


ORIGINAL ARTICLE

Double homozygosity in *CEP57* and *DYNC2H1* genes detected by WES: Composite or expanded phenotype?

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Abstract

Background: In the last few years trio-whole exome sequencing (WES) analysis has demonstrated its potential in obtaining genetic diagnoses even in nonspecific clinical pictures and in atypical presentations of known diseases. Moreover WES allows the detection of variants in multiple genes causing different genetic conditions in a single patient, in about 5% of cases. The resulting phenotype may be clinically discerned as variability in the expression of a known phenotype, or as a new unreported syndromic condition.

Methods: Trio-WES was performed on a 4-month-old baby with a complex clinical presentation characterized by skeletal anomalies, congenital heart malformation, congenital hypothyroidism, generalized venous and arterial hypoplasia, and recurrent infections.

Results: WES detected two different homozygous variants, one in *CEP57*, the gene responsible for mosaic variegated aneuploidy syndrome 2, the other in *DYNC2H1*, the main gene associated with short-rib thoracic dysplasia.

Conclusion: The contribution of these two different genetic causes in determining the phenotype of our patient is discussed, including some clinical signs not explained by the detected variants.

The report then highlights the role of WES in providing complete and fast diagnosis in patients with complex presentations of rare genetic syndromes, with important implications in the assessment of recurrence risk.

KEYWORDS

CEP57, composite phenotype, double homozygosity, *DYNC2H1*, WES

1 | INTRODUCTION

Proband-parents whole exome sequencing (Trio-WES) analysis has hugely improved the prospect of obtaining a genetic diagnosis in pediatric population, even in urgent cases

(Stark et al., 2018). Diagnostic WES also allows the detection of variants in multiple genes causing different genetic conditions in a single patient; this event has been estimated to occur at a rate of approximately 5% (Posey et al., 2017; Smith et al., 2019). In contrast to digenic inheritance, which

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requires contributing pathogenic variation at two specific loci for the manifestation of a single disease, dual molecular diagnoses represent an aggregation of independent diagnoses. The blending of two distinct disease phenotypes in a single patient may clinically suggest an apparently new phenotype, or, alternatively, the phenotypic expansion of a single known disease (Posey et al., 2017).

Here we describe the case of a boy with a complex clinical presentation in which trio-based WES analysis detected a homozygous variant of *CEP57* (OMIM*607951) allowing the diagnosis of mosaic variegated aneuploidy syndrome 2 (MVA2, OMIM #614114). This wide approach unexpectedly also identified a homozygous mutation in the *DYNC2H1* gene (OMIM*603297), the major locus of short-rib thoracic dysplasia (SRTD). In this report we discuss the manifestations due to each single genetic cause, highlighting some clinical signs that might be considered a phenotypic expansion of MVA2 and the consequent implications for the assessment of recurrence risk.

2 | CASE REPORT

The patient was the fourth child born to a healthy and consanguineous (first cousins) Moroccan couple, the family history was unremarkable. The pregnancy was characterized by ultrasound detection of atrioventricular canal (AVC) and short limbs (femur bone at 5th centile). No prenatal genetic tests were performed. The boy was born at 39 weeks of gestation by caesarean section. His birth weight, length, and occipitofrontal circumference were 2,584 g (3th centile), 42 cm (<3th centile), and 38 cm (97th centile), respectively. The Apgar scores were 6 at the 1st and 7 at the 5th minute. At 10 min of life he gradually developed apnea and respiratory distress, and an orotracheal intubation was required.

A cardiac ultrasound was performed at birth, showing a complete AVC, wide ductus arteriosus with right-to-left shunt, transverse aortic arch severe hypoplasia, and isthmus coarctation. Then, a surgical intervention of aortic arch repair by anastomosis between ascending and descending aorta through a median sternotomy and concomitant pulmonary artery bending was needed at 6 days of life. Newborn screening meanwhile allowed one to diagnose a congenital hypothyroidism and a levothyroxine replacement therapy was set up.

Transfontanellar ultrasound detected mild widened cerebral subarachnoid spaces with wide interhemispheric scissure, so a brain magnetic resonance was performed that showed benign external hydrocephalus and a thinned corpus callosum. Electroencephalogram showed only an asymmetric signal. Neurological evaluation revealed a reduced gross motility and a reduced head control to traction.

The physical examination at birth and at 3 months of life showed macrocephaly, wide anterior fontanel, high forehead with frontal bossing, hypertelorism, short neck, rhizomelic shortening of the limbs, mostly the upper ones, brachydactyly of hands and feet, preaxial polydactyly type B of the right foot, left big toe clinodactyly, and bilateral cutaneous syndactyly of II, III, and IV toes.

On suspicion of a skeletal dysplasia, a total body X-ray examination was performed, that showed macrocephaly, anomalous shape of the orbits, D10 butterfly vertebra, a supernumerary right rib, slightly bell-shaped thorax, rhizomelic shortening of the upper and lower limbs, wide distal phalanx of the first toe bilaterally, and clinodactyly of the left first toe (Figure 1).

At 4 months of life the baby still needed to be ventilated due to recurrent pulmonary, gastrointestinal, urinary and systemic infections; dosage of immunoglobulin levels when he was 3 months old revealed a deficiency, as IgA were 6 mg/dl (normal range (n.r.) 8–74 mg/dl), IgG 104 mg/dl (n.r. 231–497 mg/dl), and IgM < 4 mg/dl (n.r. 26–210 mg/dl), so periodical replacement therapy was given. Lymphocyte subpopulations analysis showed reduced values of total T cells (CD3⁺), T helper (CD3⁺CD4⁺), T cytotoxic (CD3⁺CD8⁺), and lymphocytes B (CD3⁻CD20⁺), while NK cells (CD3⁻CD16⁺CD56⁺) were normal; CD4⁺/CD8⁺ ratio was 3.73 (n.r. 1.50–2.90).

The patient needed parenteral nutrition from birth due to persistent gastric stagnation, causing abdominal distension with continuous vomiting. Enteral nutrition was never possible due to radiologically documented persistent slowed gastric emptying. At first, digestive tract X-ray studies did not detect anatomical obstacles, but at 4th month of life multiple subocclusions have been highlighted. A subsequent barium-enema X-ray revealed several colonic stenoses, and, during abdominal laparoscopy due to the detection of necrotizing enterocolitis (NEC), total colectomy and ileo-sigma anastomosis was required.

Because of the worsening of the already complex clinical course, and in order to eventually redirect the clinical management on the basis of etiological diagnosis, urgent trio-based WES analysis was performed which detected a homozygous mutation in *CEP57* gene and a homozygous mutation in *DYNC2H1* gene. Cytogenetic analysis showed a MVA karyotype consistent with molecular data.

At 5 months of life, abdomen computed tomography (CT) scan was performed due to tough weaning from total parenteral nutrition showing generalized venous and arterial hypoplasia, in particular thread-like aorta and splanchnic vessels, hypoplasia of vena cava and of right iliac vessels, and presence of multiple venous varicosities. In addition, CT detected some hepatic cystic lesions.

At 6 months of life, during a further episode of sepsis, the baby showed a serious worsening of abdominal clinical

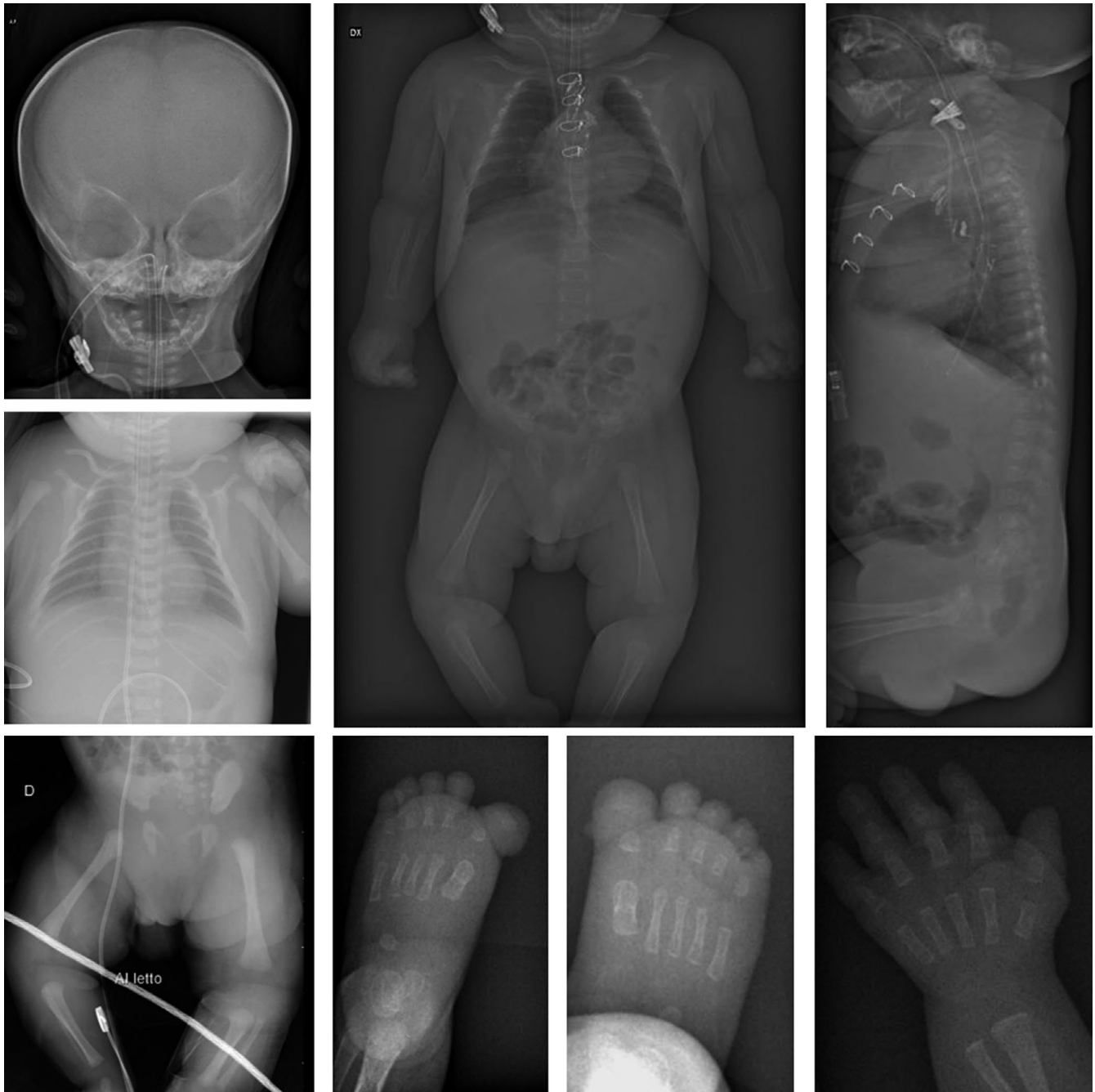


FIGURE 1 Total body X-ray examination showed macrocephaly, anomalous shape of the orbits, D10 butterfly vertebra, a supernumerary right rib, slightly bell-shaped thorax, rhizomelic shortening of the upper and lower limbs, wide distal phalanx of the first toe bilaterally, and clinodactyly of the left first toe

picture, with multiple organ dysfunction, hemodynamic instability and metabolic acidosis, and an urgent CT scan revealed the onset of renal and hepatic ischemic lesions, and adrenal glands and small bowel walls hypovolemic injuries. On the basis of the rapidly and unstoppable worsening of the clinical picture palliative cares were then set up till the exitus of the patient.

3 | MATERIALS AND METHODS

3.1 | Ethical compliance

This study was approved by ethics committee at ASST Papa Giovanni XXIII of Bergamo as part of the RARE project—Rapid Analysis for Rapid care.

3.2 | Material and methods

After genetic counseling and written informed consent, genomic DNAs of the patient and his parents were extracted from peripheral blood using commercial available DNA extraction kits. Exons and splice sites regions of the whole genome were enriched by the Agilent SureSelect Clinical Research Exome v2.0 enrichment kit (Agilent) and 150 bp paired-end sequencing was performed on the NextSeq500 platform (Illumina). Sequence reads were mapped to the reference human genome assembly (Feb. 2009, GRCh37/hg19) and analyzed by the BWA enrichment version 2.0 pipeline and a second independent in-house pipeline (Iascone et al., 2012). The variant call file, including single nucleotide variants and indels, was annotated querying variants databases, including the Genome Aggregation Database (<http://gnomad.broadinstitute.org/>), ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>) and Human Gene Mutation Database Professional (HGMD, Release 2017.4). Variants were filtered on the basis of the following criteria: effect on protein and transcript (splicing, missense, nonsense, and frameshift); patient phenotype according to the Human Phenotype Ontology classification (www.human-phenotype-ontology.org); inheritance model (autosomal recessive or de novo); minor allele frequency in general population considering the prevalence and incidence of a rare disease. Moreover, the candidate variants should have a pathogenic mechanism corresponding to the expected one for the disease. Variants were classified on the basis of ACMG guidelines (Nykamp et al., 2017; Richards et al., 2015). Visual inspection was performed with Alamut Visual Software (<http://www.interactive-biosoftware.com/alamut-visual/>) and pathogenic variants were confirmed in the proband and parents by Sanger sequencing using a second independent DNA sample. Conventional cytogenetic analysis was carried out on peripheral blood lymphocytes and skin fibroblasts according to the standard laboratory procedures, using QFQ banding method. Chromosome analysis was performed according to the European General Guidelines and Quality Assurance for Cytogenetics (Hastings, Howell, Bricarelli, Kristoffersson,

& Cavani, 2012). The data are not publicly available as parents did not give consent.

4 | RESULTS

Trio-based WES analysis generated on average 88,864,321 (range 68,525,936–107,347,433) targeted aligned reads and the range of the mean coverage of target regions was 153–241X. Over 98% of target regions were covered at least 10 X in each sample.

The analysis revealed a homozygous duplication of 11 nucleotides in *CEP57*, (Chr11(GRCh37):g.95560979_95560989dup; NM_014679.4:c.915_925dup; p.Leu309Profs*9), leading to a frameshift starting from codon 309 and ending in a stop codon 9 amino acids downstream. This variant was previously reported in MVA2 (De la Torre-García et al., 2019; Snape et al., 2011) and was present in ClinVar database as pathogenic. Both parents were heterozygous carriers of the variant.

Seventy metaphases were analyzed from lymphocyte cultures and 28% showed a male karyotype with abnormal chromosome number, being 21% near-diploid, 4% near-triploid, and 3% near-haploid. Aneuploidy appeared to be random, ranging from nullisomies to tetrasomies and involving all chromosome pairs. Multiple trisomies were the most frequent defects. Gains were preferentially observed (>4 metaphases) for chromosomes 1, 3, 4, 5, 6, 12, 15, and 22, whereas losses mostly involved chromosomes 2, 7, 9, 10, 14, and 15 (Figure 2). No cells showed structural chromosome aberrations and no evidence of premature chromatid separation was detected.

Cytogenetic analysis of 20 metaphases from fibroblasts culture showed a normal karyotype; no aneuploidy was identified, likely due to the low number of available metaphases.

Moreover, WES identified a homozygous missense variant (Chr11(GRCh37):g.103175330A > G; NM_001080463.1:c.11284A > G, p.Met3762Val) in the

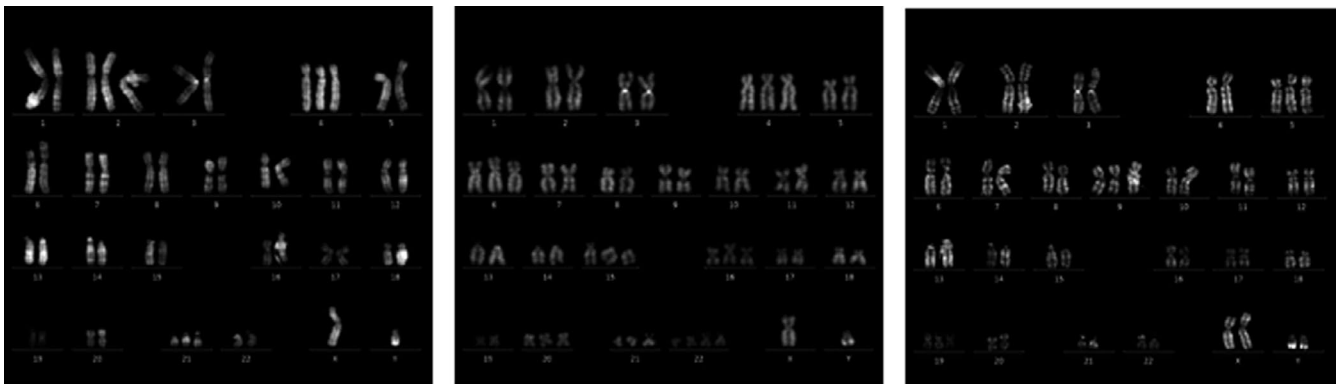


FIGURE 2 Aneuploid metaphases at cytogenetic analysis on peripheral blood lymphocytes, consistent with MVA diagnosis

DYNC2H1 gene, with both parents being heterozygous carriers. This variant was previously reported in three patients with asphyxiating thoracic dysplasia (ATD) or short rib-polydactyly syndrome (SRPS) (Baujat et al., 2013; Dagoneau et al., 2009; Zhang et al., 2018). Particularly, only one 16-month-old patient (Baujat et al., 2013) had this variant in homozygosity; he showed clinical signs consistent with ATD as short ribs with narrow thorax, trident acetabular roof, long bones shortening, respiratory neonatal distress, long-term respirator complications, and short stature, so the *DYNC2H1* variant was considered as disease causing by the authors.

CEP57 and *DYNC2H1* are located at 11q, within about 7.4 Mb each other.

5 | DISCUSSION

Mosaic variegated aneuploidy is a genetically heterogeneous autosomal recessive disease caused by mutations of three different genes: *BUB1B* (MVA1, OMIM # 257,300) (Hanks et al., 2004), *CEP57* (MVA2, OMIM # 614,114) (Snape et al., 2011), and *TRIP13* (MVA3, OMIM # 617,598) (Yost et al., 2017). The presence of constitutional mosaic aneuploidies due to gain or loss of multiple different chromosomes is the hallmark of MVA syndromes, while the related clinical phenotype can vary according to the involved causal gene. Cancer predisposition is one of the most important associations of MVA due to *BUB1B* and *TRIP13* variants, with substantial increased risk of childhood malignancies, particularly Wilms tumor, rhabdomyosarcoma, and leukemia (Hanks et al., 2004; Jacquemont, Bocéno, Rival, Méchinaud, & David, 2002; Yost et al., 2017). On the contrary none of the seven patients with *CEP57* mutation reported to date in the literature developed cancer (Brightman, Ejaz, & Dauber, 2018; De la Torre-García et al., 2019; Pinson et al., 2014) even if a recent work proved that MVA2 syndrome or haploinsufficiency in *CEP57* are associated with impaired tumor suppression (Aziz et al., 2018). Cancer proneness in MVA2 thus deserves further investigation since our patient died at 6 months of life and none of the reported MVA2 patients have reached adulthood yet. So, the careful description of additional cases, and the follow-up of the already reported patients will be important to better delineate this important aspect.

While the phenotypic spectrum of *BUB1B* MVA is now quite well outlined (Akasaka et al., 2013; Garcia-Castillo, Vasquez-Velasquez, Rivera, & Barros-Nunez, 2008; Hanks et al., 2004; Kato et al., 2017; Rio Frio et al., 2010), only few patients affected by *CEP57* and *TRIP13* MVA syndromes have been described to date.

Seven patients with *CEP57* MVA syndrome have been indeed reported in the literature, and a review (Pinson et

al., 2014) allowed one to highlight some pivotal features, as growth retardation with relative sparing of the head, normal or mildly delayed development, rhizomelic shortening of the limbs, and no cancer proneness, and some features more frequently observed as IUGR, congenital hypothyroidism, and congenital heart defects. However, as correctly pinpointed by the authors, since so few patients have been reported, the phenotypic spectrum should be better delineated and confirmed.

Our patient indeed showed the clinical MVA2 features proposed by Pinson et al. (2014), but other signs were also associated, such as preaxial polydactyly, butterfly vertebra, supernumerary rib, recurrent infections with immunodeficiency, and vascular anomalies.

As mentioned above, WES analysis also detected a homozygous *DYNC2H1* mutation complicating and confounding the reverse phenotyping.

The *DYNC2H1* gene, that encodes a component involved in the retrograde transport in the primary cilia, is the major locus of SRTD, and is associated with ATD or SRPS phenotypes (Zhang et al., 2018). SRTD with or without polydactyly refers to a group of autosomal recessive skeletal ciliopathies of variable severity that are characterized by the presence of narrow chest, short ribs, short tubular bones, and possible association with preaxial polydactyly, retinal alterations, cystic liver, and cystic kidneys. Peculiar radiological findings include handlebar clavicles, trident appearance of acetabula, and metaphyseal abnormalities of long bones.

Our patient showed some features referable to MVA2, such as IUGR, congenital hypothyroidism, and congenital heart defects, some features referable to SRTD (of which, however, major radiological features were missing, see Figure 1), like polydactyly, brachydactyly, cystic liver, and recurrent respiratory infections (Emiralioglu, Wallmeier, Olbrich, Omran, & Ozcelik, 2018), and some features that could be attributable to both the conditions, as rhizomelic shortening of the limbs and bell-shaped thorax, such as a MVA2 patient with a narrow thorax has been recently reported (De la Torre-García et al., 2019).

In addition, our patient showed other signs that have not been reported to date neither in SRTD nor in MVA2, as butterfly vertebra and supernumerary rib, recurrent systemic, urinary and gastrointestinal infections and associated immunodeficiency, and vascular malformations. It is notable that none of the *CEP57*-mutated patients reported to date has been radiologically studied in depth, so the minor skeletal findings detected in our patient might be related to MVA2, although more cases are needed to confirm this observation.

The vascular anomalies showed by our patient include generalized abdominal venous and arterial hypoplasia, and presence of multiple venous varicosities. The tissue hypoperfusion due to vascular hypoplasia of all major abdominal

vessels might be at the base of the persistent gastric stagnation and of the development of NEC and ischemic injuries detected in the liver and the kidneys. Of note, a case of MVA2 reported by Snape et al. (2011), case 638) besides atrial and atrioventricular septal defect also showed aortic coarctation, as our patient. The patient reported by Snape unexpectedly died at 3 weeks of life after cardiac surgery, so we have no further details on potential associated vascular anomalies, but we can speculate that aortic coarctation might be a sign of vascular hypoplasia, and then a negative prognostic sign in MVA2. This hypothesis needs to be investigated on further cases.

Concerning the recurrent infections, an immunodeficiency was detected. From this perspective a reevaluation of WES data has been required, but no variants associated with immunodeficiency have been detected. We hypothesized that abdominal vascular hypoplasia and consequent tissue hypoperfusion, in association with immunodeficiency, reasonably facilitated the recurrent sepsis and abdominal infections onset.

The blending of two distinct disease phenotypes in a single patient may be clinically discerned as variability in the expression of a known phenotype, or as a new unreported syndromic condition (Karaca et al., 2018; Posey et al., 2017). For example, narrow thorax has actually been reported in a case of MVA2 (De la Torre-García et al., 2019) and then considered as a possible new associated feature, however, the patient reported by De la Torre-García was molecularly diagnosed only by Sanger sequencing of MVA causal genes after the detection of MVA at karyotype, so the possible presence of multiple genetic diseases cannot be excluded. On the other hand, some of the clinical signs showed by our patient, as vascular hypoplasia and immunodeficiency cannot to date be brought under MVA2 or SRTD, so they may represent a phenotypic expansion of one of the two genetic conditions. Given the few MVA2 compared to SRTD reported cases, we hypothesized that they might be an expansion of *CEP57*-related clinical presentation.

Usually the finding of a pathogenic variant consistent with the patient phenotype stops any further genetic testing. However, single gene tests or gene panels, focused on a limited number of genes, might be potentially inadequate, hiding adjunctive genetic variations. Wide analyses, such as trio-WES, are uniquely positioned to detect potential multiple findings and could be therefore a good first-tier strategy especially in consanguineous families, where multiple genetic diagnoses are more frequent (Smith et al., 2019).

The detection by WES of multiple genetic conditions in a patient may complicate the process of reverse phenotype, since patients who received multiple diagnoses may have some clinical features imputable to either diagnosis (Smith et al., 2019). Moreover the detection of multiple inherited genetic findings in a proband has further implications in

the recurrence risk assessment since these Mendelian conditions may segregate independently. In our case, the two mutated genes are located on the q arm of chromosome 11 at about 7.4 Mb from each other. Due to the proximity of the two genes and their position in the same arm of chromosome 11, the probability of crossing over resulting in an independent segregation of the two conditions is theoretically low.

6 | CONCLUSION

This report strengthens the utility of extensive molecular workup in patients with complex clinical presentation, especially in consanguineous families (Smith et al., 2019), in which a molecular diagnosis based on a limited number of genes may not be complete, with potential implications for clinical management, recurrence risk, and genetic counseling.

Furthermore, this report highlights some unreported clinical signs, referable to *CEP57* mutations.

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CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

AUTHOR CONTRIBUTION

LiP, LaP, AP, and AC were involved in conception and design of the study and writing of the manuscript. LaP, AP, LM, BF, UG, ARL, LoP, and MP were involved in laboratory experiments and data interpretation. AS, LiP, and AC were involved in genetic counseling. EB, GM, IP, LM, and LG were involved in patient evaluations. MI revised the manuscript and made substantial scientific contributions. All authors read and approved the final version of the manuscript.

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