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PARK2 microdeletion in a multiplex family with autism spectrum disorder

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Abstract

Background: *PARK2* (PRKN; MIM*602544) encodes Parkin protein, an ubiquitin-protein ligase required for proteasomal degradation and operating in the synaptic compartments. Copy number variations (CNVs) involving *PARK2* have been associated with autism spectrum disorder (ASD). We report on a family with ASD (multiplex family) harbouring a microdeletion at chr. 6q26 causing *PARK2* disruption.

Methods: CNV analyses were performed using CGH/SNP-array platforms, and the detected microdeletion was confirmed by real-time quantitative PCR. Standardized psychometric evaluation was used for neurobehavioral characterization.

Results: We found an intragenic ~157 kb microdeletion of the chromosomal region 6q26 causing *PARK2* disruption in two male sibs with ASD and syndromic phenotype. They both had dysmorphic facial features with coarse faces, deeply set eyes with long horizontal palpebral fissures, long eyelashes and thick eyebrows, fleshy lips and mild skeletal problems. We found an intrafamilial clinical heterogeneity owing to different severity of the autism symptoms between the affected sibs: the younger one had minimally verbal autism and severe intellectual disability, whereas his older brother presented high-functioning autism and preserved speech. Parental analysis and real-time PCR using a PRKN fragment mapping within the deletion demonstrated that the deletion was inherited from their father having subthreshold features of ASD consisting with broad autism phenotype.

Conclusions: The study corroborates the hypothesis that *PARK2* aberrations may be associated with ASD and highlights correlations between CNV affecting *PARK2* and ASD in a multiplex family. We show remarkable intrafamilial variability in the severity of inherited ASD associated with *PARK2* microdeletion.

KEYWORDS
autism spectrum disorder, broad autism phenotype, multiplex family, PARK2 microdeletion

1 | BACKGROUND

Autism spectrum disorder (ASD) is a common, neurodevelopmental disturbance that affects more than 1% of population worldwide, characterized by defective social interaction and communication along with restricted, repetitive and stereotyped patterns of behaviours. Differences in the severity of nuclear symptoms and concurrent conditions such as intellectual disability and language disturbance contribute to an extreme clinical heterogeneity (Lai et al., 2014). Moreover, subthreshold features of ASD extend beyond family members into the general population, consisting with broad autism phenotype.

ASD is a highly heritable, multifactorial disorder with complex genetic basis (Klei et al., 2012). Patients with ASD have a higher burden of causative copy number variations (CNVs) than healthy controls (Bacchelli et al., 2020). In particular, CNVs were reported more often in multiplex families with at least two affected siblings (44%) than that in simplex families with only one affected child and one unaffected child (7%–10%) (Marshall et al., 2008). Multiplex families are more likely to inheriting disease-causing CNV, thus exhibiting a smaller contribution from de novo events than simplex families. Therefore, multiplex families harbouring ASD-risk genes, especially contributing to inherited risk, might deserve special interest (Leppa et al., 2016). However, the heterogeneity of the condition and dimensions of symptom severity support additional roles for polygenetic effects caused by numerous different alleles acting in summation or in a complex and unidentified pattern (Antaki et al., 2022; Klei et al., 2012).

PARK2 (PRKN; MIM*602544) encodes Parkin protein, an ubiquitin-protein ligase functioning in the covalent attachment of ubiquitin to specific substrates for proteasomal degradation. Point mutations and homozygous deletions or duplications of *PARK2* are the most common cause of autosomal recessive, early-onset Parkinson disease (PD). Heterozygous carriers of these mutations might be at increased risk for developing clinical symptoms of PD (Castelo Rueda et al., 2021; Huttenlocher et al., 2015). *PARK2* CNVs have also been identified in patients with neurodevelopmental disorders, including ASD, suggesting a pleiotropic action of the PARK2 gene in multiple brain processes (Conceição et al., 2017; Morato Torres et al., 2020). The ubiquitin-proteasome system operates in the pre- and post-synaptic compartments, regulating synaptic attributes, including neurotransmitter release, synaptic vesicle recycling in pre-synaptic terminals and dynamic changes in dendritic spines and the post-synaptic density (Glessner et al., 2009). Moreover, *PARK2* loss of function may lead to abnormal mitochondrial biogenesis and clearance, which is also considered to link to the pathology of ASD (Yin et al., 2016). Seventy-eight patients with ASD-associated *PARK2* CNV (46 deletions and 32 duplications) are counted in the medical literature (Bacchelli et al., 2020; Bitar et al., 2019; Capkova et al., 2019; Conceição et al., 2017; Girirajan et al., 2013; Glessner et al., 2009; Lovrečić et al., 2018; Pinto et al., 2014; Scheuerle & Wilson, 2011; Yin et al., 2016), whereas the frequency of *PARK2* CNV carrier in healthy control individuals has been estimated at less than 1% (Castelo Rueda et al., 2021; Huttenlocher et al., 2015).

We report clinical, molecular and neurobehavioral findings of two male sibs affected with ASD harbouring an intragenic ~157 kb microdeletion of the chromosomal region 6q26 causing *PARK2* disruption. This CNV was inherited from their father affected with broad autism phenotype. We describe the occurrence of PARK2 microdeletion in a multiplex family with autism spectrum disorder and highlight the remarkable intrafamilial variability in the severity of classical symptoms of inherited autism. The study findings will be discussed in the light of other clinically characterized patients with *PARK2* CNV carriers from the medical literature.

2 | METHODS

2.1 | Ethics considerations

This study was based solely on information and investigations that were carried out as part of the routine clinical care of patients with ASD. The proband (Sibling 1) was identified among patients with neurodevelopmental disorders in the context of routine genetic testing by using CNV analyses. Proband family members were studied for either clinical evidences or for inheritance analyses. All procedures performed were in accordance with the ethical standards of the local Ethical Committee and with the 1964 Helsinki declaration and its later amendments. Written informed consent was signed by the parents of the proband.

2.2 | Standardized measures

Cognitive levels were assessed according with age by using the intelligence quotient (IQ) on the Wechsler Intelligence Scale for Children—Fourth Edition (WISC IV) or by measuring the general quotient (GQ) through the Griffiths Mental Development Scales, 3rd edition (GMDS-III). Symptoms of ASD were established using the gold-standard tools for ASD diagnosis: Autism Diagnostic Interview—Revised (ADI-R) (Lord et al., 1994) and the Autism Diagnostic Observation Schedule, 2nd edition (ADOS-2) (Lord et al., 2012). The Social Communication Questionnaire (SCQ), designed for detecting risk for ASD, was used to evaluate communication skills and social functioning, providing a reasonable picture of symptoms severity. A cut-off ≥11 has been shown to maximize sensitivity and specificity in younger children (Moody et al., 2017). The Child Behaviour Checklist (CBCL) was used as a measure of emotional and behavioural difficulties co-occurring with ASD. Abnormal T-scores ≥60 for CBCL internalizing, externalizing and total T-scores were considered. The Conners' Parent and Teacher Rating Scale-Revised: short form (CPRS-R:S and CTRS-R:S) were used to assess inattention and hyperactivity problems and to rule out a possible ADHD comorbidity. Sensory processing difficulties, praxis and social participation were assessed using the Sensory Processing Measure (SPM) (Narzisi et al., 2022).

In order to define the possible occurrence of a broad autism phenotype, parents of the ASD siblings were assessed by the Italian version of the Autism Spectrum Quotient (AQ), that is a self-administered questionnaire validated for quantifying autistic traits in parents of children with ASD. Broad autism phenotype is defined as AQ scored between 1 and 2 SDs above the mean (AQ scores: 21–27) (Ruta et al., 2012).

2.3 | Molecular analyses

Genomic DNA was extracted from EDTA blood using NucleoSpin Blood kit (Macherey-Nagel, Düren, Germany). Genomic screening for CNV on the proband (Sibling 1) was performed using a SNP-array platform (GeneChip 6.0, Affymetrix, Santa Clara, CA, USA), following manufacturer's recommendations and analysed with ChAS software (v3.1; Affymetrix). Two hundred and seventy healthy controls belonging to the International HapMap Project were used as a reference sample in data analysis (Affymetrix). The proband's affected brother (Sibling 2) was then investigated by Array-CGH using the SurePrint G3 Custom CGH Microarray, 4x180K (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's protocol, with appropriate Agilent reference DNAs (Euro male and Euro female). The array data extraction and analysis were performed using CytoGenomics v.5.0.2.5 (Agilent Technologies, Santa Clara, CA, USA). The CNVs were identified by CMA analysis. The clinical impact of CNV was performed using several public databases including the UCSC Genome Browser, DECIPHER, ClinVar, OMIM and DGV database. The CNVs were then classified according to the American College of Medical Genetics (ACMG) recommendations (Riggs et al., 2020) using the web-based CNV classification tool (http://cnvcalc.clinicalgenome.org). CNVs classified as benign or likely benign were considered not clinically relevant and excluded from further evaluation. The deletion involving the *PARK2* gene was confirmed by real-time quantitative PCR (RT-qPCR) (primers fw: CCTGCTCCTCATTAGGGTCG and rv: GGAGTAGGCTGCTCTGTGGG) using *TERT* as a reference gene, on an ABI 7900 Sequence Detection System (Applied Biosystems, Foster City, CA) and DNA-binding dye SYBR Green (Invitrogen Corporation, Carlsbad, CA). The $2^{-\Delta\Delta Ct}$ comparative method was used for gene copy number calculation (Livak & Schmittgen, 2001). This analysis extended to parental DNA documented the paternal segregation of the rearrangement.

3 | RESULTS

3.1 | Case Presentation

The proband (Sibling 1) and his younger brother (Sibling 2) affected with ASD were born to unrelated parents (Figure 1a). Their father, aged 42, presented with pertinent findings including obesity and mild facial dysmorphism. He had normal cognitive level, unusual gaze contact, some difficulties in emotion recognition and reciprocal social communication consistent with broader autism phenotype (AQ score: 27). The mother, a 44-year old woman, had obesity and was otherwise neurologically normal. She did not complain any abnormalities of reciprocal social communication (AQ score: 15).

3.1.1 | Sibling 1

The child, 8-year-old male, was born at term following an uncomplicated pregnancy. Birth weight was 4090 g (85th–95th percentile), and birth length 55 cm (97th–99th percentile). Early developmental milestones were

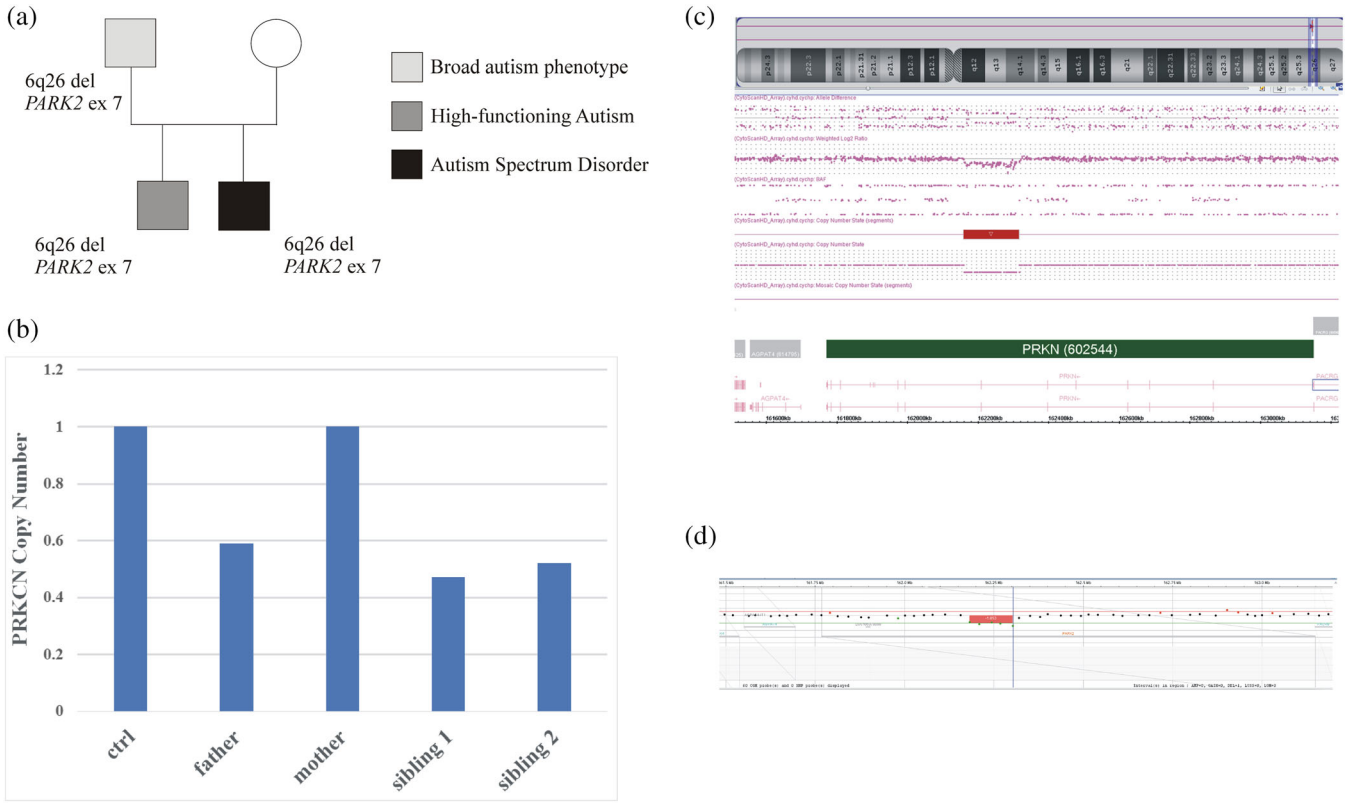

FIGURE 1 Detection of CNV at the *PARK2* locus in a multiplex family with ASD. Pedigree of the multiplex ASD family with PARK2 exonic CNV (a). An intragenic microdeletion affecting exon 7 of PRKN (NM_004562.2) was identified in the proband (Sibling 1) using SNP-array (red bar and dashed lines) (c) and in the younger affected brother (Sibling 2) using an array-CGH platform (d) The microdeletion was inherited from the father, as documented by real-time PCR using a PRKN fragment mapping within the deletion (b).

globally delayed with particular problems in social and language skills: He walked independently at 15 months; his first words were at 12 months with delayed subsequent language development.

Physical examination at age 3 showed coarse face, deeply set eyes with long horizontal palpebral fissures, long eyelashes and thick eyebrows, small ears, fleshy lips, large and spaced incisors. Moreover, he had lumbar lordosis and tapered fingers with long thumbs. He displayed social withdrawn, poor tolerance to frustrations with outbursts of hunger and self-injury behaviour. He showed restricted and repetitive interests (such as spinning objects and using electronic devices) and motor stereotypes (such as hand flapping and turning around). Psychometric evaluation using the GMDS-III showed mild delay of psychomotor development. The ADI-R, a parental interview defining the early occurrence of ASD diagnostic criteria, highlighted qualitative abnormalities in reciprocal social interaction and restricted, repetitive, stereotyped patterns of behaviours (Table 1). Standardized evaluation through the ADOS showed impaired language and communication (score:5; cut-off ≥4) along with reciprocal social interaction (score: 10; cut-off ≥7). Abdominal ultrasonography, eye examination and audiological evaluation were normal. Conventional cytogenetic analysis showed a normal male karyotype. Molecular analysis of the FMR1 gene was normal. Repeated EEG studies and biochemical investigations (including plasma amino acids analysis, serum transferrin glycoform analyses, urinary excretion of mucopolysaccharides and organic acids) were normal.

At last follow-up visit (age 8), he had obesity with weight 37.5 kg (97th–99th percentile), greater than 2 standard deviations above the WHO growth reference median. Eye contact was elusive with lateral gaze. He was distractible at times and required re-direction. The child showed fine and gross motor impairment, motor and verbal stereotypies (echolalia). Moreover, speech was slow with slightly altered prosody and literal understanding of language. Currently, he is attending the primary school, and he shows friendships selectivity and need to sameness and order, symmetry and attention to details. Formal assessment through the WISC IV scale showed normal cognitive level (Verbal Comprehension Index, VCI: 108; Perceptual Reasoning Index, PRI: 102; Working Memory Index, WMI: 79; Processing Speed Index, PSI:

TABLE 1 Molecular features of PARK2 deletion in clinically characterized patients with ASD

Patients	P1	P2	P3	P4	P5	P6
References	Yin et al., 2016	Yin et al., 2016	Yin et al., 2016	Yin et al., 2016	Scheuerle & Wilson, 2011	Conceição et al., 2017
Gender	M	M	M	M	M	M
Ethnicity	Chinese	Chinese	Chinese	Chinese	American	Portuguese
Age at diagnosis	6	2	8	8	nr	nr
Age at study time	6	15	10	10	14	nr
Clinical diagnosis	Autism	HFA	HFA	HFA	HFA, ADHD	Autism
CNV region	6q26 (162187125_ 162402914)	6q26 (162451920_ 162507690)	6q26 (162451920_ 162507690)	6q26 (162590018_ 162840211)	6q26 (162962435_ 163081102)	6q26 (161815252_ 161860054)
	Loss/exons 6–7	Loss/exon 5	Loss/exon 5	Loss/exons 2–4	Loss/exons 1–2	Loss/intron 9
Inheritance	Paternal	Paternal	Paternal	Paternal	nr	Maternal
Speech delay	−	+	+	−	−	−
Behavioural disturbance	−	−	−	−	+	nr
GQ/IQ	105	109	102	117	nr	46
ADI-R						
A. Qualitative abnormalities in reciprocal social interaction (cut-off 10)	19	11	10	5	na	Positive
B. Qualitative abnormalities in communication (cut-off V: 8, NV: 7)	10 (V)	5 (V)	8 (V)	9 (V)	na	Positive (NV)
C. Restricted, repetitive, stereotyped patterns of behaviours (cut-off 3)	7	2	3	4	na	Positive
D. Abnormality of development evident at or before 36 months (cut-off 1)	2	5	2	1	na	Positive
SCQ (cut-off: 11)	na	na	na	na	na	na
SRS (cut-off: 75)	106	109	89	50	na	na

Abbreviations: −, absent; +, present; ADHD, attention deficit/hyperactivity disorder; ADI-R, Autism Diagnostic Interview—Revised; CNV, copy number variation; GQ, general quotient; HFA, high-functioning autism; IQ, intellectual quotient; na, not assessed; nr, not reported; SCQ, Social Communication Questionnaire; SRS, Social Responsiveness Scale.

TABLE 1 (Continued)

Patients	P7	P8	P9	P10	P11	P12
References	Conceição et al., 2017	Conceição et al., 2017	Conceição et al., 2017	Conceição et al., 2017	This study (sibling 1)	This study (sibling 2)
Gender	M	M	M	M	M	M
Ethnicity	Portuguese	Portuguese	Portuguese	Portuguese	Italian	Italian
Age at diagnosis	nr	nr	nr	nr	3	5
Age at study time	nr	nr	nr	nr	8	6
Clinical diagnosis	Autism	Autism	HFA	Autism	HFA	Autism
CNV region	6q26 (161815252_161860054)	6q26 (161815252_161860054)	6q26 (162337173_162366229)	6q26 (162353308_162621707)	6q26 (162157397_162314554)	6q26 (162170536_162277522)
	Loss/intron 9	Loss/intron 9	Loss/intron 6	Loss/exons 5–6	Loss/exon 7	Loss exon 7
Inheritance	Paternal	Maternal	Maternal	Maternal	Paternal	Paternal
Speech delay	+	+	+	+	+	+
Behavioural disturbance	nr	nr	nr	nr	−	+
GQ/IQ	85	79	112	44	93	GQ < 50
ADI-R						
A. Qualitative abnormalities in reciprocal social interaction (cut-off 10)	Positive	Positive	Positive	Positive	12	16
B. Qualitative abnormalities in communication (cut-off V: 8, NV: 7)	Positive (NV)	Positive (NV)	Positive (V)	Positive	2 (V)	10 (NV)
C. Restricted, repetitive, stereotyped patterns of behaviours (cut-off 3)	Positive	Positive	Positive	Positive	8	12
D. Abnormality of development evident at or before 36 months (cut-off 1)	Positive	Positive	Positive	Positive	3	4
SCQ (cut-off: 11)	na	na	na	na	9	16
SRS (cut-off: 75)	na	na	na	na	na	na

Abbreviations: −, absent; +, present; ADHD, attention deficit/hyperactivity disorder; ADI-R, Autism Diagnostic Interview—Revised; CNV, copy number variation; GQ, general quotient; HFA, high-functioning autism; IQ, intellectual quotient; na, not assessed; nr, not reported; SCQ, Social Communication Questionnaire; SRS, Social Responsiveness Scale.

79; full-IQ: 93). Further evaluation on ADOS-2, Module 3, showed a moderate level of symptoms typical for ASD consistent with high-functioning autism. Assessment for emotional and behaviour problems revealed slightly above average scores in the CBCL subscales of internalizing disorders (withdrawn and anxiety). He scored with mild attention deficit on the Conners' scales and displayed some difficulties in 'balance and motion' and 'planning and ideas' subtests of the SPM without overt sensory profile alterations.

3.1.2 | Sibling 2

The child is a 6-year-old male, younger brother to proband sibling 1. He was born at term after an uncomplicated pregnancy with weight of 3850 g (75th–85th percentile) and length of 53 cm (95th percentile). He walked independently at 13 months and pronounced his first words at 12 months when he was noted to display some difficulties in social interaction. At examination, at age 3, he had facial dysmorphism with coarse face, low anterior hairline, hypotelorism, long horizontal palpebral fissures, long eyelashes and thick eyebrows, large ears, bulbous nose tip, thick nasal wings, long and deep lip filter and full lips. Moreover, he showed oral breathing, diffuse hair on the limbs and back, depression of the lower third of the sternum and bilateral fifth finger clinodactyly. He showed reluctance to make eye contact during natural interactions, lateral gaze, mild hypotonia, tiptoe gait and subcontinuous complex motor stereotypies with flickering and finger tapping on the mouth. Verbal language was absent with the emission of vocalizations not aimed at communication. Unusual sensory interests were also present (the child explored objects through touch and sight).

At the age of 5 years, formal developmental assessment by the GMDS-III showed delayed psychomotor development (GQ < 50; mental age: 22 months). The ADI-R highlighted abnormalities in reciprocal social interaction and in stereotyped patterns of behaviours evident at or before 36 months. Evaluation using the ADOS-2 showed a high level of symptoms typical for ASD diagnosis (Table 1).

EEG and biochemical investigations (including lysosomal enzymes analysis, serum transferrin glycoform analyses, urinary excretion of organic acids) were normal. At the age of 6 years and 6 months, body weight was 23.5 kg (50th–75th percentile); height was 127.5 cm (95th–97th percentile); and head circumference was 52 cm (25th–50th percentile). He showed motor stereotypies with restlessness and poor frustration tolerance with anger crisis and self-injury behaviour. Verbal language was absent with the production of vocalizations, not always aimed at communication. Pointing, imitative behaviour and a better understanding of social rules were present. He scored in the clinical range on the CBCL social and attention subscales. The Conners' Parent and Teacher Rating Scales pointed to the co-occurrence of symptoms consistent with attention deficit hyperactivity disorder. Assessment for sensory profile through SPM (Sensory Processing Measure) highlighted a definite dysfunction in the 'social participation', 'vision', 'hearing' and 'planning and ideas' subscales.

3.2 | Familial copy number variant characterization

SNP-array analysis showed in the proband sibling 1, a ~157-kb heterozygous deletion at locus 6q26 (arr [GRCh37] 6q26(162157397_162314554x1)) involving the exon 7 and flanking introns 6–7 of the *PARK2* gene (NM_004562.3) (Figure 1c). Although with a slight different size due to a diverse set of array probes, a similar deletion was then identified in Sibling 2 by Array-CGH analysis (arr [GRCh37] 6q26(162181982_162302687x1)) (Figure 1d). According to the RefSeq gene database, exon 7 is present in all transcript isoforms of the PARK2 gene, although *in silico* analysis predicts that the CNV would result in a frameshift leading to a premature stop codon nine amino acids downstream (p.(Arg245SerfsTer9)), either producing a truncated protein or causing the degradation of the abnormal transcript by the nonsense-mediated mRNA decay. The investigation of the deletion in all family members with real-time PCR using a PRKN fragment mapping within the deletion confirmed the rearrangement in both siblings and demonstrated its paternal inheritance (Figure 1b). According to the ACMG recommendations, this CNV was classified as a variant of uncertain significance (total score: 0.85). No other clinically significant rearrangements were identified in this family.

4 | DISCUSSION

In the present study, we describe clinical, molecular and neurobehavioral correlates in a multiplex family with ASD segregating an intragenic ~157-kb microdeletion of the chromosomal region 6q26 causing *PARK2* disruption. We found an intrafamilial clinical heterogeneity owing to different severity of the autism symptoms between the affected sibs and including the father carrying the same CNV having a broad autism phenotype. Actually, the younger sib had minimally verbal autism and severe intellectual disability, whereas his older brother

presented high-functioning autism and preserved speech (Table 1). This is consistent with concepts indicating that polygenic effects acting in summation may add to the CNV in explaining heritability and clinical heterogeneity of ASD (Klei et al., 2012). More recently, it was found that highly duplicated, copy number polymorphic sequences play a role in explaining differences in the severity of classic symptoms in ASD. This was preferentially found in multiplex versus simplex families and thus may be important to inherited forms of autism (Davis et al., 2019).

CNVs including the *PARK2* (chr6q26: 162584576–162587001) were initially associated with ASD by a large genomic-wide case–control study, attempting to identify CNV conferring ASD susceptibility in children of European ancestry. *PARK2* was significantly enriched for CNV (deletions) observed in patients with ASD ($N = 7$) but not in controls (Glessner et al., 2009). In a large sample of individuals with ASD (from the Simons Simplex Collection [SSC] and Autism Genetic Resource Exchange [AGRE]) screened through a customized targeted high density microarray, 24 CNV (12 deletions and 12 duplications) were found in PARK2 gene reaching significance in comparison with controls (Girirajan et al., 2013). Furthermore, 15 ASD patients (0.61%) with exonic *PARK2* CNV (six deletions and nine duplications) were counted in the Autism Genome Project (AGP) cohort (Morato Torres et al., 2020; Pinto et al., 2014), and 13 *PARK2* CNVs (six deletions and 7 duplications) were reported from DECIPHER database in patients with ASD (Conceição et al., 2017). However, for the large majority of patients with ASD and PARK2 gene aberrations numbered in the medical literature, no detailed clinical information was provided.

Neurobehavioral and molecular features of *PARK2* microdeletion in clinically characterized patients with ASD ($N = 12$), including the present ones, are described in Table 1.

Initially, clinical features of ASD were linked to the *PARK2* CNV (four deletions and two duplications in exons 2–7) in a sample of Chinese ASD patients in order to propose possible effects of various exonic *PARK2* CNVs (Yin et al., 2016). Based on clinical characteristics of probands harbouring deletions, it was found that *PARK2* deletions involving exons 2–4 might be less detrimental than those affecting exons 5–12. Actually, exons 5–12 map to the most conserved regions encoding highly conserved functional domains, that is, the RBR ('ring between ring fingers') domains, in the C-terminal part of the protein (Kay et al., 2010). Accordingly, the results of a *PARK2* regional analysis using DECIPHER, ClinVar and dbVar databases showed a significantly higher frequency of CNVs in the highly conserved RBR domains in patients with neurodevelopmental disorders (26.8%) than in controls (2.4%), consistent with the notion that variants targeting these protein domains (encoded in exons 5–12) might be detrimental for protein function and contribute to brain dysfunction (Conceição et al., 2017). In the same study, deletions encompassing PARK2 gene were identified in five individuals from a Portuguese ASD sample (Table 1), with a frequency of 1.5% (5/342) in this group of patients, relatively high for a variant targeting one specific gene in ASD (Conceição et al., 2017).

The analysis of a cohort of 127 ASD Italian families identified two inherited deletions overlapping exon 2 and/or exon 3 of the PARK2 gene in two families; the frequency of these *PARK2* deletions was the same in cases and controls, suggesting that they did not represent major risk factors for ASD in the two ASD families (Bacchelli et al., 2020). On the other hand, CNV duplication encompassing exon 3–4 was described in a patient with more severe autistic symptoms and worse cognitive function, suggesting that exonic 2–4 duplication might result in a more severe interference of *PARK2* expression than deletion (Yin et al., 2016).

Further insights on clinical variability associated with *PARK2* aberrations were elucidated in two patients both with ASD and syndromic phenotypes, harbouring chr6q26 microdeletion and microduplication, respectively. The one with microdeletion had dysmorphic facial features, obesity and was cognitively normal (Asperger syndrome) with attention deficit and sleep disturbance. The second one, with copy number gain, was cognitively impaired, with short stature and underweighted (Scheuerle & Wilson, 2011). The occurrence of neurodevelopmental disorders with intellectual disability in patients with overlapping 6q26 microduplication encompassing PARK2 gene was corroborated by two additional syndromic patients with developmental delay/intellectual disability, hypotonia and language impairment (Mariani et al., 2013; Palumbo et al., 2016). In addition, a diagnostic case series including 215 patients referred for ASD or developmental delay/learning disability reported one *PARK2* CNV duplication in a patient with learning disability and dysmorphic features (Roberts et al., 2014).

Chromosomal 6q26 microdeletions affecting *PARK2* and associated with ASD may be inherited from healthy parents (i.e. Bitar et al., 2019; Conceição et al., 2017; Glessner et al., 2009; Lovrečić et al., 2018; Yin et al., 2016), more rarely from affected fathers with ASD or autistic traits (Yin et al., 2016; present study).

In the present sibs, we identified a microdeletion encompassing exon 7 of the *PARK2* gene inherited from the father presenting with a broad autism phenotype. Based on *in silico* prediction analyses, we suggest that this deletion might impair protein function with exon

7 deletion inducing a frameshift and a premature stop codon nine amino acids downstream (p.(Arg245SerfsTer9)). In this regard, further studies are needed to confirm the role of this CNV at the transcript, protein and function levels. According to a multi-hit model for autism, study patients could carry partial *PARK2* deletion acting on different genetic backgrounds explaining intrafamilial heterogeneity (Lovrečić et al., 2018). Interestingly, a recent study focused on the possible *PARK2* converging mechanisms and pathways in both neurodevelopment and neurodegenerative processes (Morato Torres et al., 2020). The frequency of heterozygous CNV in *PARK2* is higher in ASD as well as in patients with Parkinson's disease (PD) compared with controls; however, the penetrance from *PARK2* CNV either in PD (Huttenlocher et al., 2015) or in ASD would not be high (Yin et al., 2016). Accordingly, *PARK2* variants would cause early-onset PD when they lead to complete loss of protein in compound heterozygous or homozygous individuals while heterozygous rearrangements would represent a moderate risk factor for ASD and PD whose penetrance and final clinical outcome is probably modulated by individual genetic background and/or epigenetic and environmental factors (Morato Torres et al., 2020). Loss of Parkin function has been shown to suppress mitochondrial biogenesis through accumulation of Parkin-interacting substrate acting as transcriptional repressor of PGC-1α (Batlevi & La Spada, 2011), a transcription co-activator linked to ASD regulating the expression of several enzymes involved in the mitochondrial fatty acid oxidation pathway, mitochondrial biogenesis, oxidative phosphorylation and energy production (Barone et al., 2021). Therefore, the impairment of the Parkin protein function, associated with *PARK2* CNV, may ultimately result in abnormal mitochondrial clearance and biogenesis, likely contributing to the pathogenesis of ASD through affecting mitochondria in particular brain region (e.g. frontal lobe) (Yin et al., 2016).

5 | CONCLUSION

This study describes a *PARK2* microdeletion segregating in a multiplex family with ASD. By thoroughly characterizing the clinical and neurobehavioral phenotypes, we highlight the remarkable intrafamilial phenotypic heterogeneity in the cognitive level, language development and autism symptoms' severity among affected members. Ultimately, the study illustrates the importance of unveiling additional genetic contributors associated with severity of the classic symptoms of inherited ASD and their potential involvement in autism risk.

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CONFLICTS OF INTEREST

The authors state no conflict of interests.

AUTHOR CONTRIBUTIONS

Rita Barone, Lara Cirnigliaro: Conceptualization, validation and writing—original draft. Lara Cirnigliaro, Silvia Valdese, Adriana Prato: Clinical investigation and formal analyses. Lucia Saccuzzo and Laura Bernardini: Molecular investigations. Marco Fichera and Renata Rizzo: Validation, writing—review. All authors critically reviewed the manuscript, participated in its revision and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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