

3q27.1 Microdeletion Causes a Clinically Recognizable Syndrome Characterized by Severe Prenatal and Postnatal Growth Restriction and Neurodevelopmental Abnormalities

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Research Article

Keywords: 3q27.1 microdeletion, 3q26q28 microdeletion syndrome, DVL3, AP2M1, PARL, CNV interpretation

Posted Date: September 2nd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-853179/v1>

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Abstract

Background

Constitutional deletions/rearrangements involving chromosome 3q are uncommon and overlapping microdeletions of chromosome 3q26-3q28 have only been reported in eight individuals. The common phenotype observed in these individuals include severe intrauterine growth restriction and postnatal growth impairment, feeding difficulties, characteristic facial features, feet abnormalities and developmental delay. The most striking clinical features shared among all reported cases is severe prenatal and postnatal growth restriction and neurodevelopmental abnormalities.

Case presentation

We identified two additional individuals with overlapping deletions and shared clinical features by high-resolution SNP oligonucleotide microarray, and refined the smallest region of overlap (SRO) to a 1.2 Mb genomic location in chromosome 3q27.1 by reviewing and comparing all published cases. We evaluated the SRO using ACMG/ClinGen current recommendations for classifying copy number variants (CNVs), and discussed the contribution of the genes deleted in the SRO to the abnormal phenotype observed in these individuals.

Conclusions

This study provides further evidence supporting the existence of a novel 3q26q28 microdeletion syndrome and suggests that haploinsufficiency of potential candidate genes, *DVL3*, *AP2M1*, and *PARL* in the SRO in 3q27.1 is responsible for the phenotype. It also demonstrates the clinical utility of the newly released ACMG/ClinGen standards for CNV interpretation.

Background

Intrauterine growth restriction (IUGR) is a condition where fetal growth did not achieve the normal growth expected for the gestational age. It is a leading cause of perinatal mortality and morbidity that needs long-term follow up due to an increase risk for future development of chronic diseases. IUGR can be caused by genetic, epigenetic, metabolic, endocrine, or environmental factors [1–4]. Specifically, there is a strong association of IUGR with chromosomal aberrations. In some micro-duplication/deletion syndrome, IUGR is a major and only manifestation [5, 6].

One of the chromosome regions associated with IUGR is located in chromosome 3q26-3q28. Patients with microdeletions in this region are rare and not well described. To date eight cases have been reported in the literature with deletions varying in size from ~ 2.0 to 8.4 Mb [7–12]. Although their breakpoints are not recurrent, these patients share an apparently distinct phenotype including IUGR, microcephaly, short stature, abnormalities of the face facial and feet, and feeding difficulties. However, the clinical significance and genetic mechanism of the 3q26q28 microdeletion are not fully established. Here we report two

unrelated individuals harboring overlapped microdeletions in this region and sharing clinical features with those reported in the literature. Using high-resolution single nucleotide polymorphism (SNP) microarrays, we narrowed the Smallest Region of Overlap (SRO) to a size of 1.2 Mb at chromosomal band 3q27.1. This SRO region contains 46 genes, 24 of which are OMIM-annotated and eight of which are associated with disease (*KLHL24*, *EIF2B5*, *DVL3*, *AP2M1*, *ALG3*, *EIF4G1*, *CLCN2*, and *THPO*).

Genome-wide assessment of copy-number variants (CNVs) is widely applied to assess the clinical significance of pre- and post-natal congenital abnormalities. However, in-between clinical laboratories, the assessment of CNV classification remains inconsistent due to lack of uniform scoring metrics. To assist clinical laboratories in the accurate and consistent classification of reporting CNVs, ACMG and ClinGen recently published technical standards for CNV interpretation [13]. We applied their recommended quantitative and evidence-based scoring framework to evaluate the deduced 1.2 Mb SRO at chromosomal band 3q27.1 and demonstrated its pathogenicity.

Case Presentation

Proband-1: Prenatal: A G1P0 woman with naturally conceived male fetus was referred for prenatal diagnosis due to second trimester abnormal ultrasound findings (Table 1): <5% abdominal circumference, absent nasal bone, placentomegaly and severe oligohydramnios. The amniotic fluid volume was low for gestational age. Fetal measurement revealed a fetal weight at less than the tenth centile, which was consistent with a diagnosis of IUGR. Fetal echocardiogram was concerning for cardiomegaly and hypoplastic aortic arch. **Postnatal:** The proband was born at 37 weeks and 3 days of gestation via normal spontaneous vaginal delivery and the birth weight was 1785 grams. Postnatal echocardiogram ruled out any cardiac anomaly and newborn screening was normal. Physical examination at 3 months of age, revealed a male infant overall small for age with all the growth parameters below the 5th centile; weight: 3.7 kg (< 3rd centile); length: 54.5 cm (< 3rd centile); occipitofrontal circumference (OFC) 38.5 cm (5th centile). The proband appeared dysmorphic, with microcephaly, mild frontal bossing, bilateral epicanthal folds, hypotelorism, posteriorly rotated ears, flat nasal bridge, micrognathia, high arched palate, left palmar simian crease, and increased muscle tone. At 6.5 years of age, he was found to have developmental, cognitive, and growth delays. He was microcephalic and still overall small for age; weight 12.7 kg (< 1st centile); height: 101.5 cm (< 1st centile), and OFC: 48 cm (< 1st centile). In addition, he was dolicocephalic and had mild bilateral elbow restrictions, flat feet, long fingers and toes, clinodactyly of the 5th toes, mild metatarsus varus and finger-like thumbs. He was making steady developmental progress and speaking in short phrases. The proband's family history was significant for a maternal cousin with autism and paternal half-brother who was autistic and nonverbal.

Proband-2

The proband was a female child of non-consanguineous parents. Prenatal ultrasound at almost 24 weeks of gestational age was concerning for IUGR where the head circumference was 3 standard deviations below the mean. An infectious workup and fetal echo were normal. At 37 weeks of gestational

age oligohydramnios was noted. The proband was born full term as small for gestational age (weight 1.725kg, length 44cm, head circumference 30cm) with a normal state newborn screen (Table 1). The proband was initially discharged without complications but was readmitted for hyperbilirubinemia that required phototherapy. At 4 months of age, she was slightly delayed in achieving milestones and not sitting without support. At 6 months of age, her growth parameters were still low with a weight of 5kg and a height of 60 cm (both 1st percentile). After 6 months, the proband only gained 1 kilogram. Her emesis and failure to thrive led to a thorough workup. Head and abdominal ultrasounds were unremarkable. Due to feeding difficulties, the proband required a nasogastric tube. Her physical examine was significant for microcephaly and plagiocephly that resolved over time. Her facial features showed epicanthal folds, wide spaced eyes, a flattened nasal bridge, and arched palate and retrognathia. Her extremities showed a larger gap between the 1st and 2nd toe as well as 5th toe clinodactyly bilaterally. Motor delays were also noted.

Results

Proband-1

SNP Oligonucleotide Microarray Analysis (SOMA) using Affymetrix GeneChip Human Mapping 6.0 SNP array and Affymetrix Chromosome Analysis Suite 3.3 (Affymetrix, Santa Clara, CA) revealed that Proband-1 harbors a 4.93 Mb deletion in genomic coordinates 183,011,106–187,947,036 (hg19) corresponding to chromosomal bands 3q27.1q28 (Fig. 1a). This deleted region contains 122 genes, 53 of which are OMIM-annotated and 20 of which are associated with disease. Maternal SOMA showed that the mother does not have the same deletion in the long arm of chromosome 3 (Fig. 1b). FISH using a BAC probe RP11-919L13 further confirmed that this deletion is present in the proband but not in the father (Fig. 1c and d) and is therefore *de novo* in origin.

Proband-2

A karyotype analysis of Proband-2 with a resolution level of 525 bands revealed a normal female chromosome complement (46,XX). SOMA in Proband-2 identified a 2.37 Mb deletion (Fig. 2a) in the chromosomal region 3q27.1q27.2, corresponding to genomic coordinates 182,950,371 – 185,324,970 (hg19). This deleted region contains 66 genes, 30 of which are OMIM-annotated and 10 of which are associated with disease. FISH using a BAC probe RP11-919L13 confirmed the presence of this deletion in Proband-2 and excluded its maternal inheritance (Fig. 2b and c). Father is unavailable for testing.

Discussion And Conclusions

In the literature, there are eight previously reported cases carrying 3q26-3q28 microdeletions with sizes of 2-8.4 Mb that overlapped with the deleted chromosomal regions in two patients from this study [7–12]. The clinical phenotype of individuals with 3q26-3q28 microdeletions is heterogeneous: IUGR, severe growth impairment, feeding problems, short stature, dysmorphic facial features, microcephaly, seizure,

eye and ear abnormalities, clinodactyly, feet abnormalities, developmental delay, intellectual disability, hypotonia, and thrombocytopenia. While there is some degree of phenotypic variability that primarily relates to the size of the deletion, the most striking clinical features shared among all reported cases are severe prenatal and postnatal growth restriction, as well as neurodevelopmental abnormalities. The clinical presentation of two patients described in this study supports the clinical profile described for other individuals in the literature (Table 1). The genotype-phenotype correlations for loss of the 3q26q28 region are, however, restricted by the fact that these individuals do not share common break points, like those generated in recurring pathogenic CNVs flanked by segmental duplications. Nonetheless, comparison of the clinical and molecular findings in Proband-1 and Proband-2 with the previous reported individuals suggests that this is a clinically recognizable microdeletion syndrome with shared clinical features. Though the precise size and position of these deletions are uncertain, it has been proposed that haploinsufficiency of dosage sensitive genes leads to defined clinical sequelae [7].

By comparing microarray findings of these ten cases, we mapped the SRO to a size of 1.2 Mb, corresponding to genomic coordinates 183,220,510 – 184,469,308 (hg19) at chromosomal band 3q27.1. This SRO region contains 46 genes, 24 of which are OMIM-annotated and eight of which are associated with disease (*KLHL24*, *EIF2B5*, *DVL3*, *AP2M1*, *ALG3*, *EIF4G1*, *CLCN2*, and *THPO*) (Table 2). Among these genes, *DVL3* is the most interesting one. Heterozygous pathogenic variants in the *DVL3* gene have been associated with autosomal dominant type III Robinow syndrome (MIM#: 616894), which shares many clinical features with the 3q26q28 microdeletion syndrome: short stature (9/9), facial dysmorphic features (10/10), epicanthal folds (7/9), nasal features (9/10), teeth abnormalities (7/8), hand abnormalities (4/10), clinodactyly (6/10), genital/urinary abnormalities (5/10). Emerging data suggest *DVL3* is a core component in the routing and transmission of canonical and non-canonical Wnt signalosome [14]. In murine, *DVL3* has been detected to express ubiquitously at E7.5, but shortly after it showed elevated expression in heart, CNS, notochord, dorsal root ganglia, branchial arches, limb buds, and somitic mesoderm [15, 16]. These findings further strengthen the role of *DVL3* in diseases by modulating Wnt signaling that is involved in cell migration and tissue morphogenesis in vertebrate development. Indeed, *Dvl3* knock out mice demonstrated partial lethality, conotruncal defects and neural tube defects, including abnormalities in cochlear cells [17]. However, all current known pathogenic variants of *DVL3* are frameshift small insertions/deletions or splice variants in the last two exons; and larger intergenic deletions of *DVL3* have not been described previously [18–20]. Furthermore, it was demonstrated by expression studies that truncating *DVL3* variants escape nonsense-mediated decay (NMD), suggesting a dominant-negative or gain-of-function disease mechanism [19–22]. Therefore, the exact contribution from loss of *DVL3* to phenotype caused by 3q26-3q28 microdeletions is still uncertain at this time.

Beside *DVL3*, the role of *AP2M1* (MIM#: 601024) in poor speech (7/9), developmental delay (9/9), hypotonia (6/9), and seizures (2/9), as well as the role of *PARL* (MIM#: 607858) in growth restriction (10/10) are of great interest. *AP2M1* has recently been associated with impaired intellectual development, poor speech and delayed walking [23]. Though a recurrent missense variant in *AP2M1* has been reported, *AP2M1* is highly intolerant to loss-of-function variant in general population with a probability of

intolerance to loss of function (pLI) of 1.0 and the Haploinsufficiency Score of 8.13. Previous studies with *Parl* knock out mouse model have shown that *Parl* plays an essential physiological role in the neurological homeostasis [24], and *Parl* deficiency results in growth retardation, cachexia, and severe atrophy of skeletal muscle, thymus, and spleen [25]. However, we think the growth phenotype caused by this SRO is predominantly overlap with *DVL3* related Robinow syndrome and further study is warranted to associate the role of *PARL* in this phenotype. The remaining OMIM genes in the SRO (*ALG3*, *CLCN2*, *EIF2B5*, *EIF4G1*, *KLHL24*, and *THPO*) are associated with autosomal recessive conditions and therefore are less likely to have major contributions to these patients' phenotype.

In order to evaluate the clinical significance of the SRO, we further assessed the deduced SRO corresponding to genomic coordinates, chr3:183,220,510 – 184,469,308 (hg19) using ACMG/ ClinGen current recommendations for classifying copy number variants (CNVs) [13]. This SRO harbor 26 protein-coding RefSeq genes (Criteria 3B, points given: 0.45). Three of them (*PSMD2*: pLI:1; HI%: 6.89; *AP2M1*: pLI:1; HI%: 8.13; *EIF4G1*: pLI:1; HI%: 9.34) have predicted haploinsufficiency score below 10% and loss intolerance (pLI) score of 1 (Criteria 2H, points given: 0.15). To the best of our knowledge, in the literatures the SRO overlap with four previously assumed (due lack of molecular confirmation for paternity and maternity) *de novo* cases (individuals 1, 2, 5, and 7 in Table 1) with phenotype that is consistent with the gene/genomic region, but not highly specific and/or with high genetic heterogeneity (Criteria 4C, points given: 0.40). Moreover, observed copy number loss is assumed *de novo* (due lack of molecular confirmation for paternity and maternity) for Proband-1 in this study (Criteria 5A, points given: 0.1). Using these recommendations as a framework, we classified the SRO as pathogenic (Total score: 1.1).

In conclusion, in this study we present two additional individuals with phenotype similar to previously reported cases with overlapped deletions. It provides further evidence supporting the existence of this novel 3q26q28 microdeletion syndrome. Additionally, our molecular cytogenetic and clinical findings defined the 1.2 Mb SRO at chromosomal band 3q27.1 as the critical region for this clinically recognizable syndrome. The refinement of this critical region suggests that deletion of at least three genes (*DVL3*, *PARL* and *AP2M1*) may contribute to anomalies observed in these individuals. At last, we demonstrated that application of new ACMG/ClinGen standards for CNV interpretation with refined molecular mapping would improve our ability for clinical diagnosis and genetic counselling of individuals harboring similar imbalance.

Abbreviations

CNV: Copy number variants; IUGR: Intrauterine growth restriction; NMD: nonsense-mediated decay; OFC: Occipitofrontal circumference; SNP: Single nucleotide polymorphism; SOMA: Single nucleotide polymorphism oligonucleotide microarray analysis; SRO: Smallest region of overlap

Declarations

Acknowledgements

We would like to thank patients and their families for their participation.

Authors' contributions

SB wrote the first draft of the manuscript. EMP and K A-Y obtained clinical information. SB, VJ, BL, and JL performed genetic analysis and data interpretation. JL reviewed and critically revised the manuscript before submission. All authors read and approved the final manuscript.

Funding

None

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethical approval and consent for participate

This study was approved by Institutional Review Board of Columbia University Medical Center. All information in this report has been de-identified in accordance with HIPAA and institutional review board regulations.

Consent for publication

Informed consent was obtained from parents of patients in this study.

Competing interests

The authors declare that they have no competing interests

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Tables

Due to technical limitations, table 1 & 2 is only available as a download in the Supplemental Files section.

Figures

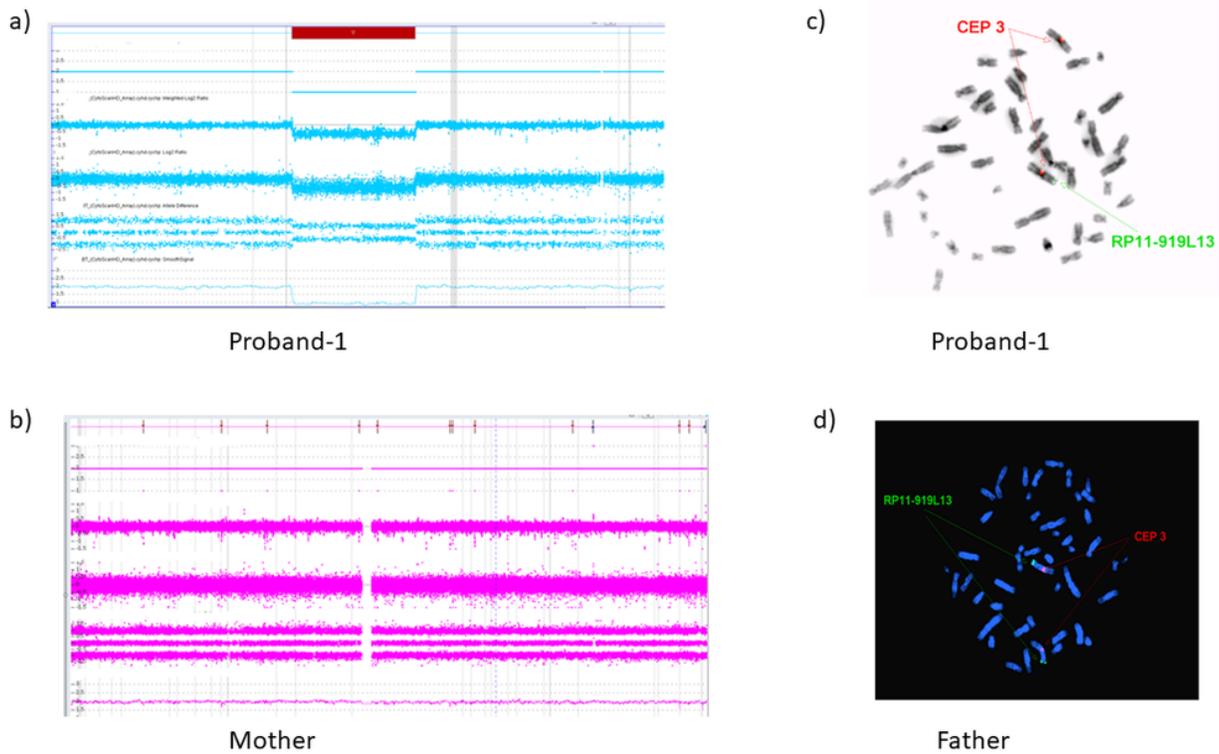


Fig.1

Figure 1

Test results for Proband-1 and his parents. a SOMA result showing the 3q27.1q28 deletion in the Proband-1. b Normal SOMA result from the mother. c FISH result using the BAC probe RP11-919L13 confirmed the presence of the deletion in Proband-1. d Normal FISH result from the father.

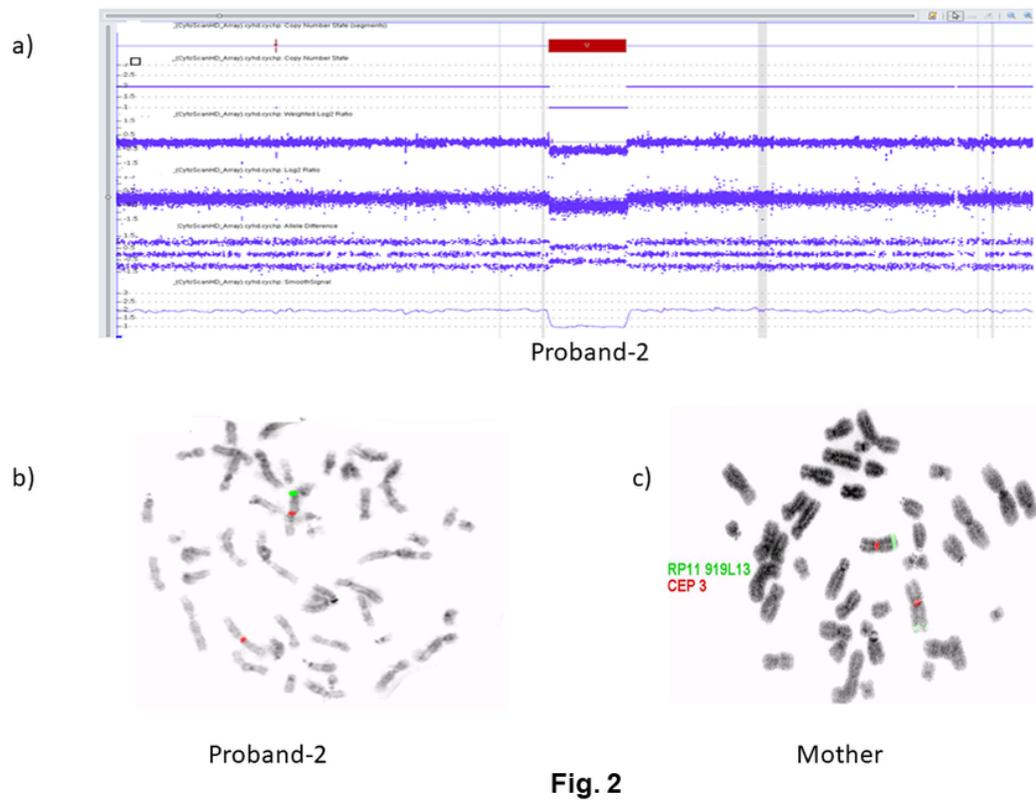


Figure 2

Test results for Proband-2 and her mother. a SOMA result showing the 3q27.1q27.2 deletion in Proband-2. b FISH result using the BAC probe RP11-919L13 confirmed the presence of the deletion in Proband-2. c Normal FISH result from the mother.

Supplementary Files

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- [Table2MCSubmission1.xlsx](#)