Genetic and Environmental Contributions to Indiscriminate Social Behavior in Institutionalized Children: Insights from Williams Syndrome

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25629

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Título do Projeto: Contribuições genéticas e ambientais no comportamento social indiscriminado de crianças institucionalizadas: *insights* da Síndrome de Williams.

Resumo: O comportamento social indiscriminado (ISB) é uma das manifestações comportamentais mais pervasivas que emergem na literatura sobre institucionalização. Existem, porém, diferenças individuais quanto à manifestação do comportamento social nas crianças institucionalizadas, o que aponta para uma possível interação gene x ambiente (GXE). Investigação prévia contribuiu com a identificação de um conjunto de genes comumente associados à psicopatologia e comportamento social nos estudos GXE. Neste estudo, propomos a análise de um gene candidato (GTF2I), localizado no cromossoma 7, que se apresenta deletado na síndrome de Williams (SW). A SW é uma perturbação neurogenética associada a um perfil socio-cognitivo único, nomeadamente um fenótipo de hiper-sociabilidade que se assemelha ao ISB observado em crianças institucionalizadas. Neste estudo participaram 126 crianças institucionalizadas em idade pré-escolar (M=4.10 anos, DP=.95), juntamente com o seu cuidador institucional. O ISB foi avaliado através da *Disturbances of Attachment Interview* e foram recolhidas amostras de saliva das crianças para genotipagem. Os níveis de cooperação e de responsividade sensível do cuidador estavam negativamente associados ao ISB. Verificou-se um efeito GXE, consistente com o modelo de duplo-risco: os genótipos TG e GG emergiram como alelos de risco para o desenvolvimento de ISB, sendo que crianças portadoras destes alelos eram as que apresentavam maiores níveis de ISB quando expostas aos menores níveis de responsividade sensível do seu cuidador. Esta investigação é pioneira na análise dos polimorfismos do gene GTF2I no estudo do ISB em crianças institucionalizadas, permitindo uma melhor compreensão sobre os mecanismos pelos quais algumas crianças institucionalizadas, mas não outras, desenvolvem ISB.

Palavras-Chave: Comportamento Social Indiscriminado; Institucionalização; GXE; Psicopatologia do Desenvolvimento.

Title of Project: Genetic and Environmental Contributions to Indiscriminate Social Behavior in Institutionalized Children: insights from Williams Syndrome.

Abstract: Indiscriminate social behavior (ISB) is the most common of social disturbed behaviors that emerge in institutionalization literature. Nevertheless, individual differences in social outcome in institutionalized children exist, which points to a possible gene x environment interaction (GXE) that may foster the heterogeneity seen in these children. Previous research has contributed with a set of genes commonly associated with psychopathology and social behavior in GXE studies. Here, we extend this research by proposing a new candidate gene (GTF2I), which microdeletion on chromosome 7 is responsible for Williams Syndrome (WS), a neurogenetic condition which main phenotype (hiper-sociability) resembles the ISB seen in institutionalized children. One hundred and twenty-six institutionalized preschoolers (M=4.10 years, SD=.95) participated along with their institutional caregiver. Child ISB was assessed with the *Disturbances of Attachment Interview* and saliva samples were provided for genotyping. Caregiver's level of cooperation and sensitive responsiveness were negatively associated with ISB. A significant GXE effect emerged consistent with the diathesis stress hypothesis: carriers of TG and GG genotype emerged as risk alleles to ISB in these children, with its carriers having the most ISB when exposed to low levels of sensitive responsiveness from their caregivers. These results are the first to include GTF2I gene in the study of ISB in institutionalization and shed new lights into why some institutionalized children, but not others, develop ISB.

Key-words: Indiscriminate Social Behavior; Institutionalization; GTF2I; GXE; Developmental Psychopathology

Index

Tables

Figures

1. Institutionalization

Institutionalization has been classified as an extreme form of social-emotional deprivation (Sheridan, Drury, McLaughlin & Almas, 2010). In many institutions, minimal physical resources, failure to adequately interact socially and emotionally with the child and unfavorable staffing patterns are commonly observed.

The detrimental consequences of institutionalization and early deprivation became clear at the beginning of the century with the Bucharest Early Intervention Project (BEIP) (Zeanah et al., 2003). The BEIP was a randomized controlled trial of foster care as an intervention for the extremely deprived Romanian institutionalized children. The main goal of this BEIP was to document the effects of early deprivation in the development of young children, and to understand the degree of recovery once children were placed in an improved and enriched environment (Zeanah et al., 2003). All 136 children and their caregiving environments (a total of 6 institutions) were subject to a comprehensive baseline assessment (Zeanah et al., 2003). After this assessment, half of the children were randomly assigned to high-quality foster care (FCG), while the other half remained in institutional care (care as usual group, or CAG) (Zeanah et al., 2003). This "natural" experimental design allowed for a better and controlled understanding of the effects of exposure to an environment of deprivation, but also of the beneficial and sometimes remediable effects of high-quality caregiving environment. Therefore, all children participated in follow-up assessments at 30, 42 and 54 months, and also at 8 years of age, and the developmental path of the above-mentioned groups (FCG and CAG) was compared to a group of never-institutionalized children (NIG) (Zeanah et al., 2003).

Several detrimental consequences have been chronicled regarding the effects of institutionalization in the different levels of child development (Zeanah et al., 2003). Among them, a deleterious effect on physical health outcomes, such as short-stature, low-weight, smaller head circumference, and abnormal

neurobiological development have been documented (Bick & Nelson, 2016); regarding cognitive development, there is a general delay, a lower IQ (between 17 to 20 IQ points lower than children raised in their families), school completion difficulties and learning disorders, and also impairments in memory and executive function when compared to biological family-reared children (Carr, Duff, & Craddock, 2018). Institutionalized children are also at higher risk of developing mental health disorders, including difficulties in emotion regulation, higher rates of anxiety, attachment disorders and disturbed social behavior such as social withdrawal or indiscriminate friendliness (Sonuga-Barke et al., 2017).

1.1. Indiscriminate Social Behavior

Evidence from the BEIP shows that two different types of disturbed social behaviors may emerge from the experience of institutional care: (1) inhibited social behavior, characterized by an emotional withdrawal behavior, which resembles a quasi-autistic phenotype, with lack of social approach and emotional reciprocity, and (2) an indiscriminately social/disinhibited behavior (Zeanah & Gleason, 2015). Empirical work has demonstrated that the latter is more frequent than the former (Zeanah Smyke, & Dumitrescu, 2002; Zeanah, 2000), and has greater validity as an independent construct (Oliveira et al., 2012).

Initial studies have linked these social disturbances with the absence or highly compromised attachment relationship of these children with their caregivers (Tizard & Rees, 1975). Indeed, both of these disturbed social behaviors are associated with two related but distinct concepts important in attachment theory (Bowlby, 1969; Ainsworth, 1978) - one of them, the attachment bond, refers to the tendency of the child to discriminate key individuals to whom they express what is called their attachment behavior, the other concept (Fearon, 2018). These attachment behaviors seek contact with a caregiver in order to achieve comfort and support and are observable when a child is anxious (Cassidy, 2008). These behaviors can broadly be characterized as signaling or communicative behaviors (e.g. crying) or as proximity and contact maintenance (e.g. reaching and clinging, respectively) (Fearon, 2018). Some of what are

considered as attachment disorders are linked to the attachment bond (or to its disruption) while difficulties related to attachment, such as insecure attachment - related to the sensitivity and responsiveness of the caregiver to the child needs (Ainsworth, 1978) - are linked to attachment behaviors (Fearon, 2018).

1.2. Attachment and ISB

Considering disinhibited social behavior as an attachment disorder has been vastly debated (see, for example, Zeanah & Gleason, 2015; Lyons-Ruth, 2015), mainly because of data showing the simultaneous presence of indiscriminate behavior and secure attachment (Bos et al., 2011; Rutter et al., 2007; Zeanah & Gleason, 2015; Zeanah, Smyke & Dumitrescu, 2002). For example, Gleason et al. (2011) found out that at 42 months, there was a negative association between secure attachment and signs of disinhibited social behavior in institutionalized children. The authors then dichotomized attachment into typical vs. atypical and showed that the latter had a moderate association with signs of indiscriminate behavior in the same group of children, although there were still some children showing signs of indiscriminate behavior who were classified as having a secure attachment. Similarly, in institutionalized toddlers, Zeanah et al. (2005) were able to find a significant correlation between ratings of attachment towards the institutional caregiver and the inhibited social behavior, but not with the indiscriminate one. Support of this disentanglement between attachment and indiscriminate behavior comes also from adoption studies. Data have shown that although children may exhibit a secure attachment towards their adoptive mother, there is still a small percentage of these children that persistently exhibit indiscriminate behavior (Marcovitch et al., 1997; O'Connor et al., 2003). Also, 47.8% of the Romanian sample adoptees that had marked disinhibited attachment also classified as having a secure attachment (Rutter et al., 2007), which favors the disentangling between attachment and the emergence and persistence of indiscriminate pattern of behavior. Indeed, the inhibited social behavior has been linked to the degree of development of

attachment to the caregiver, while the indiscriminate pattern has not (Zeanah et al., 2005; Corval et al., 2017).

These empirical observations contributed to a distinct classification of inhibited attachment behavior and indiscriminate behavior, in the latest edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V; American Psychiatric Association, 2017). As so, inhibited attachment behavior was classified as Reactive Attachment Disorders (RAD) whereas indiscriminate behavior was classified as Disinhibited Social Engagement Disorder (DSED). This reclassification of the DSED outside the attachment disorders umbrella was grounded on the core features of the disorder, as this was not considered a non-selective attachment behavior, as conceptualized by the International Statistical Classification of Diseases and Related Health Problems, 10th revision (ICD-10; World Health Organization, 2007) but rather an uncalibrated indiscriminate social behavior (Zeanah & Gleason, 2015), justifying why DSED is not conceptualized as an attachment disorder. Given the fact that we will not use such formal diagnosis, from now forward, we will refer to the indiscriminate pattern of behavior as indiscriminate social behavior (ISB). Notwithstanding, the link between attachment and the indiscriminate approach to unfamiliar adults seen in ISB should be considered, at least, as probable (Fearon, 2018), and it is most likely related to the severe break in the continuity of an attachment bond, or the lack of opportunity to establish a selective attachment bond (Rutter, Kreppner, & Sonuga-Barke, 2009). Also, Soares, Belsky, Mesquita, Osório & Sampaio (2013) defend that the establishment of a focused attachment and the quality of the bond that becomes established must be distinguished when considering the etiology and development of ISB.

1.3. Length of Institutionalization, prior and current relational experiences and ISB

Most studies have focused on the contribution of age of admission, length of institutionalization and age at adoption to explain the individual differences in outcomes observed in these children, with less deleterious effects for shorter

periods of institutionalization and more severe and harder to reverse outcomes for those exposed to institutionalization for longer periods of time. For example, Rutter et al. (2007) compared two groups of UK-adoptees, one of Romanian children who experienced harsh and neglectful conditions of institutionalization, and another group of UK adoptees that had not such experience, regarding a measure of parental report of disinhibited attachment. The authors assessed both groups at 4, 6 and 11 years of age and found that disinhibited attachment was greater in persistence in children ranging from 6 to 11 years of age, but there was a reduction over time in the frequency of this pattern among both groups, but especially in the UK adoptees. The strongest predictor of persistence of disinhibited social behavior from age 6 to age 11 was living in institutional rearing before the age of 6 months, with no significant differences being observed beyond that age (Rutter et al., 2007). Furthermore, the decreasing of ISB in children placed in foster care from the BEIP only appears to be significantly lower than the CAUG at 42 months (Bos et al., 2011), and children whose institutional exposure lasted less than 18 months had a smaller rate of disinhibited attachment (16%) than the ones whose institutional care lasted between 24 and 42 months (33%) (Rutter & O'Connor, 2004). Other adoption studies show a linearity between indiscriminate sociability at ages four and six and extension of time in institutionalization (O'Connor, Bredenkamp & Rutter, 1999; O'Connor & Rutter, 2000; O'Connor et al., 2003), as well as persistence of ISB over time and length of institutionalization (Bruce, Tarullo, & Gunnar, 2009; O'Connor & Rutter, 2000; O'Connor et al., 2003; Rutter et al., 2007) and age at adoption (Rutter et al., 2010).

However relevant and important in the institutionalization literature, this timing effect is not always present (Oliveira et al., 2012; Soares et al., 2014; Zeanah et al., 2009), and thereby there has been a focus on other variables that may work as key contributions to the development and maintenance of the negative socio-emotional outcomes of institutionalized children.

Indeed, in the study of Merz and McCall (2010), the authors compared a group of 6 to 18 years old Russian adopted children that had experienced

institutional rearing with adequate physical resources but lacking consistent and responsive caregiving, frequent changes in caregivers and low levels of caregiver-child social interaction, with two other groups of post-institutionalized children: 1) a group of post-institutionalized children from around the world with different levels of deprivation and quality of institutional settings and care (Gunnar et al., 2007), and 2) a group of severely deprived post-institutionalized Romanian children. The study concluded that, not surprisingly, children from severely deprived Romanian institutions were at greater risk of developing various kinds of behavioral problems when compared to the other two groups, but when compared to never-institutionalized children, even the less deprived institutions with good physical resources but with high child to caregiver ratios, had higher rates of attentional and externalizing problems.

The contribution of a stable and individualized care, and the emotional investment from the caregiver appears to be of the utmost importance in the etiopathogeny of ISB. For example, not having a preferred caregiver (adult with whom the child has a preferred affective relationship) predicted ISB, over and above pre-institutionally experiences of Portuguese institutionalized children aged 12- to 30-months-old (Soares et al., 2014). Similarly, being the favorite child of a caregiver seems to be a protective factor to the development and severity of ISB, given that these children exhibit lower levels of this behavior (Smyke, Dumitrescu & Zeanah, 2002). In the same study (Smyke, Dumitrescu & Zeanah, 2002), the authors developed an intervention that had as main goal improving the consistency of the caregivers during the child's waking hours, and found that the "pilot unit" (group of 29 children placed in an experimental unit with lower child to caregiver ratio) showed lower scores in ISB - measured with "*The Disturbances of Attachment Interview*", DAI (Smyke & Zeanah, 1999) - than the standard group (children on the typical unit, with poor psychosocial conditions). These results are congruent with previous research showing that pathological social behavior in institutionalized children are common even in high-quality institutions but where there is a lack of caregiver emotional investment (Tizar & Reeds, 1975; Tizard & Hodges, 1978; Lyons-Ruth et al., 2009).

Notably, more proximal interactive behaviors from the caregiver also seem to associate with children's ISB. Indeed, adoptive mother's sensitivity toward the child, as assessed in a free play activity, was associated with lower ISB in internationally adopted children from both institutions and foster care in China (Van Den Dries, Juffer, van IJzendoorn, Bakermans-Kranenburg, & Alinka, 2012). Similarly, lower sensitivity from the caregiver significantly predicted more ISB among Portuguese institutionalized toddlers (Oliveira, Fearon, Belsky, Fachada & Soares, 2014). Taking these in consideration, the quality of emotional care and the availability of a foreseeable and constant caregiver seems to be of greater importance than general deprivation in the development of ISB.

Although institutional care experience has clear associations with the socioemotional development of institutionalized children, it would be naïve to consider that individual variations on this behavior are only due to these experiences. Indeed, there is evidence showing that non-institutional factors also play a role in the pathological socioemotional development of these children, influencing thereafter the institutional experience per se. For example, Oliveira et al. (2012) found that a prenatal risk composite (i.e., maternal physical disease and maternal substance abuse during pregnancy) predicted higher levels of ISB and that a maternal emotional neglect risk composite (which included maternal emotional unavailability and psychopathology) also mediated the association between prenatal risk and ISB in institutionalized toddlers. Some previous studies support this argument by showing an association between ISB and caregiving risk and maternal psychiatric disorder in children raised in their biological families and foster care (Lyons-Ruth, Bureau, Riley, & Atlas-Corbett, 2009; Zeanah et al., 2004).

Even when taking into consideration all the above-mentioned data, one cannot fully understand the complexity behind the heterogeneity of behavioral outcomes observed in institutionalized children. Neither the consequences observed by the exposition to severe deprivation, nor the recovery after placement in foster care of institutionalized children is uniform (Rutter, Kreppner & O'Connor, 2001; Tottenham, 2012), which raises the possibility of a moderator

role of genetic variability in the effects of severe early deprivation (Bos et al., 2011).

2. Gene and Environment Studies

Taken together, attempting to explain the observed variability of outcomes in children solely in terms of environmental differences does not fully encapsulate the complexity of the child's maltreatment. Therefore, having into consideration the moderating role of genetic variability may help us to better understand how the interaction between gene and environment (GXE) leads to complex variations in phenotypes, including ISB (Bos et al., 2011; Soares, Belsky, Mesquita, Osório & Sampaio, 2013; Caspi & Moffitt, 2006; Ciccheti, 2016).

The classical diathesis-stress model of the development of psychopathology portrays that intrinsic risk characteristics of children such as their biology, genetic and/or temperament will contribute to the development of psychopathology when paired with contextual adversity (e.g., neglect, poverty) (Monrow & Simons, 1991; Zuckerman, 1999). In contrast, the differential susceptibility model in GXE studies has been signalized as a more comprehensive model to the development of complex behavioral and psychological phenotypes, including child psychopathology (Belsky, 1997; Belsky, Bakermans-Kranenburg & van IJzendoorn, 2007; Drury et al., 2012; Cicchetti, 2016). This model argues that an individual's genotype contributes to the differential responsiveness to the environment (Belsky & Pluess, 2009). Instead of "risk" or "resilient" alleles (Belsky et al., 2009), the individual either carries "plasticity alleles" - alleles that enhance outcomes when exposed to positive environments, yet confers greater vulnerability in adverse environments (Belsky, Bakermans-Kranenburg & van IJzendoorn, 2007; Belsky & Pluess, 2009) - or "fixed alleles", which are believed to show little differences in the outcomes in either positive or negative environments (Belsky et al., 2009).

Drury et al. (2012) investigated among children from the BEIP how differences in genotype of the Brain Derived Neurotrophic Factor (BDNF) and the Serotonin Transporter (5-HTTLPR) polymorphisms interacted with the

development and levels of ISB in different rearing environments. The results supported the differential susceptibility model: children carrying the short allele of the 5-HTTLPR genotype or the met allele of the BDNF genotype showed the lowest levels of indiscriminate behavior when placed in foster care and the highest levels when in the CAUG. For the other genotype (long allele of the 5-HTTLPR or the val/val genotype of the BDNF), the levels of ISB showed little alterations over time and no group and genotype interaction, suggesting that these alleles served as "fixed alleles". For the children with both the short allele of the 5-HTTLPR genotype and the met allele of the BDNF genotype (both "plasticity alleles"), the levels of indiscriminate behavior at 54 months were the greatest of all if placed in the CAUG, but the smallest when randomly placed to the FCG. This last result also supports the "cumulative genetic plasticity" prediction of the differential susceptibility model, that pose that having more than one plasticity allele augments the responsiveness to the environment (Belsky et al., 2009). The authors also found a time effect in regards to the genetic sensitivity to change of environment. The decline seen in the ISB was different between genotypes and was greater as the time experienced in a specific environment accumulated (Drury et al., 2012). Similarly, Bakermans-Kranenburg, Dobrova-Krol & van IJzendoorn (2011), in a study with both institutionally and family reared Ukrainian children (N=37) explored how different genotypes influenced the response to the difference in environments in terms of attachment security and ISB. The authors found greater attachment disorganization and lower levels of attachment security in children with the *ss* (short) or *s/* (long) genotype of the long variant of the serotonin receptor gene (5-HTTLPR), in those raised in an institution. On the other hand, homozygosity for the long allele was a protective factor against the adverse effects of institutional experience on attachment.

Although presenting differences in methodologies (i.e. sample size and experimental setting), Mesquita et al. (2015) searched for the genetic and environmental interplay between the same genotypes used in Drury et al. (2012) and ISB in a Portuguese sample of children reared in institutions and children raised in their biological families. The results showed no significant findings for

the BDNF gene, but children who were homozygous for the short 5-HTTLPR allele had the most ISB when1 reared in institution but not the least when family reared (Mesquita et al., 2015), which favors the diathesis-stress model rather than the differential susceptibility hypothesis of the relationship between ISB and 5-HTTLPR and BDNF polymorphisms seen in Drury et al. (2012).

Despite this, finding a single cause or gene to which the emergence of ISB can be attributed is highly unlikely. Having a multilevel and interdisciplinary perspective can be beneficial in looking for a broader comprehension of the etiopathogeny of ISB. Therefore, taking the developmental psychopathology perspective, studying atypical development is an informative natural model that may elucidate normal developmental mechanism, including social behavior. Considering the similarities in behavior that institutionalized children with ISB and children with Williams Syndrome share, the latter may serve as a genetic model in guiding the research of genetic contributions to the emerge of ISB (Soares, Belsky, Mesquita, Osório & Sampaio, 2013; Sampaio et al., 2017).

3. Williams Syndrome as a Model of Atypical Social Behavior

Williams Syndrome (WS) is a rare genetic disease characterized by a hemizygous deletion of 26-28 genes at the region 7q11.23 in the chromosome 7 (Korenberg et al., 2000). The region encompassing these genes is known as WS chromosome region (WSCR), and its deletion is common to ~95% of subjects (Korenberg et al., 2000). It is estimated to be about 1.6 megabases, and there are also other rare types of deletions within this region (Korenberg et al., 2000). The prevalence ranges between 1 in 7,500 (Strømme, Bjømstad & Ramstad, 2002) and 1 in 10,000 births (Pober, 2010).

This neurodevelopmental disorder has well-known features such as an elfin-shaped face, cardiovascular problems (particularly characteristic is a supravalvular aortic stenosis), transient infantile hypercalcemia, developmental and cognitive difficulties, including delayed language acquisition and motor development, visuo-spatial impairments, as well as lower IQ and physical

development, and a striking socio-emotional profile (Bellugi, Lichtenbergerm Jones, Lai, & St. George, 2000). Indeed, it is the Williams Syndrome's phenotypes of enhanced empathy, drive for social interactions, overly friendly and indiscriminate social approachability that makes the individuals with WS to be known as hypersociable (Bellugi, Lichtenbergerm Jones, Lai, & St. George, 2000; Capitão et al., 2011).

3.1. Hypersociability in Williams Syndrome

From the initial descriptions of this syndrome, patients were already described as having "outstanding loquacity and a great ability to establish interpersonal contacts" (von Armin & Engel (1964), p.376). Beuren, Apitz & Harmjanz (1962) also reported that WS patients "love everyone, are loved by everyone, and are very charming" (p.1235). Several hypotheses have emerged to explain this hypersocial behavior. One of them lies in the attentional bias that these individuals show towards any kind of social stimuli, especially human faces (Plesa Skwerer et al., 2011). In an eye-tracking study, Riby & Hancock (2009) showed how individuals with WS and individuals with Autism Spectrum Disorder fixated human and cartoon faces, compared to healthy and matched control group for age and non-verbal ability. The results showed that children with WS had considerably greater fixation in either human or cartoon faces than the other group (Riby & Hancock, 2009). In a pioneer study, Mervis et al. (2003) compared infants and toddlers with WS with matched children regarding age and mental age, and with typically developing children, regarding their gaze to either the infant's mother or a stranger. The individuals with WS spent more than twice as long gazing at their mothers when compared to the normally developed children. In the stranger session, WS children spent twice as much looking at the stranger when compared to the normally developed children, of which 78% was coded as intense gazing (Mervis et al., 2003), which goes in line with the phenotypic approachability and drive towards strangers and non-familiar people of WS (Järvinen, Korenberg & Bellugi, 2013).

Together with these cognitive-behavioral hypotheses, some authors have also proposed that this aspect of WS behavior is genetically driven. In fact, some animal models, partial deletion case studies and genetic association studies have proposed some candidate genes in the WSCR that may help to explain social variability in both typical development and WS (Berg et al., 2007; Van der Aa et al. 2009; Sakurai et al., 2010; Malenfant et al., 2011; Mervis et al., 2012). In fact, the role of chromosome 7 in social behavior is widely documented as the duplication of WSCR results in a genetic disease known as dup7q11.23 (Pober, 2010), characterized by impairments in verbal expression, delays in development, separation anxiety and some autistic-like traits, all phenotypic traits that differ from WS (Malenfant et al., 2011; Mervis et al., 2012).

3.2. The Neurogenetics of Hypersociability in WS

While it is known that the congenital pathological cardiovascular disease of WS is due to the loss of the elastin (ELN) allele (Ewart, Jin, Atkinson, Morris & Keating, 1994), less is known about the phenotypic consequences coming from the loss of other alleles, especially regarding social behavior (Pober, 2010).

Given the phenotypic similarities between WS children and Institutionalized children with ISB, studying the former may help us comprehend the contribution that genetics may have in the enduring ISB in children who do not have a diagnosis of WS. Soares, Belsky, Mesquita, Osório & Sampaio (2013) proposed that genetic and environmental factors may be necessary for the development and maintenance of ISB, above all impact of timing and low-quality care that these children are exposed to. Indeed, the authors argue that the combining effects of early deprivation experience and carrying specific Single Nucleotide Polymorphisms (SNP) within the WSCR may contribute for the manifestation of ISB. These SNP are the most common type of DNA mutation, involving a single nucleotide, which can produce changes in an amino acid sequence. SNPs have been shown to be involved in vulnerabilities to different conditions, including the ones involving the central nervous system (Allen-Brady et al., 2009; Harold et al., 2009 in Soares, Belsky, Mesquita, Osório & Sampaio,

2013; Sampaio et al., 2018). Variations within these SNPs may modulate, to some extent, the degree of impact of early life experiences and consequent differences in behavioral outcome. In particular, GTF2I gene (a gene within the region 7q11.23 in chromosome 7) has emerged as one of the most relevant for the social and cognitive phenotypes of WS (Chailangkarn, Noree & Muotri, 2018).

3.3. GTF2I as Candidate Gene

Animal studies and variations in the deletions profile of WSCR in healthy individuals support the hypothesis of Soares, Belsky, Mesquita, Osório & Sampaio (2013), suggesting a special role of GTF2I gene in the behavioral and neurocognitive profile of WS (Chailangkarn, Noree & Muotri, 2018).

In a Mouse manipulation experiment, where the author studied the effects of hemizygosity of GTF2I gene in behavior, Sakurai et al. (2010) found that the loss of one copy of the gene was associated with greater indiscriminate social interaction as indexed by a social and interaction test, where habituation and time investigating an unfamiliar mouse were greater in these heterozygous mice compared to their wild-type littermates. This points to what it seems as an incapacity of GTF2I heterozygous mice to habituate to social stimuli.

Considering that near 30% of children from the study sample of Mervis et al. (2012) who had duplication of the chromosomal region 7q11.23 were diagnosed with Separation Anxiety Disorder (SAD), while only 4% of children with WS filled the criteria for SAD, the authors generated a mice model with increased or decreased genomic copies of the GTF2I (between 1 and 4 copies of the gene), in order to assess if the SAD seen in dup7q11.23 was associated with the number of copies of GTF2I (gene from the deleted or duplicated region). The results showed that as the number of gene copies increased, also did the ultrasonic vocalizations (a measure of separation anxiety used in mouse pups) of the mouse pups when separated from their mothers (Mervis et al., 2012), suggesting an association between the duplication of GTF2I gene and separation anxiety, a contrasting phenotype to what is observed in WS.

Another line of evidence of the important role GTF2I gene plays in the socio-cognitive phenotype of WS comes from the study of Borralleras, Sahun, Pérez-Jurado & Campuzano (2015). In this study, the authors administered intracisternal GTF2I-gene therapy in mice with a complete deletion of GTF2I gene and then assessed their sociability with a direct social test. In this test, the injected mice displayed lower levels of interest in the intruder mouse container (vs. an empty container), resembling the wild-type mice behavior. Regarding anxiety behavior, the injected mice scored closer to the wild-type, with a greater proximity of results by the end of the experiment (20 minutes). Taken together, these results showed a beneficial effect (an increase of expression) of GTF2I gene administration in complete deletion mice regarding sociability and anxiety behaviors.

Crespi & Hurd (2014) also studied the role of GTF2I in social behavior by showing that healthy individuals with the AA genotype of single nucleotide polymorphisms (SNPs) rs4717907 and rs13227433 of the GTF2I gene have low social anxiety and high social communication abilities, something that resembles the WS's behavioral and socio-cognitive phenotype.

Furthermore, other studies have also been showing an association between SNPs in the GTF2I gene and alterations in the brain's structure and functioning. Jabbi et al. (2015) found a positive correlation between harm avoidance with fMRI response of the right dorsolateral prefrontal cortex to aversive social cues in healthy individuals with GTF2I rs2527367. The authors also found that this correlation was mediated by the individual's anxiety proneness (Jabbi et al., 2015). GTF2I rs13227433 AA genotype also predicted a lower bilateral reactivity from the amygdala to angry and fearful facial expression in healthy adults (Swartz et al., 2017). On the other hand, GG and GT genotypes of SNP rs13227433 have also been shown to be associated with lower levels of self-reported social anxiety and to increased reactivity to Oxytocin when presented an empathy-inducing video (Procyshyn, Spence, Read, Watson & Crespi, 2017). Together, these results point to how common genetic variation in GTF2I mediates sociability and anxiety phenotypes in healthy population.

4. Research Aims and Hypotheses

4.1. Rationale

ISB has been observed in both WS and institutionalized children. Taking into consideration the environmental differences in which both groups are reared, we hypothesize that a common genetic component that may contribute to the emergence and maintenance of this behavior in both groups. The role of GTF2I gene in social anxiety and social abilities in both healthy and WS population, as described above, makes this gene a strong candidate for the neurocognitive and behavioral phenotypes in WS. Although phenotypic similarities have been proposed between WS and institutionally reared children regarding their social behavior, the study of genetic polymorphisms within the WSCR that are associated with WS's socio-cognitive behavior has not yet been done in the context of institutionalized children who display ISB.

SNPs are genetic variations that occur in the general population and have been associated with increased vulnerability to different conditions, including the ones that affect the central nervous system (Allen-Brady et al., 2009; Harold et al., 2009 in Soares, Belsky, Mesquita, Osório & Sampaio, 2013). Previous studies have shown that the consequences of exposure to early adverse rearing conditions may be mediated by genetic factors that may foster resilience or vulnerability to the adverse environment. Variants in the GTF2I gene could moderate the degree of impact of early adverse rearing experience and explain to some extent the distinct level of expression of ISB seen in institutionalized children. Identification of this gene could help determine a potential vulnerability marker for psychopathology in children exposed to early neglect and adverse rearing environment and later contribute to preventive interventions based on experience-dependent neural plasticity and epigenome changes (Soares, Belsky, Mesquita, Osório & Sampaio, 2013). Also, having in mind experimental neuroscience may help us to better understand and conceptualize more proximal role of the nervous system to the GXE interaction between GTF2I and institutionalization.

4.2. Hypotheses

The current study has as main goal to understand the relationship between GTF2I SNPs in the ISB of institutionally reared children.

Considering the abovementioned studies that document an association between ISB and other contextual and relational factors (Smyke et al., 2007; Oliveira et al., 2012; Oliveira, Fearon, Belsky, Fachada & Soares, 2014), as well as the lack of literature on the contributions of GTF2I gene in ISB, we hypothesize that:

1. The presence of ISB symptoms will be associated with the presence of distal relational factors of early family risk and pre-institutional experiences, including experiences of parental neglect, parental abandonment and previous institutional placement;

2. Lower levels of caregiver's quality of care (operationalized in variables such as having rotating vs. fixed shifts, time spent individually with each child, number of children responsible for in one day, the type of relationship between the caregiver and the child, and the caregiver's sensitivity, cooperation and sensitive responsiveness) will be associated with increased ISB symptoms;

3. The association between caregiver's quality of care and ISB will be stronger among children with the TG and GG GTF2I rs3227433 alleles, in comparison with the same association with the TT GTF2I rs3227433 allele.

5. Methods

5.1. Sample

5.1.1. Institutionalized Children

One hundred and twenty-six institutionalized children (75 boys, 59.5%) were recruited from 27 Portuguese institutions, along with their institutional

caregivers. These institutions are also known as Temporary Care Centers, and they harbor children who were removed, by Social Services staff, from their biological family due to various reasons such as negligence, physical and psychological abuse, parental psychopathology and substance abuse, and extreme economic hardship. Children were between 36 and 78 months old (*M*=54.58, *SD*=11.10) by the time of assessment. Age of admission varied from 0 to 5 years of age (*M*=2.50, *SD*=1.343), with 7.1% of children being admitted before 12 months of age. Length of institutionalization ranged from 7 to 59 months (*M*=19.20, *SD*=11.334), with 26.2% (*n*=33) institutionalized for one or more years. Neglect was the reason of admission for the majority of our sample (108 children, 77.7%). Exclusion criteria included having severe mental or physical impairments, genetic diseases, autism spectrum disorder and being institutionalized for less than 6 months.

5.1.2. Caregivers

Eighty-seven institutional caregivers participated in this study (1 male, 1.1%). Age of caregivers ranged from 21 to 67 years (*M*=38.41, *SD*=10.87). One (1.3%) had no schooling, four (5.2%) had 4 years of education, five (6.5%) had 6 years of education, twenty-six (29.9%) had 9 years of education, twenty-six (29.9%) had a high-school diploma and 24 (27.3%) had graduated from college. Thirty-three (41.3%) did not have specific training for their role. In average, caregivers spent 34.65 minutes (*SD*=64.30) individually with each child. Fifty-six (67.5%) caregivers had rotating shifts. Regarding the number of children responsible per day, 56 (70.9%) of the caregivers had 10 or more children who were responsible for per day.

5.2. Procedure

5.2.1. Child and Caregiver Assessment

This study is part of a broader research project, that started in January 2010. Approval by the Portuguese Social Services and the National Commission

for Data Protection was obtained. The study was then presented to the staff at each institution. Written informed consent was gathered from biological parents, institution directors, and participating caregivers. Children were recruited based on their age and exclusion criteria were the presence of severe physical or mental impairments and/or genetic or neurological syndromes. After deciding which children met the criteria for participating in the study, institutional staff was consulted in order to identify the assigned caregiver to each child. Staff suggestions were then compared with the research team's judgements based on naturalistic observations of the interaction between child and caregivers, during the period of data collection. In order to characterize child's prior experiences to institutionalization, as well as children's contact with their biological family while institutionalized, information was gathered from his/her file. This information served, as well as with the staff's contribution, to complete the child's sociodemographic questionnaires. Child's medical records were assessed for physical growth data. The child's mental development was assessed by a trained examiner as well as ISB, assessed through a semi structured interview to his/her caregiver. Children's files were filled by social workers based on the information that was available to them.

5.3. Measures

5.3.1. Sociodemographic and Clinical Questionnaires

This questionnaire assesses both pre and current paths, dynamics and life contexts of the institutionalized child. It was completed with the help from a member of the technical team of the institution (e.g., social worker, psychologist, etc.) and with the access to the child's individual process. Through this questionnaire, information about (1) child's identification (i.e., sociodemographic information, date of institutionalization, reason of admission); (2) filiation (i.e., parents, siblings, household composition); (3) socioeconomic status (i.e., housing and economic situation); (4) health and developmental history of the child and

(5) kindergarten characterization and child's adaptation to it is gathered. Answers are either descriptive and/or categorical (e.g. yes/no).

Early family risk factors and pre-institutional experiences were collected through the child's files at the institution. A total of 119 (95.2%) children lived with at least one of his/her parent before institutionalization. Three risk conditions, scored as 0 (absent) or 1 (present), were assessed in order to capture sources of parental care deprivation (cf. Corval et al., 2017):

1. **Parental neglect**, which included child's experiences of physical and/or emotional neglect by her/his parents (i.e. failure to meet the physical needs of the child and her/his psychological safety and security) and that was considered as child's main reason of admission into the institution. In our sample, 108 (77.7%) of the children suffered from parental neglect and had neglect as main reason for institutional admission;

2. **Parental abandonment,** composed by experiences reflecting: i) effective abandonment by parents, ii) abandonment of the child to the care/responsibility of third-party figures, and iii) leaving the child alone for what was considered as a period of time long enough to expose child to substantial risk of harm. In the group of children composing this study, 31 (22.3%) were abandoned by their family of origin;

3. **Previous institutional placement,** which refers to previous experiences of institutionalization. In our sample, 30 (21.6%) children had been previously institutionalized.

5.3.2. Child's Mental Development

In order to assess child's general mental development, the Griffith's Mental Development Scales (1984) were used. It is composed of 6 subscales, each pertaining quotients for specific areas of development: locomotor (gross motor skills), personal-social (assesses daily-living activities, level of independence and interaction with peers), language (receptive and expressive), eye-to-hand

coordination (fine motor and visual monitoring skills), performance (visuospatial skills), and practical reasoning (understanding of moral problems and issues, mathematical reasoning and capacity to solve practical problems). Each subscale was used and calculated, resulting in a total score that reflects general development for each developmental component mentioned before. A final quotient was calculated averaging the various sub-quotients (Cronbach's a 1⁄4 .79), resulting in a global quotient of development. The mean of our sample was 97.46 (*SD* = 11.70), with a minimum of 64.82 and a maximum of 129.

5.3.3. Institutional Care

Measures of the institutional care were collected and analyzed, aiming to detect possible contributions of distal institutional characteristics (related to structural and organizational aspects of the institution; Cf. Oliveira, Fearon, Belsky, Fachada & Soares, 2014) to the children's development of ISB. For this, we considered:

1. **Institutional placement**, that gathered both the child's age of placement and length of time at the institution. Information was collected through the child's file. The mean age of placement in our sample was 2.61 years (SD = 1.365) and length of institutionalization was 17.98 months (SD = 11.491);

2. **Stability and individuality of care (SIC) offered by the Institution,** which was a composite created to measure stable and individualized care experienced by the child. A structured interview with the director of each institution was performed in order to collect staffing variables (i.e. number of caregivers that belonged to the pool of caregivers available to take care of children; average children-to-caregiver ratio; percentage of caregivers with rotating shifts). These variables were standardized to Z scores and then summed in order to create the composite;

3. **Stability and individuality of care (SIC) offered by the caregiver**, that reflected variables related to caregiver's employment. A questionnaire was

given to each caregiver participating in this study. The questionnaire is divided in three parameters: (1) caregiver data, which included questions related rotating and fixed shifts and biographical and educational questions; (2) questions related to care delivered, that assessed time spent individually with the children, type of caregiving functions and number of children responsible for, on average, in one day (later dichotomized in <10 vs. >10); (3) data related to caregiver's perspective on her functions and on institution's organization. All items were organized in a Likert scale with 5 levels (1-strongly disagree; 5-strongly agree). Three of these items (rotating vs. fixed shifts, time spent individually with the children and number of children responsible for in one day) were summed in order to create a composite. Better quality of care was reflected with a higher score in this composite.

5.3.4. Indiscriminate Social Behavior

For the assessment of ISB, we used The Disturbances of Attachment Interview (DAI; Smyke & Zeanah, 1999). It is important to note that the DAI has no formal disorder diagnosis nature. The DAI is a semi structured interview containing a total of 12 items that assess inhibited and indiscriminate social functioning, and secure-base distortions of a child, and is administered to the child's assigned caregiver. Each item is coded as 0 (=none/never), 1 (= somewhat/sometimes) or 2 (= considerable/frequently), according to the amount of evidence for the assessed behavior. For the purpose of this study, we analyzed three items (6-8) pertained to indiscriminate social behavior (e.g. "Do you think s/he would be willing to go off with a stranger?; "Does s/he check back with you or s/he one to just go off without checking back?"; "Does s/he tend to be sort of shy around strangers or is s/he one to go right up to people s/he doesn't know?"), yielding total scores ranging from 0 to 6 points. Children were classified as disinhibited if there was at least one "symptom definitely present" in one of these 3 items. All interviews were audiotaped and afterwards scored by two trained researchers. In our sample, twenty-seven (22%) children were classified as

disinhibited. Inter-rater agreement based on 37 cases was very good (ICC ric=.910).

Table 1. Child's variables

	M (SD)	*Min-Max*
Age at assessment (months)	54.58 (11.10)	36-78
Age at admission (months)	35.28 (14.88)	4-63
Time in institutional care (months)	19.20 (11.30)	7-59
Developmental Quotient	97.46 (11.70)	64.82-129
Gestational weeks	38.85 (1.83)	32-43
	Frequencies (n, %)	
Indiscriminate Social Behavior (*n*=123)	27 (22%)	
Neglect as reason of admission	108 (77.7%)	

Note. N=126 (75 boys)

5.3.5. Quality of Caregiver-child Relationship

Current experience of relational quality between child and caregiver was assessed, with the goal of identifying more proximal institutional characteristics that may influence the emergence of ISB. Here we used four measures:

1. **Classification of caregiver-child relationship**, which, after determining a key caregiver that would participate along with the child in the present study, classified each caregiver as either Assigned caregiver, Caregiver of Reference or the Preferred Caregiver of a child (cf. Oliveira et al., 2012). This information was obtained through the inquiry of institutional staff, guided by questionnaire containing the criteria that led to the classification. There are two types of answers: (1) "closed" answers (yes/no) and Likert scale with 3 levels (1- not true; 3- true almost every time). There are three different sections, the second section only being answered if there was at least one "not true" answer in section 1, and the third section only being answered if all questions from section 2 were classified as "no". After this

questionnaire, the staff classification was validated against naturalistic observations of the dyad done by trained researchers. The researcher's classification of the caregiver-child relationship was considered as final judgment when there was discrepancy. In order to be classified as Preferred Caregiver, the child should demonstrate clear signs of proximity seeking to the caregiver, especially when distress; should show separation anxiety in case of absence of the caregiver; there should also be signs of more positive responses towards this caregiver and more acknowledgement when reunited with the caregiver; the child should preferentially approach this caregiver for comfort, comparing with others caregivers. A Caregiver of Reference was someone from whom the child showed some signs of preference in comparison with others, but not as much as a Preferred Caregiver and/or was someone who was more responsible for/more frequently looked after the child. Finally, an Assigned caregiver was someone from whom the child showed no signs of preference, that is, the child did not exhibit preference from anybody at the institution. From our sample, thirty-five (25.5%) of the children had a preferred caregiver, 68 (48.9%) had a caregiver of reference and 24 (24.5%) had an assigned caregiver.

2. **Caregiver's sensitivity towards the child,** which was assessed during a fifteen-minutes interactive and video-taped task, that was divided in three episodes: i) play with a challenging toy; ii) monitoring the child during a sham questionnaire, during which the child only possess one uninteresting toy to play, with others more interesting toys to play nearby s/he is instructed not to play with and, iii) free play followed by a clean-up. Scorings of these recorded sessions were carried out using Ainsworths' Maternal Sensitivity Scales (1969), adapted to the preschool years. Two independent coders rated all cases for the sensitivity vs. insensitivity scale who were blind to the type of relationship of the dyad and to other data collected in this inquiry. The ratings evaluated the ability of the caregiver to perceive and interpret the child's cues and communication, and to

correctly respond to them. Four more aspects of caregiver sensitivity are considered: i) awareness of the child signals; ii) correct interpretation of those signals; iii) accurate response to them, and iv) promptness of response. The scores range from 1 (highly insensitive) to 9 (highly sensitive). When disagreements occurred within classification of a case, discussion was carried out until consensus was obtained. Inter-rater reliability was adequate (ICC ric =.946) for the cases classified by two researchers (n=59).

3. **Caregiver's Cooperation** based on Ainsworth (1969) Maternal Sensitivity scales. This scale focus on the caregiver's interventions with child's ongoing activity, that is, whether the caregiver breaks into, interrupts or cuts across the child's activity. Two aspects are considered when evaluating the degree of interference: i) the actual extent of physical interference during child's activity, and ii) frequency of interruptions. The scores range from 1 (Highly interfering) to 9 (Conspicuously cooperative).

4. **Caregiver's Sensitive responsiveness**, which is in line with previous studies (Baptista et al., 2014) and takes into consideration the two highly correlated scales of sensitivity and cooperation ($r=.661$, $p<.001$), by averaging the two z scores. In this study, the mean score for sensitive responsiveness was 5.15 (SD = 1.57, range 1-8).

Table 2. Caregiver's variables

	n	*M (SD)*	*Min-Max*
Age	82	38.41 (10.87)	21-67
Sensitivity	85	5.22 (1.84)	1-9
Cooperation	85	4.94 (1.71)	1-8
Sensitive responsiveness	85	5.10 (1.62)	1-8
SIC: Institution	68	-0.05 (1.62)	-2.64-2.39
SIC: Caregiver	83	0.71 (0.69)	0-2
Time dedicated to individual child care per day (min)	56	34.65 (64.3)	0-420
Number of children responsible for per day	66	11.92 (5.51)	4-31

Note. N=87 (1 male)

5.3.6. Genetic Analysis

Saliva samples were collected using Oragene DNA collection kits (DNA Genotek, Canada), and genomic DNA was isolated following manufacturers' instructions, using the standard protocol from PrepIT L2P (DNA Genotek). Sample concentration was accessed using Nanodrop technology. For the GTF2I rs13227433 and rs4717907 allele polymorphism analysis, 5 ng of DNA were used, along with the corresponding KASPar assay (LGC Genomics, UK), for a final volume of 8 μL. The thermal profile was performed as instructed by the manufacturers, in a 7500 Fast Real-Time PCR System (Applied Biosystems by Life Technology, USA).

Our sample had the following genotype frequencies for rs13227433: TG - 33.3% (n=42), TT - 63.5% (n=80), and GG - 3.2% (n=4); for the rs4717907: GA - 33.3% (n=42), GG - 63.5%, and AA - 3.2% (n=4). The distribution is in Hardy–Weinberg-equilibrium, $\chi^2(1) = 0.289$, $p = .591$. Allelic frequency for these genes is consistent with published literature and NBCI database.

The distribution of the genotypes in both SNPs were identical in our sample, something that can be explained by the fact that these two polymorphisms are in high linkage (Crespi & Procyshyn, 2017). For further analysis, it will be presented just one of the SNP (rs3227433), in order to avoid redundant results.

Given the rarity of rs13227433 GG genotype in our sample, rs13227433 GG and GT genotypes were combined and compared with TT genotypes (in line with Procyshyn et al., 2017). The GTF2I grouped genotypes proved not to be significantly associated with child ethnicity (75.4% Caucasian vs. 24.6% others), $\chi^2(1) = 3.441$, $p = .064$.

Table 3. Distribution of genetic variants subgroups as a function of sex (n, %)

SNP	***Rs13227433***	
Genotype	**TG+GG**	**TT**
Institutionalized children (N=126)	46 (36.5%)	80 (63.5%)
Sex		
Female (n=51)	21 (41.2%)	30 (58.8%)
Male (n=75)	25 (33.3%)	50 (66.7%)

6. Data Analysis

Data analysis was run with IBM® SPSS®-22 software. Descriptive statistics were firstly run in order to better characterize our sample.

To test hypotheses 1 and 2, a dichotomic version of ISB variable was used following Oosternman & Schuengel (2007) model (non-disinhibited Vs. disinhibited, i.e., children were classified as either disinhibited if there was at least one "symptom definitely present" in one of the three items of DAI disinhibited behavior scale, or as not disinhibited) and correlational tests were run to assess the association between ISB and the proximal and distal above-mentioned

relational variables. Paired samples T Tests were run with the variables that were significantly correlated with ISB. Depending on the nature of the variables analyzed, Pearson or Spearman correlations were run (depending if the assumptions needed to use parametric tests were met or not), as well as biserial point and qui-square tests.

To test hypothesis 3, the model (Model 1) was tested on macro PROCESS by Hayes (2018). A regression analysis predicting ISB was conducted using, as first step, the caregiver's level of cooperation and sensitive responsiveness and GTF2I rs13227433 genotypes (0 for TT and 1 for TG+GG). The second step included the two-way interaction between caregiver's level of sensitive responsiveness and GTF2I rs13227433 genotype. To illuminate any detected interaction, regions of significance were determined using the Johnson-Neyman technique.

7. Results

7.1. Correlations between ISB and study variables

To test the first hypothesis, qui-square analysis was run between ISB and parental neglect, parental abandonment and previous institutional placement. There were no significant results to support the first hypothesis (X^2 = .319, p = .572 for parental neglect; X^2 = 2.602, p = .107 for parental abandonment and X^2 = .460, p = .498 for previous institutional placement).

To test the second hypothesis, spearman and bivariate correlations were run between caregiver's quality of care variables and ISB. There were significant results for ISB and caregiver's levels of cooperation and sensitive responsiveness (r_{pb} = -.203, p = .025 and r_{pb} = -.178, p = .050, respectively), as well as for ISB and caregiver's type of schedule, Fisher's Test, p=.021. Within disinhibited children (n=24), 12 (50%) had caregivers with rotating shifts. Other correlations between caregiver's, child's and institution's variables and ISB were not significant (see supplemental data for further analysis).

7.2. Multiple Regression Analysis

7.2.1. Moderation of child's genotype in the association between Caregiver's cooperation scores and child's ISB symptoms

We did not observe a significant result for the overall model of regression, i.e., the GTF2I genotype was not predicting the association between ISB and caregiver's scores of cooperation, F (3,118) = 1.60, p = .193, R^2 = .061, b = -.392, t (118) = -1.62, p = .107. Additionally, there was no interaction between child's genotype and caregiver's cooperation in predicting ISB, b = -.392, t (118) = -1.624, p = .107.

7.2.2 Moderation of child's genotype in the association between Caregiver's Sensitive Responsiveness scores and child's ISB symptoms

The results of the model testing the moderator role of child GTF2I genotype in the relationship between caregiver scores of sensitive responsiveness and child ISB symptoms proved to be significant, F (3, 118) = 2.373, b = -.527, t (118) = -2.410, p = .018.

Table 4. Summary of hierarchical linear regression analysis predicting ISB

	R^2	*B*
Step 1	.077	
Caregiver's Sensitive Responsiveness		.026
GTF2Irs13227433		.248
Step 2	.050*	
Caregiver's Sensitive Responsiveness*GTF2I rs13227433		-.527*

*p<.05

To understand the nature of this significant interaction, we plotted regression slopes of caregiver sensitive responsiveness on ISB symptoms separately for the TT genotype and carriers of at least one G-allele. For GTF2I rs13227433 TG and GG allele carriers, there was a significant relationship

between caregiver sensitive responsiveness scores and child ISB symptoms, *b* = -.500, t (118) = -2.65, *p* = .009, but this was not observed for children with the TT genotype, *b* = .026, t (118) = .240, *p* = .811.

In order to illuminate the nature of this GXE interaction (diathesis-stress or differential-susceptibility model of environmental action), we conducted a "regions of significance" analysis, following Kochanska, Kim, Barry & Philibert (2011) approach. Through this technique, we are able to determine the specific values of caregiver's sensitive responsiveness below which and above the regression lines of children with the two different GTF2I genotypes (i.e. TT vs. TG and GG) differ significantly with regard to ISB symptoms. As we can see in Figure 1, with the analysis of Regions of Significance using the Johnson-Neyman technique, we observed that the slopes between GTF2I genotypes and ISB symptoms proved to be significant when caregiver's sensitive responsiveness scores were below -.927, *b* = .736, t (188) = 1.980, *p* = .050, and therefore more consistent with the *diathesis stress model*. More specifically, when exposed to lower scores of sensitive responsiveness caregiving (i.e., <-.927) TG and GG carriers scored significantly higher on ISB symptoms than TT carriers.

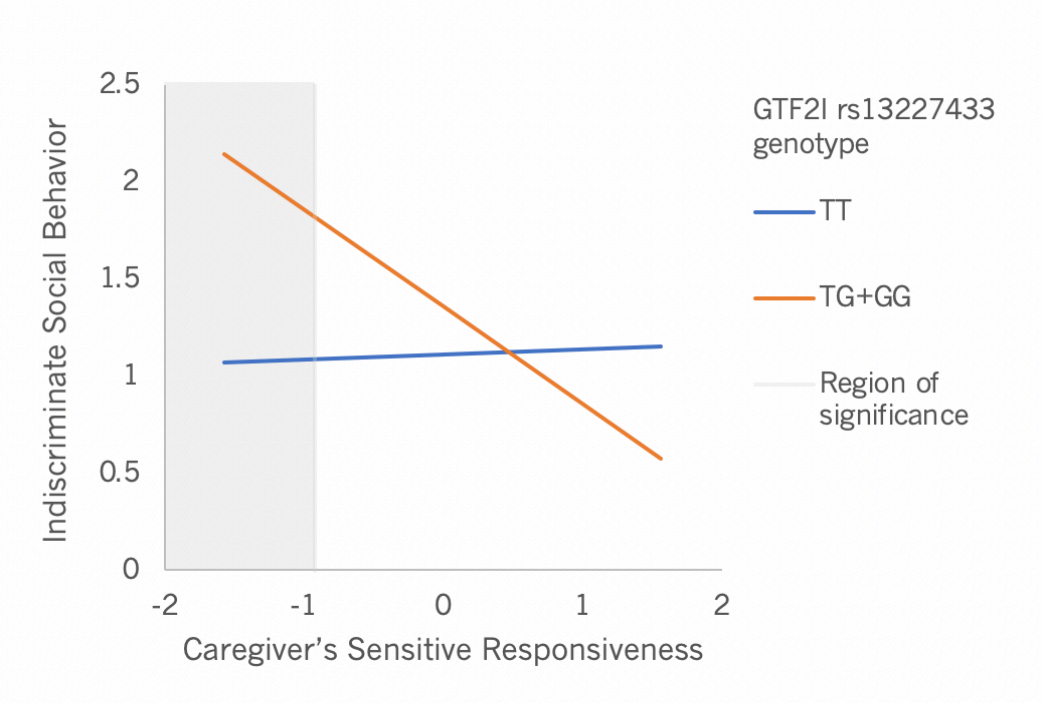

Fig. 1 Moderator role of GTF2I rs13227433 on the relation between Caregiver's sensitive responsiveness and child's indiscriminate social behavior. The shaded area represents the region of significance.

8. Discussion

The deleterious effects of institutionalization in child development has been widely documented. Although not exclusive to children raised in institutions (see for example, Lyons-Ruth, Riley, Patrick, & Hobson, 2019; Pears, Bruce, Fisher, & Kim, 2010), the presence of indiscriminate social behavior in institutionalized children is commonly observed, being persistent over time and relatively unchangeable to improvements in the environment (Guyon-Harris, Humphreys, Fox, Nelson & Zeanah, 2018; Humphreys, Nelson, Fox, & Zeanah, 2017). The current and innovative study extends previous research on the consequences of institutionalization in ISB, by exploring the genetic contributions of GTF2I gene SNPs, a candidate gene associated with WS's social behavior pattern of indiscriminate friendliness. Overall, our results document a GXE interaction on ISB with the quality of proximal institutional care factors, but no direct association between GTF2I gene SNPs and ISB was found.

From the variables included in this study that tried to capture the child's environment and relational experiences as potentially associated to ISB symptoms, only caregiver's cooperation and sensitive responsiveness towards the child emerged as significant. We found a significant negative association between caregiver's level of cooperation and sensitive responsiveness and child's ISB symptoms, that is, higher levels of responsive caregiving behaviors seem to be protective to the development of ISB symptoms in our institutionalized preschoolers. Although we expected an association between ISB symptoms and more distal relational factors such as pre-natal risk and maternal emotional neglect as observed in Oliveira et al. (2012), we did not find any of these associations in our sample. Having a preferred caregiver as a protective factor for the development of ISB symptoms proved to not be associated with ISB symptoms, a result that differs with our hypothesis and some previous work (Soares et al., 2014), but that goes in line with other works disentangling ISB and the development of attachment relationships to the caregiver (Zeanah et al., 2005).

These inconsistent results with previous research may be explained by samples' age differences, given that the above-mentioned studies worked with institutionalized toddlers, while our sample was composed by preschoolers. The development psychopathology (Cicchetti, 1984) and emotional development (Sroufe, 1997) perspectives alerts us to the fact that there are specific emotional needs that aim to be met throughout the different stages of the developmental path, and that the effect of a negative input during a specific developmental stage may have a concrete consequence, while having a different one when experienced during another developmental phase, which could explain the different associations observed between our results and the results from Soares et al. (2014) and Oliveira et al. (2012). Nonetheless, our results are in line with the importance and protective nature of current relational experiences in the context of institutionalization (Oliveira, Fearon, Belsky, Fachada & Soares, 2014; Soares et al., 2014).

A GXE interaction involving GTF2I rs13227433 SNP and caregiver's sensitive responsiveness, but not caregiver's cooperation, accounted for significant differences in ISB in institutionalized children. Notably, this interaction proved to be more consistent with the diathesis-stress model rather than the differential susceptibility hypothesis (Belsky, Bakernans-Kranenburg, & IJzendoorn, 2007; Belsky & Pluess, 2009), which was not in accordance to our predictions. Specifically, children carrying at least one G-allele were the most susceptible to lower levels of caregiver's sensitive responsiveness, exhibiting the highest levels of ISB, but not the least when experiencing highest levels of caregiver's sensitive responsiveness. Children carrying the TT genotype seemed to be little influenced by changes in caregiving environment. Our results support the diathesis-stress model rather than the differential susceptibility model. This may be explained, to some extent, by the fact that the number of children experiencing high levels (higher than 7 points out of 9) of sensitive responsiveness in our sample was relatively small (n=18), making it difficult to capture, in a statistically significant way, the "bright side" of this particular environment. Indeed, environmental sensitivity works throughout the whole spectrum of nurturing quality and not only in response to adverse rearing or traumatic experiences, and genetic components are probable to envision individual differences in this spectrum (Pluess, 2015).

This work goes not without limitations. Firstly, our sample size is considerably small for what is ideal for a GXE and candidate gene study, which compromise statistical power. Secondly, it is highly unlikely that one gene alone may account for the interaction effect we found in institutionalized children's ISB symptoms. Studying the contribution of other relevant genes related to affiliative and social behaviors, as well as other environmental factors that may contribute to the development of ISB, is important and relevant for future works, as well as replication in larger samples.

It may also be interesting to investigate other neurobiological factors associated with ISB, and how they may interact with genetic characteristics. For example, it would be interesting to investigate if differences seen in face familiarity processing in institutionalized children with ISB (Mesquita et al., 2014)

would differ between GTF2I genotypes. It might be the case that children with ISB differ in their face familiarity processing depending on their genotype, possibly working as an endophenotype underlying ISB symptoms. Another strength that could be added to the present study would be the comparison with a community group. In that way it would be interesting to see if institutionalized and community children differ not only on ISB levels but also whether or not the GXE interaction we found in institutionalized children would also emerge in a community sample. Considering a community sample would also possibly allow us to better capture the "bright side" of this GXE interaction and illuminate future work, namely elucidate some epigenetic mechanisms that may underlie the development of ISB and the experience of institutionalization in children exposed to not only low levels of current care but also exposed to pre-institutionalization risk factors that may compromise the expected neurodevelopment process and consequently normal social, emotional and cognitive child adaptation.

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Appendix

Table 5. Relation between *caregiver's, child's and institution's variables*

Variables	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1. DAI	-																				
2. Child's Sex	.000	-																			
3.Age at assessment (months)	.066	.073	-																		
4. Age at admission (years)	.061	.079	.687**	-																	
5. Time in institutional care (months)	-.087	-.061	.006	-.637**	-																
6. Developmental quotient	-.169	-.005	-.006	.029	-.025	-															
7.Neglect as reason of admission	.319	.021	-.039	-.014	.013	.035	-														
8. Parental abandonment	2.602	.001	.003	-078	.056	.122	.011	-													
9. Previous institutionalization	.460	.117	.017	-.055	.129	.185*	1.819	7.065**	-												
10.Caregiver's age	.181	.142	-.107	-.008	-.123	-.077	.141	-.052	.096	-											
11. Caregiver's academic qualifications	2.983	2.271	.162	.183	-.052	.084	.473	2.017	1.946	-.317	-										
12. Sensitivity	-.150	-.233**	.083	.096	-.060	.100	.117	-.018	-.068	-.133	.234*	-									
13. Cooperation	-.203*	-.242**	.183*	.130	-.036	.185*	.070	.047	-.005	-.136	.342**	.661**	-								
14. Sensitive responsiveness	-.178*	-.255**	.142	.133	-.061	.138	.090	.010	-.044	-.140	.307**	.922**	.893**	-							
15. Caregiver's type of schedule	7.430*	.261	.055	.120	-.171	-.009	.196	.247	1.894	.002	22.524**	-.147	-.118	-.144	-						
16. Time spent individually with each child individually	.057	.098	.157	-.019	.138	.137	.065	.199	.170	.185	.148	.060	.045	.057	-.141	-					
17. Number of children responsible for each day	1.291	1.793	.125	-.020	.208*	.103	.020	.020	3.451	-.001	8.130	.162	.077	.148	.792	.096	-				
18. Type of relationship	.510	9.519**	.008	-.062	.108	-.029	.781	3.156	7.170**	-.172	19.200*	.210*	.082	.165	6.705	-.155	9.177**	-			
19. SIC: Institution	-.015	-.113	-.294*	-.184	-.006	-.167	.039	-.214		.316**	.061	.090	.127	.116	.074	.049	-.062	-.045	-		
20. SIC: Caregiver	.315	.654	.053	-.080	.079	.045	4.837	1.621	1.863	.019	11.226	.056	.029	.050	60.765***	.407**	2.365	12.210*	-.179	-	
21. GTF2I rs13227433	.257	.806	-.053	.000	-.099	.100	2.057	.029	.033	.059	8.740	-.062	-.031	-.058	2.123	.143	1.880	1.630	-.132	.040	-

*p<.05; **p<.01;***p<.001