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A NOVEL *DLGAP2* VARIANT IDENTIFIED AFTER EXOME SEQUENCING OF AN INFANT WITH AUTISM SPECTRUM DISORDER (ASD)

UNA NUEVA VARIANTE DEL GEN *DLGAP2* IDENTIFICADA TRAS LA SECUENCIACIÓN DEL EXOMA DE UN NIÑO CON TRASTORNO DEL ESPECTRO AUTISTA (TEA)

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ABSTRACT

Autism spectrum disorder (ASD) encompasses various conditions related to neurological development alterations, affecting millions of people worldwide according to the World Health Organization. ASD displays a multifactorial etiology arising from complex interactions between genetics, epigenetics, and environmental factors. ASD diagnosis is primarily based on behavioral and neuropsychological evaluation; recent years have seen an increase in the use of various genomic analysis technologies to attempt to generate a map of potentially implicated genes. This study focused on an 8-year-old boy with a complex clinical history from birth. The patient was diagnosed at the age of four with ASD, level 3, due to difficulties in all areas of development (language, social interaction, play, cognition, and behavior). Given the severity of his phenotype, various genetic studies of increasing sensitivity were conducted including a targeted exome sequencing enriched using the Twist Human Customized Core Exome Kit. The objective of this study was to identify genetic variants potentially associated with the reported clinical diagnosis of ASD. Data were normal for karyotype and for *FMR1* gene exon 1 sequencing. Comparative genomic hybridization (CGH) array and targeted exome sequencing were also performed. These additional studies identified several genetic changes compared to reference genomes, 49.41 kb deletion (coordinates 94,621,121-94,670,533) encompassing part of the *GPC* gene in band 13q31.2, as well as heterozygous changes in the coding sequence of the *DHDDS* and *DLGAP2* genes with uncertain clinical significance according to reports from the laboratory responsible. However, the detection of the variant in the *DLGAP2* gene seems not to be a random occurrence since a relevant number of publications are pointing out a connection with ASD diagnosis. The extensive genetic characterization performed allowed the detection of several variants in his genome. Our evaluation of the clinical data and the identified genomic changes confirms the relevance of the new *DLGAP2* gene variant and are compared with the literature to suggest phenotype-genotype correlations.

Key words: autism spectrum disorder, whole exome sequencing, rare variants, *DLGAP2* gene

RESUMEN

El trastorno del espectro autista (TEA) engloba diversas patologías relacionadas con alteraciones del desarrollo neurológico que afectan a millones de personas según la Organización Mundial de la Salud. Su etiología multifactorial surge de interacciones complejas entre genética, epigenética y factores ambientales. El diagnóstico se basa principalmente en estudios conductuales y evaluación neuropsicológica pero últimamente ha habido un aumento en el uso de diversas tecnologías de análisis genómico para intentar generar un mapa de posibles genes implicados. Este estudio se centró en un niño de 8 años con una historia clínica compleja desde el nacimiento. El paciente fue diagnosticado a los 4 años con TEA, grado 3, por dificultades en todas las áreas del desarrollo (lenguaje, interacción social, juego, cognición y conducta). Por la gravedad de su fenotipo, se realizaron estudios genéticos de creciente sensibilidad, incluyendo una secuenciación dirigida de exoma, enriquecida mediante el kit *Twist Human Customized Core Exome*. El objetivo de este estudio fue identificar variantes genéticas que puedan explicar el diagnóstico TEA descrito. El cariotipo y la secuenciación de exón 1 del gen *FMR1* fueron normales. El análisis mediante hibridación genómica comparativa (CGH-array) y la secuenciación dirigida de exoma identificaron cambios genéticos, incluyendo una deleción de 49,41kb (coordenadas 94.621.121-94.670.533) que abarca parte del gen *GPC*, en banda 13q31.2, así como cambios heterocigotos en la secuencia codificante de los genes *DHDDS* y *DLGAP2*, de significado clínico incierto según los informes del laboratorio. Sin embargo, la detección de una variante en *DLGAP2* no parece algo aleatorio ya que un número relevante de publicaciones sugieren una conexión con un diagnóstico TEA. La amplia caracterización genética realizada permitió la detección de diversas variantes en su genoma. Nuestra evaluación sobre los datos clínicos y sobre los cambios genómicos identificados confirma la relevancia de la nueva variante del gen *DLGAP2* y son comparados con la literatura para sugerir correlaciones fenotipo-genotipo.

Palabras clave: trastorno del espectro autista, secuenciación del exoma completo, variantes raras, gen *DLGAP2*

INTRODUCTION

Autism spectrum disorder (ASD), commonly known as autism, comprises a group of different neurodevelopmental disorders, generally with early onset and an average age of diagnosis of five years (van 't Hof et al., 2021). Its clinical diagnosis is characterized by significant heterogeneity and consists of identifying typical deficiencies in social interaction, verbal and non-verbal communication, and preference for repetitive interests or behaviors (Singui and Smith-Hicks, 2023; Tajik-Parvinchi et al., 2023). Significantly, most individuals diagnosed with ASD also present other neurodevelopmental disorders (e.g., intellectual disability), neurological disorders (e.g., frequent manifestation of epileptic processes), and/or mild neurological motor signs, making differential diagnosis complex as many mental health and neurodevelopmental conditions present overlapping symptoms with autism (Liu et al., 2017; Peall et al., 2024).

The overall prevalence of ASD is 27.6 per 1,000 (one in 36 children) at eight years old, and it is 3.8 times more frequent in boys than in girls (4% vs. 1%) (Leow et al., 2024). However, this prevalence varies substantially across studies, depending on racial and ethnic groups (Maenner et al. 2023). Different explanations for this marked difference have been suggested, including greater public awareness and increased availability and sensitivity of diagnosis (Zeidan et al., 2022). There is evident clinical heterogeneity and developmental trajectories of underlying pathologies in ASD, even among monozygotic twins, with behavioral manifestations strongly influenced by genetic factors (Szatmari et al., 2016; Ho et al., 2022). This establishes a complex etiology for ASD, positing the theory of the existence of de novo germline mutations and rare inherited variants possibly converging on similar pathways affecting neuronal and synaptic homeostasis (Cantado et al., 2024), without excluding the presence of environmental risk factors (Chaste and Leboyer, 2012). At present, the genetic contribution to ASD is strongly supported by an increasing number of studies, with high heritability estimates, such as concordance rates of 70-90% among monozygotic twins (Ho et al., 2022; Schaefer and Mendelsohn, 2013). However, the potential influence and/or functional connection of multiple changes found in the genome of individuals with ASD with the manifestation of this disorder is still unclear. As a result, it is challenging to define specific variants directly causing ASD (Wain et al, 2018; Myers et al., 2020). Disorders with a well-defined genetic basis, such as Down syndrome (trisomy of chromosome 21), Rett syndrome (alterations in the *MECP2* gene), and several pathologies defined by expansions of tandem repeat sequences, such as Fragile X syndrome (FXS), myotonic dystrophy type 1 (DM1), or Huntington's disease (HD), have been associated with some syndromic forms of ASD, providing the first direct evidence of a genetic etiology or defect in the clinical manifestations of autism. However, a clear monogenic cause has been identified in less than 20% of patients, with most of our current knowledge about ASD genetics derived from the identification and analysis of rare variants (Bicks and Geschwind, 2024; Escudero and Sepúlveda, 2024; Krishnan et al., 2015; Angeard et al., 2018; Piras et al., 2020). The identification of rare but relevant genetic factors in individuals diagnosed with ASD has been conducted over the last decade thanks to the development of new genetic tools capable of "reading" changes in the genome with high precision. These include arrays and next-generation sequencing (NGS) (Vicari et al., 2019; Choi and An, 2021). Large-scale genomic studies using these tools have shown that approximately 20-40% of ASD cases have a defined genetic variant associated. Specifically, the presence of large genomic changes (deletions, duplications, or translocations) has been observed in 3% of ASD cases, but using arrays, the presence of copy number variations (CNVs) has been detected in 10-30% of ASD patients. These CNVs include microdeletions or micro duplications of more than 1 kb in specific regions of the genome, involving the loss or gain of genes present in these regions. Many genetic and critical genomic CNVs have been revealed in ASD and are commonly found in patients with neurodevelopmental disorders (Takumi and Tamada, 2018). The correlation between CNVs and ASD has been established for multiple loci, with the identification of potentially clinically relevant genes based on their known biological function, such as *SHANK2*, *SHANK3*, *NRXN1*, *NLGN4*, *PCDH10*, *DIA1*, *NHE926*, and *PARK2*. On the other hand, the incorporation of new genome sequencing techniques (NGS) in the study of ASD is allowing the discovery of new rare gene variants associated with this diagnosis, detecting single nucleotide polymorphisms (SNPs) in the sequence of a specific gene. Even with minimal changes, theoretically, SNPs can alter the production of specific proteins with a possible connection to ASD pathogenesis. It has been estimated that the proportion of ASD associated with SNPs is between 17% and 60% (Gaugler et al., 2014), suggesting a significant contribution through the identification of numerous variants associated with ASD that provide new insights into the genomic architecture of this pathology (Trost et al., 2022).

This article presents a clinically diagnosed case of ASD with continuous functional follow-up, for which various genetic studies have also been performed. The results have revealed specific changes in the genome of the studied individual with uncertain and/or unknown clinical significance. In-depth analysis of these changes aims to foster a better understanding of genotype-phenotype correlation in individual ASD cases and to optimize their clinical management.

MATERIALS AND METHODS

Case presentation

We present the case of an 8-year-old boy, the result of a natural pregnancy, without threats of miscarriage or preterm birth, delivered by cesarean section at 40 weeks due to lack of progression. The newborn (NB) had normal parameters (weight=3410 g, length=48.5 cm, head circumference (HC)=36.5 cm, and Apgar 9/10). The father was 41 years old and the mother 35 at the time of birth, with a single and first pregnancy, no previous miscarriages, and one live birth (GAV 1/0/1), i.e., no history of miscarriages and/or pregnancy complications (normal ultrasound series and blood pressure), normal neonatal screening for congenital diseases and otoacoustic emissions (OAE). Since birth, the child presented a distinctive clinical picture, with feeding difficulties and gastroesophageal reflux diagnosis. At 21 months of age, he underwent surgery for left cryptorchidism. He was under nephrology follow-up for intermittent hematuria until discharge at age 6, and in the Child Mental Health Unit (CMHU) from age 2, with ongoing clinical follow-up and currently on guanfacine 1 mg/day medication. At age 3, ASD was suspected due to characteristic patterns, such as a tendency for isolation, significant difficulties in speech and language development, and poor school performance. He showed no social interest, and his play was limited and stereotyped. By age 4, communication, language, and social skills were significantly underdeveloped relative to his chronological age (CA). The E. Schopler Psychoeducational Profile (PEP) showed scores ranging from one to one and a half year below his CA of 4 years (overall development= 2 years and 8 months). Finally, scores on the Reynell III Language Development Scale Module I for preverbal or single-word children were very low (comprehension scale= 1 year and 10 months and expression scale= 1 year and 9 months), surpassing the cut-off points associated with an ASD diagnosis. Based on the results of ADOS-2, ADI-R algorithm, and other assessments, a grade 3 ASD diagnosis was concluded at age 4. Over the years, the child continued to experience language difficulties, requiring supervision in daily tasks, with play remaining individual and repetitive. At age 7, spontaneous manual, digital, and postural movements appeared in various activities and routines, predominantly in the distal upper left limb. Brain MRI, EMG, and sensory and motor nerve conduction studies (ENG) showed no significant lesions, with results within normal limits. A diagnosis of Stereotypic Movement Disorder was concluded, characterized by repetitive movements without purpose or intent. At age 9, neurophysiology tests recorded a normal EEG, while quantitative EEG (qEEG) mapping indicated signs of cerebral immaturity for his age due to increased absolute (AP) and relative (RP) power values in the delta band and increased frontal-occipital coherence in the same frequency range. He also showed moderate impairments in visual and auditory attention, evidenced by increased amplitudes in Visual and Auditory Cognitive Evoked Potentials (403.14 ms/1.63µV and 377.99 ms/4.99µV, respectively, with normal values: 261.8-386.6ms/25.78µV and 248.7-373.96ms/21.3µV). At age 8, social and speech/language difficulties intensified, with challenging and aggressive behaviors (hair-pulling, pushing, slapping). His speech was repetitive and echolalic (constant and intense repetition). In a recent follow-up at age 10, it was confirmed that the child does not have reduced mobility but rather profound motor disinhibition, requiring constant adult supervision to prevent him from running off and getting hit. He needs continuous parental supervision. He still shows behavioral regulation deficits, intense frustration, crying, and complaints without apparent reason during simple activities, even when accompanied by his parents. His spontaneous speech is limited and characterized by echolalia of short phrases, with slow responses to verbal demands, and occasionally answers briefly after much insistence. Notably, early work with various speech and occupational therapy professionals, along with intense maternal collaboration in anticipating activities and school excursions as well as clinical visits, helps reduce his intense anxiety after three to four sessions, allowing him to self-regulate, control, and start collaborating and learning receptively. Under these conditions, his language shows social characteristics and he maintains better eye contact. He is currently under clinical follow-up by Neuropediatrics and CMHU, with pharmacological treatment of 20 mg/day MPH EFG (Rubifen®) and 0.5 mg/day risperidone (Risperdal®).

Karyotype

Venous blood samples (5-10 mL) were collected in sterile tubes containing heparin as an anticoagulant. The samples were transported to the laboratory at room temperature and processed within 24 hours of collection to ensure optimal cell viability, following standard protocol that included cells fixation, slide preparation (cells spreading and Giemsa staining), and microscopic analysis of metaphases, as described in Arsham et al., 2017. The karyotype was analyzed for numerical and structural chromosomal abnormalities. The analysis followed the International System for Human Cytogenetic Nomenclature (ISCN) guidelines to ensure standardized reporting.

Genomic DNA Extraction for genetic analysis

Peripheral venous blood samples (5 mL) were collected in ethylenediaminetetraacetic acid (EDTA) vacutainer tubes. Genomic DNA was extracted from all of the blood

samples using the QIAamp DNA Blood Mini Kit (Qiagen) with the Qiacube or EZ2 equipment. DNA quantification was performed with the Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) and QIAexpert System (Qiagen, Hilden, Germany).

FMR1 CGG repeat amplification

To detect mutations in the *FMR1* gene, genomic DNA (gDNA) was amplified by PCR targeting the CGG repeats in the 5' untranslated region of the *FMR1* gene. The size of the amplifies fragment was then genotyped using the ABIPRISM 3130 automated sequencer and analyzed with the GeneMapper software. The measurement range for the repeats is 5 to 200 CGG, with normal alleles considered to be approximately between 5 and 44 CGG repeats.

CGH array

The Genome-Wide Human CytoScan 750K Array (Affymetrix, CA, United States) was used to analyze genomic alterations according to the manufacturer's protocol and as summarized in Ghazali et al., 2023. Fragments of DNA (200 µg) were hybridized with pre-equilibrated Affymetrix Chip CytoScan 750K at 50 °C for 18 h. The arrays were washed and stained using GeneChip Fluidics Station 450 and Affymetrix GeneChip Command Console Software, followed by data analysis using Affymetrix Chromosome Analysis Suite (ChAS) software. The molecular karyotype (or array CGH) aims to detect chromosomal imbalances too small (e.g., deletions or duplications) to be visualized by conventional karyotyping (detection limit >5 Mb). Array CGH can detect genomic imbalances with a diagnostic resolution of 50-100 kb in an individual's genome.

Next-generation sequencing (targeted exome)

To achieve a higher sensitivity level, targeted exome sequencing was performed using next-generation sequencing (NGS), aiming to detect point variants and possible copy number variations (CNV) in the entire genome, prioritizing changes in genes associated with autism clinical phenotypes (HP:000017, HP:0000753, HP:0000729) and intellectual disability (HP:0001249, HP:0001526, HP: 0002187, HP:0002342, HP: 0006887, HP: 0006889, HP:0010864). Specifically, the coding regions (exons) of the gene and adjacent intronic regions (16 nucleotides upstream and downstream of each exon) were analyzed using the Twist Human Exome Enrichment system (Twistbioscience) and ultrasequencing (2x100bp, Novaseq 6000 platform, Illumina) and the results were analyzed with the Varsome Clinical platform. The variants detection limit was 80% with >100X and 20% with >50X coverage.

RESULTS

Based on the identified clinical picture, genetic studies were proposed for the patient by obtaining his genomic DNA and conducting various tests in chronological order, classified by increasing sensitivity, to identify the potential origin of the different pathologies observed, even before his ASD diagnosis.

At age 3, two genetic studies were conducted. First, a normal 46XY karyotype was confirmed without any evident chromosomal changes. Secondly, the 5' non-coding region of the *FMR1* gene was also analyzed, amplifying the CGG repeat size in this region via PCR. This test is regularly done because it is confirmed a connection between ASD and FXS. Between 2-6% of children diagnosed first with autism have FXS, and approximately 3% of children diagnosed first with FXS have autism. When the CGG repeat expands beyond 200 repetitions, this sequence changes to a methylated status on the cytosines, ultimately repressing *FMR1* protein expression. Sizes between 6-55 CGG repeats are considered normal within a population. In the patient under study, amplification revealed a pattern corresponding to an allele with 30 (+/-1) CGG repeats, which is within normal limits. It's important to note that this analysis does not detect other types of mutations causing FMR1 loss of function, reported in rare cases from point mutations or intragenic deletions, or by a pathogenic expansion in the adjacent *FRAXE* gene, resulting in a clinical outcome similar to FXS. An expression study of *FMR1* and/or *FRAXE* genes should be included to complement the PCR approach, fully ruling out these genes' involvement in the ASD diagnosis. The negative results from these two approaches led to broader and more sensitive exploratory genetic studies (Willemsen et al., 2011).

In this case, the clinical laboratory report provided (hg19 version for genome comparison) describes detecting a heterozygous deletion of uncertain significance in the 13q.31.2 region, with genomic coordinates (94,621,125-94,670,533) and a size of 49.41kb (at the detection limit for this technique). This region, also according to the report, contains part of the glypican 6 gene (*GPC6*, OMIM 604404) sequence. No other gene is involved. We reviewed the ClinVar repository for sequence changes described for the *GPC6* gene and its potential connection to autism or intellectual disability phenotypes. Although there are pathogenic variants of this gene, they are mainly related to autosomal recessive omodysplasia, unrelated to neurodevelopmental disorders (Bayat et al., 2020). The rest of the array results were normal, concluding that the identified deletion is highly unlikely to be clinically relevant to the described ASD clinical picture.

NGS analysis (Varsome Clinical Platform) and interpretation of the results (Gestlab-Cointec software)

did not identify any point variant described as pathogenic or likely pathogenic. However, two heterozygous gene variants were detected. Specifically, the c.995C>G(p.Ser332Ter) change in the *DHDDS* gene, and the c.2210>T(p.Thr737Met) change in the *DLGAP2* gene (Table 1). The provided report defines both identified changes as of uncertain clinical significance based on the American College of Medical Genetics (ACMG) pathogenicity criteria after consulting databases and additional literature on these genes and their association with reported clinical variants (Richards et al., 2015). In the following sections, we provide a description and evaluation of this information, along with our own additional and updated data.

For the dehydrodolichyl diphosphate synthase subunit gene (*DHDDS*, Gene ID 79947), which is involved in the synthesis and glycosylation of dolichol in the endoplasmic reticulum, a heterozygous nonsense mutation was identified, resulting in the appearance of a premature termination codon in the resulting protein's synthesis. The mutation, a cytosine (C) to guanine (G) substitution, occurs at nucleotide 995 of its mature mRNA (995C>G) and causes a premature stop codon at amino acid (aa) position serine 332 (Ser332Ter). This gene has several isoforms of the final protein, ranging in size from 240 to 334 aa. The identified mutation is located near the terminal end of its amino acid sequence, eliminating the last two aa from the sequence for those variants >332 aa. Therefore, in conjunction with its heterozygous manifestation, it seems unlikely that the observed change introduces any relevant functional alterations in the final protein or activates cellular degradation processes (nonsense-mediated decay), although *in silico* predictors for this variation suggest higher pathogenic potential compared to non-pathogenic (Varsome). This variant (annotated as rs770732789) is present in the population (gnomAD 0.0009%), recently added to the repository of annotated clinical variants (ClinVar) in February 2023, following a study in a patient with Retinitis Pigmentosa, a pathology not compatible with the symptoms developed by the patient under study. Currently, there are 369 annotated clinical variants for the *DHDDS* gene, associated with both autosomal dominant (AD) pathologies, where the patient's heterozygous state could be sufficient for possible symptom development, and recessive (AR) pathologies, where the heterozygous state would not be sufficient to cause symptoms. Initially, two de novo, nonsense, pathogenic mutations were described in this gene (R37H and R211Q) in five unrelated patients, associated with developmental delay and seizures with or without movement abnormalities (DEDSM, OMIM:617836), a dominant inheritance pathology variant (Richards et al., 2015). However, we have not identified any potential association between these and the variant found in the patient diagnosed with ASD and evaluated here, with no such clinical manifestation to date. In conclusion, it is highly unlikely that this identified genetic variation in the *DHDDS* gene has clinical relevance to the described intellectual disability as ASD clinical picture, although it is classified as a variant of uncertain significance (VUS). To entirely rule out this possibility, it is recommended to conduct a study quantifying mRNA and/or protein levels to confirm no significant change in this gene's products. Since obtaining brain tissue is unfeasible, this gene is expressed in similar amounts throughout the body, including the skin, making a study from epithelial mucosa cells equally informative (Hamdan et al., 2017).

The second variant was found in *DLGAP2* (Gene ID 9228), a gene primarily expressed in the brain and testes. Specifically, a heterozygous missense mutation was identified, involving a cytosine (C) to thymine (T) substitution at position 2210 of the mature mRNA. This change translates to the substitution of threonine (Thr) with methionine (Met) at amino acid position 737 in the amino acid sequence (Thr737Met). This mutation occurs in a central region of the primary protein isoform, which is 1055 amino acids long. This variant is identified for the first time in the *DLGAP2* gene in this work, adding to the previously 237 identified variants (ClinVar). A detailed review of the previously described variants revealed three in this gene that involve the same amino acid change (threonine to methionine) due to a single nucleotide change, but occurring in three different regions of the sequence (Thr664Met, Thr679Met, Thr941Met). These three variants were recently identified, with Thr664Met

Table 1. Genetic variants detected by targeted exome sequencing

Gen	Transmission	Genotype	Variant (c.DNA,Prot)	Classification	Consequence	DbSNP ID
DHDDS	AD/AR*	het	NM-205861.3:c.995>G NP-995583.1:p.Ser332Ter	VUS	Nonsense	rs770732789
DLGAP2	Unknonwn	het	NM-001346810.2:c.2210>T NP-001333739.1:p.Thr737Met	VUS	Missense	rs973854776

VUS: variant of uncertain significance; AD: autosomal dominant; AR: autosomal recessive

described as a benign variant, and Thr679Met and Thr941Met as VUS, so the pathogenicity of the same change identified in the ASD patient evaluated here is not immediately apparent. However, the type of change, introducing a new protein synthesis site in the sequence, and its location in the middle of the protein, suggest a more relevant role in this case, that should be further studied. Moreover, published information about this gene positions it as an interesting candidate with potential cause-effect relevance in ASD. This gene encodes a membrane protein that may play a role in synaptic organization and neuronal cell signaling, and has been described as an imprinted gene, meaning it is expressed in a parent-specific manner, in this case, only expressing variants from the father. Variations in *DLGAP2* have been described in individuals with complex autosomal dominant neurodevelopmental disorders, including ASD, schizophrenia spectrum disorder, intellectual disability, developmental coordination disorder, and attention-deficit/hyperactivity disorder (Poquet et al., 2017). Currently, there is strong evidence that the sequence of this gene frequently displays changes after the detection of genomic alterations within the chromosome 8 in individuals with ASD with severe/intellectual disability. At least 33 heterozygous variants within or including *DLGAP2* have been described in nine publications and several public databases, although not all of them have been functionally implicated. However, the number is high and continues to grow. Many of the reported variants are copy number variants (CNVs) involving *DLGAP2* along with other genes. Since the effects of the additional genes could not be ruled out as potential contributors to the patients' phenotypes, these cases were not qualified (Woodbury-Smith et al., 2020; Zarrei et al., 2019). The exome analysis report provided by the laboratory describes information on some of these studies, mentioning that this gene is relatively intolerant to loss-of-function variation (gnomAD v2.1.1).

Given all this information, the ClinGen Syndromic Disorders Gene Curation Expert (GCEP) opted to qualify cases described in the literature as evidence supporting the gene-disease relationship of variants in this gene, although they ultimately did not annotate any of the reported nonsense variants without proof of their functional impact on the protein (Cukier et al., 2014). There is indirect experimental evidence supporting the gene-disease relationship, including studies of DLGAP2 interactions with other proteins and studies with animal models. Thus, DLGAP2 interacts with DLG3, DLG4, SHANK1, SHANK2, SHANK3, NLGN4, and CASK, all factors encoded by well-known genes with variations associated with susceptibility to ASD and intellectual disability, as predicted by STRINGdb (Horner et al., 2021). Very recently, a *Dlgap2* KO mice exhibited olfactory dysfunction and impaired hippocampus-related cognitive functions, such as spatial memory, both signs of common co-occurring conditions in patients with ASD (Hsieh et al., 2023; Chen et al., 2025).

DISCUSSION AND CONCLUSIONS

All this information suggests that, although the identified genetic variant in the *DLGAP2* gene is classified as clinically uncertain in relation to the described ASD clinical picture, it would be highly interesting to conduct additional tests on the segregation of this variant and to evaluate its functionality in the patient under study, as well as in close relatives, since the implication of this gene in ASD is solid, based on other published studies. However, obtaining samples for the proposed studies would require additional familiar consents and ethical permits. To facilitate this, one option would be to attract and put together additional individuals with ASD, already reported to carry variants in the *DLGAP2* gene, for a larger study. Next experimental steps should include the design of *in silico* studies to predict the structural changes that might be caused in the protein by the introduction of the extra methionine using, for example, Alpha Fold open software (https://alphafold.com/). In case the variant identified causes relevant perturbations in the 3D structure of the protein, we would recommend the quantification of *DLGAP2* mRNA and/or protein levels to confirm whether there is a significant change in these products in the individual(s) under study. Although this second part would be challenging to execute since the gene is almost exclusively expressed in the brain and testes, with very low expression in other parts of the body, making access to relevant tissue difficult.

As a conclusion to the detailed description of this clinical case, we want to emphasize that although current traditional psychiatry research primarily relies on diagnoses based on well-characterized symptomatology and functional aspects, it is highly relevant to introduce genetic studies routinely and in parallel from the initial stages of diagnosis to support the understanding of childhood neurodevelopment processes. This should not be limited to cases with known hereditary parental pathology or immediate prenatal and postnatal evidence due to severe neonatal hypotonia, dysmorphic or malformative syndromes (such as trisomy 21, Cornelia de Lange syndrome, Phelan-McDermid syndrome, Noonan syndrome, neurofibromatosis type 1, among others). Technological advancements in genomic techniques must reach the vulnerable pediatric population.

As we have described for the *DLGAP2* gene, there are genetic factors with a high susceptibility to manifest variations in ASD phenotypes, suggesting a potential cause-effect correlation. Genetic studies will lay the groundwork for advancing to studies in which the next step is to appropriately modify these genes to

ultimately evaluate whether it is possible to modulate the pathology itself. A child correctly assessed and diagnosed with ASD should not see efforts in genetic research dwindle; rather, these efforts should serve to improve the care of the child and their family. The better the information and professional support shared, the lower the probability of parental mental health issues due to guilt, despair, and/or depression (Da Paz et al., 2018), and the fewer the errors and waste of time and money on pseudoscientific and even harmful therapies, such as chelation and hyperbaric oxygen therapy.

An important problem associated with ASD diagnosis is that when families start noticing symptoms in one of their children, it triggers a very distressing event that commonly disrupts personal and family well-being and decision-making (Medici, 2021). Finally, the inclusion of biomarkers, which are an objective way to identify and measure biological anomalies, can provide clues for the diagnosis and treatment of ASD and could predict symptom improvements from specific clinical interventions. Additionally, they can indicate if such interventions are altering or targeting an active biomedical process related to the subject's response at that time (Jensen et al., 2022).

BIBLIOGRAPHY

Angeard N., Huerta E., Jacquette A., Cohen D., Xavier J., Gargiulo M. et al. (2018) Childhood-onset form of myotonic dystrophy type 1 and autism spectrum disorder: Is there comorbidity? Neuromuscul. Disord. 28(3):216-221. doi: 10.1016/j.nmd.2017.12.006; PMID: 29361396.

Arsham M.S., Barch M.J., Lawce H.J. (2017) The AGT cytogenetics laboratory manual. John Wiley & Sons, New Jersey, United States.

Bayat A., Dunø M., Kirchhoff M., Jørgensen F.S., Nishimura G., Hove H.B. (2020) Novel Clinical and Radiological Findings in a Family with Autosomal Recessive Omodysplasia. Mol. Syndromol. 11(2):83-89. doi: 10.1159/000506384; PMID: 32655339; PMCID: PMC7325126.

Bicks L.K., Geschwind D.H. (2024) Functional neurogenomics in autism spectrum disorders: A decade of progress. Curr. Opin. Neurobiol. 86:102858. doi: 10.1016/j.conb.2024.102858; PMID: 38547564.

Cantando I., Centofanti C., D'Alessandro G., Limatola C., Bezzi P. (2024) Metabolic dynamics in astrocytes and microglia during post-natal development and their implications for autism spectrum disorders. Front. Cell. Neurosci. 18:1354259. doi: 10.3389/fncel.2024.1354259; PMID: 38419654; PMCID: PMC10899402.

Chaste P., Leboyer M. (2012) Autism risk factors: genes, environment, and gene-environment interactions. Dialogues Clin. Neurosci. 14(3):281-292. doi: 10.31887/DCNS.2012.14.3/pchaste.

Chen Y.F., Tsao C.Y., Chen Y.T., Chang H.C., Li W.Y., Chiang J.L., Chen C.F., Chen C.H., Gau S.S., Lee K.Y., Lee L.J., Wang Y.C. (2025) Altered odor perception in Dlgap2 mutant mice, a mouse model of autism spectrum disorder. Behav. Brain Res. 5;480:115365. doi: 10.1016/j.bbr.2024.115365; PMID: 39631506.

Choi L., An J.Y. (2021) Genetic architecture of autism spectrum disorder: Lessons from large-scale genomic studies. Neurosci. Biobehav. Rev. 128:244-257. doi: 10.1016/j.neubiorev.2021.06.028; PMID: 34166716.

Cukier H.N., Dueker N.D., Slifer S.H., Lee J.M., Whitehead P.L., Lalanne E., et al. (2014) Exome sequencing of extended families with autism reveals genes shared across neurodevelopmental and neuropsychiatric disorders. Mol. Autism. 5(1):1. doi: 10.1186/2040-2392-5-1; PMID: 24410847; PMCID: PMC3896704.

Da Paz N.S., Siegel B., Coccia M.A., Epel E.S. (2018) Acceptance or Despair? Maternal Adjustment to Having a Child Diagnosed with Autism. J. Autism Dev. Disord. 48(6):1971-1981. doi: 10.1007/s10803-017-3450-4; PMID: 29307036; PMCID: PMC6347022.

Escudero S.C., Sepúlveda E.M. (2024) Pragmatic competence in people with dual diagnosis: down syndrome and autism spectrum disorder. BMC Psychol. 12(1):74. doi: 10.1186/s40359-023-01508-5; PMID: 38360760; PMCID: PMC10870447.

Gaugler T., Klei L., Sanders S.J., Bodea C.A., Goldberg A.P., Lee A.B., et al. (2014) Most genetic risk for autism resides with common variation. Nat. Genet. 46(8):881-5. doi: 10.1038/ng.3039; PMID: 25038753; PMCID: PMC4137411.

Ghazali N., Rahman N.A., Kannan T.P., Ahmad A., Sulong S. (2023) Identification of copy neutral loss of heterozygosity on chromosomes 1p, 1q, and 6p among nonsyndromic cleft lip and/or without cleft palate with hypodontia. BMC Oral Health 23(1):945. doi: 10.1186/s12903-023-03464-3; PMID: 38031027; PMCID: PMC10685534.

Hamdan F.F., Myers C.T., Cossette P., Lemay P., Spiegelman D., Laporte A.D., et al. (2017) High Rate of Recurrent De Novo Mutations in Developmental and Epileptic Encephalopathies. Am. J. Hum. Genet. 101(5):664-685. doi: 10.1016/j.ajhg.2017.09.008; PMID: 29100083; PMCID: PMC5673604.

Ho A., Towheed A., Luong S., Zucker S., Fethke E. (2022) Clinical Discordance in Monozygotic Twins With Autism Spectrum Disorder. Cureus. 14(5):e24813. doi: 10.7759/cureus.24813; PMID: 35686266; PMCID: PMC9170280.

Horner A.E., Norris R.H., McLaren-Jones R., Alexander L., Komiyama N.H. (2021) Grant SGN, et al. (2021) Learning and reaction times in mouse touchscreen tests are differentially impacted by mutations in genes encoding postsynaptic interacting proteins SYNGAP1, NLGN3, DLGAP1, DLGAP2 and SHANK2. Genes Brain Behav. 20(1):e12723. doi: 10.1111/gbb.12723; PMID: 33347690; PMCID: PMC7615670.

Hsieh M.Y., Tuan L.H., Chang H.C., Wang Y.C., Chen C.H., Shy H.T., Lee L.J., Gau S.S. (2023) Altered synaptic protein expression, aberrant spine morphology, and impaired spatial memory in Dlgap2 mutant mice, a genetic model of autism spectrum disorder. Cereb. Cortex. 33(8):4779-4793; doi: 10.1093/cercor/bhac379; PMID: 36169576.

Jensen A.R., Lane A.L., Werner B.A., McLees S.E., Fletcher T.S., Frye R.E. (2022) Modern Biomarkers for Autism Spectrum Disorder: Future Directions. Mol. Diagn. Ther. 26(5):483-495. doi: 10.1007/s40291-022-00600-7; PMID: 35759118; PMCID: PMC9411091.

Krishnan K., Wang B.S., Lu J., Wang L., Maffei A., Cang J., et al. (2015) MeCP2 regulates the timing of critical period plasticity that shapes functional connectivity in primary visual cortex. Proc. Natl. Acad. Sci. USA. 112(34):E4782-91. doi: 10.1073/pnas.1506499112; PMID: 26261347; PMCID: PMC4553776.

Leow K.Q., Tonta M.A., Lu J., Coleman H.A., Parkington H.C. (2024) Towards understanding sex differences in autism spectrum disorders. Brain Res. 1833:148877. doi: 10.1016/j.brainres.2024.148877; PMID: 38513995.

Liu X., Li Z., Fan C., Zhang D., Chen J. (2017) Genetics implicate common mechanisms in autism and schizophrenia: synaptic activity and immunity. J. Med. Genet. 54(8):511-520. doi: 10.1136/jmedgenet-2016-104487; PMID: 28314733.

Maenner M.J., Warren Z., Williams A.R., Amoakohene E., Bakian A.V., Bilder D.A., et al. (2023) Prevalence and Characteristics of Autism

Spectrum Disorder Among Children Aged 8 Years - Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2020. MMWR Surveill Summ. 72(2):1-14. doi: 10.15585/mmwr.ss7202a1; PMID: 36952288; PMCID: PMC10042614.

Medici D. (2021) Falsos Tratamientos en Neurodesarrollo Infantil. Cómo identificar engaños en TEA y TDAH. Kindle eBook; https://www.amazon.es/Falsos-tratamientos-en-neurodesarrollo-infantil-ebook/dp/B08VJM4CK1 ISBN-10: B08VJM4CK1

Myers S.M., Challman T.D., Bernier R., Bourgeron T., Chung W.K., Constantino J.N., et al. (2020) Insufficient Evidence for "Autism-Specific" Genes. Am. J. Hum. Genet. 106(5):587-595. doi: 10.1016/j.ajhg.2020.04.004; PMID: 32359473; PMCID: PMC7212289.

Peall K.J., Owen M.J., Hall J. (2024). Rare genetic brain disorders with overlapping neurological and psychiatric phenotypes. Nat. Rev. Neurol. 20(1):7-21. doi: 10.1038/s41582-023-00896-x; PMID: 38001363.

Piras I.S., Picinelli C., Iennaco R., Baccarin M., Castronovo P., Tomaiuolo P., et al. (2020) Huntingtin gene CAG repeat size affects autism risk: Family-based and case-control association study. Am. J. Med. Genet. B Neuropsychiatr Genet. 183(6):341-351. doi: 10.1002/ajmg.b.32806; PMID: 32652810.

Poquet H., Faivre L., Chehadeh S.E., Mosca-Boidron A. (2017) Further Evidence for DLGAP2 as Strong Autism Spectrum Disorders/Intellectual Disability Candidate Gene. Autism-open Access 6(6): 1-8. doi:10.4172/2165-7890.1000197.

Richards S., Aziz N., Bale S., Bick D., Das S., Gastier-Foster J., et al. (2015). Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet. Med. 17(5):405-24. doi: 10.1038/gim.2015.30; PMID: 25741868; PMCID: PMC4544753.

Schaefer G.B., Mendelsohn N.J. (2013) Professional Practice and Guidelines Committee. Clinical genetics evaluation in identifying the etiology of autism spectrum disorders: 2013 guideline revisions. Genet. Med. 15(5):399-407. doi: 10.1038/gim.2013.32.

Singhi P., Smith-Hicks C. (2023) Early Diagnosis and Management of Autism Spectrum Disorder (ASD) in Low-Resource Countries-Challenges and Strategies. Indian J Pediatr. 90(4):362-363. doi: 10.1007/s12098-023-04481-4; PMID: 36800162.

Szatmari P., Chawarska K., Dawson G., Georgiades S., Landa R., Lord C., et al. (2016) Prospective Longitudinal Studies of Infant Siblings of Children With Autism: Lessons Learned and Future Directions. J. Am. Acad. Child Adolesc. Psychiatry. 55(3):179-87. doi: 10.1016/j.jaac.2015.12.014; PMID: 26903251; PMCID: PMC4871151.

Tajik-Parvinchi D., Rosenbaum P., Hidecker M.J.C., Duku E., Zwaigenbaum L., Roncadin C., et al. (2023) Construct Validity of the Autism Classification System of Functioning: Social Communication (ACSF:SC) Across Childhood and Adolescence. J. Autism Dev. Disord. 53(8):3246-3256. doi: 10.1007/s10803-022-05608-w; PMID: 35666330.

Takumi T., Tamada K. (2018) CNV biology in neurodevelopmental disorders. Curr. Opin. Neurobiol. 48:183-192. doi: 10.1016/j.conb.2017.12.004; PMID: 29331932.

Trost B., Thiruvahindrapuram B., Chan A.J.S., Engchuan W., Higginbotham E.J., Howe J.L. et al. (2022) Genomic architecture of autism from comprehensive whole-genome sequence annotation. Cell. 185(23):4409-4427.e18. doi: 10.1016/j.cell.2022.10.009; PMID: 36368308; PMCID: PMC10726699.

van 't Hof M., Tisseur C, van Berckelear-Onnes I., van Nieuwenhuyzen A., Daniels AM., Deen M., et al. (2021) Age at autism spectrum disorder diagnosis: A systematic review and meta-analysis from 2012 to 2019. Autism. 25(4):862-873. doi: 10.1177/1362361320971107; PMID: 33213190.

Vicari S., Napoli E., Cordeddu V., Menghini D., Alesi V., Loddo S., et al. (2019) Copy number variants in autism spectrum disorders. Prog. Neuropsychopharmacol. Biol. Psychiatry. 92:421-427. doi: 10.1016/j.pnpbp.2019.02.012; PMID: 30797015.

Wain K.E., Palen E., Savatt J.M., Shuman D., Finucane B., Seeley A., et al. (2018) The value of genomic variant ClinVar submissions from clinical providers: Beyond the addition of novel variants. Hum. Mutat. 39(11):1660-1667. doi: 10.1002/humu.23607; PMID: 30311381; PMCID: PMC6190575.

Willemsen R., Levenga J., Oostra B.A. (2011) CGG repeat in the FMR1 gene: size matters. Clin. Genet. 80(3):214-25. doi: 10.1111/j.1399-0004.2011.01723.x; PMID: 21651511; PMCID: PMC3151325.

Woodbury-Smith M., Zarrei M., Wei J., Thiruvahindrapuram B., O'Connor I., Paterson A.D., et al. (2020) Segregating patterns of copy number variations in extended autism spectrum disorder (ASD) pedigrees. Am. J. Med. Genet. B Neuropsychiatr Genet. 183(5):268-276. doi: 10.1002/ajmg.b.32785; PMID: 32372567.

Zeidan J., Fombonne E., Scorah J., Ibrahim A., Durkin M.S., Saxena S., et al. (2022) Global prevalence of autism: A systematic review update. Autism Res. 15(5):778-790. doi: 10.1002/aur.2696; PMID: 35238171; PMCID: PMC9310578.

Zarrei M., Burton C.L., Engchuan W., Young E.J., Higginbotham E.J., MacDonald J.R., et al. (2019) A large data resource of genomic copy number variation across neurodevelopmental disorders. NPJ Genom. Med. 4:26. doi: 10.1038/s41525-019-0098-3; PMID: 31602316; PMCID: PMC6779875.

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Protection of human and animal subjects.
The authors declare that no experiments were performed on humans or animals for this study.

CONFIDENTIALITY OF DATA

The authors declare that no patient data appear in this article. Furthermore, they have acknowledged and followed the recommendations as per the SAGER guidelines depending on the type and nature of the study.

RIGHT TO PRIVACY AND INFORMED CONSENT

The authors declare that no patient data appear in this article