Received: 14 April 2022 | Revised: 18 November 2022 | Accepted: 21 December 2022

DOI: 10.1002/mgg3.2130

Molecular Genetics & Genomic Medicine Open Access WILEY

CLINICAL REPORT

3q29 microduplication syndrome: New evidence for the refinement of the critical region

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Abstract

Background: The 3q29 microduplication syndrome is a rare genomic disorder characterized by an extremely variable neurodevelopmental phenotype usually involving a genomic region ranging from 1.6 to 1.76 Mb. A small microduplication of 448.8 Kb containing only two genes was recently described in a patient with a 3q29 microduplication that was proposed as the minimal critical region of overlap of this syndrome.

Methods: Molecular karyotyping (array-CGH) was performed on DNA extracted from peripheral blood samples using Agilent-California USA Human Genome CGH Microarray 4 × 180 K. The proband and his younger brother were further tested with a next generation sequencing (NGS) panel including genes implicated in autism spectrum disorder and in neurodevelopmental disorders. Quantitative real-time PCR was applied to verify the abnormal array-CGH findings.

Results: Here, we report on a family with two males with neurodevelopmental disorders and an unaffected sibling with a small 3q29 microduplication (432.8 Kb) inherited from an unaffected mother that involves only two genes: *DGL1* and *BDH1*. The proband had an additional intragenic duplication inherited from the unaffected father. Further testing was negative for Fragile X syndrome and for genes implicated in autism spectrum disorder and in neurodevelopmental disorders.

Conclusion: To the best of our knowledge, one of the family members here analyzed is the second reported case of a patient carrying a small 3q29 microduplication including only *DGL1* and *BDH1* genes and without any additional genetic aberration. The recognition of the clinical spectrum in patients with the critical region of overlap associated with the 3q29 duplication syndrome should prove valuable for predicting outcomes and providing more informed genetic counseling to patients with duplications in this region.

KEYWORDS

3q29 microduplication, array-CGH, CNV, minimal critical region, neurodevelopmental phenotypes

1 | INTRODUCTION

The 3q29 microduplication syndrome is a rare genomic disorder (MIM 611936), characterized by an extremely variable neurodevelopmental phenotype. Heterogenous clinical features, including autism, intellectual disability, global development delay, speech delay, learning disabilities, and seizures have been reported (Coyan & Dyer, 2020; Pollak et al., 2020; Streata et al., 2020; Tassano et al., 2018). Mild facial dysmorphism, microcephaly, obesity, ocular and cardiac defects, hypotonia, and musculoskeletal anomalies may also be present. However, patients usually demonstrate a mild clinical phenotype. Reduced penetrance is also observed, since the microduplication is often inherited from unaffected or mildly affected parents (Pollak et al., 2020).

The canonical 3q29 microduplication syndrome usually involves a genomic region ranging from 1.6 to 1.76 Mb (Coyan & Dyer, 2020; Lisi et al., 2008; Willatt et al., 2005). This interval includes 19–21 OMIM genes. Among these, *TNK2, PAK2, DLG1, BDH1*, and *FBXO45* (Tassano et al., 2018) are involved in neural development and function (Finardi et al., 2006; Kreis & Barnier, 2009; La Torre et al., 2013; Lee et al., 2017; Nakagawa et al., 2004; Saiga et al., 2009; Tada et al., 2010). More recently, a patient with a very small 3q29 duplication of 446.8 Kb including only *DLG1* and *BDH1* was described; this was proposed as the critical region of overlap (CRO) for the 3q29 duplication syndrome (Tassano et al., 2018). The identification of the critical region of a genomic disorder is an important step since it allows to better define the set of causative genes as well as genotype–phenotype correlations, allowing to differentiate subtypes according to the extent of the imbalance.

To date, only two patients are described in the literature with a 3q29 microduplication smaller than the classical size of 1.6–1.76 Mb (Coyan & Dyer, 2020; Tassano et al., 2018).

Here within, we describe the segregation of a 3q29 duplication in a family and provide new evidence supporting the existence of the CRO causing the 3q29 duplication syndrome.

2 | MATERIALS AND METHODS

2.1 | Array-CGH

Molecular karyotyping (array-CGH) was performed on DNA samples extracted from peripheral blood using Agilent-California USA Human Genome CGH Microarray 4 × 180 K (Agilent Technologies, Santa Clara, CA) according to the manufacturer's protocol. Variant calling was performed using Agilent CytoGenomics Edition 5.0.2.5 (ADM-2 algorithm; release hg19). All genomic positions were reported according to the human genome assembly (GRCh37/hg19) and subsequently confirmed using quantitative real-time PCR. The target gene sequence was selected from UCSC database and primers were designed using Primer Express 3.0 software (Applied Biosystems, Weiterstadt, Germany). The clinical interpretation of CNVs was performed according to technical standard recommendations of the American College of Medical Genetics and Genomics (ACMG), using the semiquantitative system point-based scoring metric (Riggs et al., 2020). The classification was also supported by the use of different public databases, such as DGV (Database of Genomic Variants), DECIPHER (DatabasE of genomiC varIation and Phenotype in Humans using Ensembl Resources), OMIM (Online Mendelian Inheritance in Man), UCSC (Human Genome Browsers), and internal database based on our laboratory data.

2.2 | Next generation sequencing

Next generation sequencing (NGS) analysis was performed on the proband and his younger brother using Clinical Exome Solution (CES, Sophia Genetics SA, Saint-Sulpice, Switzerland) kit according to the manufacturer's protocols. Massively parallel sequencing was performed on the MiSeq Illumina platform and data processing, filtering and base calling was performed using real-time analysis (RTA) software integrated in the MiSeq instrument (Illumina). Bioinformatic analyses were carried out on two sets of 182 and 95 genes implicated in autism spectrum disorder (ASD) and in neurodevelopmental disorders, respectively (Supplementary Table S1). Raw reads were aligned to the human reference genome (GRCh37/hg19) and variant filtering and interpretation were performed on the Sophia DDM™ platform v5.10.4 (Sophia Genetics SA) according to the ACMG criteria (Richards et al., 2015).

Triplet repeat primed PCR (TP-PCR) was performed on the proband and his younger brother using the FRAXA 1 Kit – FL (Experteam, Italy) according to the manufacturer's protocols. The detection of the CGG repeats in the 5′-non-translated (5'-UTR) region of the Fragile X mental retardation 1 (FMR1) gene was carried out using ABI Prism 310 genetic analyzer (Applied Biosystems, USA).

3 | RESULTS

The proband was a 4-year-old boy referred for genetic evaluation of neurodevelopmental delay characterized

by autistic traits (Figure 1, II-2). He is the second child of healthy non-consanguineous parents. He was born at the 38th week of a normal pregnancy by cesarean section, due to previous C-section. His birth weight was 3350 g (71st percentile) and head circumference was 31 cm.

The proband could walk unaided at 16 months and acquired sphincteric control at 4 years. During the second year, he began to show repetitive and stereotypical movements, such as moving in circles and repetitive jumps. Language development was altered: he pronounced the first words at 8 months but, at 13 months, concomitantly with an acute gastroenteritis, he showed a severe language regression with total loss of previously acquired skills. At the age of 3 years, he began attending preschool, where he showed discomfort in socialization with peers; stereotyped and repetitive motor behaviors and difficulty in expressive language were noticed. At the last neuropsychiatric evaluation performed at the age of 41 months, he showed a development quotient of 76 (Brunet–Lezine test) (Flamant et al., 2011). A neurodevelopmental disorder, characterized by an expressive language impairment, moderate intellectual disability, and a neurodevelopmental age of 24 months, consistent with ASD, was diagnosed. The child did not show any dysmorphism. The parents reported an altered sleep–wake rhythm with difficulty falling asleep and frequent night time awakenings. EEG was normal.

His younger brother (Figure 1, II-3) was seen at the age of 2 years. He was born by C-section at the 38th gestational week following an uneventful pregnancy; his birth weight was 3350 g (71st percentile). He was hospitalized in the first week due to hypoxic–ischemic distress caused by accidental ingestion of amniotic fluid. He produced his first words and walked independently at 14 months. During the preschool period, he had difficulties in social interaction. He was diagnosed with mixed developmental disorder with stereotyped behavior. Neuropsychological assessment showed a global developmental quotient of 79 with an expressive receptive language and mild psychomotor delay (Brunet–Lezine test) with atypical behaviors. Furthermore, difficult chewing and recurrent ear infections were reported. Neurological examination demonstrated oral hypotonia. He had a normal sleep–wake rhythm.

No family history of malformations, genetic diseases, intellectual disability, and neuropsychiatric disorders were reported; only a mild language delay in the maternal lineage was noted. The oldest daughter, 10 years old, had normal development (Figure 1, II-1).

The proband was found to have two CNVs detected by array-CGH (Figure 1, II-2). The first CNV was an interstitial 3q29 duplication, spanning 432.8 Kb from position 196,892,569 bp to position 197,324,567 bp. The duplication contained only two OMIM genes, *DLG1* and *BDH1*, and was inherited from the unaffected mother (Figure 1). The second CNV was an interstitial 11q22.1 duplication, spanning 397.8 Kb from position 99,728,631 bp to position 100,126,438 bp. This was an intragenic duplication encompassing nine exons of *CNTN5* (*607219) that was inherited from the unaffected father (Figure 1). Both duplications were classified as variants of uncertain significance (VUS).

Array-CGH analysis showed that his younger brother inherited only the 3q29 CNV (Figure 1, II-3), while real-time PCR analysis showed that his unaffected sister also inherited only the 3q29 duplication (Figure 1, II-1).

Further testing was negative for Fragile X syndrome and for genes implicated in autism spectrum disorder and in neurodevelopmental disorders.

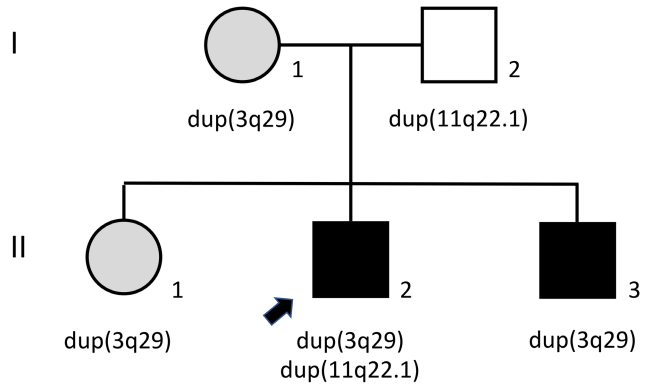

FIGURE 1 Family tree with affected proband (II-2) and his younger brother (II-3) indicated in black. Open symbols indicate unaffected individuals who do not carry the 3q29 microduplication. Individuals II-1 (proband's sister) and I-1 (proband's mother) are indicated in gray since they carry the same microduplication but do not express the phenotype

4 | DISCUSSION

The 3q29 microduplication syndrome involves a 1.6 Mb region on the short arm of chromosome 3 and is characterized by a broad range of peculiar traits encompassing both cognitive and musculoskeletal anomalies. The typical age of onset of 3q29 syndrome is within the first year of life, although Streata et al recently described an apparently late-onset case with normal development until 10 years of age (Streata et al., 2020). The smallest duplicated region associated with the 3q29 duplication syndrome was described by Tassano et al. who proposed this as the critical region for phenotypic manifestations (Tassano et al., 2018).

In this article, we report on a family segregating a duplication containing the same genes involved in the case reported by Tassano et al. (2018); the duplication is 432.8 Kb long and includes only two OMIM genes: *DLG1* and *BDH1*. It was detected in two affected brothers and in

their unaffected sister, as well as in the unaffected mother. The proband also carried a paternally inherited intragenic duplication encompassing nine exons of the *CNTN5* gene (*607219). We also tested an NGS panel specifically developed for neurodevelopmental disorders on the proband and his younger brother. In fact, an important point to consider in the analysis of complex disorders such as neurodevelopmental disorders is the epistatic effect that undetected variants might have on observed phenotypic heterogeneity. However, although multiple studies show a possible role of 3q29 duplication in neurodevelopmental disorders, most studies did not exploit NGS technology to rule out other potential genetic etiologies. In fact, only a few studies applied an NGS approach to exclude the effect of pathogenic sequence variants in genes involved in neurodevelopmental disorders on the 3q29 microduplication syndrome. Therefore, we provide additional evidence supporting the pathogenicity of the 3q29 duplication.

To date, only two cases have been described so far with this small 3q29 duplication (Coyan & Dyer, 2020; Tassano et al., 2018). To better identify the clinical phenotype associated with the candidate CRO, we also compared the clinical phenotype of the proband and his young brother with those of the two patients carrying the smallest 3q29 duplications so far detected (Coyan & Dyer, 2020; Tassano et al., 2018) (Table 1).

The first observation was that all four patients shared a clinical picture characterized almost exclusively by neuropsychiatric traits and neurodevelopmental delay, with few or no additional anomalies. None of them showed macrocephaly and generalized obesity. Intellectual disability, language delay, and behavior disorder were generally mild; the same pattern was observed for motor developmental delay. Mild facial dysmorphisms were observed in the Coyan's patient only.

The recognition of the clinical spectrum in patients with the critical region of overlap associated with the 3q29 duplication syndrome should prove valuable for predicting outcome and providing more informed genetic counseling to patients with duplications in this region. At the same time, data shown in Table 1, as well as clinical reports on more extended 3q29 duplications, highlight the high phenotypic variability among patients presenting with CNVs on this region (Ballif et al., 2008; Coyan & Dyer, 2020; Pollak et al., 2020). In particular, both the brother of the proband and the patient reported by Tassano et al. (2018) exhibited a less severe phenotype compared to the proband and to the patient reported by Coyan and Dyer (2020) who had significant language impairment and moderate to severe intellectual disability. Tassano's patient also exhibited growth impairment. However, he also suffered from celiac disease, which may have contributed to the severity of this phenotypic trait. Furthermore, our proband showed clinical features consistent with ASD. Interestingly, the latter two patients are both carriers of an additional CNV. Our proband also carries an intragenic duplication of *CNTN5* (*607219), encoding a cell adhesion molecule exclusively expressed in the central nervous system. Recent studies demonstrate a central role of this protein in brain development (Kleijer et al., 2018; Oguro-Ando et al., 2017). CNVs involving the contactin genes (CNTN) have been associated with neurodevelopmental disorders including ASD, ADHD, intellectual disability, bipolar disorder, schizophrenia, and anorexia nervosa (Oguro-Ando et al., 2017). In particular, *CNTN5* deletions specifically have been observed in patients with ASD (Mercati et al., 2017; van Daalen et al., 2011). The association between these disorders and CNVs in CNTN5 gene should be confirmed by additional studies. The patient repoerted by Coyan & Dyer (2020) carries a 17q12 duplication, which is also associated with a neurodevelopmental phenotype characterized by clinical features partially overlapping with 3q29 duplication syndrome, including facial dysmorphisms (Bierhals et al., 2013), which are reported only in this case among those described in Table 1.

To explain the clinical variability observed in patients affected by the 3q29 microduplication syndrome, Coyan and Dyer suggested that this microduplication acts as a neurosusceptibility locus (NSL) in accordance with the "two-hit" model proposed to explain the behavior of NSL loci (Coe et al., 2014; Coyan & Dyer, 2020). In this model, the heterogeneous penetrance of neurodevelopmental phenotypes associated with NSL loci might be due to the interaction of the primary variant with a second genetic or environmental hit that increases the impact of the neurodevelopmental clinical manifestations. According to this hypothesis, the additional CNVs detected in our proband and in the patient reported by Coyan et al. may explain the increased clinical severity observed in them and, more generally, the expression variability of specific phenotypic traits in the 3q29 syndrome (Coyan & Dyer, 2020).

Our results are also consistent with the analysis carried out by Pizzo and colleagues on the effects of genetic background in modulating cognitive and developmental phenotypes in individuals carrying disease-associated variants (Pizzo et al., 2019). They showed that the clinical features observed in patients who carry the same primary variant may be influenced by the coexistence of additional rare variants in the genome, suggesting a causal relationship between the genetic background and phenotypic heterogeneity. Taken together, these data suggest an important role of multiple CNVs on the clinical variability of the 3q29 microduplication syndrome.

TABLE 1 Clinical phenotype associated with the candidate CRO

Features	Patients with a CRO without any additional CNV		Patients with a CRO and additional CNV	
	Tassano et al. (2018)—case 2	Brother of proband (II-3)	Coyan and Dyer (2020) P9—Family 6	Proband (II-2)
Intellectual disability	+	mild	+	+
Language delay	+	+	+	+
Learning disabilities	+	+	+	+
Motor Developmental delay	+	+	+	+
Hypotonia	+	+	?	–
Autism/Autism-like features	–	–	?	+
Other neuropsychiatric phenotype and social disability	+	+	+	+
Epilepsy	+	–	?	–
Facial dysmorphism (Anomalies in palate, palpebral fissure)	–	–	+	–
Microcephaly	+	–	+	–
Structural brain anomalies	–	?	?	?
Musculoskeletal anomalies	–	–	?	–
Ocular anomalies	–	–	?	–
Dental problems	–	?	?	?
Ear problems	–	+	?	–
Gastrointestinal problems	+	–	?	+
3q29 duplication size	446.8 Kb	432 Kb	465.3 Kb	432 Kb
3q29 dup genomic coordinates	196,982,527–197,339,329	196,892,569–197,324,567	196,881,259–197,346,566	196,892,569–197,324,567
Additional CNVs	No	No	17q12 duplication	Intragenic duplication CNTN5 gene (11q22.1)
Genomic coordinates of additional CNVs	–	–	34,819,191–36,450,598	99,728,631–100,126,438

The uncertainty in the diagnosis and in the clinical management of the patients with 3q29 duplication syndrome may be further influenced by the observation that females and males can show a different genetic threshold for the clinical manifestation of neurodevelopmental disorders. Several studies have shown a different impact of neurodevelopmental disorders between males and females, characterized by a male/female ratio skewed toward males, on the basis of which a "female protective model" for these phenotypes was postulated (Desachy et al., 2015; Jacquemont et al., 2014). In support of this hypothesis, it has been observed that affected female probands have a higher number of additional genetic hits when compared to male probands (Pizzo et al., 2019). Interestingly, in our family, the mother and the daughter of the proband, who are carriers of the 3q29 duplication without other abnormalities, are unaffected (Figure 1, I-1 and II-1). On the other hand, Pollak et al. (2020), in a study of a cohort of patients with 3q29 duplications selected through the 3q29 registry, found no evidence for a sex-specific liability threshold. Therefeore, it is possible that the incomplete penetrance observed in our family could be related to protective factors that are not sex specific.

5 | CONCLUSION

The evidence to support the minimal critical region of overlap is questionable because pathogenicity of small duplications of 3q29 (approximately 500 kb involving the genes *DLG1* and *BDH1*) cannot be proven with current evidence. The four cases that are described have other potential explanations for the neurodevelopmental findings, II-2 has an additional intragenic duplication of the *CNTN5* gene, II-3 has history of hypoxic–ischemic distress, Tassano et al reported a patient with celiac disease and failure to thrive,

and Coyan et al reported a case with an additional 17q12 duplication. In addition, other genetic etiologies of neurodevelopmental disorders have not been completely ruled out with clinical exome or genome. On the other hand, available evidence cannot prove that small duplications of 3q29 (approximately 500 kb involving the genes DLG1 and BDH1) are benign. Reduced penetrance must be considered as well as the age of onset of the symptoms. The classification of the duplications as VUS makes sense given the prior circumstances. Finally, other studies are required to test the hypothesis about the role of genetic background and secondary variants that modify the phenotype.

Overall, in this study, we provide new evidence supporting the existence of a critical region of overlap causing the 3q29 duplication syndrome. To the best of our knowledge, the younger brother of the proband is the second reported case of a patient carrying the smallest 3q29 microduplication without any additional genetic aberration. At the same time, we also highlight the extreme phenotypic variability observed within the family under study. Our findings support the hypothesis that the genetic background plays a crucial role in modulating the penetrance of 3q29 syndrome and that the concomitant presence of secondary rare variants and/or other unknown protective factors may modify the expression of the specific clinical traits associated with this syndrome.

AUTHOR CONTRIBUTIONS

A.B., E.F., and A.M., study concept and design, wrote the article, literature search, interpretation of data, preparation and revision of article; D.P. and L.C., clinical and neuropsychiatric evaluation; V.P., R.B., L.DS, and FG performed experiments, data acquisition, and interpretation of data; A.B., E.F., and M.G., study supervision, collection of relevant clinical data, and critical revision of article.

ACKNOWLEDGMENTS

We thank the patients and their family for their participation in this study.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article (and its supplementary information files). If you have any further questions, data are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

All procedures performed in this study involving human participants were in accordance with the ethical standards of the ethics committee of the University of Calabria and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Patient's family provided written informed consent.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Bauleo, A., Pace, V., Montesanto, A., De Stefano, L., Brando, R., Puntorieri, D., Cento, L., Genuardi, M., & Falcone, E. (2023). 3q29 microduplication syndrome: New evidence for the refinement of the critical region. *Molecular Genetics & Genomic Medicine*, *11*, e2130. https://doi.org/10.1002/mgg3.2130