Novel Insights from Clinical Practice

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Robinow Syndrome and Brachydactyly: An Interplay of High-Throughput Sequencing and Deep Phenotyping in a Kindred

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Established Facts

- Robinow syndrome is a skeletal syndrome with fetal facies and genital anomalies.
- The utility of whole-exome sequencing (WES) in unrecognizable or intricate phenotypes is established.
- WES plays a role in breaking the clinical tenet of explaining a complex disorder by a monogenic inheritance.

Novel Insights

- This study contributes to the understanding of blended phenotypes in the era of comprehensive molecular genotyping.
- The relevance of deep phenotyping to allow gene prioritization is essential.
- Is it just expanding the phenotype of a monogenic disorder or dual molecular diagnosis impacting clinical manifestations?
- *DVL1*-associated autosomal dominant Robinow syndrome exibits a severe phenotype due to synergistic effect of a *BMP2* mutation – a possibility of a common interactive pathway.

Keywords
Blended phenotype · *BMP2* · *DVL1* · Reanalysis · Whole-exome sequencing

Abstract
We report a family with a spectrum of short stature, craniofacial dysmorphism, and digital anomalies in a father and 2 daughters, with the youngest (proband) displaying a severe phenotype. Clinically, autosomal dominant Robinow syndrome (ADRS) was diagnosed. Whole-exome sequencing identified a heterozygous pathogenic *BMP2* variant in the father and his daughters. The phenotype of short stature, facial dysmorphism, and skeletal anomalies with or without cardiac anomalies related to *BMP2* haploinsufficiency has some facial and digital resemblance to ADRS. Although this variant segregated in the affected members, it failed to explain the severe phenotype of the proband. A reanalysis of the girl's raw data confirmed 2 disorders: a de novo likely pathogenic *DVL1* variant implicated in ADRS and the familial

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BMP2 variant. A close interplay of high-throughput sequencing and deep phenotyping unraveled the complexities of the blended phenotype in the proband.

Robinow syndrome is a rare congenital skeletal dysplasia with dysmorphism characterized by a variable combination of short stature, mesomelia, genital hypoplasia, and distinctive fetal facies [Robinow et al., 1969]. It exhibits both autosomal dominant and recessive forms of inheritance. Mutations in genes typically encoding components of the non-canonical Wnt signaling pathway are implicated in this disorder, including *ROR2* (MIM 602337) for autosomal recessive Robinow syndrome and *WNT5A* (MIM 164975), *DVL1* (MIM 601365), and *DVL3* (MIM 601368) for autosomal dominant Robinow syndrome (ADRS).

Herein, we report a family with 2 unrelated skeletal disorders segregating in different symptomatic members. We show the impact of variation in 2 genes accounting for a blended phenotype. We further discuss the role of next-generation sequencing (NGS) technology in characterizing the familial syndromes.

Clinical Report

The proband, a 3.9-year-old girl, second born to nonconsanguineous parents, presented with short stature and dysmorphic features. She had a history of delayed motor milestones, but normal cognitive development and no feeding problems. Her height was 71.8 cm (–6.2 SD), weight was 7.8 kg (–3 SD), and her head circumference was 52 cm (+2.4 SD). The upper segment/lower segment ratio was 1.5:1. Macrocephaly with wide-open fontanelle and frontal bossing was found. Dysmorphic features included hypertelorism, downslanting prominent eyes, infraorbital crease with midface hypoplasia, flat nasal bridge, short nose, anteverted nares, supernumerary teeth, dental crowding, gum hyperplasia, high-arched palate, and a short neck. Downturned corners of the mouth and low-set ears were also observed (Fig. 1a, b). The salient skeletal abnormalities were mesomelia in both upper and lower limbs, bilateral broad great toes, brachydactyly of the 3rd and 4th fingers and all toes (Fig. 1c, d). The systemic examination was normal. Radiographs of the feet showed a bilateral absence of the middle phalanges in the 2nd–5th toes, bilateral absent distal phalanges in the 3rd toes, and bilateral hypoplastic distal phalanges in the 1st, 2nd, 4th, and 5th toes (Fig. 1e).

Her elder sister had short stature (119.5 cm, –2.2 SD) with gingival hyperplasia, dental crowding, and bilateral clinodactyly (Fig. 2a). Radiographs of the feet showed bilateral hypoplastic distal phalanges. Bilateral big toes showed a broad base of the distal phalanges and short, broad proximal phalanges (Fig. 2b). The girl's father had short stature (155.6 cm, –2.77 SD), dental crowding, and short 3rd toes (Fig. 2c, d). Radiographs of the feet showed bilaterally absent middle phalanges of the 3rd, 4th, and 5th toes. Bilateral big toes showed a broad base of the distal phalanges as well as short and broad proximal phalanges (Fig. 2e). The mother was of average height and had no dysmorphism. The clinical evaluation of this family revealed a spectrum of short stature, a recognizable craniofacial gestalt, dental crowding, and digit anomalies except for the mother. A clinical diagnosis of ADRS was made with the proband displaying severe dysmorphism and the sister and father with a milder phenotype.

Materials and Methods

Whole-exome sequencing (WES) of the siblings and parents was performed after extracting the genomic DNA from peripheral venous blood samples. Extracted DNA was used to perform targeted gene capture using the Exome Research Panel (Integrated DNA technologies). The libraries were sequenced to mean >80–100× coverage on the Illumina sequencing platform. The sequences obtained were aligned to the human reference genome (GRCh37/hg19) using the BWA program [Li and Durbin, 2010; Meyer et al., 2013] and analyzed using Picard and GATK version 3.6 [Li et al., 2009; McKenna et al., 2010] to identify variants relevant to the clinical indication. Gene annotation of the variants was performed using the VEP program [McLaren et al., 2010] against the Ensembl release 87. Clinically relevant mutations were annotated using published variants in literature and a set of disease databases – ClinVar, OMIM, and HGMD (Human Gene Mutation Database). Common variants were filtered, based on allele frequency in 1000 Genome Phase 3, ExAC (Exome Aggregation Consortium), EVS (exome variant server), dbSNP147, 1000 Japanese Genome, and our internal Indian population database. Nonsynonymous variants effect was calculated using multiple algorithms such as PolyPhen-2, SIFT, MutationTaster2, and Mutation Assessor.

Results

WES identified a heterozygous, novel nonsense variant (c.508C>T) in exon 3 of the *BMP2* gene (chr20: 6759053C>T; depth: 31×) that results in a stop codon and premature truncation of the protein at codon 170 (p.Arg170Ter; ENST00000378827) in the proband, her sister and their father, but not in the mother. We used standard variant filtration criteria such as common population variants based on allele frequency >1%, low-depth regions (<20×), low quality score, and deep intronic variants. We found the *BMP2* gene, one of the phenotypically associated genes as a loss-of-function allele in *BMP2* (PVS1), is implicated in craniofacial and skeletal features [Tan et al., 2017]. This variant is not described in the GnomAD (V2) database (PM2) and has pathogenic predictions from DANN, EIGEN, FATHMM-MKL, and MutationTaster2 computational software with no benign prediction made by any software (PP3). Based on the in

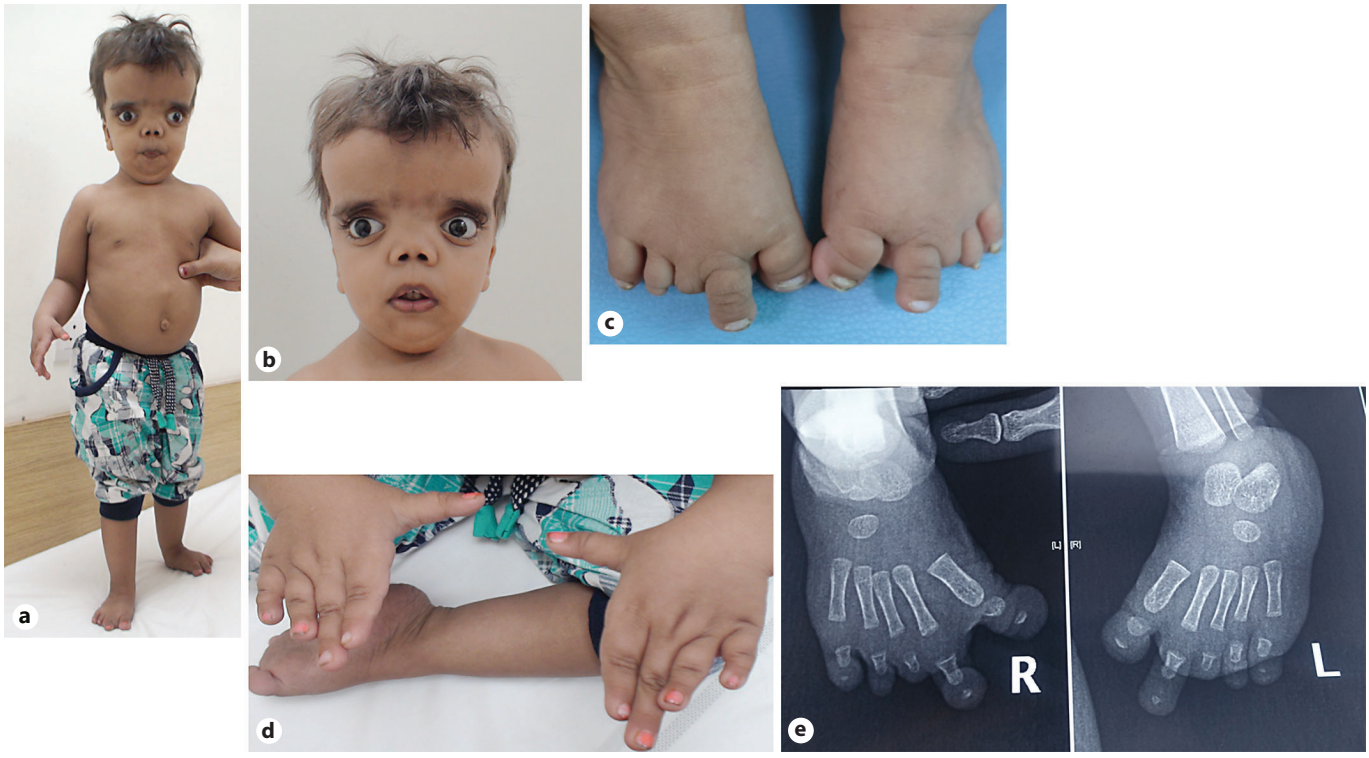

Fig. 1. a–e Photographs of the proband showing short stature, mesomelia (**a**), macrocephaly, hypertelorism, anteverted nares, dental crowding, short neck (**b**), sandal toe gap, brachydactyly of all toes (**c**), brachydactyly of third and fourth fingers, proximally placed thumbs (**d**). X-rays showing bilaterally absent middle phalanges of 2nd–5th toes, absent distal phalanx in 3rd toe as well as hypoplastic distal phalanges in the 1st, 2nd, 4th, and 5th toes (**e**).

silico predictions and the nature of the variant, it was classified as pathogenic against the American College of Medical Genetics and Genomics (ACMG) Standards and Guidelines [Richards et al., 2015]. While short stature and craniofacial, skeletal and dental features of the 3 members could be explained by the *BMP2*-related phenotype [short stature, facial dysmorphism, and skeletal anomalies with or without cardiac anomalies (SSFSC; MIM 617877)], the severity of the proband's dysmorphism was out of proportion. Hence, the genomic dataset was reanalyzed to search for Robinow or related syndromes, and the proband was found to carry a heterozygous likely pathogenic 13-bp de novo frameshift deletion in the *DVL1* gene [ENST00000378891:c.1619_1631del; p.Thr540Arg fsTer105] causative of ADRS. This was absent in the elder sibling and the parents. ClinVar reports this frameshift deletion variant as "likely pathogenic." Since there is no clinical information in ClinVar, we report this as a novel variant for *DVL1*-associated ADRS. This frameshift variant lies in a mutation cluster region (PM1) and is de novo in this family (PS2). It is not present in the GnomAD (V2) database (PM2) and a –1 frameshifting indel in *DVL1* (PVS1) is reported as a disease-causing mechanism for ADRS [White et al., 2018]. Based on the abovementioned features of the identified variant, it is reclassified as a pathogenic variant as per the ACMG Standards and Guidelines [Richards et al., 2015]. Sanger validation of the familial *BMP2* single nucleotide change and the de novo *DVL1* frameshift deletion was performed on the affected members (Fig. 3).

Discussion

Robinow syndrome is a genetically heterogeneous disorder with a distinct facial gestalt. The phenotype becomes less characteristic with age, and therefore, the diagnosis may not be as obvious [White et al., 2015]. In the family reported above, the youngest child had the typical fetal facies, while the elder sibling and father had a milder

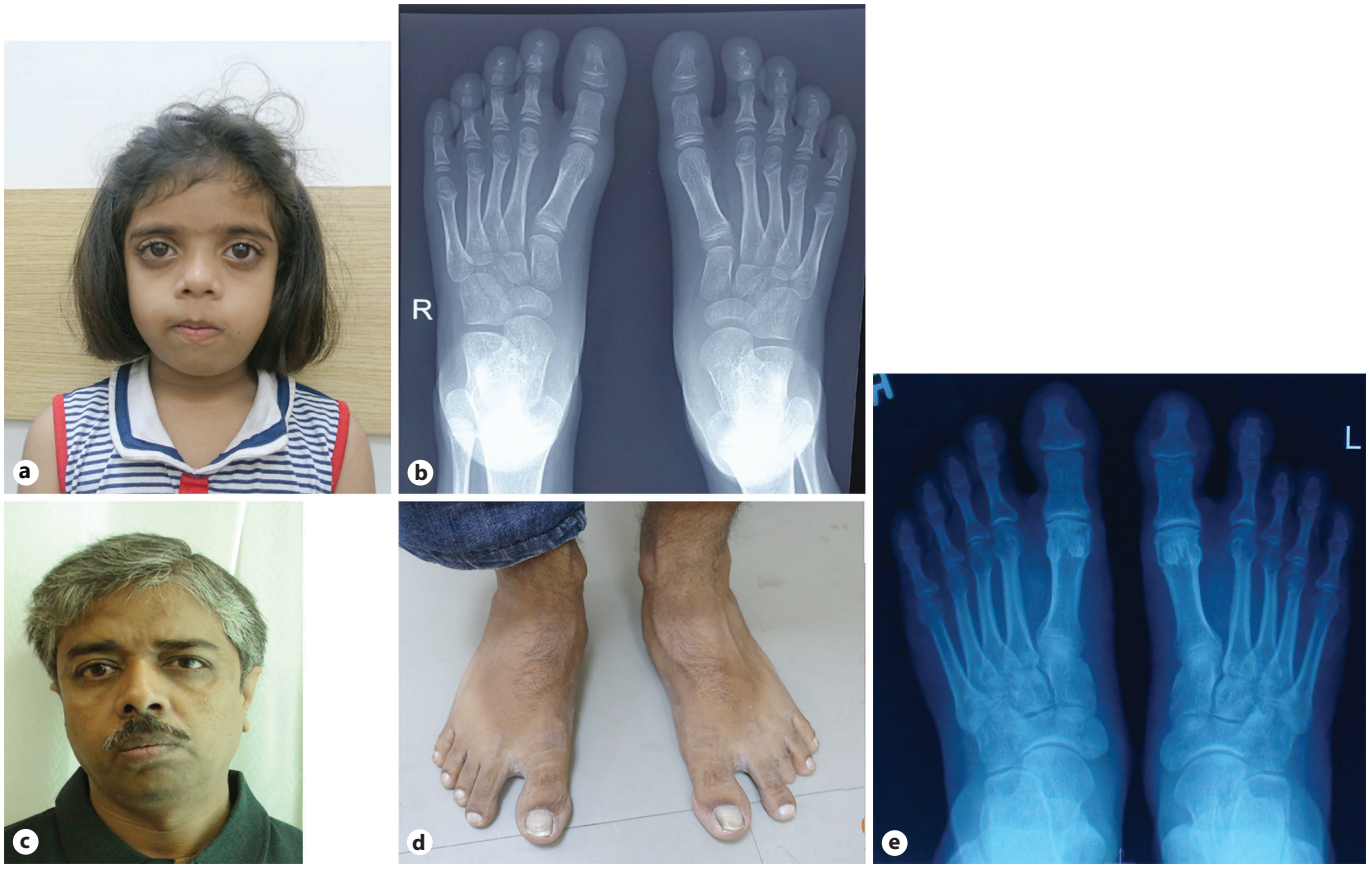

Fig. 2. a Proband's elder sibling. **b** X-ray of the sibling's foot showing hypoplastic distal phalanges. **c** Proband's father. **d** Father's feet showing sandal toe gap and short 3rd left toe. **e** Radiograph of the father's feet revealing absent middle phalanx of the 3rd toe and hypoplastic distal phalanges of all toes.

phenotype, making their clinical diagnosis less obvious. *DVL1*-associated Robinow syndrome patients have significant macrocephaly (OFC > +4 SD) and normal height [White et al., 2015]. However, these features were absent in our proband who had significant short stature at –6.2 SD and borderline macrocephaly at +2.48 SD.

Tan et al. [2017] have demonstrated that individuals heterozygous for *BMP2* truncating sequence variants or deletions display a consistent distinct phenotype characterized by short stature and skeletal features without neurological deficits. The clinical features of the 3 members from our family correlated with the SSFSC phenotype associated with *BMP2*. While many of the craniofacial, skeletal, and dental features of the 8 individuals with *BMP2* variants in the Tan et al. [2017] cohort were seen in our affected family members, the proband displayed an extended phenotype for *BMP2* genotype (Table 1).

The possibility of a contiguous gene deletion syndrome was unlikely to explain the phenotype of the proband given the missense variation in the *BMP2* gene. Expanding the phenotype of *BMP2*-related SSFSC syndrome or an additional monogenic disorder remained as possible explanations. To evaluate this, a reanalysis of the whole-exome raw data was done, and a heterozygous 13-bp de novo exonic frameshift deletion in the penultimate exon 14 of the *DVL1* gene was identified.

The era of NGS for monogenic disorders has brought about the concept of "blended phenotypes" that result from the interaction of 2 or more monogenic diseases in an individual [Posey et al., 2017]. This modifies the clinical phenotypes that traditionally were reported as extending phenotypes in established monogenic disorders. The youngest child in this family had significant facial dysmorphism that could not be attributed to the familial *BMP2* gene variant initially identified by exome sequenc-

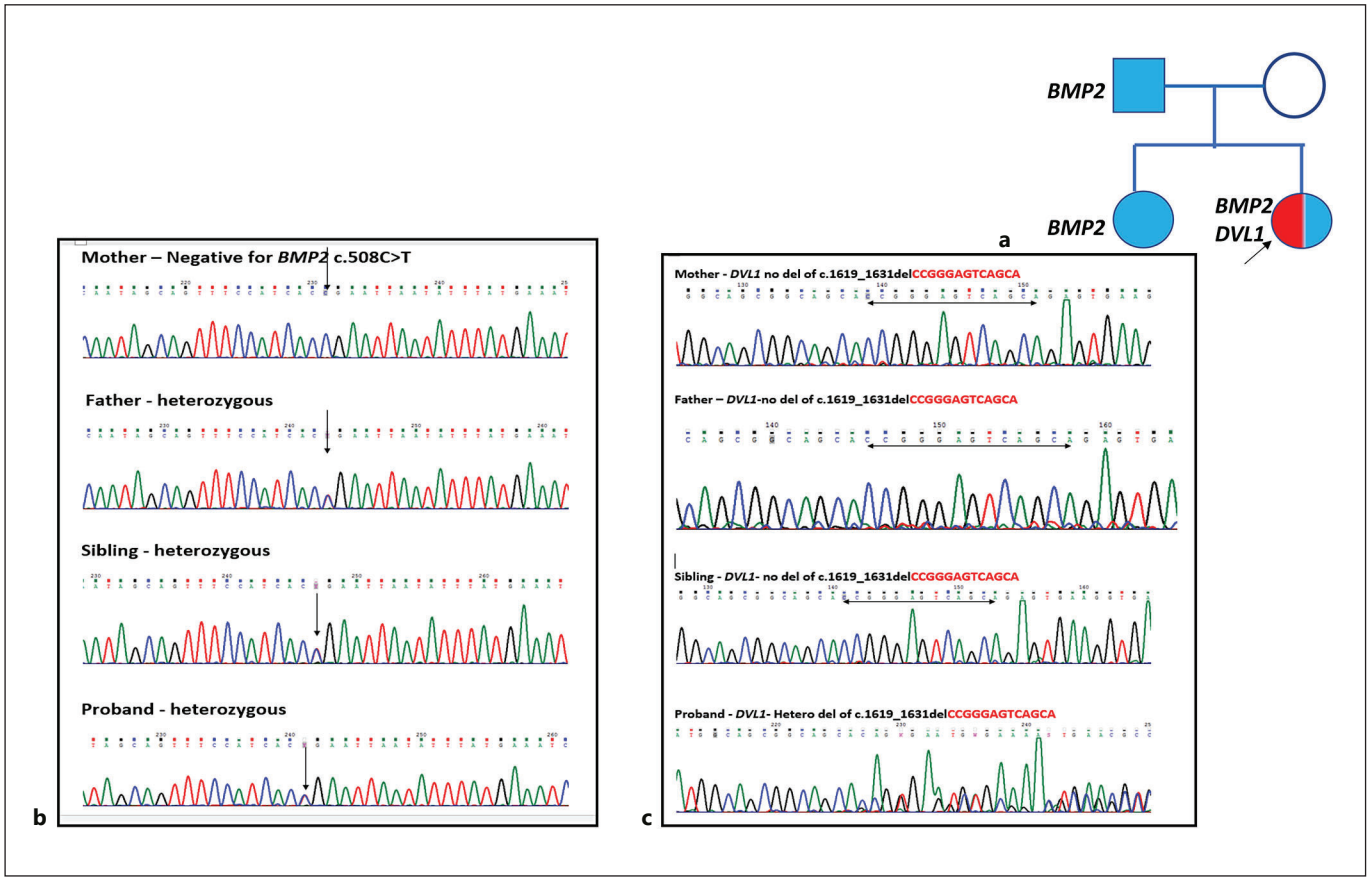

Fig. 3. a Pedigree of the family with the segregated variants. **b** Chromatograph of exon 3 of the *BMP2* gene showing a heterozygous c.508C>T mutation in the proband, sibling and father. **c** Chromatograph of exon 14 of the *DVL1* gene showing a heterozygous 13-bp frameshift deletion in the proband, but not in the sibling and the parents.

ing. We feel that overlapping pathways of *BMP2* and *DVL1* could be responsible for the blended phenotype as observed in the proband.

DVL1 encodes a segment polarity protein disheveled homolog DVL-1, which is an essential downstream mediator of the Wnt signaling pathway implicated in ADRS. Two types of Wnt signaling pathways have been characterized: the β-catenin-dependent canonical pathway and the β-catenin-independent non-canonical pathway; *DVL1* mediates both signals [White et al., 2018]. So far, all variants in *DVL1* associated with ADRS are identified in the penultimate or the last exon resulting in a –1 frameshift mutation which forms stable mRNA that escapes nonsense-mediated decay [Bunn et al., 2015; White et al., 2015, 2016, 2018]. The resultant mutant protein has a novel C terminal domain with a premature termination codon in exon 15. Bunn et al. [2015] presented data to suggest that the mechanism of these alleles is indeed a gain of function that is contingent on the coexpression of both wild-type and mutant alleles.

Bone morphogenetic proteins (BMPs) are multifunctional growth factors which belong to the transforming growth factor β (TGF-β) superfamily and exert critical actions during skeletal development [Liu et al., 2006]. Integration of BMP and Wnt signaling pathways occurs at several different molecular steps, including β-catenin and DVL. BMP promotes osteogenesis and may oppose proliferative pathways promoted by Wnt [Lin and Hankenson, 2011]. In their experimental models, Liu et al. [2006] have demonstrated that BMP-2 attenuates Wnt signaling through the formation of specific complexes between Dvl-1 and Smad1 that restricts β-catenin activation (canonical pathway) [Liu et al., 2006]. Hence, it can be pro-

Table 1. Clinical characteristics of affected individuals with *BMP2* variants and deletions

Characteristics	Tan et al., 2017[a], *n*/*N* (%)	Present study		
		proband	sibling	father
Height ≤2.0 SD	8/11 (72.7)	+ (–6.22 SD)	+ (–2.2 SD)	+ (–2.77 SD)
Broad forehead	8/12 (66.7)	+	–	–
Temporal narrowing	8/10 (80.0)	–	–	–
Synophrys	5/9 (55.6)	–	+	–
Midface hypoplasia	11/12 (91.7)	+	–	+
Low-set posteriorly rotated ears	7/12 (58.3)	+	+	+
Short nose	12/12 (100)	+	+	–
Anteverted nares	12/12 (100)	+	+	–
Thin philtrum	12/12 (100)	+	+	+
Palatal anomaly	11/11 (100)	–	–	–
Dental crowding	6/10 (60.0)	+	+	+
Anterior open bite	5/10 (50.0)	+	–	–
11 pairs of ribs	6/9 (66.7)	ND	ND	ND
Phalangeal abnormalities (mainly clinodactyly)	10/10 (100)	Hypoplasia to aplasia of multiple phalanges	Hypoplasia of distal phalanges of all toes	Aplasia of bilateral middle phalanges of 3rd–5th toes
Sandal gap	8/11 (72.7)	+	+	+
Normal intellect	11/11 (100)	+	+	+
Normal bone biochemistry	8/8 (100)	ND	ND	ND

ND, not done. [a] Only those features were compared which were present in 50% or more individuals in the Tan et al., 2017 cohort.

posed that the *BMP2* and *DVL1* variants had a synergistic effect in modifying the bone biology of the proband via the common β-catenin pathway. The sum of the disorders of the 2 pathways resulted in a more pronounced and atypical manifestation of ADRS in the proband in terms of significant short stature and extensive digital anomalies.

Indel mutation detection can be challenging in high-throughput sequencing data because of various errors associated with indel variant calling. To reduce the burden of false positives and false negatives, picking up an appropriate variant caller for indel detection becomes a necessity. This has been previously reported by White et al. [2015] and reiterates the need for reanalysis of the raw data at the *DVL1* locus in patients with a strong suspicion of ADRS and an unyielding WES result [White et al., 2015]. As frameshift indels in the penultimate and last exons of *DVL1* are the predominant mutations [Bunn et al., 2015; White et al., 2015, 2016], the variant caller has to be scripted for picking up these indels in the NGS data. This would go a long way in simplifying the diagnostic algorithm for such cases and facilitate cost-cutting by avoiding expensive Sanger sequencing as the first step in this algorithm.

Conclusion

This case highlights the importance of deep phenotyping in the era of high-throughput sequencing. It also elucidates the potential of advanced molecular technologies to identify and define blended phenotypes in patients with overlapping clinical features.

Acknowledgment

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Statement of Ethics

This study has been approved by the Institutional Ethics Committee. Informed consent was obtained from the parents for publication of this case report and accompanying images as well as molecular analysis.

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

R. Mishra designed the study and prepared the initial draft of the manuscript. V. Jain did the clinical evaluation of the case. D. Gupta performed Sanger sequencing of the identified variants. R. Saxena supervised Sanger sequencing and the analysis of the identified variants. S. Kulshreshtha did NGS reanalysis and V.L. Ramprasad supervised the initial WES of the family. I.C. Verma was in charge of overall patient management, preparation, and finalization of the manuscript providing important intellectual inputs. R. Dua Puri made the diagnosis, supervised patient work-up and management, revised and finalized the manuscript.

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