3q27.1 microdeletion causes prenatal and postnatal growth restriction and neurodevelopmental abnormalities

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Abstract

Background: Overlapping microdeletions of chromosome 3q26-3q28 have been reported in eight individuals. The common phenotype observed in these individuals include intrauterine growth restriction, short stature, microcephaly, feeding difficulties, facial dysmorphisms, limb abnormalities and developmental delay. The most striking clinical features shared among all reported cases is 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 3q27.1 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.

Keywords: 3q27.1 microdeletion, DVL3, AP2M1, PARL, 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]. Symmetric IUGR, where weight, length, and head circumference are equally affected, can be secondary to infections, chromosomal variants, or the suboptimal nutritional supply that affect the pregnancy at early stages. Asymmetric IUGR, where the weight is most affected and the head circumference is spared, occurs more commonly at later stages of pregnancy and is commonly from lack of nutrition. When IUGR is identified, the expectant mother is worked up for infectious through TORCH titers and are normally offered invasive testing with a microarray and chromosome analysis to rule out common causes of IUGR. Specifically, there is a strong association of IUGR with chromosomal aberrations. In some micro-duplication/deletion syndromes, 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, facial abnormalities, 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 classified it as a pathogenic deletion.

Case presentation

Proband-1

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. Fetal measurement revealed a fetal weight at less than the tenth centile, which was concerning for cardiomegaly and hypoplastic aortic arch. The proband was born at 37 weeks and 3 days of gestation via normal spontaneous vaginal delivery with a birth weight of 1785 g. Postnatal echocardiogram ruled out any cardiac anomaly and newborn screening was normal. By 3 months old, the proband continued to have 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). He was dysmorphic with microcephaly, mild frontal bossing, bilateral epicanthal folds, hypertelorism, posteriorly rotated ears, flat nasal bridge, micrognathia, high arched palate, left palmer simian crease, and increased muscle tone. By 18 months old he was receiving speech and feeding therapies. Gross motor skills were delayed as he was only beginning to cruise. By 30 months old, he started to walk, and by 3 years old he had 20–30 words. By 3 years of age, the proband started to walk. A neuropsychological evaluation at 4 yo demonstrated the proband was too cognitively limited to complete the exam. The proband’s family was not interested in a gastrostomy tube, and he continued to grow at <3rd percentile though he was not a picky eater. By 6 years old, the proband has not been potty trained. At the last evaluation at 8 years old, he continues to be in special education class with services and continues to have trouble with comprehension. His physical exam still demonstrated <2nd percentile for height, weight, and head circumference. Additional findings of dolichocephaly and arachnodactyly were also noted.

Proband-2

The proband is a female child of non-consanguineous Dominican descent. Prenatal ultrasounds at 24 weeks gestational age was concerning for symmetric IUGR where the head circumference was 3 standard deviations below the mean. An infectious and cardiac workup were normal. At 37 weeks gestational age oligohydramnios was noted. The proband was born full term as small for gestational age with weight, length and head circumference all <2nd percentile (weight 1.725 kg, length 44 cm, head circumference 30 cm). A post-natal TORCH panel was sent along with CMV urine analysis which were normal. She passed her newborn screen. Though the proband was discharged within 48 h, she was readmitted for hyperbilirubinemia that required phototherapy. At 6 months of age, her growth parameters were only at the 1st percentile. She continued to have failure to gain weight, prompting an admission for further workup when she was 11 months old. A nasogastric tube was placed to help with her feedings. During the admission she had a genetics evaluation. Her exam was notable for microcephaly, epicanthal folds, hypertelorism, an arched palate, and retrognathia. After her hospital discharge, the proband’s family had difficulty with follow up involving therapies and sub-specialty providers. She qualified for speech therapy but was not able to attend many of the sessions. Although she qualified for physical therapy, the family declined services once she began to walk at 20 months of age. At 3 years old she has many words but is working on sentences. She remains consistently at less than the 3rd percentile for height, weight, and head circumference. At this time, the patient is constantly encouraged to follow up with services through the school system, and to schedule appointments with appropriate subspecialty providers to help with feeding issues.
| Patient 1                          | Patient 2                          | Patient 3                          |
|----------------------------------|-----------------------------------|-----------------------------------|
| **Chromosomal regions (hg19)**   | **Chromosomal regions (hg19)**   | **Chromosomal regions (hg19)**   |
| 3q26.33-3q27.2                   | 3q26.33-3q27.2                   | 3q26.33-3q27.5                   |
| (chr3:181,648,378–185,786,898)   | (chr3:181,692,255–185,969,168)   | (chr3:183,047,473–187,212,935)   |
| **Size of deletion**             | **Size of deletion**             | **Size of deletion**             |
| 4.14 Mb                          | 4.28 Mb                          | 5 Mb                             |
| **Inheritance**                  | **Inheritance**                  | **Inheritance**                  |
| De novo                          | De novo                          | Unknown (not maternal)           |
| **Sex**                          | **Sex**                          | **Sex**                          |
| Male                             | Male                             | Female                           |
| **Age at last examination**      | **Age at last examination**      | **Age at last examination**      |
| 6 years                          | 18 years                         | 12 years                         |
| **Oligohydramnios**              | **Oligohydramnios**              | **Oligohydramnios**              |
| No                               | No                               | No                               |
| **Intrauterine growth restriction** | **Intrauterine growth restriction** | **Intrauterine growth restriction** |
| Yes                              | Yes                              | Yes                              |
| **Short stature**                | **Short stature**                | **Short stature**                |
| Yes                              | Yes                              | Yes                              |
| **Feeding problems**             | **Feeding problems**             | **Feeding problems**             |
| Yes                              | Yes                              | Yes                              |
| **Microcephaly**                 | **Microcephaly**                 | **Microcephaly**                 |
| Yes                              | Yes                              | Yes                              |
| **Cognitive abnormalities**      | **Cognitive abnormalities**      | **Cognitive abnormalities**      |
| Developmental delay, severe intellectual disability | Developmental delay, learning disability, borderline IQ | Developmental delay, intellectual disability |
| **Behavioral abnormalities**     | **Behavioral abnormalities**     | **Behavioral abnormalities**     |
| No                               | Hyperactivity                    | ADHD, an extremely friendly personality |
| **Seizure**                      | **Seizure**                      | **Seizure**                      |
| No                               | No                               | Tonic–clonic and myoclonic photo-convulsive seizures |
| **Hypotonia**                    | **Hypotonia**                    | **Hypotonia**                    |
| Yes                              | Yes                              | Yes                              |
| **Facial dysmorphisms**          | **Facial dysmorphisms**          | **Facial dysmorphisms**          |
| Yes                              | Yes                              | Yes                              |
| **Hands abnormalities**           | **Hands abnormalities**           | **Hands abnormalities**           |
| Clinodactyly (4th finger)        | No                               | Clinodactyly (5th finger)        |
| **Feet abnormalities**            | **Feet abnormalities**            | **Feet abnormalities**            |
| Pes planus, third toes overlap with fourth toes | Pes planus, abnormal foot position | Pes planus, overlapping toes     |
| **Dental abnormalities**         | **Dental abnormalities**         | **Dental abnormalities**         |
| Yes                              | Yes                              | Yes                              |
| **Heart defects**                | **Heart defects**                | **Heart defects**                |
| Patent ductus arteriosus         | No                               | No                               |
| **Other findings**               | **Other findings**               | **Other findings**               |
| Recurrent upper airway infections, inguinal hernia, mild kyphosis and pectus carinatum, joint laxity | Recurrent otitis media, conductive hearing loss, delayed puberty | Sensorineural hearing loss, severe gastroesophageal reflux, mild generalized hypertonia at 16 |
| **Diagnosis**                    | **Diagnosis**                    | **Diagnosis**                    |
| 3q26.33-3q27.2                   | 3q26.33-3q27.2                   | 3q26.33-3q27.5                   |
| (chr3:181,648,378–185,786,898)   | (chr3:181,692,255–185,969,168)   | (chr3:183,047,473–187,212,935)   |
| **Oligohydramnios**              | **Oligohydramnios**              | **Oligohydramnios**              |
| No                               | No                               | Yes                              |
| **Intrauterine growth restriction** | **Intrauterine growth restriction** | **Intrauterine growth restriction** |
| Yes                              | Yes                              | Yes                              |
| **Short stature**                | **Short stature**                | **Short stature**                |
| Yes                              | Yes                              | Yes                              |
| **Feeding problems**             | **Feeding problems**             | **Feeding problems**             |
| Yes                              | Yes                              | Yes                              |
| **Microcephaly**                 | **Microcephaly**                 | **Microcephaly**                 |
| Yes                              | Yes                              | Yes                              |
| **Cognitive abnormalities**      | **Cognitive abnormalities**      | **Cognitive abnormalities**      |
| Developmental delay, severe intellectual disability | Developmental delay, learning disability, borderline IQ | Developmental delay, intellectual disability |
| **Behavioral abnormalities**     | **Behavioral abnormalities**     | **Behavioral abnormalities**     |
| No                               | Hyperactivity                    | ADHD, an extremely friendly personality |
| **Seizure**                      | **Seizure**                      | **Seizure**                      |
| No                               | No                               | Tonic–clonic and myoclonic photo-convulsive seizures |
| **Hypotonia**                    | **Hypotonia**                    | **Hypotonia**                    |
| Yes                              | Yes                              | Yes                              |
| **Facial dysmorphisms**          | **Facial dysmorphisms**          | **Facial dysmorphisms**          |
| Yes                              | Yes                              | Yes                              |
| **Hands abnormalities**           | **Hands abnormalities**           | **Hands abnormalities**           |
| Clinodactyly (4th finger)        | No                               | Clinodactyly (5th finger)        |
| **Feet abnormalities**            | **Feet abnormalities**            | **Feet abnormalities**            |
| Pes planus, third toes overlap with fourth toes | Pes planus, abnormal foot position | Pes planus, overlapping toes     |
| **Dental abnormalities**         | **Dental abnormalities**         | **Dental abnormalities**         |
| Yes                              | Yes                              | Yes                              |
| **Heart defects**                | **Heart defects**                | **Heart defects**                |
| Patent ductus arteriosus         | No                               | No                               |
| **Other findings**               | **Other findings**               | **Other findings**               |
| Recurrent upper airway infections, inguinal hernia, mild kyphosis and pectus carinatum, joint laxity | Recurrent otitis media, conductive hearing loss, delayed puberty | Sensorineural hearing loss, severe gastroesophageal reflux, mild generalized hypertonia at 16 |
| **Diagnosis**                    | **Diagnosis**                    | **Diagnosis**                    |
| 3q26.33-3q27.5                   | 3q26.33-3q27.5                   | 3q26.33-3q27.5                   |
| (chr3:183,047,473–187,212,935)   | (chr3:183,047,473–187,212,935)   | (chr3:183,047,473–187,212,935)   |

**Table 1** Summary of clinical and genetic features of ten previously reported and present cases with 3q26-3q28 microdeletions
|                                | Bouman et al. [11]                                      | Ounap et al. [12]                                      | This study                                      | Consensus                                      |
|--------------------------------|-------------------------------------------------------|------------------------------------------------------|------------------------------------------------|------------------------------------------------|
|                                | Chromosomal regions (hg19)                            |                                                      |                                                |                                               |
|                                | 3q26.33–3q28                                         | 3q26.33–3q28                                         | 3q27.1–3q28                                    | Smallest region of overlap: 3q27.1            |
|                                | (chr3:183,220,510–189,409,266)                       | (chr3:183,220,510–189,409,266)                       | (chr3:183,011,106–187,947,036)                | (chr3:183,220,510–184,469,308)               |
|                                | Size of deletion                                      |                                                      |                                                |                                               |
|                                | 6.18 Mb                                               | 8.35 Mb                                              | 4.93 Mb                                        | 2.37 Mb                                       |
|                                |                                                        |                                                      |                                                | 1.2 Mb                                        |
|                                | Inheritance                                           |                                                      |                                                |                                               |
|                                | De novo                                               | Unknown                                              | De novo                                        | Unknown (not maternal)                        |
|                                |                                                        | Female                                               | Male                                           | 5 De novo, 5 unknown                          |
|                                | Sex                                                   |                                                      |                                                | 4 Male and 6 Female                           |
|                                | Age at last examination                               | 16 years                                             | 8 years                                        | 3 years                                       |
|                                | Fetus of 22 + 1 weeks                                 |                                                      |                                                | Age range: Fetus of 22 + 1 weeks - 18 years   |
|                                |                                                        |                                                      |                                                |                                               |
|                                | Oligohydramnios                                       |                                                      |                                                |                                               |
|                                | No                                                    | No                                                   | Yes                                            | Yes                                           |
|                                |                                                        |                                                      |                                                | 3/10                                          |
|                                | Intrauterine growth restriction                       |                                                      |                                                |                                               |
|                                | Yes                                                   | Yes                                                  | Yes                                            | Yes                                           |
|                                |                                                        |                                                      |                                                | 10/10                                         |
|                                | Short stature                                         |                                                      |                                                |                                               |
|                                | N/A                                                   | Yes                                                  | Yes                                            | Yes                                           |
|                                |                                                        |                                                      |                                                | 9/9                                           |
|                                | Feeding problems                                      |                                                      |                                                |                                               |
|                                | N/A                                                   | Yes                                                  | Yes                                            | Yes                                           |
|                                |                                                        |                                                      |                                                | 7/9                                           |
|                                | Microcephaly                                          |                                                      |                                                |                                               |
|                                | Yes                                                   | Yes                                                  | Yes                                            | Yes                                           |
|                                |                                                        |                                                      |                                                | 8/10                                          |
|                                | Cognitive abnormalities                               |                                                      |                                                |                                               |
|                                | N/A                                                   | Developmental delay, mild intellectual disability    | Developmental delay, intellectual disability  | Developmental delay                           |
|                                |                                                        |                                                      |                                                | 9/9                                           |
|                                | Behavioral abnormalities                              |                                                      |                                                |                                               |
|                                | N/A                                                   | Tics and nail biting                                  | No                                             | No                                            |
|                                |                                                        |                                                      |                                                | 4/9                                           |
|                                | Seizure                                               | N/A                                                  | No                                             | No                                            |
|                                |                                                        |                                                      |                                                | 2/9                                           |
|                                | Hypotonia                                             | N/A                                                  | No                                             | No                                            |
|                                |                                                        |                                                      |                                                | 6/9                                           |
|                                | Facial dysmorphisms                                   | Yes                                                  | Yes                                            | Yes                                           |
|                                |                                                        |                                                      |                                                | 10/10                                          |
|                                | Hands abnormalities                                   | Bilateral clinodactyl (5th finger)                   | Clinodactyl (5th finger)                       | Arachnodactyl                                 |
|                                |                                                        |                                                      |                                                | No                                            |
|                                |                                                        |                                                      |                                                | 6/10                                          |
|                                | Feet abnormalities                                    | Bilateral club feet                                  | Mild left Club foot                            | No                                            |
|                                |                                                        |                                                      |                                                | 6/10                                          |
|                                | Dental abnormalities                                  | N/A                                                  | Yes                                            | N/A                                           |
|                                |                                                        |                                                      |                                                | 7/8                                           |
|                                | Heart defects                                         | Atrial septal defect, coarctation                     | Supravalvular aortic and pulmonary stenosis    | No                                            |
|                                |                                                        |                                                      |                                                | 4/10                                          |
|                                | Other findings                                        | Clitoromegaly                                        | Brain atrophy in frontal lobe, sensorineural hearing loss, hypertonia and increased deep tendon reflexes at 16 | Hypertonia since 3 months, dolichocephaly    |
|                                |                                                        |                                                      |                                                | Hyperbilirubinemia                            |
|                                |                                                        |                                                      |                                                | Various                                       |
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 (arr[hg19] 3q27.1q28(183,011,106_187,947,036)x1; Additional file 1: Fig. S1a). 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 (Additional file 1: Fig. S1b). FISH using a BAC probe RP11-919L13 further confirmed that this deletion is present in the proband but not in the father (Additional file 1: Fig. S1c and d) and is therefore de novo in origin. No other CNVs except for those commonly seen in normal populations were detected in this individual.

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 (Additional file 1: Fig. S2a) in the chromosomal region 3q27.1q27.2, corresponding to genomic coordinates 182,950,371–185,324,970 (arr[hg19] 3q27.1q27.2[182,950,371_185,324,970]x1). 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 (Additional file 1: Fig. S2b and c). Father is unavailable for testing. No other CNVs except for those commonly seen in normal populations were detected in this individual.

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, postnatal growth impairment, feeding problems, short stature, dysmorphic facial features, microcephaly, seizure, dental and limb abnormalities, developmental delay, intellectual disability, hypotonia, and thrombocytopenia. Alternatively, in the Decipher database, several individuals harboring the deletions (range of 1.8–2.7 Mb) in this region has been reported to exhibit variety of phenotypes including intraterine growth retardation, short stature, microcephaly, facial dysmorphisms, hypotonia, developmental delay, and cardiac defects (DECIPHER ID: 317983, 276986, and 323724). 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 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).

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 (Fig. 1). Deletions overlapping with this region are absent from population databases including gnomAD SVs v2.1 (controls) and DGV Gold Standard [14, 15]. 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) (Additional file 2 Table S1). 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), teeth abnormalities (7/8), and hand abnormalities (6/10). Emerging data suggest DVL3 is a core component in the routing and transmission of canonical and non-canonical Wnt signalasome [16]. 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 [17, 18]. 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 [19]. 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 [20–22]. 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 [21–24]. 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 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 [25]. 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 [26], and Parl deficiency results in growth retardation, cachexia, and severe atrophy of skeletal muscle, thymus, and spleen [27]. 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 harbors 26 protein coding RefSeq genes (Criteria 3B, points given: 0.45). Three of them, PSMD2, AP2M1, and EIF4G1 are predicted to be haploinsufficiency genes by the gnomAD pLI score [28], the gomAD LOEUF score [29], and the DECIPHER HI index [30] (Additional file 2: Table S1; Criteria 2H, points given: 0.15). To the best of our knowledge, in the literature 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 in chromosome 3q26q28 region. It provides further evidence supporting the existence of this novel 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 microdeletion 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 utilized the new ACMG/ClinGen standards for CNV interpretation with refined molecular mapping that improved 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.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s13039-022-00587-0.

Additional file 1: Figure S1. 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 S2. Test results for Proband-2 and her mother. a SOMA result showing the 3q27.1q28 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.

Additional file 2: Table S1. Summary of protein coding genes within the 1.2 Mb in chromosome 3q27.1 (XLSX 13 KB).

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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.

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

Declarations
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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