ADHD traits assessed using the Adult ADHD Self-Report Scale (ASRS-V1.1).

ASD traits were measure in mothers and fathers using the Autism-Spectrum Quotient (AQ). ASD traits in parents were not associated with an increased risk of ADHD or ASD in children. (Table 2.6).

Table 2.6 Binary logistic regression analysis for ASD traits in parents divided by clinical group

	ADHD group		**ASD group**	
	OR (95%CI)	p	OR (95%CI)	p
ASD traits mothers	0.00		0.00	
ASD traits fathers	0.00		0.00	

Adjusted by sex of the child, mother's educational level and social class. p <0.05.
ASD traits assessed using the Autism-Spectrum Quotient (AQ).

The Alabama Parenting Questionnaire (APQ) was applied to mothers and fathers to assess parenting through five dimensions. In the regression analysis of mother scores, the dimension "Inconsistent discipline" was associated with an increased risk of ADHD (OR=1.36; 95% CI, 1.04-1.78), and "Involvement" dimension was associated as a protective factor for ADHD (OR=0.80; 95% CI, 0.65-0.99); the dimensions "Positive parenting", "Poor monitoring/supervision", and "Corporal punishment" were not associated with ADHD. The analyses of the dimensions were not associated with ASD (Table 2.7).

Table 2.7 Logistic regression analysis for parenting in mothers divided by clinical groups

	ADHD group		**ASD group**	
	OR (95%CI)	p	OR (95%CI)	p
Involvement	0.80 (0.65-0.99)	**0.04**	0.89 (0.75-1.06)	0.18
Positive parenting	0.86 (0.59-1.24)	0.42	0.86 (0.58-1.28)	0.45
Poor monitoring/supervision	1.08 (0.94-1.23)	0.26	1.22 (0.91-1.63)	0.18
Inconsistent discipline	1.36 (1.04-1.78)	**0.02**	1.03 (0.80-1.34)	0.79
Corporal punishment	0.73 (0.45-1.20)	0.22	0.00	0.10

Adjusted by sex of the child, mother's educational level and social class. $p < 0.05$.
Parenting assessed using the Alabama Parenting Questionnaire (APQ)

The regression analysis of the Alabama Parenting Questionnaire (APQ) in fathers was not significant. The dimensions in fathers scores were not associated with an increased risk of ADHD or ASD in children. (Table 2.8).

Table 2.8 Logistic regression analysis for parenting in fathers divided by clinical groups

	ADHD group		ASD group	
	OR (95%CI)	p	OR (95%CI)	p
Involvement	0.84 (0.63-1.11)	0.22	0.85 (0.63-1.16)	0.31
Positive parenting	1.24 (0.80-1.91)	0.34	1.58 (0.76-3.28)	0.22
Poor monitoring/supervision	0.97 (0.74-1.28)	0.85	1.13 (0.85-1.50)	0.40
Inconsistent discipline	1.53 (0.92-2.55)	0.10	1.07 (0.68-1.67)	0.78
Corporal punishment	58532037,64 (0.00)	0.10	0.44 (0.01-14.14)	0.65

Adjusted by sex of the child, mother's educational level and social class. $p < 0.05$.
Parenting assessed using the Alabama Parenting Questionnaire (APQ).

The Symptom Check-list-90-R (SCL-90-R) was used to assess general psychological distress and psychiatric symptoms in mothers and fathers of children through three global indices and nine symptomatic dimensions or subscales.

The regression analysis in mother's score showed an association between "Hostility" subscale and an increased risk of ADHD (OR=1.19; 95% CI, 1.02-1.39), as well as for the global scale "Positive Symptom Distress Index (PSDI)" (OR=1.13; 95% CI, 1.02-1.25). There is no association between the other global indices or symptomatic dimensions in mother's score and an increased risk of ASD in children. (Table 2.9).

Table 2.9 Logistic regression analysis for psychological distress and psychiatric traits in mothers divided by clinical group

	ADHD		ASD	
	OR (95%CI)	p	OR (95%CI)	p
Somatization	1.04 (0.96-1.13)	0.32	1.10 (0.99-1.23)	0.08
Obsessive-compulsive	1.09 (0.99-1.11)	0.06	1.07 (0.96-1.19)	0.19
Interpersonal sensitivity	1.07 (0.98-1.16)	0.12	1.09 (0.97-1.23)	0.15
Depression	1.05 (0.96-1.15)	0.29	1.10 (0.10-1.22)	0.06
Anxiety	1.05 (0.97-1.13)	0.25	1.07 (0.97-1.17)	0.20
Hostility	1.19 (1.02-1.39)	**0.03**	1.11 (0.97-1.28)	0.14
Phobic anxiety	1.04 (0.94-1.16)	0.42	0.88 (0.69-1.12)	0.29
Paranoid ideation	1.08 (0.98-1.18)	0.10	1.07 (0.97-1.18)	0.15
Psychoticism	1.07 (0.94-1.21)	0.31	1.18 (0.99-1.41)	0.06
Global Severity Index (GSI)	1.06 (0.98-1.16)	0.13	1.10 (0.10-1.29)	0.06
Positive Symptom Total (PST)	1.06 (0.98-1.14)	0.12	1.06 (0.98-1.15)	0.17
Positive Symptom Distress Index (PSDI)*	1.13 (1.02-1.25)	**0.02**	1.12 (0.98-1.28)	0.09

Adjusted by sex of the child, mother's educational level and social class. $p < 0.05$.
Assessments using the Symptoms check list (SCL-90-R).
*PSDI: the level of symptoms adjusted by the number of symptoms.

The Symptom Check-list-90-R (SCL-90-R) was used to assess general psychological distress and psychiatric symptoms in mothers and fathers of children through three global indices and nine symptomatic dimensions or subscales.

The regression analyses of the global indices and symptomatic dimensions in father's scores were not associated with an increased risk of ADHD or ASD in children. (Table 2.10).

Table 2.10 Logistic regression analysis for psychological distress and psychiatric traits in fathers divided by clinical group

	ADHD		ASD	
	OR (95%CI)	p	OR (95%CI)	p
Somatization	1.08 (0.94-1.25)	0.27	0.97 (0.83-1.14)	0.71
Obsessive-compulsive	1.88 (0.85-4.14)	0.12	0.94 (0.78-1.14)	0.54
Interpersonal sensitivity	30.90 (0.00)	0.10	0.97 (0.75-1.24)	0.80
Depression	1.09 (0.87-1.36)	0.48	0.86 (0.67-1.11)	0.26
Anxiety	1.12 (0.89-1.42)	0.34	0.90 (0.73-1.10)	0.29
Hostility	1.09 (0.88-1.36)	0.42	0.91 (0.66-1.26)	0.58
Phobic anxiety	1.07 (0.77-1.49)	0.68	1.02 (0.68-1.52)	0.92
Paranoid ideation	1.08 (0.90-1.29)	0.39	0.86 (0.69-1.07)	0.18
Psychoticism	1.13 (0.91-1.39)	0.26	0.69 (0.39-1.20)	0.19
Global Severity Index (GSI)	1.16 (0.94-1.42)	0.16	0.91 (0.75-1.12)	0.38
Positive Symptom Total (PST)	1.15 (0.94-1.40)	0.16	0.93 (0.81-1.08)	0.37
Positive Symptom Distress Index (PSDI)*	1.14 (0.90-1.43)	0.27	1.03 (0.83-1.27)	0.81

Adjusted by sex of the child, mother's educational level and social class. $p < 0.05$.
Assessments using the Symptoms check list (SCL-90-R).
*PSDI: the level of symptoms adjusted by the number of symptoms.

2.3 Discussion

Attention-Deficit/Hyperactivity Disorder (ADHD) and Autism Spectrum Disorder (ASD) are multifactorial disorders caused by genetic and environmental risk factors (Faraone & Larsson, 2019; Ornoy et al., 2015; Waltes et al., 2019).

A variety of environmental factors have been studied for ADHD and ASD. Prenatal, perinatal, and postnatal exposure to environmental factors have been associated with an increased risk of ADHD and ASD in offspring; however, there are still inconsistencies in the studies.

The present study explored a wide variety of environmental risk factors in a sample of children and adolescents with a diagnosis of ADHD and ASD. The information on exposure to environmental factors was collected retrospectively through a questionnaire and scales were applied to parents of children.

In this study, prenatal and postnatal environmental risk factors were related to ADHD in the sample. The ADHD sample exposed to a cesarean delivery was associated with an increased risk of ADHD in offspring. ADHD children exposed to postnatal risk factors such as formula feeding, high consumption of sugar and/or artificial colors, family adverse situations, verbal or psychological aggression, and bullying at school, were associated as well to an increased risk of ADHD.

Other environmental factors related to parenting and parent's characteristics, such as inconsistent discipline and hostility by the mother, were also associated as a risk of ADHD, and involvement, which is a parenting dimension to measure how parents are involved in children activities, was associated as a protective factor of ADHD.

Immune mediated pathologies in family (first and second degree-relatives), such as celiac disease, arthritis, multiple sclerosis, and psoriasis, were associated with an increased risk of ADHD and ASD. ADHD or ASD traits in parents of children were no associated as a risk of ADHD and ASD.

Cesarean delivery compared with natural childbirth was associated as a risk factor for neurodevelopmental disorders including ADHD (Curran et al., 2015; Zhang et al., 2019).
Some studies have hypothesized the biological mechanisms of cesarean delivery in neurodevelopmental disorders by altering immune development through the perturbation of bacterial colonization, disturbing the immune and sensory activation by the lack of stress response or modifying epigenetic regulation in DNA methylation (Zhang et al., 2019).

Feeding in ADHD sample is an important factor in children health from birth, formula feeding compared with breastfeeding and high consumption of sugar and/or artificial colors were associated with the disorder. However, the mechanisms between dietary and ADHD are still not clear. Natural childbirth has been associated as a protective factor for ADHD in offspring, and could be related with increased levels of oxytocin during breastfeeding (Carter, 2003) or neurotrophic factors present in breast milk that could help in brain development, which are absents in formula milk (Serpero et al., 2012).

Children with a diagnosis of ADHD are surrounded by a family environment of stress and psychological aggression, hostility, and inconsistent discipline, and have been exposed to bulling at school, which is another kind of violence against children. Children exposed to emotional abuse in foster care, especially males, have an increased risk of ADHD, and prolonged exposure to abuse increases the probability of ADHD symptoms to persist (González et al., 2019). Adverse experiences and maltreatment may be linked to changes in brain functioning due stress to the exposure (Gul & Gurkan, 2018). However, maternal hostility related to ADHD may be also explained by genetic, or by a contribution of both genetic and environmental factors, suggesting that ADHD symptoms have an impact in mother and son hostility (Lifford et al., 2009).

This study did not find common environmental factors exposure in ADHD and ASD, and the only factor in common was the immune mediated pathologies in family in first and second degree-relatives (Nielsen et al., 2017; Scott et al., 2017).

Parental ADHD is also associated with an increased risk of ADHD in offspring (Millichap, 2008), and was found as elevated in first-degree relatives (American Psychiatric Association, 2013). However, in this study ADHD traits in parents were not associated with an increased risk of ADHD in children at the time of the measures.

Special attention should be given to the environmental factors linked to ADHD in this study, due to the association found to ADHD in children and adolescents. Interventions aimed at avoiding or regulating exposure to these factors could help in the prevention and intervention of this disorder.

Chapter 3

3. Research at University of Groningen: "Interplay between polygenic risk scores and maltreatment and different forms of abuse in attention-deficit/hyperactivity.

This chapter explains the research carried out at the University of Groningen, the Netherlands, at the Child and Adolescent Psychiatric Unit, University Medical Center Groningen, using the Avon Longitudinal Study of Parents and Children (ALSPAC), a prospective birth cohort study with a sample of 15,454 pregnancies of women recruited in Bristol, England, from 1990 to 1992.

Rational:

The aim of this study was to investigate whether polygenetic risk scores for Attention-Deficit/Hyperactivity Disorder (ADHD) were linked to maltreatment and whether genetic liability and maltreatment mediate each other's effects in relation to ADHD traits. Attention-Deficit/Hyperactivity Disorder is a multifactorial disorder that involves genetics and environmental factor. Studies about the association between ADHD and maltreatment have been conducted, however, studies involving genetic and environment are still missing and need to be expanded. For this purpose, a genetic-environment study was conducted using a sample of children of the ALSPAC prospective birth cohort study. Information about the environmental factors was collected longitudinally through questionnaires, and genotyping of children were conducted. ADHD traits were measured by mother-reported symptom scores of ADHD, using the Development and Well-Being Assessment (DAWBA) interview at age 8. Maltreatment was measured using mother-reported questionnaires of different aspects of abuse and malparenting from birth up to age 8. ADHD polygenetic risk scores (PRS) were derived from the most recent genome-wide association study of ADHD, from the Psychiatric Genomic Consortium (PGC). Gene-environment correlation (rGE), and mediation analysis were conducted for the genetic-environmental analysis.

3.1 Methods

3.1.1. Objectives

General objective

To investigate whether genetic risk for ADHD is linked to maltreatment and whether genetic liability and maltreatment mediate each other's effects in relation to ADHD traits.

Specific objectives

- To conduct a gene-environment correlation (rGE) analysis to investigate whether genetic risk for ADHD was linked to maltreatment.

- To carry out a mediation analysis to investigate whether genetic liability and maltreatment mediate each other's effects in relation to ADHD traits.

3.1.2 Type of study

The Avon Longitudinal Study of Parents and Children (ALSPAC), known to its participants as "Children of the 90s", is a prospective birth cohort study (Boyd et al., 2013; Golding et al., 2001). A cohort study may also be referred to as a longitudinal study. The definition of cohort study is the follow-up of individuals over time. This type of study often requires a large sample and a follow-up of several years. Prospective means looking forward in time; if a survey measuring exposure is done now, the occurrence of cases will be in the future (Bruce et al., 2017).

ALSPAC is a collaborating of the European Longitudinal Study of Pregnancy and Childhood (ELSPAC), and was designed to determinate how the genotype and the environment are combine to influence the health and development of an individual (Golding et al., 2001).

Children born from these pregnancies and their families have been observed for more than 25 years. Data collected through questionnaires and clinical assessment visits provides information about environmental and genetic factors that affects health and development across the life course (Boyd et al., 2013; Fraser et al., 2013; Golding et al., 2001).

Ethical approval was obtained from the ALSPAC Ethics and Law Committee and from the Local Research Ethics Committees.

3.1.3 Sample

The initial ALSPAC sample consisted of 14,541 pregnancies of women recruited from Bristol, England, during 1990–1992. When the oldest children were around 7 years old, the total sample increased as a result of including initial cases that had failed to join the study originally. The total ALSPAC sample after the age of 7 consists in 15,454 pregnancies (Boyd et al., 2013; Fraser et al., 2013).

The current study used a genotype sample of N=8941 children from the ALSPAC study. Data for the 8 maltreatment-related phenotypes was available for at least 3725 children, but up to 6844. Children with data of the Development and Well-Being Assessment (DAWBA) interview at age 8 were included in the study.

3.1.4 Measures

The Development and Well-Being Assessment (DAWBA) was used to assess ADHD traits in children and was applied when children were aged approximately 8. The Development and Well-Being Assessment (DAWBA) is a validated instrument that consists of a semi-structured interview applied to parents of children aged 5-16, and is used for the diagnosis of psychiatric disorders in children and adolescents based on DSM-IV criteria (A. Goodman et al., 2011; R. Goodman et al., 2000).

To assess ADHD traits in children and adolescents, parents of children completed 18 items about attention, hyperactivity and impulsivity. Parents of children were asked about level of activity and concentration of the child in the last six months compared with other children of the same age. Items were rated as following: No more than others=0, A little more than others=1, A lot more than others=2. The total score was obtained by the sum of all items, and the scores ranges from 0 to 36. Is it also possible to calculate scores for inattentive and hyperactive-impulsive traits separately, and the scores ranges from 0 to 18 for each dimension (R. Goodman, 2001).

3.1.5 Data Collection

Information for ALSPAC study was collected through multiple sources from early pregnancy (Golding et al., 2001), and include:

- Self-completion questionnaires to mothers, partners, and children.
- Medical and educational records.
- Environmental measures in a subsample of houses to measure air pollution levels, magnetic radiation, and noise.
- In-depth interviews and examination of subgroups and the controls.
- Biological sample from the mother, the partner, and the child.

Questionnaires and genetic data:

Questionnaires were first pilot-tested on about 100 parents, including mothers and partners in prenatal and postnatal stages, and a validity process for the information collected was carried out comparing details about medical history with medical records (over 95% agreement), psychological scales were compared with clinical examinations (with high correlation) (Golding et al., 2001).

Questionnaires were designed for mothers and their partners to be completed prenatally and postnatally and were also designed for children. A total of four questionnaires were designed to be completed by mothers during pregnancy, depending on the time of enrollment to the study, and two questionnaires were designed to be completed by their partners.
Several questionnaires were designed to be completed postnatally by mother, partners and children in multiple time points from birth (Golding et al., 2001). Follow-up has been focused mainly on the offspring (Boyd et al., 2013).

ALSPAC follow-up includes 59 questionnaires, and 9 clinical assessment visits. The information collected contains a wide range of phenotypic and environmental measures, as well as biological samples, genetic data including DNA on 11.343 children, genome-wide on 8.365 children, and complete genome sequencing on 2000 children, additionally epigenetic data (methylation sampling on 1.000 children) (Boyd et al., 2013).

In ALSPAC study, mothers were the participants recruited, but the goal of the study was to determine ways in which genotype and environmental characteristics influence health and development in both children and parents. For this reason, additional funding has been obtained afterwards to collect mother's additional data to complete genome-wide analyses, to complete extraction of obstetric data, and to carry out four mothers ´s follow-up assessments. In this way, additional ALSPAC mother's data is a source of information about women's reproductive health as well as the impact in mother's future health and their children. Genome-wide data is available on approximately 10.000 mother–offspring pairs, as well as phenotypic data on mothers and their offspring (Fraser et al., 2013).

Additional funding to collect data on fathers, siblings and the next generation (children of the children of the 90s) makes ALSPAC a multigenerational cohort that provides data about intergenerational transmission of health and well-being (Fraser et al., 2013).

3.1.6. Inclusion and exclusion criteria.

The inclusion and exclusion criteria for ALSPAC study were defined as follows (Boyd et al., 2013; Golding et al., 2001).

Inclusion criteria

- Pregnant women resident in Avon, Bristol, while pregnant.
- Women with delivery date expected between 1st April 1991 and 31 December 1992.
- Women who were resident in the area and had completed the questionnaire for the third trimester of pregnancy before leaving the area.

Exclusion criteria

- Pregnant women not resident in the Avon study area.
- Pregnant women who were resident in the study area but left shortly after enrollment.

3.1.7 Recruitment and procedures

A variety of methods were used to enroll pregnant women in the study (Golding et al., 2001), including:

- Posters with the logo of the study inviting interested pregnant women to contact the study team were distributed in strategic places usually visited by pregnant women such as shops, libraries, preschool playgrounds, antenatal clinics.

- The ALSPAC team approached pregnant women when attended for routine ultrasound examinations.

- The Hospital sent information to the pregnant women with their booking information.

- The local community midwives during the first-time interview with pregnant women provided information about the study and a card to send for further information.

- Local and national covered in the press, including radio and television.

- The ALSPAC team approached non-enrolled mothers after birth during maternity hospital visits.

- By sending brochures to the mother explaining the study and confidentiality of the data collected, informing them that the collected data would also be linked to the medical records, unless the mother did not give the permission, and also inform about the signed authorization needed to analyze biological samples.

3.1.8 Main outcome: ADHD traits (Phenotype)

ADHD traits were measured by mother-reported symptom scores of ADHD, using the Development and Well-Being Assessment (DAWBA) interview at age 8 (R. Goodman et al., 2000). DAWBA is a validated instrument and consists of structured parent interviews complemented with teacher questionnaires (R. Goodman et al., 2000). ADHD-traits were calculated based on the sum of the severity rating (0, 1 or 2) for 18 items representing the DSM-IV ADHD symptoms. Range scores for parent's version is 0-36.

3.1.9 Genotype and polygenic risk scores

Genotyping of ALSPAC children was conducted by the Wellcome Trust Sanger Institute, Cambridge, UK and the Laboratory Corporation of America, Burlington, NC, US subcontracted by 23andMe. Genome-wide genotyping was performed using the Illumina HumanHap550 chip and standard quality control included SNP call rate (0.95), subject call rate (0.97), Hardy-Weinberg equilibrium test (P cut-off 5.00E-07), minor allele frequency (0.01), and autosomal heterozygosity (removal of outliers). Imputation was carried out using Impute2 (v2.2.2) and the 1000 Genomes phase 1 v.3 reference panel. Only SNPs with an Impute Information score >0.8 were retained. More details regarding genotyping procedures are provided elsewhere (Riglin et al., 2017). Individual genome-wide genotype data was available for 8941 ALSPAC children.

Polygenic risk scores (PRS) for ADHD were calculated using PRSice2 software (Choi & O'Reilly, 2019) and the latest available GWAS of ADHD (Demontis et al., 2019), which included a total of 20,183 ADHD-cases and 35,191 controls. A total of 4,791,369 SNPs were available in both the GWAS summary statistics and in the individual-level genotyping data from ALSPAC and could be used for calculating PRS. Clumping based on linkage disequilibrium (LD) was applied using the PRSice2 default settings (i.e. an R2-threshold of 0.1 and a bidirectional window of 250 kb). After LD-clumping, a total of 135,489 independent SNPs remained. For inclusion of SNPs into the PRS, 8 'broad' p-value thresholds were considered, i.e. 0.0001, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 and 1, and subsequently used the threshold at which the ADHD PRS showed the strongest association with ADHD traits for the PRS analyses in relation to maltreatment.

3.1.10 Environmental factors

Child maltreatment definition consisted of physical and emotional abuse and malparenting assessed at different time points from birth to 8 years. Data for measure physical abuse was available at 1.6, 1.9, 2.6, 2.9, 3.6, 4.9, 5.1, 5.9, 6.1, 6.9 years, and to assess emotional abuse at 0.8, 1.9, 2.9, 5.1, 6.1 years.

Malparenting was defined as "hitting of", "shouting at", or a "hostile attitude" from parents to child, and data was available at 1.6, 1.9, 2.6, 2.9, 3.6, 3.11, 4.9, 5.9, 6.1, 6.6, 6.9, 7.1, 7.9 years. Maltreatment was constructed by a global measure of physical abuse, emotional abuse and malparenting across the multiple time points.

Binary variables were created based on mother reported assessment indicating presence or absence of physical abuse, emotional abuse, hitting of, shouting at and hostile attitude towards the child across to multiple time points, as well as for the global measures of maltreatment and malparenting.

Maltreatment as mentioned consisted of different aspects of abuse and malparenting, rated by the mother between birth and the age of 8 years. When at any time point one of these forms of abuse occurred and was rated as affecting the child "much" or "moderate", this subtype of abuse was coded as being present. Maladaptive parenting included hitting, shouting or a hostile attitude ("hostility") towards the child. When at any time point one of these forms of malparenting occurred "often" or "sometimes", this subtype of malparenting was coded as being present. If any form of abuse or any form of malparenting was present, maltreatment as an overarching construct was coded as being present. The definitions of maltreatment used were similar to previous ALSPAC studies (Lereya et al. 2015; Ruisch et al. 2019). More details about the data are available through a fully searchable data dictionary of all the ALSPAC-variables (available at http://www.bristol.ac.uk/alspac/researchers/our-data/).

3.1.11 Statistical analyses

To confirm previously reported associations, first was investigated whether ADHD PRS and maltreatment – both as an aggregated construct and the different subtypes separately – would predict ADHD traits. Given the slightly skewed and overdispersed distribution of ADHD traits, a negative binomial regression was used as implemented in the R package "MASS" (Venables & Ripley, 2002; R Core Team, 2018), to perform PRS analyses in relation to this outcome measure.

To investigate to what extent effects of the PRS and/or environmental factors in relation to the ADHD-related traits were mediated by one another, gene-environment mediation analyses was performed. For gene-environment mediation analyses, the R package 'mediation' was used (Tingley et al., 2014).

Because of the small number of children exposed to sexual abuse (N=13 in the total sample with both genotyping and phenotypic data), this subtype of abuse was not considered separately, but only as part of the aggregated constructs of (any type of) abuse and maltreatment. Therefore, a total of 8 maltreatment-related phenotypes was considered. The Bonferroni-corrected alpha for these first analyses was accordingly set to 0.05 / (8 PRS p-value thresholds + 8 maltreatment-related variables) = 3.125E-03.

Subsequently, it was investigated whether ADHD PRS were linked with the aforementioned 8 maltreatment-related phenotypes, representing maltreatment both as aggregated constructs and different subtypes. Given the binary coding of the maltreatment-related variables, logistic regression analysis was used, also performed in R (R Core Team, 2018). As mentioned in the previous section, the PRS p-value threshold at which the PRS was most strongly associated with ADHD traits was used. For these analyses, the Bonferroni-corrected alpha was set to 0.05 / 8 maltreatment-related variables = 6.25e-03.

Subsequently, mediation analysis was performed, using the R package "mediation" (Choi & O'Reilly, 2019), to investigate whether ADHD PRS (using the p-value threshold at which the PRS showed the strongest association with ADHD traits) and maltreatment (as an aggregated construct and separate forms) would mediate each other's effects in relation to ADHD traits. Mediation analysis were only performed when ADHD PRS significantly were linked with maltreatment (for the aggregated construct or separate forms). It was investigated whether (part of) the effect of ADHD PRS in relation to ADHD traits was mediated by maltreatment, and also whether (part of) the effect of maltreatment in relation to ADHD traits was mediated by ADHD PRS. The Bonferroni-corrected alpha was therefore set to 0.05 / (2 * N maltreatment-related variables significantly associated with ADHD PRS). Standard errors for the indirect path (mediation effect) were calculated using 10,000 Monte Carlo draws for quasi-Bayesian approximation.

Included as covariates in all analyses were sex of the child and the first 10 genetic principal components. Furthermore, Veall-Zimmermann Pseudo-R2 estimates were computed using the R-package "DescTools" (R Core Team, 2018).

Sensitivity analyses

It was investigated whether the ADHD PRS at other p-value thresholds, representing partially different sets of SNPs, showed a stronger association with maltreatment (as an aggregated construct and separate forms) to investigate potential underfitting of these analyses.

3.2 Results

Descriptive statistics

A total of 8941 subjects were genotyped and from these, data for the 8 maltreatment-related phenotypes was available for at least 3725, but up to 6844 children. Sex distribution was about equal. Descriptive statistics of ALSPAC are provided in Table 3.1.

Table 3.1 Descriptive statistics of ALSPAC

Variable	N total	N (%) or mean (SD)
Physical abuse	4430	602 (13.59%)
Sexual abuse	4624	23 (0.50%)
Emotional abuse	5227	548 (10.48%)
Any abuse	4482	965 (21.53%)
Hitting	4054	1185 (29.23%)
Shouting	4337	430 (9.91%)
Hostility	6844	568 (8.30%)
Any malparenting	4069	1709 (42.00%)
Any maltreatment	3725	2373 (63.70%)
Sex of the child (males)	8795	4505 (51.22%)
ADHD traits (DAWBA)	6061	4.88 (6.76), range 0-36

ADHD PRS and maltreatment in relation to ADHD traits

ADHD PRS significantly predicted ADHD-traits across all the 8 p-value thresholds, except 0.0001. When more SNPs were added to the PRS, the strength of association with ADHD traits increased up to p-value threshold 0.2 (58323 LD-clumped SNPs, P=6.47E-13, pseudo-R2=0.94%, N=6061). Therefore, this p-value threshold was used for subsequent analyses. Adding SNPs to the PRS beyond a p-value threshold 0.2 resulted in slightly less or a similar model fit and strength of association. Figure 1 (a) and Table 3.2 provide an overview of results.

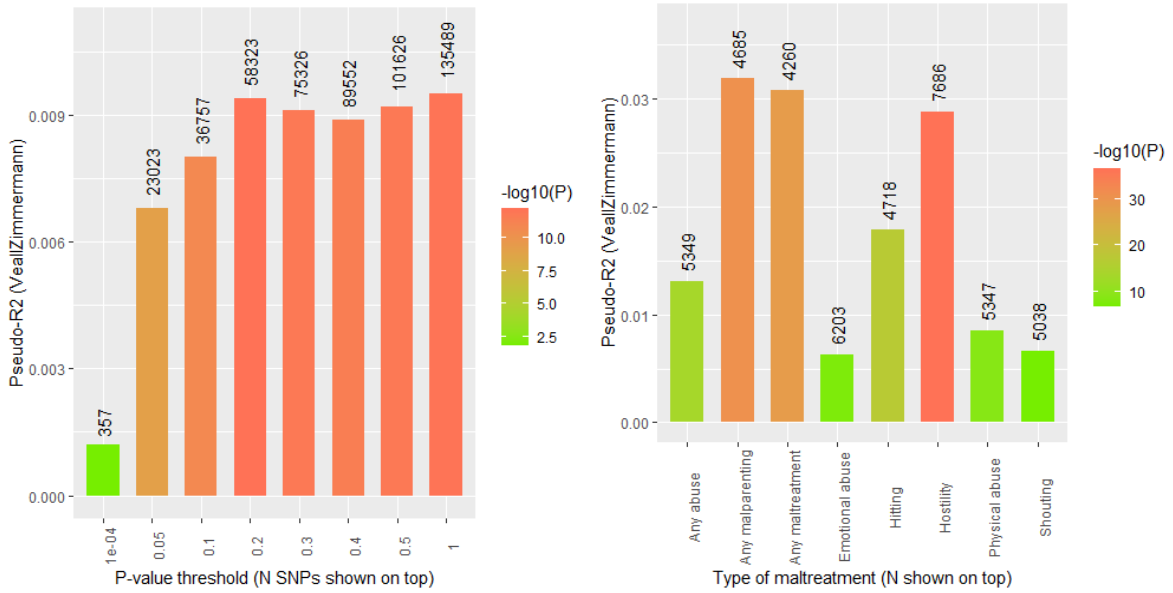
Both maltreatment as an aggregated construct (pseudo-R2=3.08%, P=2.80E-29, N=4260) and all separate subtypes of maltreatment predicted ADHD traits. From the subtypes of maltreatment, the strongest association was between hostility and ADHD traits (P=2.51e-37, pseudo-R2=2.88%, N=7686). Figure 1 (b) and Table 3.2 provide an overview of results.

Table 3.2 ADHD PRS and maltreatment in relation to ADHD traits (mother rated at age 8 years).

Predictor	Outcome	N	B (SE)	P	Pseudo-R2
ADHD PRS (N SNPs)					
PT 0.0001 (357 SNPs)	ADHD traits (8 year)	6061	37.23 (15.15)	1.40E-02	0.12%
PT 0.05 (23023 SNPs)		6061	1127.39 (184.15)	**9.23E-10**	0.68%
PT 0.1 (36757 SNPs)		6061	1683.43 (250.29)	**1.75E-11**	0.80%
PT 0.2 (58323 SNPs)		6061	2503.75 (348.21)	**6.47E-13**	0.94%
PT 0.3 (75326 SNPs)		6061	2984.51 (422.49)	**1.62E-12**	0.91%
PT 0.4 (89552 SNPs)		6061	3380.16 (485.99)	**3.52E-12**	0.89%
PT 0.5 (101626 SNPs)		6061	3833.85 (542.29)	**1.55E-12**	0.92%
PT 1 (135489 SNPs)		6061	5088.34 (710.72)	**8.10E-13**	0.95%
Maltreatment					
Physical abuse	ADHD traits (8 year)	5347	0.40 (0.07)	**1.23E-09**	0.85%
Emotional abuse		6203	0.38 (0.07)	**1.87E-08**	0.63%
Any abuse		5349	0.41 (0.05)	**2.27E-14**	1.31%
Hitting		4718	0.39 (0.05)	**8.37E-18**	1.79%
Shouting		5038	0.36 (0.07)	**1.49E-07**	0.66%
Hostility		7686	0.84 (0.07)	**2.51E-37**	2.88%
Any malparenting		4685	0.49 (0.04)	**1.60E-31**	3.19%
Any maltreatment		4260	0.51 (0.04)	**2.80E-29**	3.08%

All analyses were ran as negative binomial regression and were adjusted for sex (ADHD PRS, maltreatment) and the first 10 genetic principal components (ADHD PRS).

Figure 1 ADHD PRS (left) and maltreatment (right) in relation to ADHD traits (mother rated at age 8 years).



All analyses were ran as negative binomial regression and were adjusted for sex (ADHD PRS, maltreatment) and the first 10 genetic principal components (ADHD PRS).

ADHD PRS in relation to maltreatment

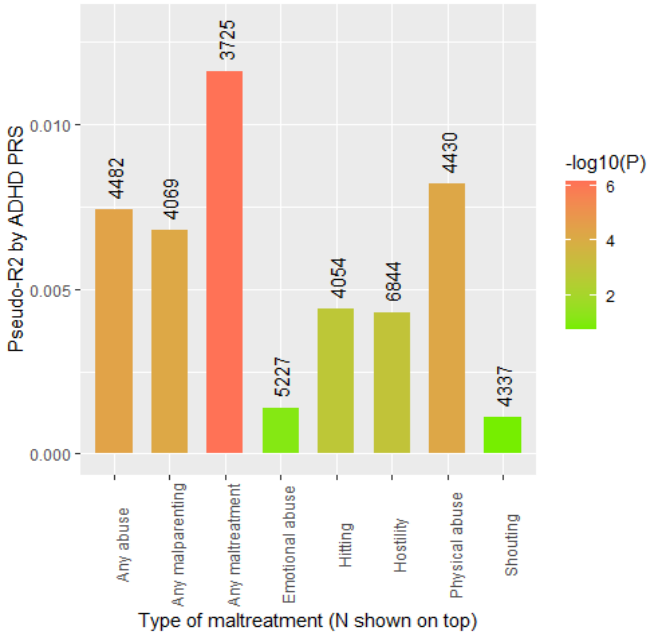
ADHD PRS (at p-value threshold 0.2) were linked to maltreatment as an aggregated construct (P=6.30E-07, pseudo-R^2=1.16%, N=3725) and to multiple of the subtypes of maltreatment (physical abuse, hitting, hostility, and the aggregated constructs of any abuse and any malparenting). Table 3.3 and Figure 2 provide an overview of results.

Table 3.3 ADHD PRS (p- value threshold 0.2) in relation to different types of maltreatment (mother reported up to age 8 years).

Predictor	Outcome	B (SE)	N	P	Pesudo-R2
ADHD PRS (PT 0.2)	Physical abuse	3097.88 (769.07)	4430	**5.62E-05**	0.82%
	Emotional abuse	1360.34 (786.43)	5227	8.37E-02	0.14%
	Any abuse	2622.05 (635.06)	4482	**3.65E-05**	0.74%
	Hitting	1903.62 (603.18)	4054	**1.60E-03**	0.44%
	Shouting	1181.13 (879.26)	4337	1.79E-01	0.11%
	Hostility	2498.79 (762.14)	6844	**1.04E-03**	0.43%
	Any malparenting	2225.75 (554.33)	4069	**5.94E-05**	0.68%
	Any maltreatment	2975.84 (597.33)	3725	**6.30E-07**	1.16%

All analyses were ran as negative binomial regression and were adjusted for sex and the first 10 genetic principal components.

Figure 2 ADHD PRS (p value threshold 0.2) in relation to different types of maltreatment (mother reported up to age 8 years).



All analyses were ran as negative binomial regression and were adjusted for sex and the first 10 genetic principal components.

Mediation among ADHD PRS and maltreatment in relation to ADHD traits

Because both maltreatment as an aggregated construct and five subtypes of maltreatment were significantly linked with ADHD PRS, a mediation analysis was performed using these 6 phenotypes. Because both mediation effects of maltreatment were investigated in the relation between ADHD PRS and ADHD traits and mediation effects of ADHD PRS in the relation between maltreatment and ADHD traits, the Bonferroni-corrected significance threshold was set to $0.05 / (6 * 2) \approx 4.167E-03$.

Multiple significant mediation effects among ADHD PRS and maltreatment were observed. The effect of maltreatment as an aggregated construct in relation to ADHD traits was significantly partially mediated by ADHD PRS (mediation P<2.00E-16, 4.18% of the total effect). The effect of hitting in relation to ADHD traits was also significantly partially mediated by ADHD PRS (mediation P=1.80E-03, 3.84% of the total effect). The effect of hostility in relation to ADHD traits was also partially mediated by ADHD PRS (mediation P=6.00E-04, 2.79% of the total effect). The effect of malparenting in relation to ADHD traits was also significantly partially mediated by ADHD PRS (mediation P<2.00E-16, 3.48% of total effect). Table 3.4 provides an overview of results.

Table 3.4 Mediation analysis of ADHD PRS (P value threshold 0.2) and maltreatment aggregated construct and subtypes in relation to ADHD traits.

Predictor	Mediator	Outcome	N	Estimate (proportion)	Mediation P
Physical abuse	**ADHD PRS (PT 0.2)**	ADHD traits	4149	0.11 (4.81%)	1.20E-02
Any abuse			4140	0.09 (3.75%)	1.70E-02
Hitting			3585	0.10 (3.84%)	**1.80E-03**
Hostility			5765	0.17 (2.79%)	**6.00E-04**
Any malparenting			3575	0.11 (3.48%)	**< 2.00E-16**
Any maltreatment			3255	0.12 (4.18%)	**< 2.00E-16**
ADHD PRS (PT 0.2)	**Physical abuse**	ADHD traits	4149	0.03 (3.55%)	1.50E-01
	Any abuse		4140	0.04 (3.97%)	1.70E-01
	Hitting		3585	0.07 (7.44%)	6.50E-02
	Hostility		5765	0.07 (8.91%)	5.90E-02
	Any malparenting		3575	0.10 (10.72%)	2.60E-02
	Any maltreatment		3255	0.10 (9.57%)	2.10E-02

All analyses were ran as negative binomial regression and were adjusted for sex and the first 10 genetic principal components.

Sensitivity analysis investigating other p-value thresholds in the relation between ADHD PRS and maltreatment

While investigating other p-value thresholds in the relation between ADHD PRS and maltreatment (as an aggregated construct and different subtypes), the strength of association increased (slightly) at a p-value threshold of 0.1 (for ADHD PRS in relation to hitting, hostility, any malparenting and any maltreatment). Table 3.5 provides an overview of results.

Table 3.5 Sensitivity analysis investigating other p-value thresholds in the relation between ADHD PRS and maltreatment

ADHD PRS	Outcome	B	SE	N	P	Pseudo-R2
PT 0.0001 (357 SNPs)	Physical abuse	24.61	33.23	4430	4.59E-01	0.03%
PT 0.05 (23023 SNPs)		1260.09	405.35	4430	1.88E-03	0.49%
PT 0.1 (36757 SNPs)		1969.51	550.00	4430	3.42E-04	0.65%
PT 0.2 (58323 SNPs)		3097.88	769.07	4430	5.62E-05	0.82%
PT 0.3 (75326 SNPs)		3378.50	931.87	4430	2.88E-04	0.66%
PT 0.4 (89552 SNPs)		3756.61	1069.71	4430	4.45E-04	0.62%
PT 0.5 (101626 SNPs)		4386.21	1195.02	4430	2.42E-04	0.68%
PT 1 (135489 SNPs)		5531.29	1569.53	4430	4.25E-04	0.63%
PT 0.0001 (357 SNPs)	Emotional abuse	-3.98	34.03	5227	9.07E-01	0.00%
PT 0.05 (23023 SNPs)		733.15	416.07	5227	7.81E-02	0.15%
PT 0.1 (36757 SNPs)		869.38	564.10	5227	1.23E-01	0.11%
PT 0.2 (58323 SNPs)		1360.34	786.43	5227	8.37E-02	0.14%
PT 0.3 (75326 SNPs)		1458.97	953.88	5227	1.26E-01	0.11%
PT 0.4 (89552 SNPs)		1893.32	1097.30	5227	8.44E-02	0.14%
PT 0.5 (101626 SNPs)		2179.63	1225.91	5227	7.54E-02	0.15%
PT 1 (135489 SNPs)		3163.92	1608.60	5227	4.92E-02	0.18%
PT 0.0001 (357 SNPs)	Any abuse	24.99	27.55	4482	3.64E-01	0.04%
PT 0.05 (23023 SNPs)		1224.32	335.66	4482	2.65E-04	0.58%
PT 0.1 (36757 SNPs)		1668.60	454.55	4482	2.42E-04	0.58%
PT 0.2 (58323 SNPs)		2622.05	635.06	4482	3.65E-05	0.74%
PT 0.3 (75326 SNPs)		2875.31	770.28	4482	1.89E-04	0.60%
PT 0.4 (89552 SNPs)		3363.94	884.95	4482	1.44E-04	0.63%
PT 0.5 (101626 SNPs)		3897.70	989.05	4482	8.12E-05	0.67%
PT 1 (135489 SNPs)		5170.64	1298.77	4482	6.86E-05	0.69%
PT 0.0001 (357 SNPs)	Hitting	26.88	26.30	4054	3.07E-01	0.05%
PT 0.05 (23023 SNPs)		717.27	318.03	4054	2.41E-02	0.22%
PT 0.1 (36757 SNPs)		1414.19	431.44	4054	1.05E-03	0.47%
PT 0.2 (58323 SNPs)		1903.62	603.18	4054	1.60E-03	0.44%
PT 0.3 (75326 SNPs)		2134.37	732.10	4054	3.55E-03	0.38%
PT 0.4 (89552 SNPs)		2173.32	842.12	4054	9.86E-03	0.29%
PT 0.5 (101626 SNPs)		2599.91	939.76	4054	5.66E-03	0.34%
PT 1 (135489 SNPs)		3297.82	1230.52	4054	7.36E-03	0.32%

PT 0.0001 (357 SNPs)	Shouting	20.00	38.57	4337	6.04E-01	0.02%
PT 0.05 (23023 SNPs)		503.09	465.19	4337	2.79E-01	0.07%
PT 0.1 (36757 SNPs)		1046.73	630.38	4337	9.68E-02	0.16%
PT 0.2 (58323 SNPs)		1181.13	879.26	4337	1.79E-01	0.11%
PT 0.3 (75326 SNPs)		1364.99	1067.99	4337	2.01E-01	0.10%
PT 0.4 (89552 SNPs)		1556.66	1230.02	4337	2.06E-01	0.09%
PT 0.5 (101626 SNPs)		2064.49	1374.68	4337	1.33E-01	0.13%
PT 1 (135489 SNPs)		2611.17	1799.49	4337	1.47E-01	0.12%
PT 0.0001 (357 SNPs)	Hostility	5.91	33.08	6844	8.58E-01	0.00%
PT 0.05 (23023 SNPs)		1390.99	402.77	6844	5.53E-04	0.48%
PT 0.1 (36757 SNPs)		2084.96	549.23	6844	1.47E-04	0.58%
PT 0.2 (58323 SNPs)		2498.79	762.14	6844	1.04E-03	0.43%
PT 0.3 (75326 SNPs)		3047.83	925.38	6844	9.89E-04	0.43%
PT 0.4 (89552 SNPs)		3245.75	1064.27	6844	2.29E-03	0.37%
PT 0.5 (101626 SNPs)		3714.65	1187.49	6844	1.76E-03	0.39%
PT 1 (135489 SNPs)		4787.05	1556.13	6844	2.10E-03	0.38%
PT 0.0001 (357 SNPs)	Any malparenting	36.93	24.14	4069	1.26E-01	0.10%
PT 0.05 (23023 SNPs)		986.85	291.79	4069	7.19E-04	0.48%
PT 0.1 (36757 SNPs)		1693.02	396.89	4069	1.99E-05	0.77%
PT 0.2 (58323 SNPs)		2225.75	554.33	4069	5.94E-05	0.68%
PT 0.3 (75326 SNPs)		2541.90	672.87	4069	1.58E-04	0.60%
PT 0.4 (89552 SNPs)		2700.78	774.42	4069	4.88E-04	0.51%
PT 0.5 (101626 SNPs)		3196.89	864.99	4069	2.19E-04	0.58%
PT 1 (135489 SNPs)		4041.70	1133.15	4069	3.61E-04	0.54%
PT 0.0001 (357 SNPs)	Any maltreatment	51.46	26.04	3725	4.81E-02	0.18%
PT 0.05 (23023 SNPs)		1457.94	315.18	3725	3.73E-06	1.00%
PT 0.1 (36757 SNPs)		2159.88	428.22	3725	4.56E-07	1.19%
PT 0.2 (58323 SNPs)		2975.84	597.33	3725	6.30E-07	1.16%
PT 0.3 (75326 SNPs)		3421.70	724.64	3725	2.34E-06	1.04%
PT 0.4 (89552 SNPs)		3792.83	834.47	3725	5.49E-06	0.97%
PT 0.5 (101626 SNPs)		4488.44	933.30	3725	1.52E-06	1.08%
PT 1 (135489 SNPs)		5947.37	1225.30	3725	1.21E-06	1.10%

3.3 Discussion

The aim of the present study was to investigate whether polygenetic risk scores (PRS) for ADHD were linked to maltreatment and different forms during childhood, and whether genetic liability for ADHD and maltreatment mediate each other's relation with ADHD traits. PRS was created based on the latest available GWAS of ADHD (Demontis et al., 2019) and tested for different forms of gene-environment interplay using the individual-level genome-wide genotype and phenotypic data from the well-powered ALSPAC cohort. Most importantly, it was found that ADHD PRS are linked with different forms of maltreatment, including physical abuse and hostility between the mother and child.

First, it was confirmed previously reported associations of ADHD PRS and maltreatment with ADHD traits in the ALSPAC children (Craig, Bondi, O'Donnell, Pepler, & Weiss, 2020; Taylor et al., 2019; Tistarelli, Fagnani, Troianiello, Stazi, & Adriani, 2020). Genetic sharing between ADHD as a clinical disorder and ADHD traits in the population was confirmed and also was investigated the link between different subtypes of maltreatment and ADHD in more detail. Although all of the 8 (more or less overlapping) maltreatment-related phenotypes showed a strong association with ADHD traits in the sample, the most strong association was found for hostility between the mother and child. Malparenting (i.e. hitting or shouting or hostility) showed the largest effect size in relation to ADHD traits, although the effect of maltreatment as an aggregated construct (i.e. any type of malparenting or abuse) was similar. Emotional abuse and shouting at the child by the mother showed the smallest effects. These results point to differences between several aspects of child maltreatment in relation to ADHD, which can be of both scientific and clinical value when evaluating children with ADHD. However, although robust associations of maltreatment with ADHD (and other psychopathology) have been reported, possible confounding due to genetic factors remains a key issue to be addressed (Tistarelli et al., 2020).

The analysis showed that there are clear (positive) gene-environment correlations involving genetic variation related to ADHD and different forms of maltreatment. Another way of describing these results is that ADHD and maltreatment share part of their genetic background. Similar to ADHD, maltreatment itself may represent a complex genetic phenotype, and a certain

proportion of SNPs that contribute to genetic liability for ADHD also contribute to genetic liability for maltreatment. Indeed, maltreatment could represent a heritable trait that is passed on from parents to children, only manifesting itself as (a vulnerability for) externalizing or behavioral problems during childhood. Evidence for maltreatment as a distinct (phenotypic) trait across major forms of psychopathology has been postulated previously (Teicher & Samson, 2013). Such a trait could be related to altered neurodevelopment, as suggested by a twin study that showed that a reported link between maltreatment and multiple neurodevelopmental disorders was explained mostly by common genetic factors among twins (Dinkler et al., 2017). As such, the link between maltreatment and ADHD PRS may also be mediated by (undiagnosed) ADHD of the parents. Improvement of ADHD treatment for (biological) parents of maltreated children may therefore also prove beneficial for the children themselves (Dinkler et al., 2017). Further, while ADHD PRS and ADHD traits represent a (genetic liability for a) behavioral phenotype of the child itself, maltreatment and its different subtypes represent an external environment to the child. One can raise the questions whether or not this environment is to a certain degree also evoked by the behavior of the child and, hence, its genetic liability for ADHD. In other words, currently observed gene-environment correlations could have an evocative nature (Knafo & Jaffee, 2013). In this case, optimizing ADHD treatment for the child, would also contribute to decreasing the likelihood of being exposed to maltreatment. Summarizing, genetic liability for ADHD may be linked to maltreatment for different underlying reasons, however, the clear gene-environment correlation may implicate that optimizing treatment for ADHD for both children and parents may prove beneficial for the maltreated child.

Furthermore, by investigating the relation between ADHD PRS and the different subtypes of maltreatment, it was gained more detailed insights into the gene-environment correlation involving maltreatment as an overarching construct. Although all of the subtypes of maltreatment were linked with ADHD traits, not all of these were linked with ADHD PRS, suggesting that genetics contributes differently to the relation of these types of maltreatment with ADHD. The strongest gene-environment correlations were observed for physical abuse and malparenting (any type, i.e. hitting, shouting or hostility between the mother and child). Interestingly, a previous family study also already pointed to the significant role of genetic contributions to hostile parent-child interactions (even involving both the father and the mother) (Lifford et al., 2009). Considering that such hostility also appeared to show the strongest association with ADHD traits, this may suggest that this component of maltreatment plays a

particular role in ADHD, and also is subject to genetic sharing with ADHD. In contrast, emotional abuse and shouting were not related to ADHD PRS, pointing to the possibly more environmentally driven nature of these forms of maltreatment.

It was found that the relation between maltreatment and ADHD traits was partially mediated by ADHD PRS. More concretely, a small but significant part of the link between maltreatment and ADHD is mediated by genetic liability for ADHD. The relation between ADHD PRS and ADHD traits was not significantly mediated by maltreatment. This suggests that "confounding" of the link between maltreatment and ADHD traits by genetics is more pronounced than such effects the other way around. However, a similar pattern in the data could be observed, with malparenting and maltreatment as aggregated constructs reaching nominal significance. This may indicate that some part of the effect of genetic liability for ADHD could be mediated by maltreatment.

Chapter 4

4. General Discussion

4.1 Discussion

In chapter 2, the aim of the study was to identify environmental risk factors in ADHD and ASD population, both samples were patients of the Child and Adolescent Neuropsychiatric Unit at the "Antonio Cao" Hospital, Cagliari, and were compared with a control sample of children and adolescents without disorders, patients of Pediatric Units at the "San Michele" Hospital, Cagliari.

In this study, several environmental factors were explored retrospectively through a questionnaire, and statistical analysis was conducted to identify association between the environmental factors and ADHD and ASD. The results suggested association between ADHD and some environmental factors in early age such as cesarean delivery, and formula feeding. Furthermore, other postnatally factors were associated as well with an increased risk of ADHD including stressful life events, children consumption of sugar and colors, emotional aggression, and bullying; other factors related to mothers were also identified as risk factors such as hostility, and inconsistent discipline. In ASD sample, the analysis showed no association between most of the environmental factors, however, an association was found between ASD and immune mediated pathologies in family in first and second degree-relatives, as well as was found in ADHD sample.

To answer the question regarding common risk factors between the disorders, these results suggested no common risk factors between ADHD and ASD, however, an association was found between the disorders and immune mediated pathologies in family. ADHD and ASD despite being neurodevelopmental disorders involving genetic and environmental factors, as well as being frequently comorbid, no evidence of common environmental risk factors was found between ADHD and ASD in this study.

These findings suggest different effects on exposure to the environmental factors in these disorders. The results suggested a relation between exposure to some environmental factors and ADHD, which is consistent with some findings in other studies, but no association was found between environmental exposure and ASD, suggesting mainly a genetic effect in this sample.

In chapter 3, the aim of the study was to investigate whether genetic risk for ADHD is linked to maltreatment and whether genetic liability and maltreatment mediate each other's effects in relation to ADHD traits.

In this study, a sample of children of the ALSPAC prospective birth cohort study was used. The Development and Well-Being Assessment (DAWBA) was used for ADHD traits, and a maltreatment construct was created based on exposure of the child to malparenting, physical and emotional abuse, and hostility between mother and child; genetic data of the children was analysed to calculate the ADHD polygenetic risk scores.

The gene-environment correlation (rGE) analysis was conducted to investigate whether genetic risk for ADHD was linked to maltreatment. The analysis showed that ADHD-PRS predicted ADHD traits, maltreatment and their different forms including: malparenting, hostility between mother and child, and physical abuse.

The gene-environment correlation analysis suggested genetic influences in environmental exposure in maltreatment, malparenting, hostility between mother and child, and in physical abuse. The genetic liability for ADHD is linked with different aspects of maltreatment (Dinkler et al., 2017; Jaffee & Price, 2007). The mediation analysis was carried out to investigate whether genetic liability and maltreatment mediate each other's effects in relation to ADHD traits. The relation between ADHD PRS and ADHD traits was not significantly mediated by maltreatment.

Genes affect environments indirectly, via behavior and personality characteristics (Jaffee & Price, 2007). A genetic liability of the child was predicted in the genetic correlation analysis; however, a genetic liability of the parents may be associated with child maltreatment. Gene-environment correlation refers as well to the association between an individual's genetically influenced behavior and the reaction of those in the individual's environment to that behavior. (Jaffee & Price, 2007).

Psychological abuse was associated with an increased risk of ADHD in the environmental study, but no association was found between emotional abuse and polygenetic risk scores in relation to ADHD traits. These findings suggest an important environmental role of psychological aggression related to ADHD.

Maternal hostility was related to ADHD in the environmental study, and parent's hostility in the gene-environment study was associated with polygenetic risk scores. Therefore, hostility in parents seems to have a genetic base, however, a gene-environment study needs to be conducted with the environmental sample to amplify the results. Maternal hostility linked to ADHD as an environmental risk factor, may be also explained by genetic, or by a contribution of both genetic and environmental factors (Lifford et al., 2009).

Genetic and environmental factors are involved in the etiology of neurodevelopmental disorders. The results in the study showed some environmental factors that are associated with an increased risk of ADHD, however, a heritability association between parental ADHD and ADHD in offspring was not found in the environmental study.

Immune mediated pathologies in first and second degree-relatives were also related with ADHD and ASD samples in the environmental study. However, more studies need to be conducted to figure out the mechanism of this factor.

ADHD polygenetic risk scores were correlated with maltreatment regarding ADHD traits in the gene-environment study, suggesting genetic influences in environmental exposure. More gene-environment studies need to be carried out with parents of children with ADHD to measure the genetic contribution related to maltreatment in ADHD population.

4.2 Limitations

- Parental scales missing in the case control study, specially fathers' scales.

- The limitation of missing genetic data in the case control study which could provide a more complete analysis involving genetic and environmental factors and their relation with ADHD and ASD.

- Lack of an accurate ASD assessment to conduct genetic and environmental analysis in ASD population in ALSPAC study.

4.3 Conclusion

Genetic and environmental factors have been linked to the etiology of neurodevelopmental disorders. This thesis investigated environmental risk factors in ADHD and ASD in Italian samples to identify risk factors in each group and between the groups. The analysis identified mainly postnatal risk factors related to ADHD sample and no common factors were found between the groups, except for the immune mediated pathologies in first and second degree-relatives that were related with both ADHD and ASD samples.
Further studies must be conducted for comparison of additional samples of patients suffering from ADHD as well as from ASD with high and low functioning, including in the analysis both environment and genetic factors.

In addition, the other aim of this thesis was to investigate a genetic correlation between polygenetic risk scores and maltreatment and different forms of abuse in relation to ADHD traits using a birth cohort study of a British sample. The analysis conducted showed the important contribution of polygenetic risk scores in relation to maltreatment in ADHD traits. The vulnerability of children with ADHD traits to be exposure to maltreatment seems to be related to genetic behavioral characteristics of the disorder as well as genetic behavioral characteristics of parents.
These findings suggest that further genetics studies about maltreatment in children with ADHD need to be conducted in parents of children to get a better understanding of this complex system.

The studies conducted for this thesis suggested an association between environmental factors and an increased risk of ADHD, and a genetic liability of ADHD polygenetic risk scores in relation to ADHD traits and maltreatment. These findings extend the knowledge about the relation between environmental risk factors and genetic liability in the ADHD population.

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