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Delineation of the Genetic Architecture and Clinical Polymorphism of 3q29 Duplication Syndrome: A Review of the Literature and a Report of Two Novel Patients With Single-Gene *BDH1* Duplications

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ABSTRACT

Background: Chromosome 3q29 duplication syndrome is a rare chromosomal disorder with a frequency of 1:5000 in patients with a neurodevelopmental phenotype. The syndrome is characterized by phenotypic polymorphism and reduced penetrance.

Methods: Patients were investigated by performing a cytogenetic analysis of GTG-banded metaphases, aCGH with the SurePrint G3 Human CGH Microarray 8×60K, qPCR, FISH, and WES.

Results: Here, we report five new patients with atypical duplications overlapping with the 3q29 duplication syndrome region and no other genetic findings. In two patients, duplications were found in the single *BDH1* gene, a candidate gene for the 3q29 duplication phenotype. For the first time, we delineated and described the smallest minimal critical region, including the single *BDH1* gene; in our patients, this region was associated with ASD, heart defects, biliary tract dysfunction, and obesity. The frequencies of the pathological phenotypes in duplication carriers reported in the literature were calculated and compared with those in patients with 3q29 deletions. Most of the phenotypes were observed in both groups but were significantly less common among individuals with 3q29 duplications. Mirrored phenotypes in patients with duplications and deletions included overweight and weight deficit. Schizophrenia, generalized anxiety disorder, and recurrent ear infections were unique phenotypes of patients carrying deletions.

Conclusion: Chromosome 3q29 duplication syndrome is characterized by a complex genetic architecture and clinical polymorphism.

1 | Introduction

Chromosome 3q29 duplication syndrome (OMIM 611936) (http://omim.org) was first described by Lisi et al. (2008). The authors identified a 3q29 duplication through a combination of methods (fluorescence *in situ* hybridization [FISH], array comparative genomic hybridization [aCGH] with bacterial artificial chromosome [BAC] clones, and single-nucleotide polymorphism [SNP] arrays) in five family members over three generations. A proximal break occurred between the SNPs rs11926771 (base position 197,145,041) and rs6797622 (base position 197,223,225), whereas a distal breakpoint occurred between the SNPs rs11922324 (base position 198,832,486) and rs6583248 (base position 198,910,079) (NCBI Build 35). In 2011, Kaminsky et al. (2011) reported that the frequency of 3q29 duplication among patients with idiopathic developmental delay (DD), intellectual disability (ID), dysmorphic features, multiple congenital anomalies, autism spectrum disorder (ASD), or clinical features suggestive of a chromosomal syndrome was significantly greater than that among apparently healthy individuals (1:1969 vs. 1:10118, respectively). In 2020, the frequency of 3q29 duplication among patients with a neurodevelopmental phenotype was shown to be lower (1:5000), whereas among apparently healthy individuals, it remained almost unchanged (1:10000) (Coyan and Dyer 2020).

The following clinical features, based on the family described by Lisi et al. (2008), are associated with 3q29 duplication syndrome: microcephaly, a round or long face, short and downslanting palpebral fissures, large eyes, a bulbous and short nose, a wide nasal bridge, excessive hand creases, pes planus, a low posterior hairline, obesity, cognitive delay, and mild to moderate mental retardation (OMIM 611936). Therefore, this syndrome was first identified based on the segregation of a 3q29 duplication among affected members of one family in three generations.

To date, 22 studies on 3q29 duplication syndrome have been published; among them, 12 papers describe individual patients with genomic coordinates of the aberration (date of access August 07, 2024; Bauleo et al. 2023; Coyan and Dyer 2020; Fernández Jaén et al. 2014; Lawrence et al. 2017; Lisi et al. 2008; Massier et al. 2024; Ormond et al. 2024; Schilter et al. 2013; Streata et al. 2020; Tassano et al. 2018; Viñas-Jornet et al. 2018; Vitale et al. 2018). The aberrant region is flanked by almost identical low-copy repeats (LCRs), which indicates that nonallelic homologous recombination is the most likely mechanism for rearrangement formation. The canonical 3q29 duplication syndrome region is 1.6–1.76 Mb and includes the following candidate genes involved in neural development and functioning: *TNK2* (OMIM 606994), *PAK2* (OMIM 605022), *DLG1* (OMIM 601014), *BDH1* (OMIM 603063), and *FBXO45* (OMIM 609112) (Bauleo et al. 2023). The minimal overlapping region (MOR), delineated by Tassano et al. (2018), Coyan and Dyer (2020), and recently by Bauleo et al. (2023), includes two genes: *DLG1* and *BDH1*. Bauleo et al. (2023) reported a family of healthy nonconsanguineous parents with three children. All the children had dup3q29, 432.8 kb in length, containing the same two genes, *DLG1* and *BDH1*, which was inherited from an apparently healthy mother. The first daughter was healthy. The second and third children, both boys, suffered from neurodevelopmental delay with autistic traits. The younger boy also had another intragenic duplication encompassing nine exons of the *CNTN5* gene (11q22.1, OMIM 607219) that was inherited from the apparently healthy father.

More individual patients, rather than families, are described in the papers listed below. Ballif et al. (2008) identified 19 patients with dup3q29 of different sizes among 14 698 individuals with developmental disabilities. Among the 17 patients investigated with high-resolution microarrays, 12 aberrations ranging in size from 200 kb to 2.4 Mb were identified. The other five patients appeared to carry reciprocal duplications of the 3q29 microdeletion region that were flanked by LCRs. Parental DNA was available for only 10 patients; the duplications in two patients were *de novo* aberrations. Clinical information was available for seven individuals. Goobie et al. (2008) described four families with individuals with dup3q29. Coyan and Dyer (2020) summarized data on 16 individuals from the literature and 11 new patients from nine families; eight patients had the typical 1.6 Mb 3q29 duplication and three had atypical aberrations partially overlapping with the classical region with sizes of 0.5 Mb (a child) and 0.7 Mb (affected child and apparently healthy mother). Pollak et al. (2020) reported a cohort of 31 individuals with dup3q29. The age of the participants ranged from 0.3 to 52.2 years (mean of 10.0 ± 10.8 years). The data were obtained from the 3q29 registry (https://3q29.com). In the latest paper by Massier et al. (2024), 46 patients from 31 families were included: 23 individuals with recurrent duplications (including 6 parents), 10 with overlapping duplications > 1 Mb (including 2 mothers), and 13 with duplications < 1 Mb (including 3 mothers). An additional genetic analysis was reported for 15 individuals with recurrent duplications and duplications larger than 1 Mb: three had single nucleotide variants of uncertain clinical significance, one had a pathogenic 15q11.2 deletion, and the remaining 11 had a negative ID next generation sequencing (ID NGS) panel or exome sequencing (ES). Among the 10 individuals with less than 1 Mb of overlapping duplications, seven were carriers of unbalanced translocations, four of which were pathogenic deletions and three of which were variants of uncertain significance.

Here, we described five new patients in four families with dup3q29 ranging from 232 to 486 kb in size; based on the analysis of our own results and published data, we delineated for the first time the smallest MOR, including only the *BDH1* gene, and compared the phenotypes of carriers of reciprocal microdeletions and microduplications at the 3q29 locus.

2 | Materials and Methods

2.1 | Ethical Compliance

The study was approved by the local Ethics Committee of the Research Institute of Medical Genetics, Tomsk National Research Medical Center of the Russian Academy of Sciences (#15 from February 28, 2023).

2.2 | Materials

The families of patients were referred to the Medical Genetic Center (Genetic Clinic), Research Institute of Medical Genetics, Tomsk NRMC. The cytogenetic, molecular cytogenetic and molecular

genetic studies were performed at the Core Medical Genomics Facility of the Tomsk National Research Medical Center (NRMC) of the Russian Academy of Sciences using the resources of the biocollection "Biobank of the population of Northern Eurasia" of the Research Institute of Medical Genetics, Tomsk NRMC.

Peripheral blood was collected in tubes containing EDTA for molecular genetic analyses and in tubes containing heparin for banding cytogenetics.

2.3 | Cytogenetic Analyses

Banding cytogenetic analysis was performed on GTG-banded metaphases from peripheral blood lymphocytes from the patients.

2.4 | Array-Based Comparative Genomic Hybridization (aCGH) Analyses

aCGH was performed using the SurePrint G3 Human CGH Microarray 8×60K (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's recommendations. The overall median spacing was 41 kb. Labeling and hybridization of the patient and reference DNA (#5190-4370, Human Reference DNA, Agilent Technologies) were performed using enzymatic labeling and hybridization protocols (v. 7.5, Agilent Technologies). Array images were acquired with an Agilent SureScan Microarray Scanner (Agilent Technologies). Data were analyzed using CytoGenomics software (v. 5.3.0.14) (Agilent Technologies) and the publicly available Database of Genomic Variants (DGV) (http://projects.tcag.ca/variation). The clinical significance of the copy number variants (CNVs) was interpreted according to American College of Medical Genetics and Genomics (ACMG) recommendations (Riggs et al. 2020).

2.5 | Quantitative Real-Time PCR

Target sequences within the duplicated 3q29 chromosomal region and specific primers for quantitative real-time PCR were selected using Primer 3 software (Table 1) (https://bioinfo.ut.ee/primer3-0.4.0/). The presence of 3q29 microduplications was assessed using genomic DNA from the peripheral blood lymphocytes of the patients and their relatives with the AriaMx Real-Time PCR System (Agilent Technologies). The reference DNA used was #5190-4370 (human reference DNA, Agilent Technologies). *HEXB*, which encodes the β subunit of hexosaminidase and is located on chromosome 5q13, was used as the control gene (Table 1). Real-time PCR was performed using 25 ng of DNA (10 ng/μL), 2.5 μL (1 μM/L) of forward and reverse primers, 12.5 μL of 2× BioMaster HS-qPCR SYBR Blue (BioLabMix, Novosibirsk, Russia), and RNase-free water to a total volume of 20 μL (per well). The real-time PCR conditions were as follows: an initial incubation at 95°C for 10 min, followed by 40 cycles of 15 s at 95°C, 30 s at 60°C, and 30 s at 72°C. Three technical replicates were run for each sample. The CT values obtained for the test and reference (control) DNA amplifications with primers for the test and reference genes were analyzed using the following calculations: average value for three Ct, logQT test primer = (Ct test DNA − Ct reference DNA)/slope, logQT control primer = (Ct test DNA − Ct reference DNA)/slope, (logQT test primer − log QT control primer), and fold change $= 10^{\text{logQT test primer}-\text{logQT control primer}}$. Fold change values were used to construct a chart.

2.6 | Fluorescence *In Situ* Hybridization (FISH)

FISH was performed for Patient 3.1 due to her short stature using the commercial probes "Short Stature (Xp22)/SEX" for the *SHOX* gene (Xp22) and centromere-specific DXZ1 (Kreatech-Leica, Germany) on metaphase chromosomes from the cultured lymphocytes of the probands following the standard protocol.

FISH was performed for Patient 4.1 due to multiple café-au-lait spots on the skin using the commercial probes "NF1 (17q11)/MPO (17q22)" for the *NF1* gene (Kreatech-Leica, Germany) on metaphase chromosomes from the cultured lymphocytes of the probands following the standard protocol.

2.7 | Whole-Exome Sequencing

Whole-exome sequencing (WES) was performed for 11 individuals: Family 1 (Patient 1.1 and his mother), Family 2 (Patients 2.1 and 2.2), Family 3 (Patient 3.1 and both parents), and Family 4 (Patient 4.1, his sibling and their parents).

TABLE 1 | Primers for real-time PCR.

Region	Primer	Sequence
5q13.3	HEXB F	5'-CCGGGCACAATAGTTGAAGT-3′
	HEXB R	5'-TCCTCCAATCTTGTCCATAGC-3′
3q29	IQCGex12 F	5'-CTTAGGGCTTCCTCATACCTTG-3'
	IQCGex12 R	5'-ACTCAAGTTTCAATGCGTCACT-3'
	LMLNex16 F	5'-CATTTTAAGGTCCCAGTTGTCA-3'
	LMLNex16 R	5'-AACCTACCTGTTTGCCAGTTTC-3'
	LINC00885ex2 F	5′-CTCCTCTACCCAGCCAGTAAC-3'
	LINC00885ex2 R	5′-GGTGTTGGTGCTTTATCTTGGA-3'
	BDH1ex5 F	5′-AGCCATCTCCGACATGATCTAC-3'
	BDH1ex5 R	5′-CTTCCTGGCATGGTGGATAATC-3'

For WES, the patients' DNA was analyzed on a NextSeq 2000 Sequencing System (Illumina Inc., San Diego, CA, USA) with an average coverage of ×81. Targeted enrichment was performed using the Agilent Sure Select All Exon v8 Kit (Agilent, Santa Clara, CA, USA) for sample preparation.

Sequencing data were analyzed using the GATK Best Practices Germline short-variant discovery pipeline. Reads were aligned to the reference human genome sequence (hg38), with post-processing alignment, variant calling, quality filtering, and annotation of the identified variants by the canonical transcript of each gene. Variants that did not meet the quality criteria were excluded from further analysis. The pathogenicity of the variants was determined according to the ACMG recommendations (Green et al. 2013). The reads had a length of 2×101 bp. The median reliability of nucleotide determination was greater than that of Q30. For all the samples, the median coverage was no less than 77×, and the mean coverage was no less than 85.8×.

2.8 | Statistical Analysis

The chi-squared test was used to compare the frequencies of phenotypes induced by reciprocal deletion and duplication of the 3q29 locus (Table 5). The results were considered statistically significant at $p < 0.05$.

3 | Results

3.1 | Case Description

3.1.1 | Family 1

Patient 1.1 was a 5-year-old boy. The family was referred to the Genetic Clinic due to developmental and speech delays in the child. The child was from a first pregnancy and was born in the 42nd week of gestation. The karyotype (lymphocytes) was 46,XY. His birth weight was 3920 g (75th centile), and his birth length was 55 cm (95th centile). Developmental milestones were appropriate for the patient's age (held head up at 2 months, sat at 7 months, and walked at 10.5 months). The child said his first word when he was 1-year-old, but after 1.5 years, he had learned no new words. The vocabulary at the time of the examination was 7 words, and he exhibited no phrased speech. Several phenotypic abnormalities were observed, including a dolichocephalic skull, forehead hemangioma, epicanthus, large protruding ears, high palate, cylindrical chest, and postural defects. At high temperatures, the child would lose consciousness. Magnetic resonance imaging indicated no pathology.

The aCGH analysis revealed a 3q29 duplication in Patient 1.1 in the chromosome 3q29 duplication syndrome region (OMIM 611936; arr[GRCh37] 3q29(197608333_197840339)×3) (Figure 1, Table 2). The 3q29 duplication was verified by real-time PCR with primers

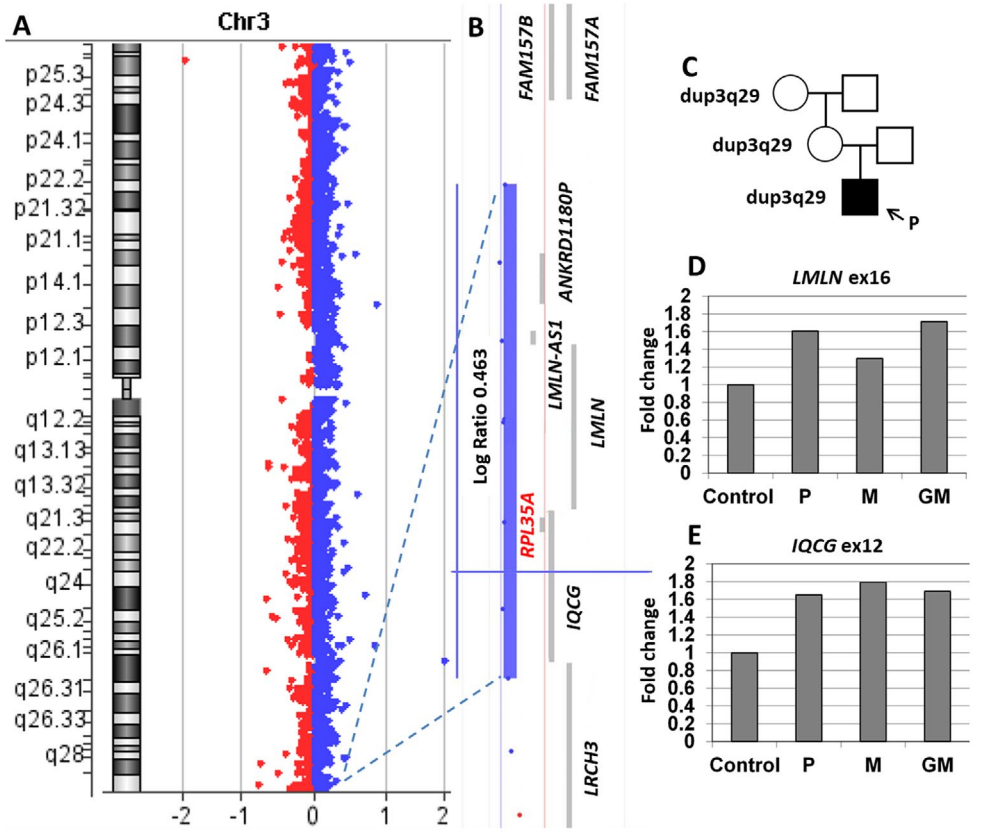

FIGURE 1 | Molecular cytogenetic results and pedigree of Family 1. (A) aCGH profile of chromosome 3 in Patient 1.1. (B) Gene content of the duplicated 3q29 region (blue). (C) Pedigree of Family 1. (D) qPCR results obtained using primers for exon 16 of the *LMLN* gene. (E) qPCR results obtained with primers for exon 12 of the *IQCG* gene. X axis—control DNA and examined individuals; Y axis—fold change in the copy number of a DNA region compared with the control. GM, grandmother; M, mother; P, patient 1.1.

TABLE 2 | Results of the molecular cytogenetic analysis of patients and their relatives.

Individuals	aCGH and qPCR results, according to ISCN2020 (McGowan-Jordan, Hastings, and Moore 2020)	Size, kb	Genes	Clinical significance of the CNV, according to ACMG2020 (Brandt et al. 2020; Riggs et al. 2020)
Family 1, Patient 1.1	arr[GRCh37] 3q29(197608333_197840339)×3 mat	232	*IQCG*, *RPL35A*, *LMLN*, *ANKRD18DP*	VUS
Family 1, mother	arr[GRCh37] 3q29(197574293_197840339)×3 mat	266	*LRCH3*, *IQCG*, *RPL35A*, *LMLN*, *ANKRD18DP*	VUS
Family 1, grandmother	arr[GRCh37] 3q29(197574293_197840339)×3	266	*LRCH3*, *IQCG*, *RPL35A*, *LMLN*, *ANKRD18DP*	VUS
Family 2, Patient 2.1	arr[GRCh37] 3q29(195633970_196120090)×3 mat	486	***TNK2***, *TFRC*, *SLC51A*, *PCYT1A*, *TNK2-AS1*, *TCTEX1D2*, *SDHAP1*, *LINC00885*, *ZDHHC19*, *UBXN7*, *TM4SF19-TCTEX1D2*, *TM4SF19-AS1*	LP
Family 2, Patient 2.2	arr[GRCh37] 3q29(195633970_196120090)×3 mat	486	***TNK2***, *TFRC*, *SLC51A*, *PCYT1A*, *TNK2-AS1*, *TCTEX1D2*, *SDHAP1*, *LINC00885*, *ZDHHC19*, *UBXN7*, *TM4SF19-TCTEX1D2*, *TM4SF19-AS1*	LP
Family 2, mother	arr[GRCh37] 3q29(195633970_196120090)×3	486	***TNK2***, *TFRC*, *SLC51A*, *PCYT1A*, *TNK2-AS1*, *TCTEX1D2*, *SDHAP1*, *LINC00885*, *ZDHHC19*, *UBXN7*, *TM4SF19-TCTEX1D2*, *TM4SF19-AS1*	LP
Family 3, Patient 3.1	arr[GRCh37] 3q29(197052877_197310451)×3 dn	257	*LINC02012*, ***BDH1***	VUS
Family 4, Patient 4.1	arr[GRCh37] 3q29(197052877_197310451)×3 dn	257	*LINC02012*, ***BDH1***	VUS

Note: (Brandt et al. 2020; Riggs et al. 2020). Genes that are discussed in the literature as potentially related to abnormalities in the functioning of the nervous system are listed in bold font.
Abbreviations: LP, likely pathogenic; VUS, variant of uncertain significance.

for exon 12 of the *IQCG* gene and exon 16 of the *LMLN* gene. The duplication was inherited from the apparently healthy mother and grandmother. An aCGH analysis was performed with an Agilent 60K array to identify the borders of the 3q29 duplication and the presence of possible additional modifying CNVs in the mother and grandmother. No modifying CNVs were found (Table 2). No pathogenic or likely pathogenic single nucleotide variants associated with the patient's phenotype were detected by WES.

3.1.2 | Family 2

Patients 2.1 and 2.2 were 5-year-old girls from a twin pregnancy. The family was referred to the Genetic Clinic due to developmental and speech delays in the twin girls. The patients were born at 38 weeks of gestation via cesarean section. The patients were born from a second pregnancy; the first pregnancy ended with miscarriage. The karyotype of both patients (lymphocytes) was 46,XX. Their birth weights were 2470 g (3rd–5th centiles) and 2650 g (5th centile), and their birth lengths were 49 cm (50th centile) and 50 cm (50th centile). The Apgar score was 9/9 for both patients. Developmental milestones were appropriate for their age (held head up at 1.5 months, sat at 6 months, and walked at 12 months). In the first year of life, the patients spoke no identifiable words and only babbled. The patients had no eye contact in the first year of life. The girls did not respond to their names, exhibited no tactile contact, and slept very poorly. Their mother reported these unusual behaviors in the first year of life, and a psychiatrist diagnosed autism. The complaints at the time of the examination (5 years old) were as follows: speech delay (separate words and simple phrases, dyslalia), partial understanding of speech, communication difficulties, food selectivity, stereotyped movements, hyperactivity, and behavioral dysregulation.

Duplications of the region associated with chromosome 3q29 duplication syndrome (OMIM 611936) were detected in Patients 2.1 and 2.2 (arr[GRCh37] 3q29(195633970_196120090)×3) (Figure 2, Table 2). The duplication was verified by real-time PCR with primers for exon 2 of the *LINC00885* gene. The duplication was inherited from the apparently healthy mother. An aCGH analysis was performed with an Agilent 60K array to identify the borders of the 3q29 duplication and the presence of possible additional modifying CNVs in the mother. No modifying CNVs were found (Table 2). WES did not reveal any pathogenic or likely pathogenic single nucleotide variants associated with the patient's phenotype.

3.1.3 | Family 3

Patient 3.1 was a 5-year-old girl. The pediatrician referred the family to the Genetic Clinic due to the short stature of the patient. She was born at the 39th week of gestation. The karyotype (lymphocytes) was 46,XX. Her birth weight was 3320 g (25–50th centiles), and her birth length was 52 cm (75th centile). The child was from a fifth pregnancy; the first pregnancy ended with induced abortion. The patient had two older sisters and one younger sister who were all healthy; the eldest sister was from another marriage. The patient was diagnosed with right-sided torticollis and a patent foramen ovale at birth. Until 1 year of age, the child grew and developed according to her age. The patient began to walk when she was 1-year-old, after which hip dysplasia was diagnosed, which was corrected with the help of spacers and plaster. Phrased speech developed at age one and a half.

The patient was examined by a geneticist at age 5 years. The development of the child was appropriate for her age. The intelligence of the girl was typical. The patient's weight was 15.5 kg (10th centile), and her height was 100 cm (5th centile). Due to her short stature, a deletion of the *SHOX* gene was suspected and excluded by FISH; the karyotype was 46,XX.ish Xp22(SHOX,DXZ1)×2;nuc ish Xp22(SHOX,DXZ1)×2[50]. The phenotype of the proband included a prominent forehead and occiput, exophthalmos, hypertelorism of the eyes, blue sclera, prominent supraorbital ridges, wide nasal bridge, cleft ala nasi, microretrognathia, gothic palate, short neck, funnel chest, nipple hypertelorism, and flat feet.

A single-gene duplication of the *BDH1* gene, a candidate gene for chromosome 3q29 duplication syndrome (OMIM 611936), was detected in Patient 3.1 (arr[GRCh37] 3q29(197052877_197310451)×3) (Figure 3, Table 2). The duplication was verified by real-time PCR with primers for exon 5 of the *BDH1* gene. The duplication was *de novo*. No modifying CNVs were found (Table 2). No pathogenic or likely pathogenic single nucleotide variants associated with the patient's phenotype were identified using WES.

3.1.4 | Family 4

Patient 4.1 was an 11-year-old boy. The family was referred to the Genetic Clinic due to DD and autistic signs in the child. The karyotype (lymphocytes) was 46,XY. The child was born from a fourth pregnancy. The first pregnancy ended with the birth of a boy who, according to his mother, suffered from delayed speech development. The second and third pregnancies ended in induced abortions. The birth weight of the proband was 3300 g (25th centile), and his birth length was 52 cm (50th centile). His Apgar score was 8/9. At birth, the child was diagnosed with right-sided cryptorchidism. The motor development of the boy in the first year of life corresponded to his age. He spoke his first word before 1 year of age. At the age of 1.5 years, the boy suffered from acute otitis media. One week after recovery, the child experienced sudden regression of speech, lost eye contact, and became "on his own." From the age of three, the child was observed by a psychiatrist and diagnosed with childhood autism. Since the age of 5 years, the boy has been observed by a neurologist–epileptologist and was diagnosed with symptomatic focal epilepsy, occipital epilepsy, focal vegetative seizures, early childhood autism, and obesity. At the age of 6, the boy was diagnosed with a congenital heart defect (coarctation of the aorta), and balloon angioplasty was performed. After surgical correction of the defect, the epileptic seizures stopped. From the age of 8, the child was observed by a gastroenterologist and diagnosed with biliary tract dysfunction with dyscholia. Ultrasound revealed hepatomegaly.

At age 10, the patient's weight was 70 kg (> 97th centile), and his height was 152 cm (97th centile). The phenotypic manifestations included protruding ears, clinodactyly of the little toes, and valgus deformity.

At age 11, the patient's weight was 84 kg (> 97th centile), and his height was 164 cm (> 97th centile). His body mass index was 31.23,

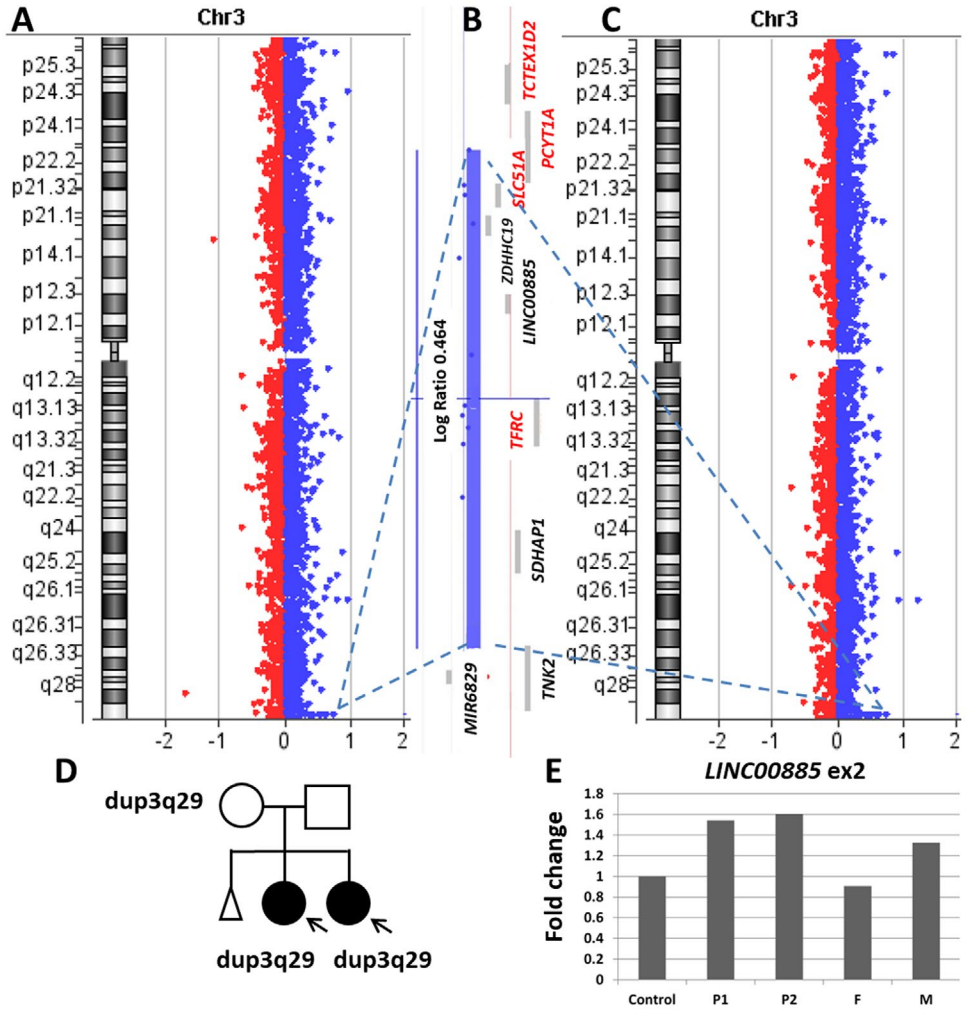

FIGURE 2 | Molecular cytogenetic results and pedigree of Family 2. (A) aCGH profile of chromosome 3 in Patient 2.1. (B) Gene content of the duplicated 3q29 region (blue). (C) aCGH profile of chromosome 3 in Patient 2.2. (D) Pedigree of Family 2. (E) qPCR results obtained with primers for exon 2 of the *LINC00885* gene. X axis—control DNA and examined individuals; Y axis—fold change in the copy number of a DNA region compared with the control. F, father; M, mother; P1, patient 2.1, P2, patient 2.2.

and his body type was proportional. His constitution is hypersthenic. His muscle tone was diffusely symmetrically reduced. Two café-au-lait spots were present on the skin of the abdomen that were irregular in shape and 1–3 cm in length. Two spots of up to 4–5 cm in length were present above the left shoulder blade. Many small spots that were irregular in shape were present on the left knee. An *NF1* deletion was excluded by FISH analysis (nuc ish 17q11(NF1×2)[50]). The patient used simple sentences in conversation and exhibited echolalia. The boy was learning English as a second language, and English speech seemed to be easier for him than native Russian. He could partially understand addressed speech, but repetitions of what has been said were needed.

A single-gene duplication of the *BDH1* gene, similar to that in Patient 3.1, was detected in Patient 4.1 (arr[GRCh37] 3q29(197052877_197310451)×3) (Figure 4, Table 2). This finding was verified by real-time PCR with primers specific for exon 5 of the *BDH1* gene. The duplication was *de novo*. No additional modifying CNVs were found (Table 2), nor were pathogenic or likely pathogenic single nucleotide variants associated with the patient's phenotype.

3.2 | Characteristics of the 3q29 Region

All 3q29 duplications found in our patients overlapped with a known region of chromosome 3q29 duplication syndrome but had different coordinates and genetic contents. In two families, the smallest duplication was found to affect the only protein-coding gene associated with nervous system function, *BDH1*–3-hydroxybutyrate dehydrogenase 1 (OMIM 603063). Therefore, we aimed to delineate the MOR for duplications in our study and those reported in the literature (Table 3). In three studies describing patients with dup3q29, the exact coordinates of the breakpoints of the aberrations were not reported; therefore, we were not able to include these patients in the analysis to delineate the minimal critical region (Ballif et al. 2008; Goobie et al. 2008; Pollak et al. 2020). Patients described by Coyan and Dyer (2020) were also excluded because the clinical features of each individual were not provided and four subjects had additional genetic findings, some of which were pathogenic. A patient described by Ormond et al. (2024) from a monozygotic twin pair who suffered from bipolar disorder (BP) and carried a recurrent 1.69 Mb 3q29 duplication revealed by WGS was not included, as a detailed

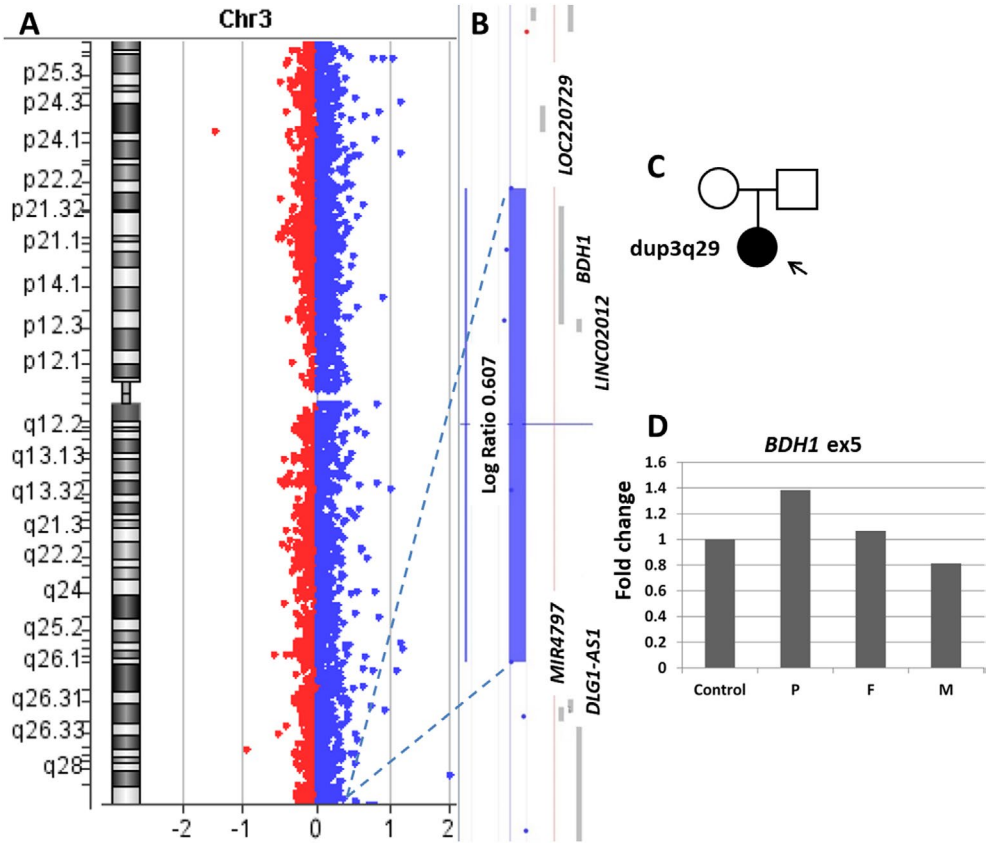

FIGURE 3 | Molecular cytogenetic results and pedigree of Family 3. (A) aCGH profile of chromosome 3 in Patient 3.1. (B) Gene content of the duplicated 3q29 region (blue). (C) Pedigree of Family 3. (D) qPCR results obtained with primers for exon 5 of the *BDH1* gene. X axis—control DNA and examined individuals; Y axis—fold change in the copy number of a DNA region compared with the control. F, father; M, mother; P, patient 3.1.

description of the affected individual in the paper, other than BP, was not provided. The second twin was healthy with a balanced karyotype.

By plotting all the data on the chromosome 3 ideogram, we were able to identify two MORs—MOR1 and MOR2 (Figure 5).

MOR1 ([GRCh37] 3q29(195751853_196120090)×3) included the following genes: *TFRC*, *LINC00885*, *ZDHHC19*, *SLC51A*, *PCYT1A*, *TCTEX1D2*, *TM4SF19*, and *UBXN7*. The OMIM genes with disease-associated variants are listed below. The *TFRC* gene encodes the transferrin receptor, which is important for cellular iron uptake (OMIM 190010) and is a known blood barrier transporter. The receptor is required for erythropoiesis and neurological development (NCBI, Gene ID 7037) (http://www.ncbi.nlm.nih.gov/gene). The homozygous missense variant Y20H in the *TFRC* gene causes immunodeficiency-46 (IMD46), which is characterized by severe childhood infections (OMIM 616740). *ZDHHC19* is a palmitoyl acyltransferase (OMIM 618671). The *ZDHHC19* gene is homologous to the *ZDHHC9* gene, which encodes an enzyme called palmitoyltransferase and has been described to be involved in X-linked syndromic intellectual developmental disorder, Raymond type (OMIM 300799). *SLC51A* encodes an organic solute transporter (OMIM 612084) and participates in neurosteroid transport in the brain (neurons of the cerebellum and hippocampus, and Purkinje cells) (Grube, Hagen, and Jedlitschky 2018). The homozygous variant Q186X in the *SLC51A* gene is associated with progressive familial intrahepatic cholestasis-6 (PFIC6), which is characterized by elevated liver transaminase levels, cholestasis, and congenital diarrhea (OMIM 619484). *PCYT1A* encodes phosphocholine cytidylyltransferase (OMIM 123695), in which homozygous or compound heterozygous mutations are associated with spondylometaphyseal dysplasia with cone–rod dystrophy characterized by postnatal growth deficiency resulting in a profound short stature, rhizomelia with bowing of the lower extremities, platyspondyly with anterior vertebral protrusions, progressive metaphyseal irregularity and cupping with shortened tubular bones, and early-onset progressive visual impairment associated with pigmentary maculopathy and electroretinographic evidence of cone–rod dysfunction (OMIM 608940). In mice, *Pcyt1a*−/− zygotes fail to form blastocysts, do not develop past embryonic day 3.5 (E3.5), and fail to implant. *In situ* hybridization of E11.5 embryos revealed that *Pcyt1a* is expressed ubiquitously, with the highest level occurring in the fetal liver (Wang et al. 2005). *TCTEX1D2* encodes a light chain that appears to stabilize retrograde intraflagellar transport and the dynein complex (OMIM 617353). Cytoplasmic dyneins function in intracellular motility, including retrograde axonal transport, protein sorting, organelle movement, and spindle dynamics (NCBI, Gene ID 255758). Homozygous or compound heterozygous variants in the *TCTEX1D2* gene are associated with short-rib thoracic dysplasia-17 with or without polydactyly (SRTD17), characterized by a constricted thorax, short ribs, shortened tubular bones, and a 'trident' appearance of the acetabular roof (OMIM 617405). Short-rib polydactyly syndromes are also accompanied by extraskeletal phenotypes, including kidney, liver, eye, heart, and brain defects (Schmidts et al. 2015). In human brain tissue, the *TM4SF19* gene

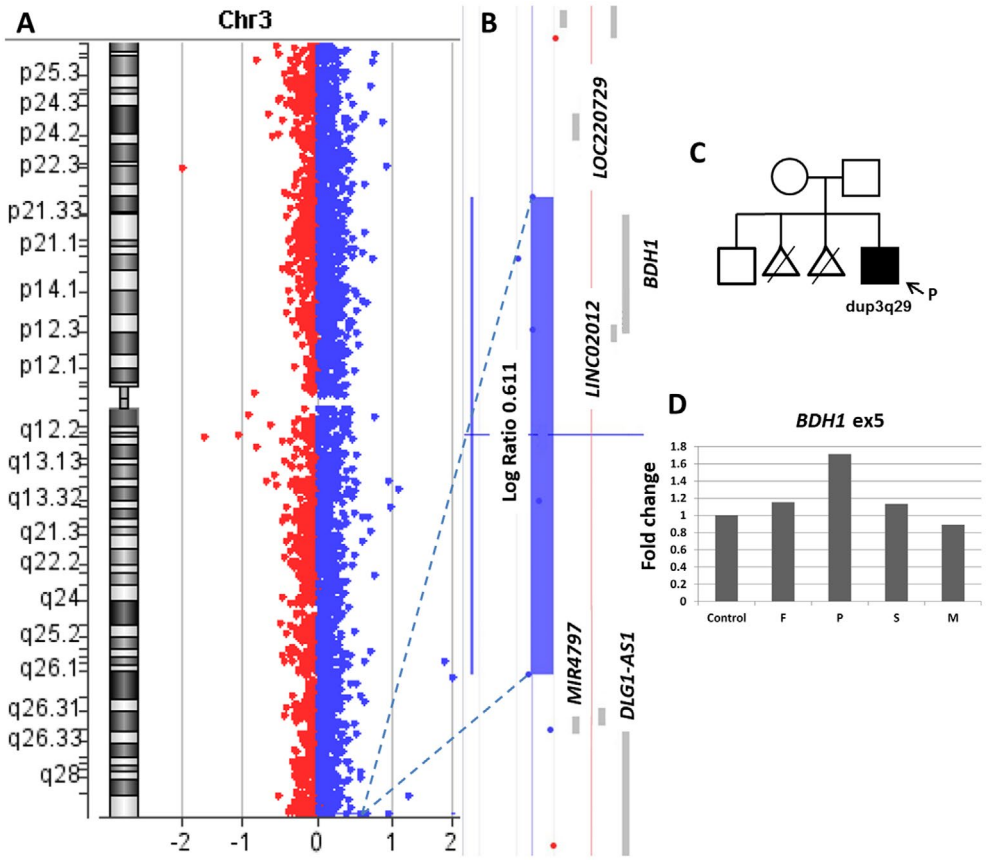

FIGURE 4 | Molecular cytogenetic results and pedigree of Family 4. (A) aCGH profile of chromosome 3 in Patient 4.1. (B) Gene content of the duplicated 3q29 region (blue). (C) Pedigree of Family 4. (D) qPCR with primers for exon 5 of the *BDH1* gene. X axis—control DNA and examined individuals; Y axis—fold change in the copy number of a DNA region compared with the control. F, father; M, mother; P, patient 4.1; S, sibling.

TABLE 3 | Coordinates of 3q29 microduplications in patients from the literature and from this study.

Paper	Case no.	GRCh37
Coordinates of 3q29 duplication syndrome from DECIPHER		
		chr3:195726835–197344663
Coordinates of 3q29 duplications in patients from the literature		
Lisi et al. (2008)	Study 1 Case 1 (S1C1)	chr3:195656731–197421769
Schilter et al. (2013)	Study 2 Case 1 (S2C1)	chr3:195383130–197550799
Fernández Jaén et al. (2014)	Study 3 Case 1 (S3C1)	chr3:195731956–197339329
Lawrence et al. (2017)	Study 4 Case 1 (S4C1)	chr3:195495220–197851986
Viñas-Jornet et al. (2018)	Study 5 Case 1 (S5C1) (trip-mat-pat)	chr3:196022728–196515371
Vitale et al. (2018)	Study 6 Case 1 (S6C1)	chr3:195747856–197339329
Tassano et al. (2018)	Study 7 Case 1 (S7C1)	chr3:195633970–197532175
	Study 7 Case 2 (S7C2)	chr3:196892527–197339329
Streata et al. (2020)	Study 9 Case 1 (S9C1)	chr3:195979518–197638922
Bauleo et al. (2023)	Study 10 Case 1 (S10C1)	chr3:196892569–197324567
	Study 10 Case 2 (S10C2)	chr3:196892569–197324567

(Continues)

TABLE 3 | (Continued)

Paper	Case no.	GRCh37
Massier et al. (2024)	Study 11 Case 1 (S11C1)	chr3:195623619–197317244
	Study 11 Case 2 (S11C2)	chr3:195738406–197332150
	Study 11 Case 3 (S11C3)	chr3:195740357–197310451
	Study 11 Case 4 (S11C4)	chr3:195740357–197310451
	Study 11 Case 5 (S11C5)	chr3:195740357–197317103
	Study 11 Case 6 (S11C6)	chr3:195740357–197317103
	Study 11 Case 7 (S11C7)	chr3:195747856–197339329
	Study 11 Case 8 (S11C8)	chr3:195747856–197398813
	Study 11 Case 9 (S11C9)	chr3:195747856–197398813
	Study 11 Case 10 (S11C10)	chr3:195747856–197339329
	Study 11 Case 11 (S11C11)	chr3:195747856–197339329
	Study 11 Case 12 (S11C12)	chr3:195747856–197339329
	Study 11 Case 13 (S11C13)	chr3:195747856–197339329
	Study 11 Case 14 (S11C14)	chr3:195747856–197339329
	Study 11 Case 15 (S11C15)	chr3:195747856–197339329
	Study 11 Case 16 (S11C16)	chr3:195747856–197339329
	Study 11 Case 17 (S11C17)	chr3:195908864–197310451
	Study 11 Case 18 (S11C18)	chr3:195908864–197310451
	Study 11 Case 19 (S11C19)	chr3:195425875–197608392
	Study 11 Case 20 (S11C20)	chr3:195425875–197608392
	Study 11 Case 21 (S11C21)	chr3:195456487–197310451
	Study 11 Case 22 (S11C22)	chr3:196639961–196860980
	Study 11 Case 23 (S11C23)	chr3:196787361–197597264
	Study 11 Case 24 (S11C24)	chr3:196860721–197387399
	Study 11 Case 25 (S11C25)	chr3:196892527–197339329
	Study 11 Case 26 (S11C26)	chr3:197023214–197324783
	Study 11 Case 27 (S11C27)	chr3:197023214–197324783
	Study 11 Case 28 (S11C28)	chr3:197031290–197339329
	Study 11 Case 29 (S11C29)	chr3:197031290–197339329
	Study 11 Case 30 (S11C30)	chr3:197052877–197310451
	Study 11 Case 31 (S11C31)	chr3:197052877–197310451
	Study 11 Case 32 (S11C32)	chr3:197052877–197310451
	Study 11 Case 33 (S11C33)	chr3:197074890–197324783
	Study 11 Case 34 (S11C34)	chr3:197209837–197339329
Coordinates of 3q29 duplications in patients from the present study		
Present study	Case 1 (Patient 1.1)	chr3:197608333–197840339
	Case 2 (Patient 2.1)	chr3:195633970–196120090
	Case 3 (Patient 2.2)	chr3:195633970–196120090
	Case 4 (Patient 3.1)	chr3:197052877–197310451
	Case 5 (Patient 4.1)	chr3:197052877–197310451

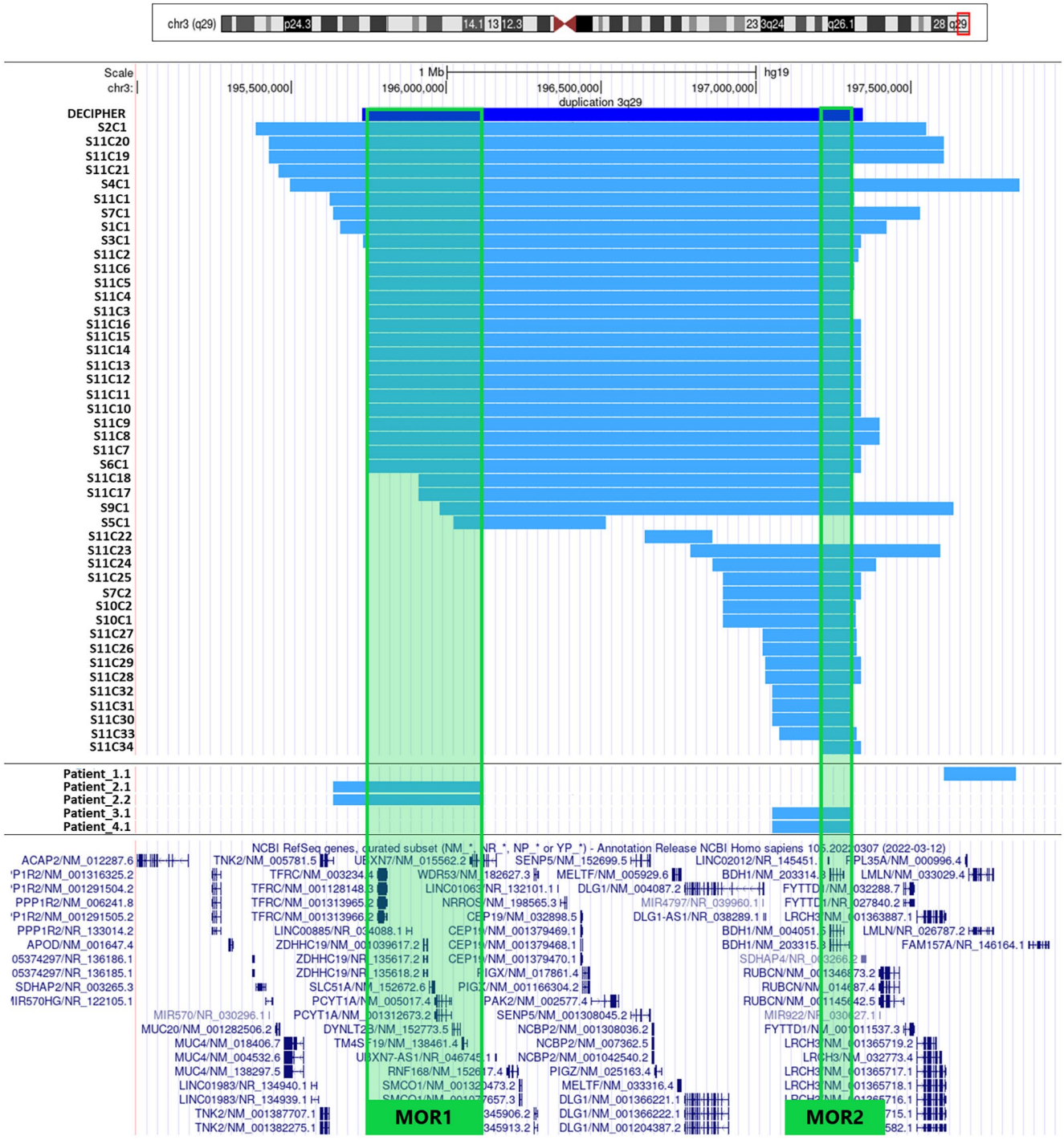

FIGURE 5 | Map of 3q29 microduplications with minimal overlapping regions. MOR, minimal overlapping region.

is expressed at high levels in the parietal lobe, occipital lobe, hippocampus, pons, white matter, corpus callosum, and cerebellum. TM4SF19 is a member of the transmembrane 4 L six family, which functions in various cellular processes, including cell proliferation, motility, and adhesion via interactions with integrins (NCBI, Gene ID 116211). *UBXN7* enables ubiquitin binding activity and ubiquitin protein ligase binding activity (NCBI, Gene ID 26043).

MOR2 ([GRCh37] 3q29(197121593_197310451)×3) included only the *BDH1* gene. This gene encodes a mitochondrial membrane enzyme with an absolute and specific requirement for phosphatidylcholine (OMIM 603063).

We also summarized the clinical features of the patients and their relatives with 3q29 duplications for whom the exact coordinates of the aberrations were known; patients with additional genetic findings were not included (Tables 3 and 4). Two frequencies were calculated: common (for duplications regardless of the coordinates and size) and a frequency for recurrent and overlapping duplications > 1 Mb.

Sixty-four individuals in total were analyzed (patients and their affected or healthy relatives who were carriers of dup3q29). In a family reported by Lisi et al. (2008) and in Family 2 in the present study, miscarriages during anamnesis

TABLE 4 | Frequency of clinical features in individuals with dup3q29.

Feature	Lisi et al. (2008), $n=5$[a]	Schilter et al. (2013), $n=1$[a]	Fernández Jaén et al. (2014), $n=1$[a]	Lawrence et al. (2017), $n=1$[a]	Viñas-Jornet et al. (2018), $n=1$	Vitale et al. (2018) $n=3$	Tassano et al. (2018), $n=2$[a]
Small for gestational age	3/5	N/A	1	1	N/A	N/A	1/2
DD, ID	3/3	0	1	N/A	1	0	2/2
Language delay	0	0	1	N/A	0	0	2/2
ASD	0	0	0	N/A	0	0	1/2
ADHD	0	0	0	N/A	0	0	0
Epilepsy	0	0	1	0	0	0	1/2
Microcephaly	4/5	0	0	1	0	0	1/2
Macrocephaly	1/5	0	1	0	0	0	0
Structural brain anomalies	N/A	0	0	1	N/A	N/A	0
Ocular anomalies	0	1	0	0	0	0	1/2
Musculoskeletal anomalies	2/5	0	1	1	1	0	2/2
Congenital heart disease	1/5	0	1	0	0	0	0
Anomalies of the genitourinary system	1/5	0	0	1	0	0	0
Dental anomalies	0	0	0	N/A	1	0	1/2
Conductive hearing loss	0	0	1	0	0	0	0
Overweight/obesity	3/4	0	1	0	1	3/3	0
Weight deficit	0	0	0	0	0	0	0
Dysphagia	0	0	0	0	0	0	0
Palpebral fissure anomalies	2/5	0	1	1	1	0	0
Nasal anomalies	4/5	0	1	1	0	0	0
Excessive hand creases	2/5	0	1	0	0	0	0
Diabetes	1/5	0	0	0	0	2/3	0
Hypertension	1/5	0	0	0	0	2/3	0
Dyslipidemia	0	0	0	0	0	2/3	0
Melanoma	0	0	0	0	0	1/3	0
Hemangioma	1/5	0	0	0	0	0	0
Café-au-lait spots	0	0	0	0	0	0	0
Myelomeningocele	0	0	0	1	0	0	0
Chronic pain	0	0	0	0	0	0	0
Sleep disorder	0	0	0	0	0	0	0
Additional CNVs	0	0	0	0	0	0	0

Note: Viñas-Jornet et al. (2018) described a patient with a trip3q29 mat-pat. Vitale et al. (2018) described a family in which two sons had the same phenotype as their father, but dup3q29 was inherited from their mother, who was only overweight; the father did not carry dup3q29. Streata et al. (2020) described a patient with a late onset of the disease (10 years) and a severe phenotype. Patient with pathogenic 15q11.2 deletion and patients with unbalanced translocations and single nucleotide variants from the study by Massier et al. (2024) were excluded.

Abbreviations: ASD, autism spectrum disorder; DD, developmental delay; ID, intellectual disability; N/A, information is not available.

[a]Recurrent and overlapping duplications > 1 Mb.

[b]Atypical duplications < 1 Mb. The common frequency was calculated regardless of the coordinates and size of the duplications.

Streata et al. (2020), $n=1$[a]	Bauleo et al. (2023), $n=4$[b]	Massier et al. (2024), $n=21$[a]	Massier et al. (2024), $n=13$[b]	Present study, $n=8$[b]	Common frequency	Frequency of recurrent and overlapping duplications > 1 Mb	p
0	0/4	1/21	0/13	1/8	15/56 (27%)	14/31 (45%)	0.082
1	2/4	15/21	6/11	4/8	35/57 (61%)	23/34 (68%)	0.549
1	2/4	1/19	0/10	4/8	11/55 (20%)	5/33 (15%)	0.569
0	1/4	2/4	3/9	3/8	10/39 (26%)	3/18 (17%)	> 0.05
0	0	0	2/9	0	2/56 (4%)	0	—
0	0	2/21	0	1/8	5/59 (8%)	4/34 (12%)	> 0.05
0	0	0	0	N/A	6/45 (13%)	6/28 (21%)	0.365
0	0	1/21	0	N/A	3/53 (6%)	3/53 (6%)	1
1	0	N/A	1/1	0	3/19 (16%)	2/7 (29%)	> 0.05
0	0	N/A	1/9	1/8	4/36 (11%)	2/15 (13%)	> 0.05
1	0	3/21	1/13	3/5	15/58 (26%)	11/36 (31%)	0.621
0	0	2/19	0	2/8	6/59 (10%)	4/34 (12%)	> 0.05
0	0	0	0	0	2/61 (3%)	2/36 (6%)	> 0.05
0	0	0	1/13	0	3/60 (5%)	2/35 (6%)	> 0.05
0	0	2/21	0	0	3/61 (5%)	3/36 (8%)	> 0.05
0	0	2/21	1/13	1/5	12/57 (21%)	10/35 (29%)	0.412
0	0	0	0	1/8	1/61 (2%)	0	—
1	0	0	0	0	1/61 (2%)	1/36 (3%)	> 0.05
1	0	0	0	0	6/61 (10%)	6/36 (17%)	0.324
1	0	0	0	1/5	8/58 (14%)	7/36 (19%)	0.154
0	0	0	0	0	3/61 (5%)	3/36 (8%)	> 0.05
0	0	0	0	0	3/61 (5%)	3/36 (8%)	> 0.05
0	0	0	0	0	3/61 (5%)	3/36 (8%)	> 0.05
0	0	0	0	0	2/61 (3%)	2/36 (6%)	> 0.05
0	0	0	0	0	1/61 (2%)	1/36 (3%)	> 0.05
0	0	0	0	1/5	2/58 (3%)	1/36 (3%)	> 0.05
0	0	0	0	1/5	1/58 (2%)	0	—
0	0	0	0	0	1/61 (2%)	1/36 (3%)	> 0.05
1	0	0	0	0	1/61 (2%)	1/36 (3%)	> 0.05
1	1/4	0	0	2/5	4/58 (7%)	1/36 (3%)	> 0.05
0	1/4	0	0	0	1/61 (2%)	0	—

TABLE 5 | Phenotypes induced by reciprocal deletion and duplication of the 3q29 locus.

Categories	Phenotypes	3q29 duplication syndrome	3q29 deletion syndrome	*p*
Common	ASD	3/18 (17%)	12/32 (38%)	>0.05
	Language/speech delay	5/33 (15%)	25/39 (64%)	**<0.001**
	ID/DD	23/34 (68%)	11/32 (34%)	**0.007**
	Ocular anomalies	2/15 (13%)	19/32 (59%)	**<0.05**
	Dental anomalies	2/35 (6%)	13/32 (41%)	**<0.05**
	Congenital heart disease	4/34 (12%)	15/32 (47%)	**<0.05**
	Musculoskeletal anomalies	11/36 (31%)	49/57 (86%)	**<0.001**
	Macrocephaly	3/53 (6%)	1/15 (6%)	>0.05
	Microcephaly	6/28 (21%)	7/15 (46%)	0.086
Mirrored	Weight deficit	N/A	26/32 (81%)	N/A
	Overweight	10/35 (39%)	N/A	N/A
Unique	SZ	N/A	20–40 fold increased risk[a]	N/A
	GAD	N/A	26/67 (28%)	N/A
	Recurrent ear infections	N/A	Frequent	N/A

Note: p, significance level, $p < 0.05$ is shown on bold.
Abbreviations: ASD, autism spectrum disorder; GAD, generalized anxiety disorder; ID, intellectual disability; N/A, not available; SZ, schizophrenia.
[a]Mulle (2015).

occurred. Unfortunately, the karyotype of the embryos was not investigated. These miscarriages may also have been carriers of dup3q29 inherited from one of the parents. Coyan and Dyer (2020) identified dup3q29 in three families during the prenatal period. However, two fetuses had additional genetic aberrations consistent with the indications for testing. One fetus had a pathogenic variant in the *EFTUD2* gene consistent with a diagnosis of mandibulofacial dysostosis, and the indication for testing was microcephaly. The second product of conception was tested due to recurrent pregnancy loss, and trisomy 21 (a known risk factor for miscarriage) was identified. The third fetus had anencephaly, a severe phenotype inconsistent with a reduced penetrance of dup3q29.

According to our summarized data on the clinical presentations of patients with dup3q29 presented in Table 4, the following symptoms are the most common (among all patients and patients with recurrent and overlapping duplications >1 Mb, respectively): DD/ID (61% and 68%), musculoskeletal anomalies (26% and 31%, including abnormal muscle tonus, gait anomalies, high-arched palate, and pes planus), overweight/obesity (21% and 29%), ASD (26% and 17%), structural brain anomalies (16% and 29%), microcephaly (13% and 21%), language delay (20% and 15%), nasal anomalies (14% and 19%), ocular anomalies (11% and 13%), palpebral fissure anomalies (10% and 17%), epilepsy (8% and 12%), congenital heart disease (10% and 12%), and sleep disorders (7% and 3%). Importantly, 45% of the individuals with recurrent and overlapping large duplications were small for gestational age. Although some features are more common in patients with recurrent and large overlapping duplications (small for gestational age, DD/ID, musculoskeletal anomalies, overweight/obesity, structural brain anomalies, microcephaly, nasal anomalies, epilepsy, and heart disease), no statistically significant differences were observed compared with the common frequency in the general group. ASD, language delay, and sleep disorders slightly prevailed in the general group. ADHD and café-au-lait spots were described only in patients with atypical 3q29 duplications (Table 4), as well as BP (Ormond et al. 2024). Therefore, the symptoms from summary Table 4 partially overlap with those mentioned in the Clinical Synopsis for chromosome 3q29 duplication syndrome in the OMIM database (OMIM 611936). The features observed in patients with recurrent and large overlapping duplications also significantly complement the known symptoms; musculoskeletal, brain, and heart anomalies, as well as epilepsy and sleep disorders, which occur with a frequency of 3%–31%, are not represented in the Clinical Synopsis.

Additional CNVs or SNVs were identified in 16 families (Bauleo et al. 2023; Coyan and Dyer 2020; Goobie et al. 2008; Massier et al. 2024). Bauleo et al. (2023) identified an additional dup11q22.1 in Patient S10C2. This intragenic duplication (397.8 kb) encompassing nine exons of the *CNTN5* gene (11q22.1, OMIM 607219) was inherited from the unaffected father. The authors classified it as a variant of uncertain significance (VUS). A 3q39 duplication in this family was inherited by three children (a healthy daughter and two affected sons) from an unaffected mother. All three siblings reported by Coyan and Dyer (2020) had a 928 kb 2p11.2 deletion involving *REEP1* (OMIM: 609139). This gene is associated with neuropathy, spastic paraplegia, and spinal muscular atrophy. The proband from another family had a 17q12 duplication that is considered a neurosusceptibility locus. The authors suggest an additive effect of dup3q29 and dup17q12. Goobie et al. (2008) reported all CNVs were detected in

their patients. Most of them, except dup8p23.1 (407 kb) and dup6q24.2-q24.3 (2.6 Mb), are polymorphic CNVs present in the general population. Dup8p23.1 was interpreted as likely pathogenic because the proximal end of this gene overlapped with the region associated with 8p23.1 duplication syndrome. Patients with dup3q29 and dup8p23.1 shared some clinical features with subjects with 8p23.1 duplication syndrome. The dup6q24.2-q24.3 region in another patient included the *UTRN* gene, which, according to the authors, could be related to his tetramelia. Massier et al. (2024) identified additional genetic aberrations in individuals from nine families. In one family, both the patient and his mother carried an SNP of uncertain clinical significance in the *TRRAP* gene along with recurrent dup3q29. In the second family, a proband additionally had a VUS SNP in the *PHF6* gene. One additional patient carried a pathogenic 15q11.2 deletion corresponding to chromosome 15q11.2 deletion syndrome (OMIM 615656). Individuals from six families had unbalanced translocations.

4 | Discussion

Information on patients with dup3q29 was obtained from approximately two dozen articles, some of which were based on the 3q29 registry (https://3q29.com). In the canonical region (1.6–1.72 Mb), five candidate genes associated with neural development have been described. The MOR was identified by three research groups (Bauleo et al. 2023; Coyan and Dyer 2020; Tassano et al. 2018) and included two genes, *DLG1* and *BDH1*. In this study, we describe five new patients with atypical 3q29 duplications, two of which revealed aberrations affecting only the *BDH1* gene that comprises a single-gene MOR (MOR2) for the first time (Figure 5).

The *BDH1* gene encodes a mitochondrial membrane enzyme with an absolute and specific requirement for phosphatidylcholine. It is expressed in the developing murine cortex, has been implicated in aging and Alzheimer's disease, and may be responsible for ketone metabolism within the brain (Lee et al. 2017; Semeralul et al. 2006). The *BDH1* mRNA level was decreased significantly in the cortex of a mouse model of Alzheimer's disease (Zhang et al. 2023). Ketone metabolism is an essential process for normal heart and liver functions. An increase in ketone metabolism and the expression of the ketogenic enzyme β-hydroxybutyrate dehydrogenase 1 (BDH1) has been demonstrated in mice and humans with heart failure (Aubert et al. 2016; Bedi Jr. et al. 2016). BDH1 is the last enzyme in the process of hepatic ketogenesis and the first enzyme in the process of ketolysis in extraliver organs. Using *Bdh1*-deficient mice, Otsuka et al. (2020) demonstrated that systemic BDH1 deficiency was well tolerated under normal dietary conditions but manifested during fasting with hepatic steatosis, indicating the importance of ketogenesis for maintaining the lipid energy balance in the liver. Xu et al. (2022) reported that *Bdh1* knockdown led to reactive oxygen species (ROS) overproduction and ROS-induced inflammation and apoptosis in LO2 cells, whereas *Bdh1* overexpression protected LO2 cells from lipotoxicity by inhibiting ROS overproduction. Hepatic *Bdh1* also exhibits a developmental expression pattern, increasing in the brain and liver from birth to weaning (Cotter, Schugar, and Crawford 2013). Abnormal phenotypes are associated with reduced protein expression, and no information is available showing that the overexpression of the gene is related to any pathology.

Two patients from our study with *de novo* single-gene *BDH1* duplications presented variable phenotypes. Patient 3.1 presented with some dysmorphic features, skeletal abnormalities, and low weight and height. The intelligence and speech of the patient were intact, which is not typical for patients with chromosome 3q29 duplication syndrome. At birth, the patient was small for gestational age and had a minor heart defect (patent foramen ovale). Patient 4.1 was diagnosed with ASD and obesity. He also had epilepsy and congenital heart defects (coarctation of the aorta). After surgical correction of the heart defect, the epileptic seizures stopped. The patient was also diagnosed with biliary tract dysfunction with dyscholia and hepatomegaly.

Massier et al. (2024) described seven individuals from four families (patients and their parents who were carriers of dup3q29) who carried a single *BDH1* gene duplication. The only features described were DD (2/7), ID (1/5), learning disabilities (1/5), neuropsychiatric disorders (4/6), bilateral retinal coloboma (1/7), and dental dysgenesis (1/7). In two families, the duplication was inherited from the apparently healthy mothers. This gene is considered a candidate for proper brain, heart, and liver function. Symptoms associated with liver or heart problems are not presented in the article. However, whether the function of these organs was assessed in carriers of the *BDH1* duplication is not clear.

Duplications of the entire gene are usually interpreted as benign or likely benign variants (Brandt et al. 2020). The *BDH1* gene is not predicted to be triplosensitive; in contrast, it is haploinsufficient. Currently, how increased expression of this gene can cause this disease remains unclear, but the pathogenic effect may be related to the microduplication itself. As shown in our previous study of the 3p26.3 microduplication affecting a single *CNTN6* gene, increasing the copy number, especially in the subtelomeric region, led to a downregulation of gene expression, probably due to heterochromatinization of the duplicated area (Gridina et al. 2018).

Two additional atypical duplications at the 3q29 locus were observed in our patients. These duplications allowed us to delineate MOR1. MOR1 includes several genes potentially related to abnormal neuronal function, namely, *TFRC*, *ZDHHC19*, *SLC51A*, *TCTEX1D2*, and *TM4SF19*. ID was the most common feature of patients with dup3q29 (68%). *PCYT1A* and *TCTEX1D2* are potentially associated with skeletal anomalies.

Patient 1.1 carried another atypical 3q29 duplication, which, however, did not overlap with the canonical chromosome 3q29 duplication syndrome region. Moreover, two patients described by Lawrence et al. (2017) and Coyan and Dyer (2020) had duplications involving this region. This region does not contain genes associated with any known pathological phenotype except for the *RPL35A* heterozygous variant associated with DBA5, Diamond–Blackfan anemia-5 (OMIM 612528). However, information about such distal duplications is important because, as data accumulate, it may indicate the existence of an additional telomeric breakpoint for dup3q29.

Chromosome 3q29 deletion syndrome (OMIM 609425) is reciprocal to duplication syndrome and has the same genomic coordinates. Willatt et al. (2005) were the first to describe six patients with 3q29 deletion and identified the syndrome. Eight papers on del3q29 included analyses of the 3q29 registry (Glassford et al. 2016; Klaiman et al. 2023; Mak et al. 2021; Murphy et al. 2018; Pollak et al. 2019, 2023; Sanchez Russo et al. 2021; Wawrzonek et al. 2022). The frequencies of different phenotypes associated with the deletion calculated from the registry are included in Table 5 and were compared with the corresponding frequencies in patients with reciprocal recurrent duplications from Table 4. According to Golzio and Katsanis (2013), the most frequent phenotypes induced by reciprocal del3q29 and dup3q29 were classified into three categories (Table 5). Phenotypes such as ASD, language/speech delay, micro/macrocephaly, ocular and dental anomalies, heart problems (hypoplastic right heart, patent ductus arteriosus, tricuspid stenosis, ventricular septal defect, and arterial–venous malformation), and musculoskeletal anomalies (high-arched and cleft palate; chest deformities; long, thin, and tapered fingers; pes planus; medial rotation of the medial malleolus; abnormal hallux; abnormal nonhallux toes; abnormal muscle tonus; and gait anomalies) were common among patients with both deletions and duplications at the 3q29 locus, although language/speech delay and ocular, dental, heart and musculoskeletal pathologies were described significantly more frequently in individuals with deletions. Surprisingly, the DD/ID phenotype was more common in patients with duplications ($p = 0.007$). The rates of micro- and macrocephaly were not significantly different between the two groups. The mirrored phenotypes "weight deficit" and "overweight" were specific to patients with del3q29 and dup3q29, respectively; however, weight deficit was diagnosed in Patient 4.1 from the present study with an atypical duplication of the single *BDH1* gene. Schizophrenia, generalized anxiety disorder, and recurrent ear infections were unique to patients with 3q29 deletions. However, Ormond et al. (2024) recently described a patient with BP and canonical 3q29 duplication. Psychosis is one of the symptoms of schizophrenia and BP. The pathogenic effect of the deletion is assumed to be more likely caused by haploinsufficiency of the involved genes, whereas the effect of the duplication, which does not always manifest, often depends on the presence of additional factors.

The phenotypic variability and reduced penetrance of 3q29 duplication are discussed in the literature and are believed to be characteristics of neurosusceptibility loci (NSLs). These NSLs may manifest according to the "two-hit" model, that is, when they are affected by additional genetic aberrations and/or environmental factors. The epistatic effects of some genetic variants (those that may be undetected) on the phenotypic heterogeneity of neurodevelopmental disorders have also been discussed (Coyan and Dyer 2020). However, few publications have described additional genetic aberrations in patients with 3q29 duplication (Bauleo et al. 2023; Coyan and Dyer 2020; Goobie et al. 2008; Massier et al. 2024). Bauleo et al. (2023) described an additional dup11q22.1 inherited from the unaffected father that encompassed nine exons of the *CNTN5* gene; this gene is important for brain development and is associated with neurodevelopmental disorders. In four subjects, in addition to dup3q29, Coyan and Dyer (2020) described pathogenic variant in the *EFTUD2* gene associated with mandibulofacial dysostosis, trisomy 21, 2p11.2 deletion (928 kb), and 17q12 duplication. The deletion at the 2p11.2 locus overlapped with the region of larger deletions and was associated with DD and ID. Duplication of 17q12 was also considered an NSL. Overlapping clinical features have been reported between 17q12 and 3q29 duplications. Two patients described by Goobie et al. (2008) carried potentially pathogenic dup8p23.1 and dup6q24.2-q24.3, which are associated with 8p23.1 duplication syndrome and tetramelia, respectively. Massier et al. (2024) reported additional genetic findings in individuals from nine families: SNPs of uncertain clinical significance in the *TRRAP* and *PHF6* genes, a pathogenic 15q11.2 deletion associated with chromosome 15q11.2 deletion syndrome, and unbalanced translocations. In three of the five patients and their mothers who carried a single-gene *BDH1* duplication, no additional genetic variants were identified through ES. No other genetic analyses were performed for the remaining two subjects with *BDH1* duplication. In our patients, no additional pathogenic or likely pathogenic CNVs or SNVs or CNVs interpreted as VUSs were found.

A milder manifestation and a more pronounced incomplete penetrance of the duplication may also be due to the coordinates and size of the duplicated region. Twenty-four of 64 subjects (38%) with known coordinates of the aberration had atypical duplications, which allowed us to delineate two MORs (Figure 5). Atypical aberrations were identified in seven of the 64 patients with deletions (11%) ($p < 0.001$) (Figure S1). The MOR for deletions corresponds to the critical region in 3q29 deletion syndrome (OMIM 609425). When analyzing CNV boundaries, researchers must consider that they are influenced by the array resolution and the position and number of oligonucleotide DNA probes on the array. Therefore, the coordinates and sizes of CNVs determined by a chromosomal microarray analysis are relative.

Overall, in this study, we described four new families with 3q29 duplications. By reviewing the literature, the frequencies of pathological phenotypes in duplication carriers were calculated and compared with those in patients with 3q29 deletions. Common phenotypes were observed between both groups but were slightly less common among individuals with 3q29 duplications. The mirrored category included overweight for patients with dup3q29 and weight deficit for patients with del3q29. The unique phenotypes SZ, GAD, and recurrent ear infections were observed only in patients with del3q29. We also delineated and described two MORs for 3q29 duplication. The smallest MOR included only the *BDH1* gene, which is important for normal brain, heart, and liver functions. In two of our patients with single-gene *BDH1* duplications, ASD, heart defects, biliary tract dysfunction, and obesity were described. The phenotypic variability and reduced penetrance of 3q29 duplication may be due to atypical aberrations, as well as additional genetic and/or environmental factors. The main limitation of the study is the lack of functional data.

Author Contributions

K.A.A. analyzed the data and wrote the manuscript. L.M.E. and V.O.Y. performed the aCGH analysis; F.D.A. performed the real-time PCR analysis; F.E.A., Z.I.Z., and Z.A.A. performed WES; S.O.A., B.E.O., P.V.V., R.E.G., and A.A.A. performed genetic consultations; C.A.D. performed the FISH analysis, T.N.B. performed the cytogenetic analysis, and L.I.N. participated in the conceptualization and discussion of the manuscript.

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Consent

Informed consent for the analyses and publication was obtained from the parents of the patients.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

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

Web Resources

Database of genomic variants [http://projects.tcag.ca/variation].

NCBI Gene Database [http://www.ncbi.nlm.nih.gov/gene].

Online Mendelian Inheritance in Man [http://omim.org].

Primer3 (v. 0.4.0) [https://bioinfo.ut.ee/primer3-0.4.0/].

3q29 Registry [https://3q29.com].

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.