Further Evidence for DLGAP2 as Strong Autism Spectrum Disorders/Intellectual Disability Candidate Gene

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Abstract

Autism spectrum disorders are classified as neurodevelopmental disorders characterised by diminished social communication and interaction. The core symptoms typically coexist with other medical conditions such as intellectual disability. The involvement of rare copy number variations of varying expressivity and penetrance as risk factors in autism spectrum disorders/intellectual disability phenotypes has been highlighted in large series. The DLGAP2 gene, whose glutamatergic postsynaptic density product may play a role in synaptogenesis and plasticity, has been identified as a novel candidate on the basis of 2 de novo duplications in sporadic non-syndromic autism spectrum disorders/intellectual disability males. It has also been suggested that increased DLGAP2 gene expression may contribute to the pathogenesis of schizophrenia spectrum disorders. Based on these results and after fine phenotyping of another patient with a de novo duplication involving DLGAP2 and presenting with autism spectrum disorder intersecting early-onset schizophrenia spectrum disorder, we gathered an international series of 9 cases (6 families) via international data sharing. Four sporadic males presented with autism spectrum disorders and one had other neurodevelopmental disorders. A family with 4 females displayed intellectual disability (2/4) and specific learning disorder (2/4). This study supports the hypothesis that rare copy number variations encompassing DLGAP2 with incomplete penetrance and variable expressivity could predispose to a broad range of early-onset neurodevelopmental disorders trajectories including autism spectrum disorders/intellectual disability, highlighting the existence of common predisposing factors to these overlapping phenotypic spectrums.

Keywords: Autism spectrum disorders; Array-CGH; Copy number variation; Intellectual disability; Neurodevelopmental disorders; DLGAP2

Abbreviations: CNV: Copy Number Variation; ASD/ID: Autism Spectrum Disorders/Intellectual Disability; EO-SSD: Early-Onset Schizophrenia Spectrum Disorder; ID: Intellectual Disability; EO-ND: Early-Onset Neurodevelopmental Disorders

Introduction

Autism Spectrum Disorders (ASD) comprise a wide range of heterogeneous early-onset persistent neurodevelopmental conditions characterised by significant impairments in reciprocal social interaction, diminished verbal and non-verbal communication and repetitive restricted stereotyped behaviour [1]. The core symptoms of ASD typically coexist with other medical conditions such as Intellectual Disability (ID), seizures, abnormality in sensory stimuli...
Patients and Methods

French array-CGH network and European Decipher database

A collaborative study was set up to colligate all of the French cases carrying an 8p23.3 microduplication including a part or the entire DLGAP2 gene. The decipher database was also interrogated and allowed us to find new cases in Australia (patient 5) and England (patients 6 to 9).

Cytogenetic and molecular analyses

Patients 1 to 4 were studied within the French array-CGH network. These platforms used either the Human Genome CGH Microarray 44, 60, 180K from Agilent (Agilent Technologies, Santa Clara, CA, USA) or the Illumina HumanHap300 array (Illumina, San Diego, CA, USA) according to the manufacturer's protocol. For patients 1 to 3, data were processed either with Cytogenomics (V2.7.22) software or with feature extraction (FE10.7.1.1) and Genomic Workbench (V5.0.1.4) software (Agilent Technologies) in the Hg19 genome assembly. For patient 4, image analysis and automated CNV calling was performed using Genomewide v.2010.3 and CNV Partition v.3.1.6. When 8p23.3 microduplication was identified through array-CGH, the confirmation and parental studies were performed using quantitative PCR (qPCR).

Patients 5 to 9 were studied with a 60K Oligo-arrays (BlueGnome) International Standard Cytagenomic Array (ISCA) and data were analysed with either BlueMulti (V2.5) or (V2.2) software. Test DNA was referenced against same-sex control DNA. In Australia, a second array (Agilent technologies, 15K custom design via e-array website) was performed to confirm this chromosomal alteration in the proband and parental studies were performed using FISH with the probe RP11-439C15 obtained from The Centre of Applied Genomics, Hospital for Sick Children, Toronto, Canada. Informed consent was obtained for all tested patients.

Multidisciplinary phenotyping methods for index patient 1

Global neurodevelopmental phenotyping was performed thanks to a multidisciplinary assessment platform. The patient and his parents underwent retrospective semi-structured trio-interviews regarding their personal and familial medical, developmental and life-event history, and especially by the psychiatrist about ASD features, according to Autism Diagnostic Interview-Revised (ADI-R). Communication and reciprocal social interaction on the one hand, as well as interests, play and behaviour patterns on the other hand were explored. Verbal and non-verbal tasks tested intellectual ability (Wechsler Intelligence Scale for Children – Fourth Edition, WISC-IV), attention and executive functions, and academic skills. Speech phenotyping of receptive and expressive language, as well as phonological, lexical and syntactic levels, aimed to exclude specific language impairment. After orality (orofacial praxia and swallowing) and qualitative assessment of pragmatic difficulties, different tools, such as Peabody Picture Vocabulary Test-Revised (PPVT-R) and the Oral Written Language Memory Attention Battery Test (L2MA) were used to assess oral and written language components. Psychomotor phenotyping outlined sensori-motor functioning, sensory integration, gross and fine motor skills and body image disorders. Social cognition and psycho-affective phenotyping was performed. Theory of Mind investigation comprised first and second order False-belief tasks (Sally Ann and Smarties) and one Recognition of Faux Pas or Social Blunders Test of Baron-Cohen. Projective Psychodiagnostic Assessment was performed using the Rorschach Comprehensive System by Exner and the Thematic Apperception Test by Social Cognition and Object Relations Scale. After this multidisciplinary assessment, a DSM-5° diagnosis was also made.

Results

Patient 1 (P1)/Family 1 (F1) (France, decipher 268436)

This thirteen-year-old teenage male of European ancestry, was the only child of a non-consanguineous couple with a negative family history for ID/ASD (Figure 1). He was born at term following a normal pregnancy. Birth measurements were 3400g for weight, 47 cm for length and 35 cm for occipito-frontal circumference (OFC). Apgar scores were 8/10. There was no significant psychomotor delay in achieving expected milestones. He could sit at 8 months and could walk independently at 14 months. He had normal growth. At the sensory level, he presented no hearing deficiency but divergent
strabismus, treated by surgery and oculomotor therapy. Hypersensitivity to noise was noticed. No speech delay was reported. He pronounced his first words at 20 months and first sentences at 2 years old. At 3, his language was quantitatively and qualitatively appropriate for his development age. Before the age of 4-5 years old, he used effective verbal and non-verbal communication tools. From the age of 8-9 years old, he started using sentences about inappropriate issues, with neologisms, echolalia and hermetic speech and had developmental disorders in the fields of communication and socialization, behaviour and interests. He was potty trained for the daytime at 3 years and for night-time at 6 years. A postural deficiency syndrome was diagnosed and corrected using prism glasses, custom fit orthotics and weekly physiotherapy. There was no history of epilepsy. From admission to pre-school onwards, he presented a hindered relationship with peers. This was responsible for progressive withdrawal. The care and schooling trajectory comprised an individual care plan that combined day care in a psychiatric institution for children and mainstream schools with a classroom assistant.

The clinical examination showed mildly dysmorphic features, including puffy eyelids and cheeks, thick eyebrows and slightly down-slaing palpebral fissures. There were no malformations. The neurological examination identified minor abnormalities such as hypermetria without ataxia. The orofacial praxis examination revealed functional difficulties, including poor control of the food bolus process during deglutition despite compensatory strategies, breathing through the mouth and saliva suction during speech.

Trio interviews revealed no sleeping disorders, but feeding and oral disorders. Indeed, selective eating patterns according to texture, colour and taste were adopted since food diversification. During the face-to-face clinical interview, the boy showed difficulty maintaining eye contact. The speech evaluation revealed theatricalized prosody, inappropriate melodic patterns, delivery and voice volume. He failed to respect turn-taking and displayed a logorrhoeic monologue of long complex unfinished sentences laden with comments and digressions about favourite issues. This generic automatic disembodied speech was not meaningful outside the context, thus supporting a pragmatic language disorder. He did not understand instructions that were reworded for easier understanding or even repeated, taking them literally at face value, and answered inadequately.

The assessment of thinking processes found a massive invasion of cognitive functions by pervasive non-elaborated imaginary processes threatening to break with reality. During psychometric tests, the index patient was distracted by repetitive patterns of interest so that it was necessary to make breaks in order to refocus on tasks with precise space and time references. Very low scores with heterogeneous verbal and non-verbal reasoning skills were obtained using the WISC-IV test. Attention and executive disorders were identified. These included a significant lack of cognitive flexibility, difficulties in conceptualization and in facing complex task by developing coping skills, mental fatigability and sustained/selective attention and memory deficit.

In terms of language, average-low receptive and expressive levels were noticed. He displayed difficulties in telling a chronologically
ordered story and in reporting personal experiences in everyday life coherently. Narrative formulations got bogged down with details. The patient had difficulties with spelling and morphosyntax during dictation, with slow and clumsy writing, reading and drawing, as well as delayed logical-mathematical visuospatial and visuoconstructural skills. Standardized tests revealed oral and written comprehension difficulties with idiomatic expressions, irony metaphor and sarcasm, polysemy and underlying meanings of words.

In terms of psychosocial evaluations, he demonstrated ritualistic, restricted, repetitive patterns of behaviour and interests. He showed a search for immutability and continuity, an insistence on sameness, and difficulty coping with unexpected changes in time schedules daily. Motor stereotypes, including light body balancing and hand flapping during emotional overloads, had disappeared. No self-injurious behaviour, tantrums, or aggression toward others were noticed. There was no excessive attachment to or non-functional handling of current objects. Poor social cognition and heterogeneous theory-of-mind status were identified. In cases of objective events, he was able to attribute to others mental states that may have differed from his own, but had limited understanding of background, second-degree history and social causality. Projective Psychodiagnostic Assessment using the Rorschach Comprehensive System by Exner and the Thematic Apperception Test highlighted pre-psychotic psycho-affective vulnerability. Indeed, he was found to have porosity of physical and psychic envelopes, and a lack of physical and psychological containing comforting earthly body. Thus, a body image disorder, a fragile identity and sense of self and disorganization vulnerability associated with archaic fears and an unstable emotional state. His storytelling was disjointed and confused with the occurrence of incongruous events. He showed close identification with known imaginary cartoon and video game characters, and unclear distinction between fact and fiction. He suffered from impaired judgment and reasoning, as well as denial of reality mechanisms.

<table>
<thead>
<tr>
<th>Family</th>
<th>Sex</th>
<th>Age</th>
<th>Country of residence</th>
<th>Growth</th>
<th>Age at first words</th>
<th>Epilepsy</th>
<th>Sleeping disorder</th>
<th>Eating disorder</th>
<th>Facial dysmorphism</th>
<th>Neurological soft signs</th>
<th>DSM-5™ diagnosis</th>
<th>ASD/EO-SSD</th>
<th>SLD/ADHD/DCD</th>
<th>ASD/ID</th>
<th>ASD/ID</th>
<th>ASD/ID</th>
<th>ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>M</td>
<td>13</td>
<td>France</td>
<td>Normal [-2 SD] Microcephaly [-3.5 SD]</td>
<td>20 months</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>ASD/ID</td>
<td>Severely delayed speech</td>
<td>Significant speech delay</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Patient 2</td>
<td>M</td>
<td>11</td>
<td>France</td>
<td>Normal [+2.5 SD]</td>
<td>24 months</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>ASD/ID</td>
<td>Severe motor delay</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 3</td>
<td>M</td>
<td>5</td>
<td>France</td>
<td>Normal</td>
<td>4 years</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ASD/ID</td>
<td>ASD/ID</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Patient 4</td>
<td>M</td>
<td>5</td>
<td>Australia</td>
<td>Short stature</td>
<td>No words</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>ASD/ID</td>
<td>ASD/ID</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 5</td>
<td>M</td>
<td>7</td>
<td>England</td>
<td>Short stature</td>
<td>12 months</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ASD/ID</td>
<td>ASD/ID</td>
<td></td>
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<tr>
<td>Patient 6</td>
<td>F</td>
<td>5</td>
<td>France</td>
<td>Short stature</td>
<td>24 months</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ASD/ID</td>
<td>ASD/ID</td>
<td></td>
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</table>

Table 1: Clinical and molecular patients’ profiles, ADHD: Attention Deficit Hyperactivity Disorder; ASD: Autism Spectrum Disorder; DCD: Developmental Coordination Disorder; ID: Intellectual Disability; SLD: Specific Learning Disorder; EO-SSD: Early-Onset Schizophrenia Spectrum Disorder [American Psychiatric Association, DSM-5™, 2013], For each patient are combined phenotypically main developmental features and DSM-5™ diagnosis, genotypically CGH-Array result, genes and inheritance pattern.
Integrated global neurodevelopmental and psychiatric phenotyping identified ASD without ID but intersecting EO-SSD. Array-CGH studies revealed a 1.8 Mb de novo 8p23.3p23.2 duplication involving the DLGAP2 gene (Table 1).

**Patient 2 (P2)/Family 2 (F2) (Achropuce network, France)**

This eleven year old pre-adolescent male of European ancestry was the first child of a non-consanguineous couple with a negative family history for ID/ASD (Figure 1). He had a healthy younger brother. He was born at term following a normal pregnancy with standard birth weight (3700 g), but was quickly followed for endocrinologically-unexplained growth retardation (~2 SD) and microcephaly (~3.5 SD). During the history-taking, the parents reported eating disorders such as food selectivity without oro-facial dyspraxia, walking at 18 months and delayed cycling. The physical examination showed facial dysmorphism and corrected hypermetropia. The neurological assessment highlighted slightly impaired motor skills in single-leg standing and clumsiness and slowness in walking along a line. Patient 2 repeated a grade because of learning difficulties in written language (reading, writing, and spelling). The speech test identified oral difficulties in understanding complex instructions, as well as phonological and morphosyntactic abnormalities. He had no logicomathematical disorder and even had strong arithmetic skills. Behaviourally, he was described as sociable but hyperactive, impulsive and inattentive. Emotionally, he probably had secondary anxiety depressive and sleep disorders. The diagnosis of syndromic Neurodevelopmental Disorders with Attention Deficit Hyperactivity Disorder (ADHD), Specific Learning Disorder (SLD), and Developmental Coordination Disorder (DCD), but without the ASD/ID profile was made. The care and schooling trajectory comprised accommodated mainstream school combined with targeted rehabilitation. Array-CGH diagnosed a 220 kb microduplication of the short arm of chromosome 8. qPCR analysis revealed that the duplication was inherited from the apparently unaffected father (Table 1).

**Patient 3 (P3)/Family 3 (F3) (Achropuce network, France, decipher 282449)**

This five year old male child was the only child of a Franco-Chinese couple with a negative family history for ID/ASD (Figure 1). He was born at term following a normal pregnancy with standard parameters. He was breast-fed for 5 months and dietary variety was started at 7 months. He had no eating, digestive or sleeping disorders. He walked at 12 months, the age at which he started to use double syllables. The developmental trajectory was marked by speech and communication delay, which may have been worsened at 18 months by his mother leaving home. He pronounced his first words at 4 years old after having been potty trained between 3½ and 4 years. Since the parental separation, he rarely saw his mother and lived with his father, who was an educational assistant with two years of higher education. The physical examination of patient 3 highlighted normal growth, collapsed arches of the feet necessitating orthopaedic insoles and no neurological abnormalities. Behaviourally, he showed furtive eye contact and social withdrawal. He exhibited echolalia and a narrow interest in cars, letters and numbers that he stereotypically aligned. He appeared poorly tolerant of changes in daily habits but was never aggressive towards himself or others. The diagnosis of syndromic ASD/ID phenotype was raised. He benefited from multidisciplinary rehabilitation and took part in an institutional integration care project.

Array-CGH revealed a 325 kb microduplication of the short arm of chromosome 8. qPCR analysis revealed that the duplication was inherited from the apparently unaffected father (Table 1).

**Patient 4 (P4)/Family 4 (F4) (Achropuce network, France)**

This five year old male child was the youngest of five siblings born to a Malian father and a Sierra Leonean mother, both of whom had, been living and working in France for years. There was a negative family history for ID/ASD (Figure 1). He was born at term following a normal pregnancy with standard birth parameters. Gross motor acquisitions (sitting, walking) were not delayed. The first parental concerns at 18 months focused on the lack of reciprocal interaction (no response to forename, avoiding eye contact). The developmental trajectory was marked by non-verbal (gestual, visual) and verbal (no verbal language, no identifiable phonemes pronounced) communication deficits. Clinically and behaviourally, patient 4 showed stereotypies, hand flapping, social withdrawal and unadjusted expression of emotions during play. At the physical examination at the last follow-up at age 5, height was +2.5 SD, weight was +3 SD, OFC was +0.5 SD, and the child had a hyper pigmented spot on the scalp. There was no facial dysmorphism, and no neurological abnormalities or seizures. The parents reported an isolated sensitivity to music and dance, difficulties in falling asleep, which required sedative medication, but no eating disorders. The diagnosis of ASD/ID with overgrowth was raised. He was referred to a Specialized Day Hospital for children with ASDs.

SNP-array diagnosed a 191 kb microduplication of the short arm of chromosome 8. qPCR analysis in the parents revealed that the duplication was inherited from the apparently unaffected father. A neuropsychiatric standardized structured interview of the father did not identify any neuropsychiatric disorder (Table 1).

**Patient 5 (P5)/Family 5 (F5) (decipher 256571, Australia)**

This five year old male child of European ancestry was the only child of a non-consanguineous couple with a negative family history for ID/ASD (Figure 1). He was born at term after a normal pregnancy with standard weight. He had normal growth, but facial dysmorphism including a low anterior hairline, hypertelorism, arched eyebrows and down-slanting palpebral fissures. Sitting was acquired at 7 months, walking at 14 months. He showed a particular sensory processing disorder (hypersensitivity to noise) and had suffered from intractable (myoclonic, tonic and focal) seizures since the age of 4 years, as well as sleeping and eating disorders. He was potty trained but lost it in the course of secondary developmental regression. He used very few effective non-verbal and verbal communication tools, and had severely delayed speech with echolalia. He showed withdrawal towards peers, motor stereotypies, restricted patterns of interest and intolerance to changes in daily habits with tantrums. The diagnosis of syndromic ASD with an overlapping ID phenotype was made. He benefited from a 'First-chance Early Childhood Intervention Program'.

Array-CGH diagnosed a 720 kb microduplication of the short arm of chromosome 8. Parental FISH studies confirmed the microduplication, inherited from the apparently unaffected mother (Table 1).
Patients 6-9 (P6-9)/Family 6 (F6) (decipher 253999, 256497 and 279764, England)

Patient 6 (not on decipher), was the second child of non-consanguineous parents, both of European ancestry (Figure 1). Her perinatal history was marked by polyhydramnios during pregnancy. At 10 months, truncal hypotonia and a deficit of visual reciprocal attention were noticed. Walking was acquired at 2 years. In terms of language, she had significant speech delay, which impaired both comprehension and expression skills, and stutterers. At the age of 7, she was still in nappies at night, had a poor sleep pattern (very late asleep), necessitating medication with melatonin. Behaviourally and emotionally, she showed outbursts of anger and tantrums. The physical examination identified a short stature, a small, upturned nose, a broad nasal tip and short 5th fingers. Patient 6 only carried the 8p duplication and presented with mild ID without assessed ASD (Table 1).

Patient 7 (decipher 253999), her sister (Figure 1), was born at term following a normal pregnancy with a standard birth weight and with no neonatal concerns. In terms of feeding, she disliked lumpy foods when weaning and only ate purées until the age of 4. She was able to sit unsupported at 16 months and walk at 3 years with an unsteady gait and frequent falls. She was unable to go down stairs, or to run or jump. In terms of language, she had a significant speech delay, which impaired both comprehension and expression skills. She sometimes tended to babble rather than use intelligible speech. At the age of 4.5 years, she could count to 3. At the age of 6, she was still in nappies at night. She had behavioural problems with no sense of danger, aggression towards her siblings and bad temper tantrums. She had a poor, unsettled sleep pattern with nocturnal waking treated by melatonin. On examination, she had a short stature and generalized joint laxity, and walked with bent knees. She avoided eye contact, was very shy and “emotional” and frequently burst into tears. She had short 5th fingers, spindle shaped fingers and mild facial dysmorphism with a small nose, prominent lower lip and dimple on the chin. No epilepsy was noticed. Subject 7, whose phenotyping profile without assessed ASD, also presented with a de novo 22q11.21 duplication arr[hg19] 22q11.21(18,628,048-21,540,318)x3 dn added to the 8p duplication.

Patient 8, their mother (decipher 256497) (Figure 1), only carried the 8p duplication and presented with Specific Learning Disorder. Physical examination of patient 8 identified a short stature, a high forehead and prominent chin.

Patient 9, the maternal grandmother (decipher 279764) (Figure 1) had the 8p duplication, but also had a 2p21 microdeletion containing SLC3A1 and PREPL (arr[hg19] 2p21(44,359,271-44,433,438)x1). She presented with Specific Learning Disorder with no noticeable dysmorphic phenotype.

Discussion

Over the past decade, important advances in the genetics of ASD have emerged from studies of CNVs using whole-genome microarrays [19]. CNVs play an important role in susceptibility to ASD, which are often mediated by deletion or duplication of genes involved in synaptic function and structure.

Here we report on 6 families with 2 DLGAP2 duplications and 4 DLGAP2 disruptions with a whole range of neurodevelopmental disorders. Our findings suggest that DLGAP2 gene give rise to a broad phenotypic neurodevelopmental and psychiatric spectrum: ADHD, SLD, and DCD coexisting in one patient (P2), isolated SLD in 2 patients (P8 and P9), Isolated ID in 2 patients (P6 and P7), ASD/ID phenotype in 3 patients (P3, P4 and P5) and ASD without ID intersecting EO-SSD in index patient (Table 1 and Figure 1). Indeed, the shared genetic etiology between different neurodevelopmental and psychiatric disorders has now been largely demonstrated [8,20].

In our cohort, one CNV was de novo whereas five were inherited from mildly affected or apparently unaffected parents suggesting that they may confer susceptibility rather than play a monogenic role in neurodevelopmental pathogenesis. Our results are in favor in the “multiple hit model” for ASD, as demonstrated with the 16p11.2p2.2 microdeletion [12] for a large range of neurodevelopmental diseases. The additional hits could be CNVs, coding or regulatory mutations, as well as environmental events influencing the phenotype. All the CNV described here, except the one in patient 2, also include the CLN8 and ARHGEF10 genes. CLN8 is known to cause neuronal ceroid lipofuscinoses, a clinically and genetically heterogeneous group of neurodegenerative disorders caused by homoyzogous or compound heterozygous mutations in the CLN8 gene. ARHGEF10, Rho guanine nucleotide exchange factor (GEF) 10 is highly expressed in the central and peripheral nervous systems. In affected members of a family with slowed motor and sensory nerve conduction velocities with autosomal dominant inheritance, Verhoven et al. [21-23] identified a heterozygous missense mutation in a conserved region of the ARHGEF10 gene. The authors suggested that ARHGEF10 played a role in developmental myelination of peripheral nerves. These genes may have an effect on the phenotype of patients. Besides to the DLGAP2 duplication or disruption, patient 7 also presented with the common 22q11.21 duplication that likely contributes to the patient’s phenotype.

Other sporadic patients with duplication overlapping DLGAP2 have been reported in the literature (Figure 1). Marshall et al. [5] first reported de novo duplication with breakpoints intersecting the DLGAP2 gene in an autistic patient with below average language. His brother also had features of ASD, but did not carry the CNV which can emphasize on to the susceptibility factor theory. Pinto et al. [13] reported the case of a sporadic non-syndromic ASD male, carrying a de novo 8p23.3 duplication overlapping the 5’ end of DLGAP2 and revealed DLGAP2 as a new ASD locus. Besides, several patients were reported with DLGAP2 microdeletions and ASD [8,9]. Chien et al. [24,25] detected some common and rare genetic variants of DLGAP2 that may have implication in the pathogenesis of ASD, but they alone may not be sufficient to lead to clinical phenotypes. Li et al. [18] reported rare variants in DLGAP2 associated with an increased expression of this gene in patients with ASD and Lissiov et al. [26] reported a de novo missense mutation (chr8:g.1626547G>c, NM_001277161.1:c.2174G>C) affecting a conserved amino acid and predicted as deleterious in a male with ASD. Taken together, these studies and our series, suggest that the DLGAP2 gene is likely a common susceptible gene between schizophrenia and autism. Interestingly, our patient P1 met the criteria for both disorders. Further reports in humans and functional studies in animal models are needed to support these findings and determine the risk magnitude.

DLGAP2 (MIM 605438) product may play a role in brain glutamatergic synaptogenesis and plasticity. Our cohort is of interest since DLGAP2 belongs to the postsynaptic density complex, a protein rich specialization at the postsynaptic membrane critical for effective neural transmission and synaptic maturation. High throughput
techniques have revealed that the encoded proteins of many suspected ASD genes are located at the postsynaptic density, making this pathway a hotspot for ASD-causing mutations. At postsynaptic density, DLGAP2 could be an adapter component linking ion channels to the subsynaptic cytoskeleton in interactions with the scaffolding SHANK2 and SHANK3 proteins, which are key players in ASD pathophysiology [2,27,28]. DLGAP2 is a direct binding partner of the NRXN/NLGN/SHANK protein complex, one of the first pathways to emerge in the etiology of ASD. This complex seems to be a conserved evolutionary mechanism in regulating social behaviour and cognition. Jiang-Xie et al. demonstrated that DLGAP2-/- mice have exacerbated aggressive behaviour, whereas SHANK mutant mice have a social withdrawal phenotype. Using biochemical, electrophysiological and ultrastructural studies, they found that DLGAP2-/- mice exhibited pronounced synaptic deficits in the orbitofrontal cortex (OFC), a region that plays a critical role in behavioural inhibition and in regulating aggressive drives. They also demonstrated that DLGAP2-/- mice have a shorter and thinner postsynaptic density complex in the OFC, indicating that there are less excitatory inputs into the neurons in this region [29].

Conclusion

In conclusion, regarding DLGAP2 de novo duplications in ASD there are, to our knowledge, two previous reports [5,13] and this study add a third event. Our study supports the hypothesis that rare CNVs encompassing the DLGAP2 gene are associated with an increased risk of developing a range of cognitive and psychiatric disorders but larger case-control studies and animal models are needed to determine the autism risk in the presence of DLGAP2 duplication. This study also highlights the importance of community databases for sharing patients and relevant scientific information.

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References
