HHS Public Access

Author manuscript

J Endocrinol Invest. Author manuscript; available in PMC 2021 December 22.

Published in final edited form as:

J Endocrinol Invest. 2021 August ; 44(8): 1775–1782. doi:10.1007/s40618-020-01497-x.

Assessment of children in the autistic spectrum disorder that carry the Thr92Ala-DIO2 polymorphism

A. A. e Marcondes[1], **T. G. B. Gomez**[1], **T. T. Ravache**[1], **A. Batistuzzo**[1], **F. B. Lorena**[1,2], **C. S. de Paula**[1], **R. Lowenthal**[3], **A. C. Bianco**[4], **M. O. Ribeiro**[1]

[1]Developmental Disorders Program, CCBS, Center of Biological Science and Health, Mackenzie Presbyterian University, Rua da Consolação, 930 Bld. #28, São Paulo, SP 01302-907, Brazil

[2]Federal University of Sao Paulo, São Paulo, Brazil

[3]Santa Casa de São Paulo School of Medical Sciences, São Paulo, Brazil

[4]Section of Endocrinology, Diabetes and Metabolism, Department of Medicine, University of Chicago, Chicago, IL, USA

Abstract

Introduction—A polymorphism in the type 2 deiodinase (Thr92Ala-DIO2) gene has been associated with behavioral and cognitive dysfunction as well as neurodegeneration and oxidative stress in the central nervous system.

Objective—To test whether the minor allele (Ala92) frequency (MAF) is increased in children in the autism spectrum disorder (ASD), and whether carriers of the minor allele exhibit more severe symptoms and/or worse adaptive behavior.

Study design—ASD children were evaluated at baseline and yearly throughout the study by psychologists using the following tools: autism behavior checklist, Vineland Adaptative Behaviour Scales II, non-verbal intelligence test SON-R $2^{1/2}$–7, SON-R 6–40, Weschler scale for intelligence, and autism treatment evaluation checklist.

Settings—Academic outpatient mental health facility in Sao Paulo, Brazil.

✉M. O. Ribeiro, miriamribeiro@mackenzie.br.

Supplementary Information The online version of this article (https://doi.org/10.1007/s40618-020-01497-x) contains supplementary material, which is available to authorized users.

Conflict of interest Dr. Antonio C. Bianco is a consultant for Allergan Inc, Synthonics Inc and BLA Technology LLC. Dr. Miriam O. Ribeiro has received the research grant from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP proc. N° 2017/18277-0) and Pró-Reitoria de Extensão (PROEX Proc n° 1133/2019). Alice Batistuzzo and Alyna A. e Marcondes recieved a scholarship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). The authors also acknowledge Program of Developmental Disorder of Presbyterian University Makenzie and the ASD Reference Unit CAISM-Vila Mariana for providing infrastructure for completing the work.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual's parent participants included in the study.

Participants—ASD boys and girls younger than 18 years of age. 132 consecutive ASD children, mostly boys (~ 80%); ~ 50% was classified as verbal. Exclusion criteria were coexistence of sensory and/or physical impairment, or any associated genetic syndromes.

Results—Median follow-up was for an uninterrupted period of 937 days (139–1375 days), which did not vary significantly among the genotypes. The MAF was 47% in ASD patients vs. 51% in a local reference population with similar ethnic background; the clinical severity and progression were not affected by the minor allele. Carriers of the minor allele exhibited higher adaptive behavior in the domains “daily living skills” and “communication”, which correlated positively with the dose of the minor allele.

Conclusion—The MAF is not different in ASD children, but carriers of the Thr92Ala-DIO2 polymorphism exhibited higher adaptive behavior.

Keywords

Austism Spectrum Disorder; D2; Polymorphism; Vineland

Introduction

A group of three selenoenzymes metabolize thyroid hormone (TH) inside and outside the thyroid gland. By removing specific iodine moieties from the TH molecules, these enzymes change the biological activity of TH molecules, both enhancing or terminating TH action. Thyroxine (T_4) is the main product of the thyroid gland and the type 2 deiodinase (D2) catalyzes the activation of T_4 to T_3, increasing intracellular levels of the active TH [1]. Whereas D2 is expressed in a number of tissues, its presence in the central nervous system (CNS) is particularly relevant, as > 50% of the T3 in the brain is locally generated via the D2 pathway. D2 is expressed in glial cells and, as T3 exits these cells it acts in a paracrine fashion to induce T3-responsive genes in neighboring neurons [2].

Humans can be carriers of a DIO2 polymorphism (Thr92Ala-DIO2) that results in a single aminoacid change in D2, where Ala substitutes Thr at position 92 [3]. The frequency of this polymorphism varies between 12 and 36% depending on the ethnic background of the population [3–5]. Whereas in its original description carriers of the Thr92Ala-DIO2 were found to have increased BMI and glucose intolerance [3], subsequent small studies suggested a positive association with intellectual disability, lower IQ, bipolar disorder, and schizophrenia [6–9].

Ala92-D2 has been linked to transcriptional changes as detected in cell models and in the temporal pole of carriers of the Thr92Ala-DIO2 polymorphism [10]. These changes were associated with ectopic D2 distribution to the Golgi apparatus, in addition to its regular presence in the endoplasmic reticulum. In addition, cells expressing Ala92-D2 exhibit increased cellular oxidative stress, and endoplasmic reticulum (ER) stress with an active unfolded protein response (UPR) [10]. A mouse carrier of the Thr92Ala-Dio2 polymorphism exhibited similar transcriptome modifications in the CNS, including ER stress and decreased TH signaling in selected brain areas [10, 11]. These features were associated with cognitive deficits and reduced motivation for physical activity. Whether

these deficits are present in human carriers of the Thr92Ala-DIO2 polymorphism is subject of intense investigation, which has led to conflicting results [12].

Autistic spectrum disorder (ASD) is a neurodevelopmental condition characterized by deficits in communication and social interaction and the presence of restricted and repetitive patterns of behavior [13]. In these patients, there are no signs of neurodegenerative disease but patients may exhibit markers of oxidative stress that correlate positively with the severity of the disease [14, 15]. ASD patients also exhibit increased levels of inflammation markers, such as interleukins and tumor necrosis factors, accompanied by an increase in central and peripheral inflammatory responses [16], which could exacerbate the oxidative stress [15]. Therefore, here we tested whether carrying the Thr92Ala-DIO2 polymorphism affects children in the ASD.

Methods

Settings and subjects

Individuals with autistic spectrum disorder—Prospective study with 132 ASD outpatients being followed at the ASD Reference Unit CAISM-Vila Mariana in São Paulo, Brazil. The IRB protocol was approved by the National Research Ethics Committee (CONEP 1.578.245). During the period of Jan 2014 through May 2017, consecutive patients were screened during the first visit and subsequently diagnosed as having ASD according to Diagnostic and Statistical Manual of Mental Disorders (DSM-5) by a multidisciplinary team of experts. All ASD patients were clinically evaluated for attention deficit hyperactive disorder (ADHD) as a co-morbidity.

At baseline, patients were evaluated through a series of tests (see below) to determine the IQ and severity of the symptoms. Assessment data were limited to 124 autism behavior checklist (ABC), 120 VABS-II, 127 initial ATECs, 115 final ATECs, and 58 IQ assessments. Subsequently, all patients were enrolled on behavioral therapy, multidisciplinary assistance and medical consultations. Reassessment occurred on a yearly basis through Autism Treatment Evaluation Checklist (ATEC); only the last assessment was considered in the study.

Neurotypical individuals—As indicated, a local reference population was also studied, which exhibited a similar ethnic background as ASD groups. This reference population consisted of 50 healthy, neurotypical children with ages between 6 and 17 years, and were recruited in mainstream school during June–October 2018.

Exclusion criteria and informed consent—Exclusion criteria were sensory and/or physical impairment, or any associated genetic syndromes. Through the respective caregivers, all individuals were offered the possibility of enrolling in the study. Patient's caregivers gave informed consent prior to enrollment. Patients were removed from the study if we were unable to collect oral mucosa swab sample.

Study outcomes

Primary

1. To determine the minor allele (Ala92-DIO2) frequency (MAF) in children in the ASD.

Secondary

1. To determine whether carrying the Thr92Ala-DIO2 polymorphism affects adaptive behavior in children in the ASD.
2. To determine whether children in the ASD that are carriers of the Thr92Ala-DIO2 polymorphism have more severe symptoms.

Measuring tools

Autism behavior checklist (ABC)—The severity of the symptoms in the ASD group was determined considering the symptomology and functionality of communication using the Autism Behavior Checklist (ABC) and a consultation with a speech therapist. ABC consists of a questionnaire composed of 57 questions that evaluate the severity of autistic symptoms and whose scores range from 1 to 4 (1 for the behaviors most weakly related to autism and 4 for the behaviors most strongly related to autism). The questions relate to five spheres of behavior: sensory stimulation, relationship, use of the body and objects, language and personal/social. The sum of the scores determines the probability of an ASD diagnosis for that patient; the higher the score, the greater the severity of symptoms. The four levels of severity are: low, mild, moderate and severe [17]. A secondary measure of severity was the ability of communication defined by whether the child was verbal or non-verbal. The criterion was to speak more than 10 words with functionality. This evaluation was carried out by two independent speech therapists specialized in ASD.

Vineland Adaptive Behavior Scale II (VABS-II)—The adaptive functioning of ASD patients was assessed using the VABS-II. This is a semi-structured interview conducted with a parent to obtain ratings of children's adaptive functioning across three domains: Communication (Expressive, Receptive and Written), Daily Living skills (domestic, personal and community), and Socialization (Interpersonal relationships, Play and Leisure time and Coping Skills). The interview consists of 577 questions to be answered by the main caregiver of the patient. Answers should indicate whether the patient behaves as described by the evaluator frequently, sometimes / partially or never. In addition, the interviewee may respond that the patient has not had the opportunity to express the indicated behavior or that he/she does not know whether the behavior is present. The assessment is corrected for the child's age at the time of the interview according to predefined standards, and scores are obtained by domain, subdomain and global score, which allow determining the child's level of development in relation to what is expected for their age group age [18, 19]. The higher the score, the less severe the impairment of adaptive functioning is.

Nonverbal Intelligence Test (SON)—According to the cognitive/communication skills and age of the patients, the intelligence assessment was performed using Nonverbal Snijders-Oomen Intelligence Test (in versions SON-R 2.5–7 and SON-R 6–40), or Weschler Intelligence Scales (in versions WISC III, WISC IV and WAIS III). SON assesses intelligence with a focus on the individual's learning ability [20]. The minimal need for verbal instructions that can be replaced by statements, allows estimating total IQ without

underestimating the result due to poor understanding of verbal instructions and poor vocabulary [20].

Weschler Scales for intelligence (WISC)—This assessment is important for the diagnostic process, allowing a complete and careful analysis of the cognitive abilities of children, adolescents and adults, according to the version of the test used [21], 22. The WISC III and WISC-IV aim at evaluating the intelligence of individuals between 6 and 16 years of age [23–25]. For the assessment of adults, the Weschler Intelligence Scale for Adults, 3rd edition (WAIS III), was used, comprising 14 subtests to evaluate people aged between 16 and 89 years [22].

Autism Treatment Evaluation Checklist (ATEC)—ATEC was used before and 12 months after a regular treatment received when attending the ambulatory to assess whether the polymorphism impacts the ASD patient response to treatments and interventions. ATEC is an instrument that allows assessing the patient's performance in response to different types of treatments and intervention models. It consists of 77 questions divided into 4 areas: Area I, Discourse, Language and Communication [14]; Area II, Sociability [20]; Area III, Cognitive and Sensory Awareness [18] and Area IV, Health, Physical and Behavior [25] [12]. Thus, it is possible to calculate the difference (ΔATEC) between the scores obtained after an intervention period (final ATEC) and those obtained in the first assessment (initial ATEC), that is, ΔATEC = final ATEC – initial ATEC. Higher scores indicate greater losses, which allow to admit that the patient's improvement is verified when ΔATEC < 0 [26].

Genotyping

Oral mucosal cells were collected with a sterile swab and genomic DNA extracted using a commercially available kit (A&A Biotechnology, Gdańsk, Poland). DNA extraction was performed with NaCl (Abrão et al. 2005). The TaqMan® Pre-Designed SNP Genotyping Assays kit for the Thr92Ala-DIO2 polymorphism and its respective Master Mix (TaqMan® Universal Master Mix II, with UNG) from Thermo Fisher (USA) were used. SNPs were coded by as non-polymorphic homozygous (TT), polymorphic homozygous (AA), and heterozygous (TA). To examine the main effect of the Thr92Ala-DIO2, the three genotypes were considered separately under the additive model. All members of the care team and study participants were blinded to the genotype results.

Statistical analysis

Power analysis—Patients were stratified in three groups according to their DIO2 genotype, i.e. TT, AA, TA. Considering the prevalence of 12–36% (midpoint 24%) for Thr92Ala-DIO2 [4, 27] and ~ 1.5% for ASD in the population [28], we estimated the target population to be 10,800 children/young adults. The 2010 National Census (Brazilian Institute of Geography and Statistics [29] indicates that there are about 3.2 million individuals aged 0–19 years. Thus, assuming a sampling error of 7.6%, and having a cohort of 132 subjects, we calculated that the sample size reaches 100% power to detect a significant difference using a binomial test and a threshold level of 5% ($p < 0.05$). Furthermore, 50 typical children were added to the study as a control group for the

purpose of comparison. Therefore, the sample is sufficient to estimate the prevalence of the Thr92Ala-DIO2 polymorphism in the ASD population.

Descriptive statistical analyses were performed to obtain the sex distribution among participants, as well as the polymorphic allele in the sample, the verbal competence and the age range of the participants. A Chi-square test was performed to assess whether there was a difference among the genotypes regarding the distribution of verbal and non-verbal patients, and the distribution of the severity of symptoms assessed by ABC. Each variable was analyzed for data distribution characteristics, and normal distribution was tested by the D'Agostino–Pearson test. The averages obtained in each group for the ABC, VABS-II, IQ and ATECs assessments were compared using one-way ANOVA followed by Tukey's post-test, for variables with normal distribution, and Kruskal–Wallis test followed by Dunn's post test for the remainder variables. For IQ analyses, the total IQ provided by each of the scales was used as a way to standardize the data obtained from different instruments. The correlations between ABC vs IQ, ABC vs VABS-II, ABC vs ATEC, and ABC vs ΔATEC were tested for each group using the Pearson's correlation tests for samples with normal distribution and Spearman's correlation for the remainder variables. Data were analyzed using the GraphPad Prism 7.05. A $p < 0.05$ was considered significant.

Results

Assessment of children in the autistic spectrum disorder

MAF in carriers of Thr92Ala-DIO2 polymorphism—146 children were interviewed during the study period and 14 were eliminated based on exclusion criteria. Families of the remaining 132 children were offered participation and accepted enrollment in the study. All children completed the study with no dropouts. They were mostly male, younger than 18 years of age; approximately half was verbal (Table 1). Genotyping revealed a distribution of 30% TT, 46% AT, and 24% AA, with a MAF of 47%, which is similar to the local neurotypical reference population (chi-square $p > 0.05$ vs. ASD patients).

Thr92Ala-DIO2 polymorphism and adaptive behavior

The VABS II is an acceptable instrument in the diagnosis of intellectual and developmental disabilities. It provides an assessment of personal and social skills employed in daily living activities. VABS II consists of a three-domain structure: Socialization, Daily Living and Communication skills, and, each with its respective subdomains. The Global Adaptive Level, which reflects the scores obtained in the three VABS II domains, was not different among the three children's genotypes (Fig. S1A) but a weak positive correlation was found between the dose of the minor allele (TT = 0; TA = 1; AA = 2) and the total score ($p = 0.025$; $r = 0.2$) (Table 2).

The analyses of the Socialization domain and its subdomains Interpersonal Relationship, Play and Leisure Time and Coping Skills revealed no differences across the three genotypes (Fig. S1B–E), but a weak positive correlation was observed between the dose of the minor allele and the scores in Play and Leisure Time ($p = 0.05$; $r = 0.18$) (Table 2).

In contrast, AA ASD children had higher scores in the domain Daily Living Skills (Fig. 1a) and its subdomain Personal (Fig. 1b), but no differences were observed in the Domestic and Community subdomains (Fig. S2A–B). In addition, weak positive correlations were observed between the dose of the minor allele and the scores in Daily Living Skills (p = 0.01; r = 0.22) and Community (p = 0.02; r = 0.22) (Table 2).

AA ASD children also had higher scores in the domain "Communication" (Fig. 1c) and its subdomain Receptive (Fig. 1d), and weak positive correlations were observed between the dose of the minor allele and these scores (p = 0.01; r = 0.23) (Table 2); no effect of the Thr92Ala-DIO2 polymorphism was observed in Expressive and Written subdomains (Fig. S2C–D), or correlations between the doses of the minor allele (Table 2).

Thr92Ala-DIO2 polymorphism and clinical severity

A subgroup analysis was performed to determine whether carrying the Thr92Ala-DIO2 polymorphism affected the clinical severity of children in the ASD. The distribution of ABC scores was not affected by patients' genotype (Fig. S3A). In addition, the ABC scores were used to group the children as "low", "mild", "moderate" and "severe" (Table 3), and the respective MAFs were 12.2%, 3.7%, 12.2% and 18.9%, not different among groups (chi-square $p > 0.05$). Furthermore, no differences in MAF were observed when ASD patients were grouped in verbal and non-verbal categories (Table 4), or when the IQ test scores were plotted against genotypes (Fig. S3B).

Thr92Ala-DIO2 polymorphism and clinical progression

Patients were assessed yearly for a median uninterrupted period of 937 days (min 139 and max 1375 days), which did not vary significantly among the genotypes (Fig. S3C). The last, most recent assessment was used as "end of treatment". Baseline ATEC scores were not affected by patients' genotype (Fig. S3D), and neither were the changes in ATEC score over time (ΔATEC) (Fig. S3E): 50% of the TT patients exhibited a drop in ATEC score (improvement), whereas in AT patients, the drop in ATEC scores was 56% and in AA patients, it was 44%. Furthermore, the ATEC scores at the end of the study were also not affected by patients' genotype (Fig. S3F).

ADHD co-morbidity in children in the ASD

The frequency of ADHD in children in the ASD was assessed clinically and found to be present in 44 children (36%). Nonetheless, the MAF of Ala92Dio2 was similar in the groups of children with and without ADHD co-morbidity, i.e. 47.7 vs. 47.2%, respectively.

Discussion

The present studies revealed that although the Thr92Ala-DIO2 MAF was not increased in children in the ASD, or associated with clinical severity or progression of the disease, carrying the allele was associated with better performance in two domains of adaptive behavior, i.e. Daily Living Skills and Communication. A weak correlation between the dose of the minor allele and the intensity of these effects was observed as well.

While the study was appropriately powered to address the primary outcome, the findings of superior adaptive behavior exhibited by carriers of the minor allele should be considered exploratory. Although statistically significant differences were identified, definitive conclusions should wait until future studies designed and powered to address these specific issues are performed. Nonetheless, the present findings rely on the relative homogeneity and size of the cohort, which was followed for 2.5 years (median), as well as the in-depth psychological assessment.

A transcriptome analysis of the human temporal pole of the CNS revealed that carriers of the Thr92Ala-DIO2 polymorphism exhibit signs of ER stress and UPR [11]. Indeed, evidence of ER stress and UPR was obtained in multiple brain areas of mice carrying the Thr92Ala-Dio2 polymorphism [10]. That Thr92Ala-DIO2 is associated with ER stress and UPR is likely to be clinically relevant. For example, African-American carriers of the Thr92Ala-DIO2 polymorphism are at a higher risk for developing Alzheimer's disease [5], a condition in which ER stress has been implicated as an etiological factor [30]. Another relevant finding in the brains of mice carrying the Thr92Ala-Dio2 polymorphism was a reduction in thyroid hormone signaling in the striatum, hippocampus and pre-frontal cortex, which was associated with changes in humor and behavior [10]. This suggests that carriers of the Thr92Ala-DIO2 polymorphism are potentially at risk of developing/worsening diseases in which these two pathogenetic mechanisms, i.e. ER stress and disruption of thyroid hormone signaling, play a role.

A post-mortem analysis of different brain areas of 5 patients with ASD revealed signs of ER stress and UPR activation in the cerebellum, hippocampus and pre-frontal cortex [31]. Thus, it made sense to look at a potential association between Thr92Ala-DIO2 polymorphism and ASD. Nonetheless, after studying a cohort of 132 ASD children, we found no evidence that the MAF is increased in ASD or is associated with a more severe clinical presentation. We did find however, good evidence that children in the ASD exhibit improved adaptive behavior. A mouse carrying the Thr92Ala-Dio2 polymorphism is systemically euthyroid but exhibits a pattern of T3-responsive gene expression in the brain consistent with localized reduction in thyroid hormone signaling. These animals exhibit higher exploratory activity in the open field and in the elevated plus maze, with more risk-assessment behavior when compared with control mice [11]. They also exhibit higher mobility during the tail-suspension studies, which indicates lesser depressionlike behavior. Nonetheless, once settled in their home-cage, Thr92Ala-Dio2 mice refrain from physical activity and exhibit sleepiness and impaired short-term memory [11].

The findings in the Thr92Ala-Dio2 mouse suggest reduction in the anxiety level and a decreased tendency for depressive behavior, which is reminiscent of what was observed in carriers of the Thr92Ala-DIO2 polymorphism treated with levothyroxine [32]. While these specific mood changes were not assessed in the present investigation, it is notable that the performance in the VABS-II assessment was negatively affected by anxiety and depression in ASD children [33, 33, 34]. Further studies should clarify whether any of these elements played a role in the superior adaptive behavior exhibited by the ASD children carriers of the Thr92Ala-DIO2 polymorphism.

The disruption in thyroid hormone signaling observed in carriers of the Thr92Ala-DIO2 polymorphism is constitutive and thus could have substantial impact in brain development and function. For example, several studies have shown that children with inactivating mutations in the thyroid hormone receptor beta (TRb) exhibit an increased frequency of ADHD [34–36], which happens to be a common ASD co-morbidity [37]. In fact, ADHD and ASD partly overlap in their pathophysiology and phenomenology in SOCIALIZATION and COMMUNICATION domains. The mechanistic underpinnings of the association between ASD and ADHD remain unknown, but neuroimaging studies identified similar structural alterations in both cortical and subcortical areas of children with ASD or ADHD. Nonetheless, we failed to identify an association between ASD, ADHD and Thr92Ala-DIO2 in the present cohort, given that the MAF was similar in ASD patients with or without ADHD.

In conclusion, despite previous reports of the association between the Thr92Ala-DIO2 polymorphism vs. lower IQ and bipolar disorder in children, in the present investigation, we failed to detect an increased MAF in a cohort of children with ASD. The severity of ASD was also not affected in carriers of the Thr92Ala-DIO2 polymorphism. Future studies should clarify what if any role the Thr92Ala-DIO2 polymorphism plays in the better adaptive level exhibited by these patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

AB is a consultant for Allergan Inc, Synthonics Inc and BLA Technology LLC; the other authors have nothing to disclose.

Funding

Funding was provided by FAPESP (2017/24615-5), PROEX (1133/2019) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

References

1. Bianco AC, Dumitrescu A, Gereben B, Ribeiro MO, Fonseca TL, Fernandes GW et al. (2019) Paradigms of dynamic control of thyroid hormone signaling. Endocr Rev 40(4):1000–1047 [PubMed: 31033998]
2. Freitas BC, Gereben B, Castillo M, Kallo I, Zeold A, Egri P et al. (2010) Paracrine signaling by glial cell-derived triiodothyronine activates neuronal gene expression in the rodent brain and human cells. J Clin Investig 120(6):2206–2217 [PubMed: 20458138]
3. Mentuccia D, Proietti-Pannunzi L, Tanner K, Bacci V, Pollin TI, Poehlman ET et al. (2002) Association between a novel variant of the human type 2 deiodinase gene Thr92Ala and insulin resistance: evidence of interaction with the Trp64Arg variant of the beta-3-adrenergic receptor. Diabetes 51(3):880–883 [PubMed: 11872697]
4. Dora JM, Machado WE, Rheinheimer J, Crispim D, Maia AL (2010) Association of the type 2 deiodinase Thr92Ala polymorphism with type 2 diabetes: case-control study and meta-analysis. Eur J Endocrinol 163(3):427–434 [PubMed: 20566590]

5. McAninch EA, Rajan KB, Evans DA, Jo S, Chaker L, Peeters RP et al. (2018) A common DIO2 Polymorphism and Alzheimer disease dementia in African and European Americans. J Clin Endocrinol Metab 103(5):1818–1826 [PubMed: 29481662]
6. Taylor P, Okosieme O, Sayers A, Pearce E, Gregory J, Lazarus J et al. (2014) Effect of low thyroid hormone bioavailability on childhood cognitive development: data from the Avon Longitudinal Study of Parents and Children birth cohort. Lancet 383:S100
7. Guo TW, Zhang FC, Yang MS, Gao XC, Bian L, Duan SW et al. (2004) Positive association of the DIO2 (deiodinase type 2) gene with mental retardation in the iodine-deficient areas of China. J Med Genet 41(8):585–590 [PubMed: 15286152]
8. He B, Li J, Wang G, Ju W, Lu Y, Shi Y et al. (2009) Association of genetic polymorphisms in the type II deiodinase gene with bipolar disorder in a subset of Chinese population. Prog Neuropsychopharmacol Biol Psychiatry 33(6):986–990 [PubMed: 19427350]
9. Colak A, Akan G, Oncu F, Yanbay H, Acar S, Yesilbursa D et al. (2013) 1508—association study of the dio2 gene as a susceptibility candidate for schizophrenia in the Turkish population; a case-control study. Eur Psychiatry 28:1 [PubMed: 21920709]
10. McAninch EA, Jo S, Preite NZ, Farkas E, Mohacsik P, Fekete C et al. (2015) Prevalent polymorphism in thyroid hormone-activating enzyme leaves a genetic fingerprint that underlies associated clinical syndromes. J Clin Endocrinol Metab 100(3):920–933 [PubMed: 25569702]
11. Jo S, Fonseca TL, Bocco B, Fernandes GW, McAninch EA, Bolin AP et al. (2019) Type 2 deiodinase polymorphism causes ER stress and hypothyroidism in the brain. J Clin Investig 129(1):230–245 [PubMed: 30352046]
12. Bianco AC, Kim BS (2018) Pathophysiological relevance of deiodinase polymorphism. Curr Opin Endocrinol Diabetes Obes 25(5):341–346 [PubMed: 30063552]
13. Diagnostic and statistical manual of mental disorders: DSM-5™, 5th ed (2013)
14. El-Ansary A (2016) Data of multiple regressions analysis between selected biomarkers related to glutamate excitotoxicity and oxidative stress in Saudi autistic patients. Data Brief 7:111–116 [PubMed: 26933667]
15. Zhang QB, Gao SJ, Zhao HX (2015) Thioredoxin: a novel, independent diagnosis marker in children with autism. Int J Dev Neurosci 40:92–96 [PubMed: 25433158]
16. Siniscalco D, Schultz S, Brigida AL, Antonucci N (2018) Inflammation and neuro-immune dysregulations in autism spectrum disorders Pharmaceuticals (Basel) 11(2):56
17. Marteleto MR, Pedromônico MR (2005) Validity of Autism Behavior Checklist (ABC): preliminary study. Braz J Psychiatry 27(4):295–301 [PubMed: 16358111]
18. Carter AS, Volkmar FR, Sparrow SS, Wang J-J, Lord C, Dawson G et al. (1998) The Vineland Adaptive Behavior Scales: supplementary norms for individuals with autism. J Autism Dev Disord 28(4):287–302 [PubMed: 9711485]
19. Vineland adaptive behavior scales: Interview edition, survey form manual. Circle Pines MN: American Guidance Service. https://link.springer.com/referenceworkentry/10.1007%2F978-1-4419-1698-3_255#howtocite
20. Laros J, Jesus G, Karino C (2013) Validação brasileira do teste não-verbal de inteligência SON-R 2½-7[a]. 1677–0471 12:233–242
21. Figueiredo V, Pinheiro S (1998) Teste de inteligência WISC-III Adaptando para a população brasileira 1. Psicologia Escolar e Educacional (Impresso) 2
22. Fernandes Lopes RM, Welter Wendt G, Rathke SM, Alves Senden D, Ferreira Da Silva RB, Lima Argimon IID (2012) Reflexões teóricas e práticas sobre a interpretação da escala de inteligência wechsler para adultos. Acta Colombiana de Psicología 15:109–118
23. Cruz MBZ (2005) WISC III: Escala de Inteligência Wechsler para crianças: Manual. Avaliação Psicológica 4:199–201
24. Vidal F, Figueiredo V, Nascimento E (2011) A quarta edição do WISC americano. Avaliação Psicológica 10:205–207
25. Macedo M, da Mota M, Mettrau M (2017) WISC-IV: Evidências de Validade para Grupos Especiais de Superdotados" WISC-IV. Revista Psicologia em Pesquisa 11:65–73

26. Mahapatra S, Vyshedsky D, Martinez S, Kannel B, Braverman J, Edelson SM et al. (2018) Autism treatment evaluation checklist (ATEC) norms: a “growth chart” for ATEC score changes as a function of age. Children (Basel) 5(2):1–12
27. Mentuccia D, Thomas MJ, Coppotelli G, Reinhart LJ, Mitchell BD, Shuldiner AR et al. (2005) The Thr92Ala deiodinase type 2 (DIO2) variant is not associated with type 2 diabetes or indices of insulin resistance in the old order of Amish. Thyroid 15(11):1223–1227 [PubMed: 16356084]
28. Christensen DL, Braun KVN, Baio J, Bilder D, Charles J, Constantino JN et al. (2018) Prevalence and characteristics of autism spectrum disorder among children aged 8 years—autism and developmental disabilities monitoring network, 11 Sites, United States, 2012. MMWR Surveill Summ 65(13):1–23 [PubMed: 30439868]
29. IBGE (2010). https://censo2010.ibge.gov.br/. Accessed 12 Dec 2020
30. Wilson EL, Metzakopian E (2020) ER-mitochondria contact sites in neurodegeneration: genetic screening approaches to investigate novel disease mechanisms. Cell Death Differ. 10.1038/s41418-020-00705-8
31. Dong D, Zielke HR, Yeh D, Yang P (2018) Cellular stress and apoptosis contribute to the pathogenesis of autism spectrum disorder. Autism Res 11(7):1076–1090 [PubMed: 29761862]
32. Peterson S, Fonseca TL, Schuff KG, McAninch E, Bianco AC, Samuels MH (eds) (2019) Effect of THR92ALA-DIO2 polymorphism on cognition, mood and health status among LT4 treated hypothyroid individuals. In: 89th annual meeting of the American Thyroid Association; 2019 October 2019; Chicago, IL: Mary Ann Liebert, Inc
33. Davidsson M, Hult N, Gillberg C, Särneö C, Gillberg C, Billstedt E (2017) Anxiety and depression in adolescents with ADHD and autism spectrum disorders; correlation between parent- and self-reports and with attention and adaptive functioning. Nord J Psychiatry 71(8):614–620 [PubMed: 28836480]
34. Hallett V, Lecavalier L, Sukhodolsky DG, Cipriano N, Aman MG, McCracken JT et al. (2013) Exploring the manifestations of anxiety in children with autism spectrum disorders. J Autism Dev Disord 43(10):2341–2352 [PubMed: 23400347]
35. Gadow KD, Devincent CJ, Pomeroy J, Azizian A (2005) Comparison of DSM-IV symptoms in elementary school-age children with PDD versus clinic and community samples. Autism 9(4):392–415 [PubMed: 16155056]
36. Hauser P, Zametkin AJ, Martinez P, Vitiello B, Matochik JA, Mixson AJ et al. (1993) Attention deficit-hyperactivity disorder in people with generalized resistance to thyroid hormone. N Engl J Med 328(14):997–1001 [PubMed: 8450877]
37. Al Mohareb O, AlMalki MH, Mueller OT, Brema I (2018) Resistance to thyroid hormone-beta co-existing with partially empty sella in a Jordanian male. Endocrinol Diabetes Metab Case Rep 2018:18–0104. 10.1530/EDM-18-0104
38. Torre P, Bertoli M, Di Giovanni S, Scommegna S, Conte C, Novelli G et al. (2005) Endocrine and neuropsychological assessment in a child with a novel mutation of thyroid hormone receptor: response to 12-month triiodothyroacetic acid (TRIAC) therapy. J Endocrinol Investig 28(7):657–662 [PubMed: 16218051]
39. Li YJ, Xie XN, Lei X, Li YM, Lei X (2020) Global prevalence of obesity, overweight and underweight in children, adolescents and adults with autism spectrum disorder, attention-deficit hyperactivity disorder: a systematic review and meta-analysis. Obes Rev

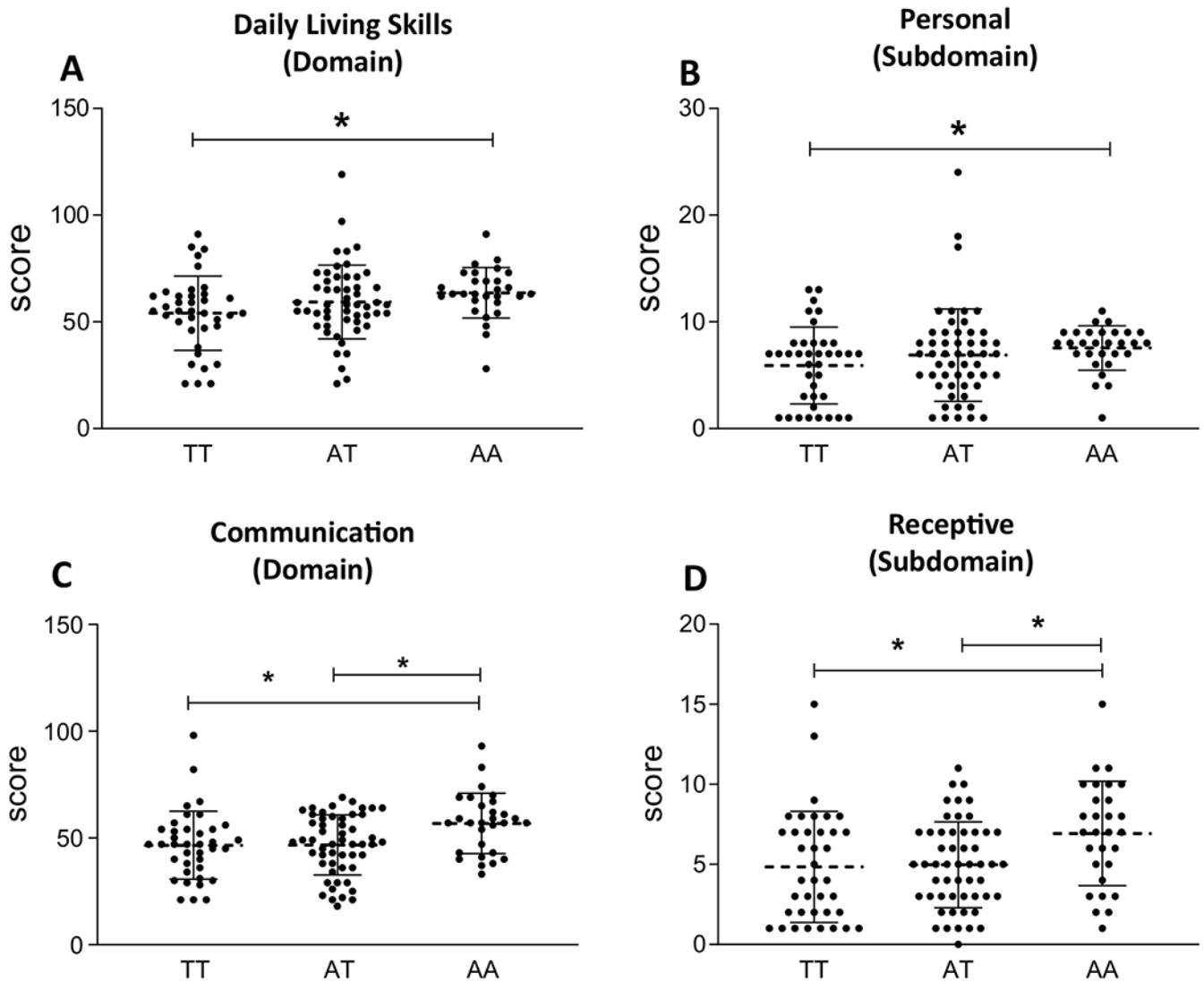

Fig. 1.

Test scores in children in the ASD. VABS-II in **a** daily living skills domain; **b** personal subdomain; **c** communication domain; **d** receptive subdomain. Results were grouped according to genotyping as TT (Thr92-DIO2), AT and AA (Ala92-DIO2). Data were analyzed using one-way ANOVA with Tukey's post-test. *$p < 0.05$

Table 1

Demographics and clinical characteristics of 132 enrolled ASD children

	n **(%)**
Gender	
Male	106 (80.3)
Female	23 (19.7)
Age (years)	
5–11	69 (52.3)
12–17	49 (37.1)
> 18	14 (10.6)
Communication	
Verbal	69 (52.3)
Nonverbal	63 (47.7)
Genotype	
TT	39 (29.5)
AT	61 (46.2)
AA	32 (24.3)

Table 2

Correlation of dose of minor allele vs. scores for adaptive behavior of ASD children

	p	r
Global adaptive level	0.025	0.2
Socialization	NS	0.02
Subdomains	NS	0.07
Interpersonal relationship		
Play and leisure time	0.05	0.18
Coping skills	NS	0.17
Daily living skills	0.01	0.22
Subdomains	NS	0.17
Personal		
Domestic	NS	0.16
Community	0.02	0.22
Communication	0.009	0.24
Subdomains	0.01	0.23
Receptive		
Expressive	NS	0.15
Written	NS	0.14

Data are the Pearson's *r* coefficient correlation and *p* value (statistical significance was set to $p < 0.05$)

NS not-significant

Table 3

Genotype distribution according to severity of autistic symptoms

Genotype	Low (%)	Mild (%)	Moderate (%)	Severe (%)
TT	6.5	1.6	8.1	14.5
AT	11.3	4.1	9.7	20.2
AA	6.5	1.6	7.3	8.9

Low, mild, moderate and severe are different degrees of symptoms severity according to the ABC assessment test

Table 4

Genotype distribution for ASD children according to ability to communicate

Genotype	Verbal (%)	Nonverbal (%)
TT	15.9	13.6
AT	21.9	24.2
AA	14.5	9.9

Assignment to “verbal” vs “nonverbal” categories was made through clinical assessment