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Novel Insights from Clinical Practice

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Genomic Characterization of a Rare, de Novo Unbalanced ins(3;1)(p25.3;q21.3q23.3) in a Female Child with Multiple Congenital Anomalies

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Established Facts

- "Simple" 1-way interchromosomal insertions involving an interstitial 1q segment are exceptional.
- Regardless of their length, pure partial trisomy 1q, involving or overlapping our patient's duplication, has shown a broad range of prenatal and postnatal clinical manifestations. However, a common core of clinical features including developmental delay (DD)/intellectual disability (ID), craniofacial anomalies, and limb defects may be recognized.

Novel Insights

- We describe a previously unreported 1-way interchromosomal insertion (3;1) involving the pure gain of 1q21.3q23.3 that causes typical (e.g., microcephaly, DD/ID, and facial dysmorphism) and atypical (i.e., interauricular communication, small feet with bilateral deep plantar creases, syndactyly of II-IV toes, and mild pachyonychia of all toes) clinical manifestations associated with this region.
- Our results further expand the clinical spectrum of duplications involving 1q21.3q23.3, evidence the high phenotypical heterogeneity among similar carriers, and suggest the involvement of a set of duplicated genes (including *LMNA*, *USF1*, *VANGL2*, *LOR*, and *POGZ*) to account for most clinical findings in our patient.
- The apparent disruption of a promoter region and a topologically associated domain (TAD) by the insertion also suggests reconfiguration/position effects on other genes (e.g., *CPNE9* and/or *BRPF1*) in distal 3p, plausibly contributing to the patient's phenotype.

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Abstract
"Simple" 1-way interchromosomal insertions involving an interstitial 1q segment are rare, and therefore, their characterization at the base pair level remains understudied. Here, we describe the genomic characterization of a previously unreported de novo interchromosomal insertion (3;1) entailing an about 12-Mb pure gain of 1q21.3q23.3 that causes typical (microcephaly, developmental delay, and facial dysmorphism) and atypical (interauricular communication, small feet with bilateral deep plantar creases, syndactyly of II-IV toes, and mild pachyonychia of all toes) clinical manifestations associated with this region. Based on our analyses, we hypothesize that the duplication of a subset of morbid genes (including *LMNA*, *USF1*, *VANGL2*, *LOR*, and *POGZ*) could account for most clinical findings in our patient. Furthermore, the apparent disruption of a promoter region (between *CPNE9* and *BRPF1*) and a topologically associated domain also suggests likely pathogenic reconfiguration/position effects to contribute to the patient's phenotype. In addition to further expanding the clinical spectrum of proximal 1q duplications and evidencing the phenotypical heterogeneity among similar carriers, our genomic findings and observations suggest that randomness – rather than lethality issues – may account for the paucity of "simple" interchromosomal insertions involving the 1q21.3q23.3 region as genomic donor and distal 3p25.3 as receptor. Moreover, the microhomology sequence found at the insertion breakpoint is consistent with a simple nonhomologous end-joining mechanism, in contrast to a chromothripsis-like event, which has previously been seen in other nonrecurrent insertions. Taken together, the data gathered in this study allowed us to inform this family about the low recurrence risk but not to predict the reproductive prognosis for hypothetical carriers. We highlight that genomic-level assessment is a powerful tool that allows the visualization of the full landscape of sporadic chromosomal injuries and can be used to improve genetic counseling.

Chromosomal insertions are relatively uncommon rearrangements that introduce a chromosomal segment elsewhere in the genome. They can be inter- or intrachromosomal, but the former are the most common and usually involve a nonhomologous receptor chromosome. Although the underlying molecular mechanisms have not been clearly established and may be diverse, some nonrecurrent insertions, which have been genomically characterized, appear to have resulted from chromothripsis-like replication-related repair mechanisms that sometimes concurred with a trisomy rescue in the postzygotic stage. As previously observed, these events imply unexpected complexity and might lead to inversions, deletions, duplications/triplications, and/or even gene disruptions at the receptor site. Additionally, the insertion junctions' signature can exhibit blunt ends, short microhomologies, or short microinsertions [Gu et al., 2016; Kato et al., 2017].

Either full trisomy 1 or pure large 1q duplications (e.g., 1q11qter, 1q21qter, or 1q25qter) are seemingly not compatible with fetal development and postnatal survival [Machlitt et al., 2005; Sifakis et al., 2014]. Conversely, individuals with a pure smaller duplication (e.g., in 1q11q22, 1q12q23, 1q31q41, 1q31.1q32.1, 1q32qter, or 1q42qter) present a better chance of survival [Mertens et al., 1987; Schorry et al., 1998; Sillén et al., 1998; Percesepe et al., 2007; Balasubramanian et al., 2009; Otake et al., 2009]. To date, more than 200 cases with a pure partial 1q duplication with breakpoints mostly at 1q21.1, 1q25, 1q32, and 1q42 (commonest) have been documented [Percesepe et al., 2007; Balasubramanian et al., 2009; Dolcetti et al., 2013; Sifakis et al., 2014]. Approximately 20 other pure duplications (between 1 and 15 Mb in size) within or overlapping 1q21.3q23.3 have been described in the DECIPHER and ISCA databases [Firth et al., 2009].

Here, we describe a previously unreported interchromosomal insertion (3;1) entailing a pure gain of the segment 1q21.3q23.3 into 3p25.3 and causing typical and atypical clinical manifestations in the affected child. In addition to thoroughly analyzing the insertion site by whole-genome sequencing (WGS), we revised other overlapping insertions/duplications and the associated phenotypes.

Patient and Methods

Case Report

The girl (Fig. 1) was born at 37 weeks by cesarean section following an uneventful pregnancy. At birth, her weight and length were 2,840 g (6th centile) and 50 cm (44th centile), respectively. Her parents, aged 25 (mother) and 30 years, were healthy and nonconsanguineous and denied other affected relatives. At physical examination (age of 8 months), the patient showed a weight, length, and occipitofrontal circumference of 6.5 kg (9th centile), 68 cm (62nd centile), and 39.5 cm (1st centile), respectively. At the current age of 3.2 years (Fig. 1C) the patient showed microcephaly

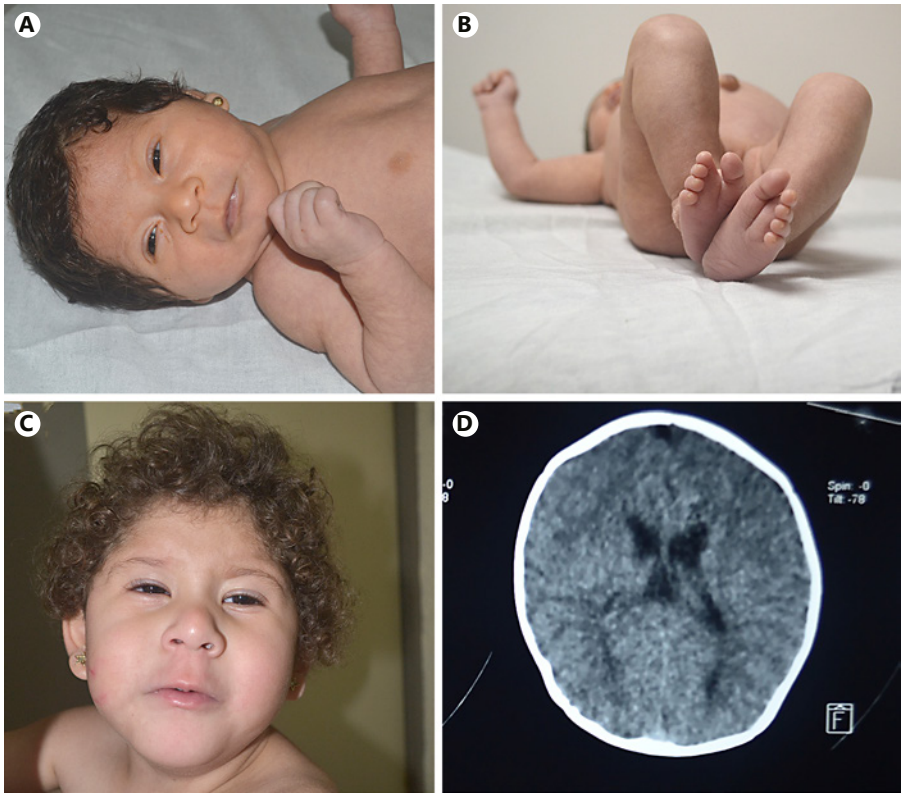


Fig. 1. Photographs of the patient at the age of 1 month (**A**) and 3.2 years (**B**). **A** Appraisable facial features include round face with prominent cheeks, wide forehead, narrow palpebral fissures, short nose with anteverted nostrils, long philtrum, and prominent columella. **B** Note the bilateral deep plantar crease. **C** The patient also presents curly, brown hair. **D** Cranial axial CT image (at the age of 7 months), suggesting a typical sign ("racing car sign") for agenesis/hypoplasia of the corpus callosum.

(44 cm, average 11 months), a length of 90.5 cm (40–50th centile), flat occiput, curly and brown hair, slightly prominent metopic, narrow forehead, telecanthus, right epicanthus, long eyelashes, ptosis of the left eyelid, high nasal root, broad nasal tip, hypoplasia of nasal wings, long philtrum, prominent upper lip, high palate, micrognathia, malformed ears (cupped, thickened helix and very large lobe), low anterior and posterior hairline, short neck, pectus excavatum, inverted left nipple, small hands (9.7 cm, <3rd centile), palmar folds and bilateral 5th finger nail hypoplasia, narrow and small feet (13 cm, 3rd centile) with bilateral deep plantar creases between I and II toes, overlapping toes (2nd finger on 3rd), syndactyly of II-IV toes, mild pachyonychia of all toes, and hypertrichosis in the back. Hand X-rays showed bilateral short metacarpal bones and phalanxes. The patient also presented developmental delay (DD). A cranial CT scan suggested hypoplasia of the corpus callosum (HCC) (Fig. 1D). Echocardiography showed an interauricular communication.

Cytogenetics and Array-Based Comparative Genomic Hybridization (aCGH)

Chromosome analyses of the patient and her parents were performed on GTG-banded metaphases obtained from 72-h cultured peripheral blood lymphocytes. Under informed parental consent, the patient's genomic DNA was analyzed using the SurePrint G3 Hmn CGH + SNP 4× 180K Microarray Kit according to the manufacturer's instructions (protocol v7.3; Agilent Technologies, Santa Clara, CA, USA). The array was scanned on a SureScan scanner (Agilent Technologies) and analyzed with the aberration detection method (ADM-2 algorithm) from Agilent CytoGenomics software v.4.0.3. Finally, in order to illustrate a potential interaction among genes related to imbalances, we used STRING database v11.0.

Fluorescence in situ Hybridization

To validate the chromosomal rearrangements, we used predesigned FISH probes (Cytocell, Abbott). Whole chromosome painting probes (red) were used for chromosome 1 paints (LPP 01R, Cytocell). Vysis subtelomere probes for 3p (D3S4559, green; genomic coordinates chr3:184,320–368,406; GRCh37/hg19) and 3q (D3S4560, red; genomic coordinates chr3:196,942,073–197,037,915; GRCh37/hg19) were also used (Abbott).

Whole-Genome Sequencing

Paired-end WGS (30×) was performed to pinpoint the breakpoint of the insertion site. Briefly, the patient's library was prepared from 350 ng of genomic DNA using the Nextera DNA Flex Library Prep Kit (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. Sequencing was performed on a Novaseq 6000 instrument with S4 Reagents kit (Illumina). The WGS analyses included mapping to the GRCh37 reference genome and variant calling (i.e., single nucleotide variants, small indels, copy number variants, and structural variants) using the Whole Genome Sequencing app v8.0.1 from Illumina. Alignments were visualized in the Integrative Genome Viewer (IGV) program v2.8.2 to identify rearrangements and/or copy number variants. To search for disruptions of topologically associated domains (TADs), we used the TAD knowledge base (TADKB) [Liu et al., 2019]. Additionally, the Circos plot tool (by clicO FS program) [Cheong et

al., 2015] was used to visualize interchromosomal rearrangements. The karyotype and track files to generate this visualization were according to the GRCh37/hg19 assembly.

Results

The patient's G-banded karyotype was 46,XX,add(3)(p25) (Fig. 2A). Because no parental rearrangement was found, a de novo origin of the 3p+ was inferred. Subsequently, the aCGH results exposed a duplication of about 12 Mb at 1q21.3q23.3 (genomic coordinates 150,530,678–162,497,945; GRCh37/hg19) (Fig. 2B). FISH analyses with chromosome 1 and 3p subtelomeric probes confirmed both the chromosome 1 origin of the extra segment and the retention of the 3p subtelomere on the 3p+ (Fig. 2A). This duplication encompasses 494 genes, of which 271 correspond to OMIM entities, and approximately 65 are annotated as morbid. At this resolution level, there was no other imbalance nor could the precise breakpoint be observed on the receptor chromosome. However, WGS mapped an intergenic breakpoint with discordant reads at 3p25.3 (between genomic coordinates chr3:9,772,046–9,772,047) (Fig. 2C; 3A, B). The break-

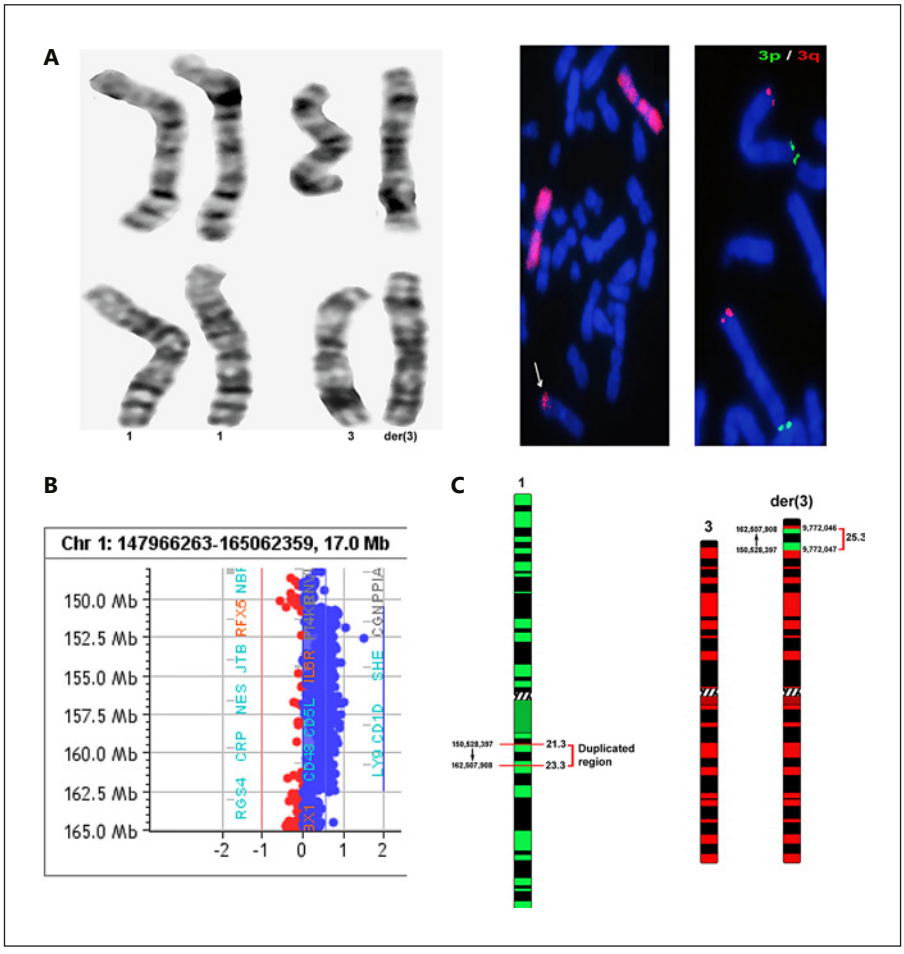
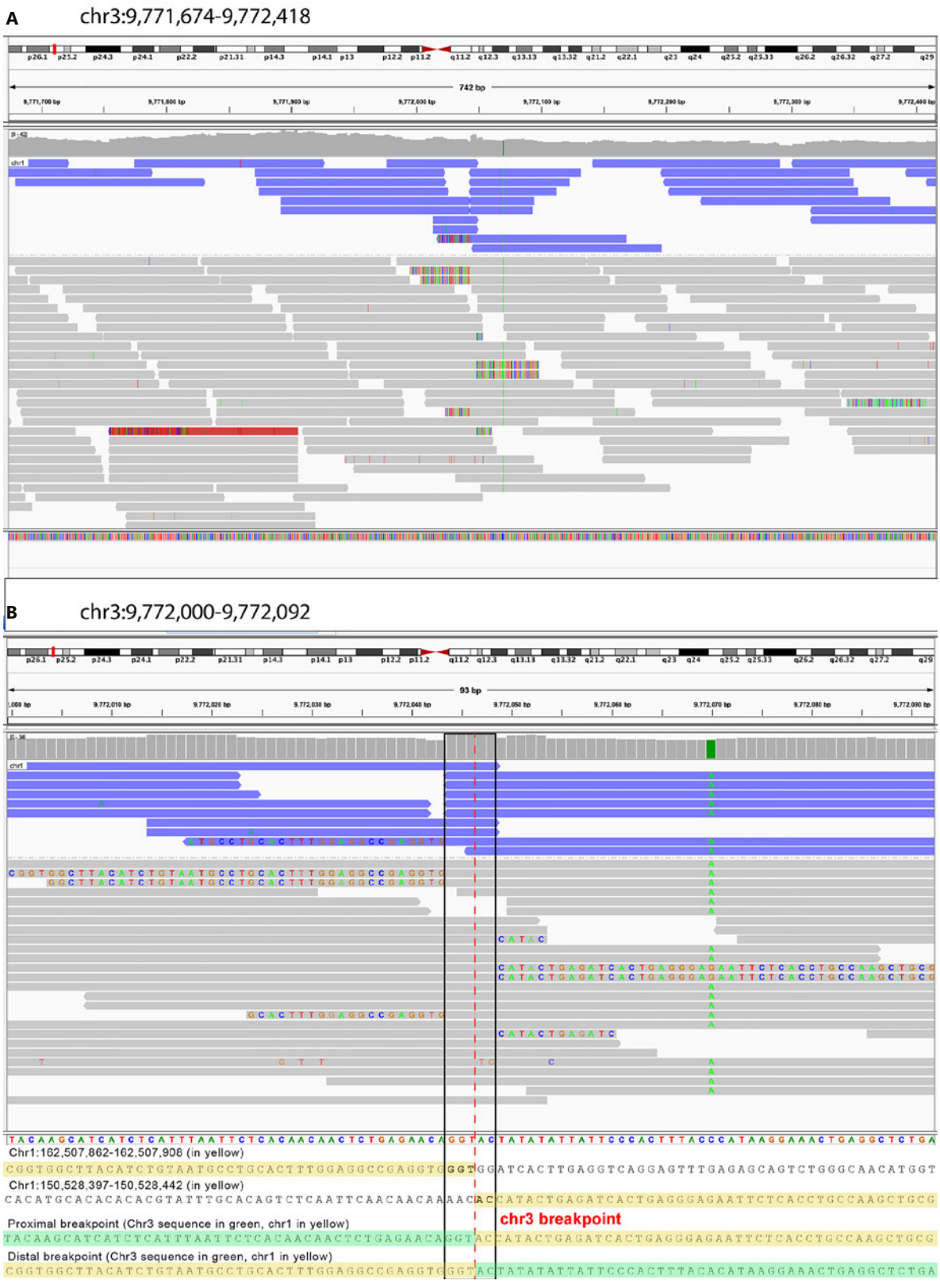


Fig. 2. Cytogenetic, aCGH, and WGS findings of the patient. **A** On the left, 2 pairs of each G-banded chromosome 1 and 3 are shown. Upper and lower rows show both normal chromosomes 1 (left) and normal and rearranged chromosome 3 (right). On the right, both painting results exhibiting painted homologous chromosomes 1 (red) and a portion on 3p stained with the specific chromosome 1 probes (arrow) and chromosomes 3 retaining their subtelomeric 3p (green)/3q (red) regions (farthest right) are depicted. **B** Partial $\log_2$ratio and gene view panel showing an interval of interest with an evident gain of 1q21.31q23.3 (right-biased blue probes, mean $\log_2$ratio = 0.491). **C** Chromosome 1 (green) and 3 (red) ideograms showing normal and derivative chromosomes. Here, the precise breakpoints of both the duplicated region and the insertion site at 3p25.3 can be appreciated as provided by WGS. Arrows show the orientation of the duplicated segment in the normal chromosome and in the der(3).

Fig. 3. Overview of the read alignment around the insertion point in chromosome 3. **A** Window of 742 bp around the insertion point. Reads whose mate aligns to chromosome 1 are shown in blue and soft-clipped bases in reads that do not fully align to chromosome 3 and that indicate the breakpoint are shown in colors. **B** Zoomed-in alignment. The proposed breakpoint is indicated in a dotted red line and is located between GRCh37 chr3:9,772,046–9,772,047. The region around the insertion point with overlapping bases to the endpoints of the inserted region is indicated in a box. Below, the sequences around both breakpoints in chromosome 1 are indicated, and the inserted region, which matches the soft-clipped reads, is indicated in a yellow box. At the bottom, both the proximal and distal sequences are shown that correspond to chromosome 3 (green) and the inserted sequence of chromosome 1 (yellow).

(For figure see next page.)



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ends displayed by IGV were chr1:162,507,908-chr3:9,772,046 and chr1:150,528,397-chr3:9,772,047. Thus, the duplication terminal breakpoint (162,507,908) was orientated toward 3p distal and the duplication proximal breakpoint (150,528,397) toward 3p proximal, i. e., the insertion was inverted regarding its original orientation in chromosome 1 [but normal in orientation relative to the centromere of der(3)]. Indeed, the duplication breakpoints by WGS were about 12.2 kb larger than those observed by aCGH (chr1:150,530,678–162,497,945). Upstream (452 bp), the closest gene to the mapped breakpoint was *CPNE9*, whereas downstream (1.3 kb), it was *BRPF1*. According to the Ensembl database and repeat masker filter (UCSC genome browser), the breakpoint disrupted a promoter region (ENSR00000148304) partially spanning both genes and a SINE-class repetitive element (MIRb), respectively, and included a small (5 bp) microhomology sequence (3 bp to the breakpoint in chr1:162,507,908 and 2 bp to the breakpoint in chr1:150,528,397) (Fig. 3B). Both genes appear to be within a TAD (Domain_ID: 10, genomic coordinates 9,380,000–10,380,000; includes 29 genes) as provided from Hi-C data of the GM12878 cell line (10/5 kb resolution) [Liu et al., 2019]. No imbalance was noticed around the insertion site. The final ISCN [2016] karyotype was: seq[GRCh37] der(3)ins(3;1)(p25.3;q21.3q23.3) g.[chr3:9,772,046_9,772,047inschr1:150,528,397_162,507,908].

Finally, a pathways/terms enrichment analysis from a protein-protein interaction network built with 66 morbid genes (including *BRPF1*) showed statistical significance (FDR <0.05) for intellectual disability (*GATAD2B, BRPF1, ARHGEF2, ASH1L, POGZ, KCNJ10, PRUNE*), ichthyosis (*LOR, FLG, GBA*), and osteoclast differentiation (*CTSK, FCGR2A, FCGR2B, FCGR3A, FCGR3B*) terms (online suppl. Fig. 1; for all online suppl. material, see www.karger.com/doi/10.1159/511234).

Discussion

While the distal 1q region has an apparently increased susceptibility to translocate [Misceo et al., 2009; Watanabe et al., 2016], "simple" intrachromosomal or interchromosomal insertions involving more proximal 1q segments are exceptional [Stoll et al., 1984; Muneer et al., 1991; Duba et al., 1997; Schorry et al., 1998; Utine et al., 2007; Kang et al., 2010; Quinonez et al., 2012]. So far, just 5 different 1q segments inserted into a nonhomologous chromosome have been documented (online suppl. Fig. 2) [Stoll et al., 1984; Muneer et al., 1991; Schorry et al., 1998; Utine et al., 2007; Kang et al., 2010]. Except for chromosome 4, which has been seen twice [Stoll et al., 1984; Schorry et al., 1998], the other receptor chromosomes have been different. Of these cases, one insertional translocation was balanced, but its unbalanced segregation products (namely monosomy or trisomy 1q12q21.3) seemingly accounted for several abortions in the healthy carrier [Muneer et al., 1991]. The remaining 4 patients with duplications overlapping 1q21.3q23.3 or downstream of this region survived. Interestingly, a duplication of 1q12q23 appeared not to be lethal. The postnatal survival of the remaining 4 patients with a duplication overlapping 1q21.3q23.3 or a more distal segment, also observed in a child with a non-insertional duplication of 1q12q23 [Otake et al., 2009], suggests that the lifespan of these patients could also depend on the level of alteration in the receptor chromosome. In this regard, our WGS results showed that the insertion breakpoint was intergenic and did not involve further imbalances. Although this may not be innocuous (due to the disruption of a gene promoter and a TAD), it was not lethal for our patient.

Regardless of their length, pure partial trisomy 1q involving or overlapping our patient's duplication has shown a broad range of prenatal and postnatal clinical manifestations [Sifakis et al., 2014]. However, a common core of clinical features including DD/intellectual disability (ID), craniofacial anomalies, and limb defects may be recognized. Although this fact suggests the involvement of the same gene(s) or genes with similar and/or complementary biological functions within 1q, a clear genotype-phenotype association has remained elusive. Our patient's clinical picture matches this phenotypic core, but it also includes interauricular communication, small feet with bilateral deep plantar creases between I and II toes, syndactyly of II-IV toes, and mild pachyonychia of all toes as new findings for interstitial pure 1q duplications overlapping the present one. Of note, just 2 cases (DECIPHER patients 4663 and 251161) involving a pure duplication similar to the present one but with clinically heterogeneous features have been reported (revised at UCSC Genome Browser and DECIPHER databases) (Table 1; online suppl. Fig. 3).

The lack of solid evidence of triplosensitivity for most genes within the 1q21.3q23.3 duplicated region (ClinGen Dosage Sensitivity Map Database) may partially account for the difficulty in establishing genotype-phenotype correlations in this and other cases. Nevertheless, among 65 morbid genes within this region, *LMNA*, *USF1*, and *VANGL2* present haploinsufficiency scores

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Table 1. Clinical features of patients involving duplication within or overlapping 1q21.3q23.3

	Present case	Patient 4663 (DECIPHER)	Patient 251161 (DECIPHER)	Patient 248281 (DECIPHER)	Patient 341850 (DECIPHER)	Patient 2, Utine et al. [2007]	Case III-2[a], Fichera et al. [2014]	Sifakis et al. [2014]	Sowinska-Seidler et al. [2018]
Karyotype	46,XX,add(3)(p25)	46,XY	46,XY	46,XX	46,XY	46,XY,ins(18;1) (q22;q23q31.1 32)	46,XY	46,XX,dup(1) (pter→q31::q31→q12::q31→qter)	46,XY
Duplicated region	1q21.3q23.3	1q21.2q23.3	1q21.2q23.3	1q21.1q22	1q21.1q21.3	1q23q31.1 32	1q22	1q21q31	1q22q23.1
Neurological	DD, delayed language development	ID, delayed language development, seizures, stereotypy	ID	ID, dyscalculia	NR	NR	Behavioral disturbances and global DD, hyperactivity and opposition- defiant disorder (ODD), aggressive and destructive behavior	NR	Global DD, severe muscular hypotonia
Craniofacial	Microcephaly, flat occiput, prominent metopic, telecanthus, right epicanthus, long eyelashes, ptosis of the left eyelid, nasal root high, broad nasal tip, hypoplasia of nasal wings, long philtrum, prominent upper lip, high palate, micrognathia, dysplastic ears helices, low anterior and posterior hairline	Microcephaly, strabismus	Micrognathia, persistent pupillary membrane	Abnormality of the eye, ptosis	NR	Long face with a long and smooth philtrum, micrognathia with glossoptosis and a high palate, narrow palpebral fissures, hypertelorism with a wide and flat nasal bridge, bilateral over-folded upper helices	NR	Hypertelorism, downslanting palpebral fissures, severe retromicrognathia, flat nasal bridge, and anteverted nares, small low set and abnormal ears	Prominent metopic ridge, trigonocephaly, lambdoid craniosynostosis, prominent occiput, hypotelorism, shallow orbits, low-set, high arched palate, posteriorly rotated ears
Limbs	Small hands, palmar folds hypoplasic, bilateral 5th finger nail hypoplastic, narrow and small feet, bilateral deep plantar creases between I and II toes, overlapping toes of II on III toes, syndactyly of II-IV toes, and mild pachyonychia of all toes	Hallux valgus	Camptodactyly	NR	NR	Long fingers and toes	NR	Adducted thumbs, camptodactyly, overlapping fingers, short curved femora, prominent heels and clenched toes	NR
Cardiovascular	Interauricular communication	NR	Cardiomyopathy, mitral regurgitation	NR	NR	Patent ductus arteriosus and patent foramen ovale, which disappeared	NR	NR	NR
Other defects	Short neck, pectus excavatum, inverted left nipple, hypertrichosis in the back	Scoliosis	Proportionate short stature, scoliosis, lipodystrophy	Abnormality of the skin, pectus excavatum, enuresis	NR	NR	Physical and neurological examinations were unremarkable	Broad neck and prominent thorax	NR
Neurological malformations	Hypoplasia of corpus callosum	NR	NR	NR	NR	Deeper frontal sulci and suspected region of nodular heterotopia	Normal	Ventriculomegaly, hypoplastic cerebellum	Hypoplastic corpus callosum, abnormal development of the white matter and Chiari malformation, premature fusion of the metopic suture, unilateral right-sided lambdoid craniosynostosis
Molecular technique	Karyotype/aCGH/FISH/WGS	NR	NR	NR	NR	Karyotype/FISH	*FMR1* gene molecular analyses and screening for neurometabolic disorders were normal Microarray analysis MLPA assay	aCGH FISH	aCGH qPCR

For further details and/or a complete view of all DECIPHER patients/duplications within or overlapping the present one, please see online suppl. Fig. 2 and https://decipher.sanger.ac.uk/search/patients/results?q=1%3A150528397-162507908. [a] This patient was the index case of a family with a 1q22 microduplication. DD, developmental delay; ID, intellectual disability; NR, not reported.

(HI) <10% (1.71, 4.99, and 8.70, respectively), suggesting potential mono- and perhaps triplosensitivity. Of these, *LMNA*, with the highest HI score, encodes for nuclear lamina proteins. Lamin proteins are required for multiple biological processes, including normal development of peripheral nervous system and skeletal muscle, osteoblastogenesis and bone formation, and cardiac homeostasis (GeneCards database). Interestingly, submicroscopic chromosomal imbalances (1 deletion, 2 duplications) involving this gene have been observed in patients with diverse phenotypes, including microcephaly, HCC, DD/ID, dysmorphic features, and cardiac defects (with only the deletion implicated), but a direct association remains to be defined [Fichera et al., 2014; Aleksiūnienė et al., 2018; Sowińska-Seidler et al., 2018]. The fact that our patient also presents with these features may help to confirm that this gene is also triplosensitive and associated with these phenotypes. Despite this, other genes such as *USF1*, *VANGL2*, *NES* (HI = 5.29, but not yet morbid), *MEF2D* (HI = 11.76, but not yet morbid), and *POGZ* (HI = 29.96) could account – together or not with *LMNA* – for HCC and/or DD (and perhaps other features) in this and other patients [Sowińska-Seidler et al., 2018]. Supporting this notion, the proband reported by Fichera et al. [2014], without involving a duplication of any of these genes, exhibited DD but not HCC. *USF1* is an important transcription factor for different processes such as normal brain functioning and lipid metabolism, and it regulates multiple key genes, including *FMR1*, *Slc12a5* (KCC2b isoform), and apolipoproteins. For the latter, it has been observed that induced *USF1* overexpression decreases HDL and VLDL cholesterol levels in female mice [Wu et al., 2010]. Nevertheless, although slightly lower, as compared to the reference values, the levels of these lipoproteins in this patient were otherwise normal. *VANGL2* is involved in the development of the neural plate, whereas *NES* and *MEF2D* participate in normal neuron development (in mice) [Sowińska-Seidler et al., 2018]. *POGZ* plays a role ensuring adequate mitotic cell cycle progression, and defects in this gene have been related to syndromic DD and thin corpus callosum (MIM 616364). In addition to the abovementioned genes, the protein products of several other genes appeared to be functionally related among them and associated to ID, ichthyosis, and osteoclast differentiation (online suppl. Fig. 1). Strikingly, from all interstitial duplication cases involving or overlapping 1q21.3q23.3 revised here, only DECIPHER patient 251161 (mitral regurgitation) and patient 2 (patent ductus arteriosus and patent foramen ovale, which spontaneously resolved) described in Utine et al. [2007] exhibited a cardiac defect, denoting reduced penetrance and variable expressivity (Table 1).

Although we did not find any other likely pathogenic imbalances or gene disruption in the receptor site, we cannot rule out reconfiguration/breakpoint effects. Position effects from chromosomal rearrangements have been discussed elsewhere [Aristidou et al., 2018]. These effects may disturb gene regulatory regions, such as highly conserved TADs, promoters, and enhancers when a breakpoint does not specifically disrupt exons/introns. In this case, the insertion breakpoint is relatively close to the 3′ UTR of *CPNE9* and 5′ UTR of *BRPF1* and appears to disrupt a promoter and a TAD (online suppl. Fig. 4). *CPNE9* belongs to the copine gene family, whose products are calcium-dependent lipid-binding proteins. CPNE9 plays a role in dendrite formation of melanocytes and has been related to Renpenning Syndrome 1, characterized by ID, unspecific facial dysmorphism, and heart defects. *BRPF1* encodes a bromodomain, PHD finger and chromo/Tudor-related Pro-Trp-Trp-Pro (PWWP) domain-containing protein, which is a component of the MOZ/MORF histone acetyltransferase complexes that function as transcriptional regulators. Heterozygous mutations in *BRPF1* have been associated with an ID disorder with dysmorphic facies and ptosis (MIM 617333). Therefore, regulatory disruptions in these or perhaps other genes within this TAD could have also contributed to the patient's phenotype. Similar to other nonrecurrent rearrangements [Kato et al., 2017], the presence of short microhomology at the insertion breakpoint suggests a nonhomologous end-joining mechanism to explain the present rearrangement. Although this mechanism is increasingly being linked to chromothripsis(-like) events, we did not find evidence of the complexity associated with this phenomenon. Finally, the disruption/involvement of a SINE-class repeated element in this case is comparable with what has been observed in a few other simple rearrangements [Schluth-Bolard et al., 2019].

In conclusion, our findings and observations further expand the clinical spectrum of 1q duplications, evidence the high phenotypical heterogeneity among similar carriers, and, due to the presence of repeated elements in the region, suggest simply randomness - rather than differential survival - to account for the paucity of interchromosomal insertions involving the 1q21.3q23.3 region as genomic donor and distal 3p25.3 as receptor. However, the large number of genes involved in this and most 1q duplications along with the lack of triplosensitivity studies

and plausible reconfiguration/position effects on other genes in distal 3p still prevents us from reaching a more precise genotype-phenotype association. Taken together, the data gathered in this study allowed us to inform this family about the low recurrence risk but not to predict the reproductive prognosis for hypothetical carriers. However, we highlight that genomic-level assessment is a powerful tool that allows the visualization of the full landscape of sporadic chromosomal injuries and can be used to improve genetic counseling.

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Statement of Ethics

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. A local ethics committee approved this study. The patient's parents authorized the use of biological and photographic material for research purposes through informed consent.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

M.M.R.-S. and R.A.L.-A. performed aCGH and contributed to the data interpretation. G.H.-Z. performed GTG-banding karyotypes and contributed to the FISH analyses and interpretation. M.L.O.-A., G.P.-G., C.P.-O., and C.I.J.-V. recruited the subject and performed the clinical description and analysis. C.D.R.-E. and C.C.-G. provided the WGS experiments and data analysis. C.C.-F. and M.L.O.-A. conceived the study and wrote the paper. All authors revised the manuscript and approved the final version.

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