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CASE REPORT

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Expanding *OBSL1* Mutation Phenotype: Disproportionate Short Stature, Barrel Chest, Thoracic Kyphoscoliosis, Hypogonadism, and Hypospadias

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We present a Pakistani kinship afflicted with a syndrome with features including short stature, reduced sitting height, orofacial symptoms including prominent forehead and thick eyebrows, short and broad thorax, and variable features such as long philtrum, short broad neck, barrel chest, thoracic kyphoscoliosis, hypogonadism, and hypospadias. Phenotypic variation even within different sibships was considerable. The unique combination of the phenotypic characteristics prompted us to determine the shared homozygosity regions in patient genomes and the pathogenic variants by next generation technologies like single nucleotide polymorphism (SNP) genotyping and whole exome sequencing (WES). Through these analyses, we detected homozygous *OBSL1* c.848delG (p.Gly283AlafsTer54) as the causal variant. Biallelic variants in *OBSL1* are known to cause Three M Syndrome 2 (3M2), a rare disorder of growth retardation with characteristic facial dysmorphism and musculoskeletal abnormalities. Affected members of the family do not have the 3M2 hallmark features of dolichocephaly, hypoplastic midface, anteverted nares, low nasal bridge, pectus excavatum, sacral hyperlordosis, spina bifida occulta, anterior wedging of thoracic vertebrae, prominent heels, and prominent talus. Moreover, they have some variable features not typical for the syndrome such as round face, disproportionate short stature, barrel chest, thoracic kyphoscoliosis, hypogonadism, and hypospadias. Our study facilitated genetic diagnosis in the family, expanded the clinical phenotype for 3M2, and unraveled the considerable clinical variation within the same kinship. We conclude that unbiased molecular analyses such as WES should be more integrated into healthcare, particularly in populations with high parental consanguinity, given the potential of such analyses to facilitate diagnosis.

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Abbreviations: 3M2S, Three M syndrome 2; ACMG, American College of Medical Genetics; ID, intellectual disability; MIM, Mendelian inheritance in Man; NGS, next generation sequencing; SNP, single nucleotide polymorphism; WES, whole exome sequencing.

Keywords: dwarfism, facial dysmorphism, musculoskeletal abnormalities, kyphoscoliosis, consanguinity, Pakistani family

Author Contributions: SMalik and AT conceived and designed the study; RMKS performed field work, family recruitment and data collection; MK and AT analyzed the data; SMumtaz, SMalik, and AT interpreted the data and secured funding for this study; All authors read and agreed on the last version of manuscript. [1]These authors contributed equally.

INTRODUCTION

Rare disease refers to diseases with low incidence, <1 in 2,000 by European definition. All rare diseases together affect 5-8% of the individuals in the populations investigated; it is estimated that over 300 million individuals worldwide are affected with a rare disease [1]. Moreover, rare diseases are responsible for up to approximately 35% of mortality cases within the first year of life [2]. Yet, the scientific knowledge on many of these diseases remains limited, and a better understanding of rare diseases is crucial for improved diagnosis and treatment options. Next-generation sequencing (NGS) technologies have provided a comprehensive and robust platform to extract high quality genetic data from kinships and are very promising in elucidating the molecular genetic bases of rare diseases [3].

Three M Syndrome (3MS) is an extremely rare disorder with autosomal recessive inheritance [4]. Miller et al. (1975) described two siblings born to first-cousin parents who had low birth weight, short stature, narrow facies, grooved lower anterior thorax, and clinodactyly, and the disorder was named 3MS after the shared initial of the describing researchers: Miller, McKusick, and Malvaux [5]. However, later it was discovered that the first case of the disorder might have been reported in 1972 by Fuhrmann et al. (1972) [6]. Approximately 200 cases have been reported so far, but the exact prevalence and incidence remain unknown (Orphanet, 2023). Biallelic variants in *CUL7*, *OBSL1*, and *CCDC8* are reported to be causal for 3MS (3M1, 3M2, and 3M3, respectively), *CUL7* variants being the most common cause and account for approximately 78% of all cases [7]. The proteins coded by these three genes form a large complex, known as 3M complex, which has been demonstrated to have a role in key cellular processes such as microtubule and genome integrity, and it has been proposed that disturbance of these key processes due to aberrant 3M complex proteins underlies the pathology in 3MS [8]. Obscurin-like protein 1 (OBSL1) was demonstrated to functionally regulate CUL7, along with CCDC8, and to play a role in the localization of CUL7 in neurons.

OBSL1 is a 1896-amino acid protein which functions as a cytoskeletal adaptor. The encoding gene, *OBSL1,* has 22 exons, and many variants in this gene have been reported to be responsible for 3M2 [9-17]. *OBSL1*-related 3MS was first reported in 2009 (MIM-612921), in 18 cases from 10 families [11]. To date, in total at least 22 different homozygous *OBSL1* variants have been reported in 56 cases in 46 families (Appendix A: Supplementary Table 1): 14 homozygous truncating variants leading to the truncation of the protein in 35 families [7,9-17], four homozygous splicing variants in five families [9,10], three missense variants in five families [10,11,15], and one nonframeshift duplication of one amino acid in one family [7]. In addition, 10 other truncating variants were reported in compound heterozygosity in nine families where the other allele was the common c.1273dupA variant [7,11,16]. 3M2 is characterized by severe prenatal and postnatal growth retardation, characteristic orofacial dysmorphisms, and mild to severe skeletal abnormalities. Intrauterine growth retardation and delayed bone maturation lead to considerably reduced birth length and weight. Post-natal growth retardation is drastic; in adults height is typically -4 to -6 SD [7]. Short stature is always proportionate, where the lengths of the limbs are proportional to the body [13]. However, head circumference is usually normal, giving an impression of relative macrocephaly. Distinctive facial features include triangular face, frontal bossing, midface hypoplasia, long philtrum, prominent ears, anteverted nares, fleshy nasal tip, high-arched palate, full fleshy lips, and delayed eruption and malocclusion of teeth [10]. Some cases also present skeletal findings including delayed bone age, long and slender tubular bones, and tall vertebral bodies where the anterior-posterior and transverse diameters are shortened [7,18]. Additional variable musculoskeletal findings reported include short neck, square shoulders, short thorax, thin ribs, lumbar hyperlordosis, winged scapulae, small narrow pelvis, joint hypermobility, pes planus, and prominent heels [7]. Intelligence is reported to be normal in all cases except for one case with mild intellectual disability (ID) [4].

Affected members of Pakistani kinship we present have short stature but the facial dysmorphism is different from features observed in 3M2. Patients also have additional features not reported for 3M2. In different sibships of the kinship there was intra-familial clinical variation with regard to the syndromic features besides the extent of short stature. As the disease did not seem similar to any known syndrome, we launched disease gene search by employing SNP-based genetic mapping and whole exome sequencing (WES) and detected a homozygous truncating variant in *OBSL1* in affected members of the family.

MATERIALS AND METHODS

Participants

This family originates from Southern Punjab, Pakistan. In the four-generation pedigree with several consanguineous loops, a total of 12 participants including six affected individuals were physically examined with the help of local physicians (Figure 1A). Photographs of four affected individuals (312, 401, 406, and 409), anthropometric measurements of six (303, 312, 401, 406, 407, and 409), and complete body roentgenograms of

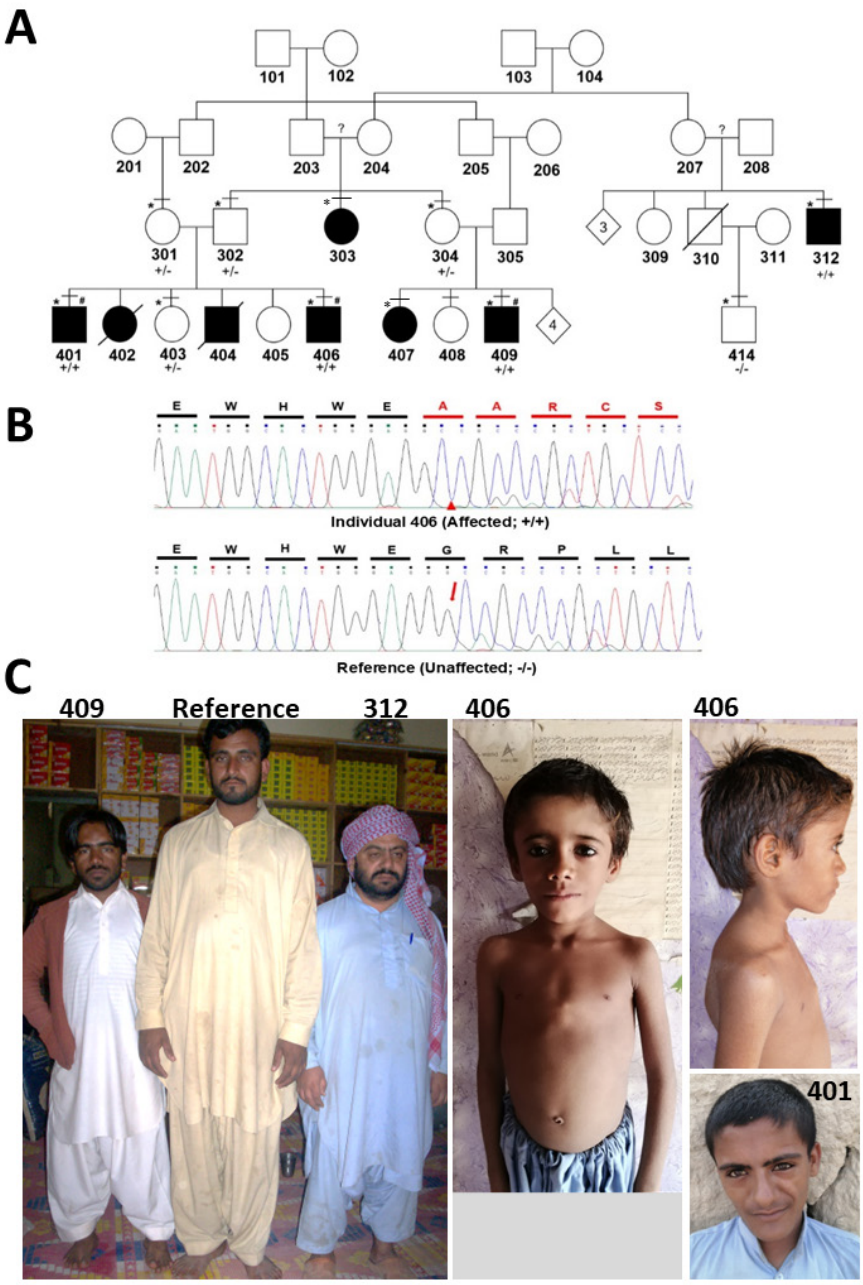

Figure 1. **Affected individuals**. **A. Pedigree of the kinship.** *, DNA available for genetic studies; horizontal bar above symbol, physical examination was performed; #, exome data available; ?, distant consanguinity is known but not shown; +, mutant allele; -, wild-type allele. **B. Electropherograms for OBSL1 c.848delG frameshift variant**. The site of the deleted nucleotide is marked with a triangle and the reference nucleotide is shown with an arrow. +, mutant allele; -, wild-type allele. **C. Pictures of affected individuals 312, 401, 406 and 409**. Features common to 406 and 401 are facial dysmorphism, prominent ears, proportionate short stature, short and broad thorax and protruding sternum. Features common to 409 and 312 are round face, prominent forehead, thick eyebrows, short neck, short and broad thorax, barrel chest, disproportionate short stature, and limbs of normal lengths for age. Reference is unaffected 414 with height 175 centimeters.

two (406 and 409) were obtained. All materials and data were collected according to the declaration of Helsinki II. The study protocol was approved by Institutional Review Board of Quaid-i-Azam University (DAS-1071) and the Istanbul Technical University Human Research Ethical Review Board (MBG.22/2014).

Genetic Analysis

Inheritance pattern for the disease in the kinship was assumed to be autosomal recessive, given that parents of all affected individuals were unaffected and consanguineous. For the genetic studies, DNA was extracted from blood samples of 11 participants from two generations of the kinship (Figure 1A).

Genotype data for >710,000 SNP markers for pooled DNA samples of three affected individuals (312, 401, and 407) from different branches were generated using Illumina Human OmniExpress-24 BeadChip. To identify homozygous regions as candidate disease loci, homozygosity mapping was performed with HomozygosityMapper to detect regions of homozygosity shared by the affected individuals only. Regions with homozygosity scores higher than 80% of the maximum and >1 Mb were selected. SNP genotype data were aligned in Microsoft Excel for manual inspection of the regions to investigate shared homozygosity. Homozygosity was ascertained and delineated via exome sequence data in the final single shared region. The region was scrutinized for candidate genes through GeneDistiller (as described in [19,20]).

Exome sequencing was performed for three affected individuals from two different sibships (401, 406, and 409) using the Agilent SureSelect Target Enrichment System and the Illumina HiSeq2000 platform. Briefly, it involved genomic DNA extraction, DNA fragmentation, adopter ligation, adopter-tagged DNA library preparation, pre-capture DNA library amplification, capturing the DNA library, captured library indexing, and target-enrich library amplicons (Agilent). The UCSC Genome Bioinformatics site was retrieved for downloading of human reference genome sequence (assembly GRCh37/hg19). BWA (Burrows-Wheeler Aligner) program was used to align the pair-end reads to the reference genome and the final alignment was generated in SAM (Sequence Alignment/Map) format. Quality control (QC) on raw data was through FASTX-Toolkit. The detailed bioinformatics pipeline followed to analyze the generated data is described elsewhere [19,20]. In the single candidate region, rare (frequency <0.01 in all populations in gnomAD, ExAC and 1000 Genomes) and novel variants that are present in all three exomes, possibly homozygous (alternate depths >0.6) and possibly altering protein sequence (nonsynonymous, truncating, deletion/duplication, and splicing) were considered. Variants found to be not rare (>0.01) in public databases (1000 Genome and gnomAD that has at least 10,000 Pakistani exomes) and our in-lab exome files were excluded. Sanger sequencing was carried out to validate the single candidate variant and to investigate its segregation in the family. *In silico* tools MutationTaster2 and CADD were used to predict the pathogenicity of the variant, as those tools can be applied to frameshift variants.

RESULTS

Clinical Findings

Unaffected participants examined did not exhibit any symptoms of 3M2. All affected individuals had prenatal or postnatal low weight, short stature (<1 percentile), reduced sitting height (<1 percentile), prominent forehead, thick eyebrows, and short and broad thorax (Tables 1, 2, 3; Figure 1C). Radiographic evaluation of affected cousins 406 and 409 revealed slender long bones, hypoplastic pectoral girdle, flared metaphyses, terminal phalangeal tufts in fingers, tall and columnar lumbar vertebrae, small pelvis, flared iliac wings with small obturator foramina, patellar dislocation, narrowly aligned tibiae and fibulae, and hypoplasia of mid and terminal phalanges of toes (Table 2; Figure 2). Variable findings in affected members of the family and a comparison of radiologic findings in 406 and 409 justify grouping into two:

Proportionate short stature with characteristic facial features: Siblings 401 (16 years old) and 406 (8 years old) constitute this group. Parents declared that short stature is congenital and siblings had swollen abdomens in childhood. We assessed the short stature as proportionate type and the head size normal for age and sex. Characteristic orofacial features included triangular face, frontal bossing, fleshy tip of nose, long philtrum, full lips, prominent and pointed chin, and prominent ears (Table 1; Figure 1C). The musculoskeletal features included protruding sternum, joint hypermobility, clinodactyly of fifth fingers, and pes planus. Additional features were growth retardation, hypogonadism and hypospadias, and one of them (401) additionally had low IQ (Appendix A: Supplementary Material).

Radiographic evaluation of 406 revealed hypoplastic pectoral girdle, flared metaphyses and terminal phalangeal tufts in upper limbs, patellar dislocation, narrowly aligned tibiae-fibulae, and hypoplasia of mid and terminal phalanges of toes in lower limbs (Figure 2). Features not in common with affected cousin 409 of 406 are dysplastic elbow joints, shortened ulnae, clinodactyly of 5th horizontal and thin ribs, hip dislocation, short and deformed femoral necks, and delayed bone age (Table 2; Figure 2).

Disproportionate dwarfism without facial dysmorphism: The four relatives in this group, namely, siblings 407 (26 years old) and 409 (22 years old), and father's

Table 1. Clinical Features of Affected Individuals

Variables	Patient						Feature in OMIM
	303	**312**	**401**	**406**	**407**	**409**	
Age (years), sex	60, F	42, M	16, M	8, M	26, F	22, M	-
IQ	Normal	Normal	Low	Normal	Normal	Normal	Normal
Growth retardation	–	–	+	+	–	–	+
Short stature	+	+	+	+	+	+	+
Low prenatal or postnatal weight	+	+	+	+	+	+	+
Facial features							
Dolichocephaly	–	–	–	–	–	–	+
Spina bifida occulta	–	–	–	–	–	–	+
Triangular and conical face	–	–	+	+	–	–	+
Round face	+	+	–	–	+	+	–
Frontal bossing	–	–	+	+	–	–	+
Prominent forehead	+	+	+	+	+	+	+
Midface hypoplasia	–	–	–	–	–	–	+
Thick eyebrows	+	+	+	+	+	+	–
Fleshy nasal tip and bulbous nose	–	–	+	+	–	–	+
Long philtrum	+	–	+	+	+	+	+
Full lips	–	–	+	+	–	–	+
Delayed eruption of teeth, malocclusion	–	–	–	–	–	–	+
Enamel hypocalcification	–	–	–	–	–	–	+
Pointed, prominent chin	–	–	+	+	–	–	+
Prominent ear	+	–	+	+	+	+	+
Musculoskeletal							
Short broad neck	+	+	–	–	+	+	+
Short broad thorax	+	+	+	+	+	+	+
Barrel shaped chest	+	+	–	–	+	+	+
Pectus excavatum	–	–	–	–	–	–	+
Deformed and protruding sternum	–	–	+	+	–	–	+
Thoracic kyphoscoliosis	+	+	–	–	+	+	+
Joint hypermobility	–	–	+	+	–	–	+

Clinodactyly of fifth fingers	–	–	+	+	–	+	–	+	–	–
Pes planus	–	–	+	+	–	+	–	+	–	–
Prominent heels	–	–	–	–	–	–	–	+	–	–
Other										
Swollen abdomen in childhood	?	–	+	+	?	+	–	–	–	–
Genitourinary anomalies: Hypogonadism and hypospadias	NA	–	+	+	NA	+	–	–	–	–

+, feature present; –, feature absent; NA, not applicable; ?, unknown

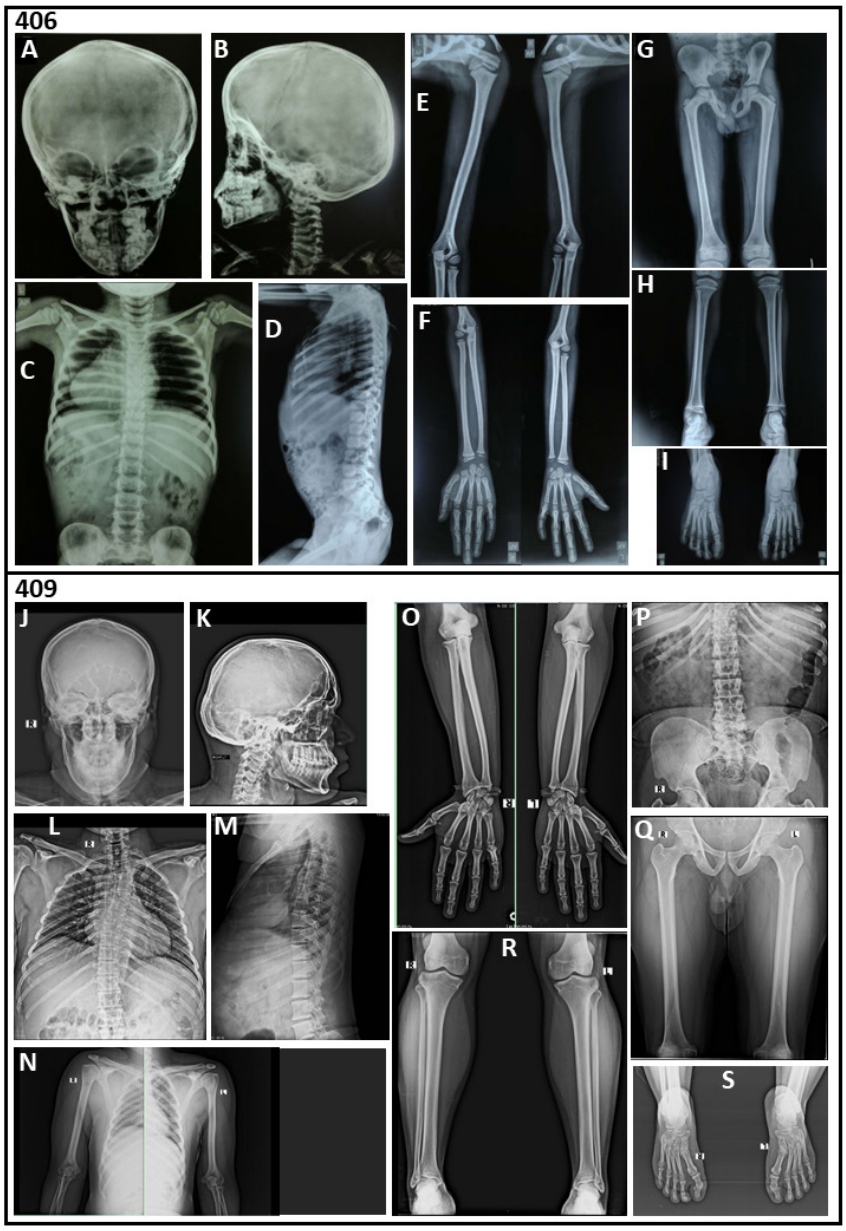

Figure 2. **X-rays of affected individual 406**. **A-B**. Frontal bossing and prominent forehead; **C-D**. Short and broad thorax, slender, thin and horizontal ribs, tall and columnar vertebral bodies in lumbar region; **E**. Slender long bones with slightly delayed age, hypoplastic pectoral girdle, dysplastic elbow joint; **F**. Shortened ulna, immature and cone-shaped epiphyses; fused carpals, terminal phalangeal tufts in fingers, clinodactyly of fifth fingers; **G**. Small pelvis and dislocated hips, flared iliac wings, small obturator foramina, deformed and short femoral necks, patellar dislocation; **H**. Narrowly aligned tibiae and fibulae; **I.** Immature and cone-shaped epiphyses, terminal phalangeal tufts in toes. **X-rays of affected individual 409**. **J-K**. Frontal bossing; **L-M-N**. Thoracic kyphoscoliosis, tall and columnar lumbar vertebrae, irregular upper and lower endplates, hypoplastic pectoral girdle, short humarii; **O**. Slender long bones, flared metaphyses, fusion of carpals, deformed middle phalanges of index fingers, deformed proximal phalange of 5th finger in right hand, terminal phalangeal tufts in fingers; **P-Q**. Small pelvis, flared iliac wings, small obturator foramina, short femora; **R**. Short and narrowly aligned tibiae and fibulae, patellar dislocation; **S**. Terminal phalangeal tufts in toes.

Table 2. Radiographic Findings for Patients 406 and 409

Features	Patient	
	406	**409**
Age (years), sex	8, M	22, M
Long bones are slender	+	+
Hypoplastic pectoral girdle	+	+
Dysplastic elbow joint	+	−
Shortened ulna	+	−
Deformed proximal phalange of 5th finger (right)	−	+
Deformed middle phalange of index fingers	−	+
Flared metaphyses	+	+
Terminal phalangeal tufts in fingers	+	+
Fusion of carpals	+	+
Clinodactyly of 5th fingers	+	−
Short and broad thorax	+	+
Slender, thin and horizontal ribs	+	−
Irregular upper and lower endplates; thoracic kyphoscoliosis	−,−	+,+
Tall and columnar vertebral bodies (lumbar)	+	+
Small pelvis, especially pubis and ischium	+	+
Dislocated hips	+	−
Flared iliac wings, small obturator foramina	+,+	+,+
Femoral necks deformed and short	+	−
Patellar dislocation	+	+
Narrowly aligned tibiae and fibulae	+	+
Mid and terminal phalangeal hypoplasia in toes	+	+
Bone age slightly delayed	+	−

+, feature present; ++, severe manifestation; −, feature absent

Table 3. Anthropometric Measurements of Patients

Features	303	312	401	406	407	409
Age (years), sex	60, F	42, M	16, M	8, M	26, F	22, M
Standing height*	125 (<1)	143 (<1)	117 (<1)	98 (<1)	128 (<1)	145 (<1)
Sitting height#	68 (<1)	76 (<1)	55 (<1)	50 (<1)	65 (<1)	77 (<1)
Head circumference^	52 (>50)	57 (>50)	50 (>30)	52 (>50)	61 (>50)	56 (>50)
Neck circumference	33	42	27	24	37	38
Chest circumference	NA	94	57	51	NA	88
Arm span	125	148	119	110	123	143

Percentiles are given in parentheses. All measurements are in cm; NA, not assessed.
* National Center for Health Statistics. Available at: https://www.cdc.gov/growthcharts/.
# Burton R. The Sitting-Height Index of Build, (Body Mass)/(Sitting Height)^3, as an Improvement on the Body Mass Index for Children, Adolescents and Young Adults. Children (Basel, Switzerland), 2018; 5(2), 30. https://doi.org/10.3390/children5020030
^ Rollins, J. D., Collins, J. S. & Holden, K. R. United States head circumference growth reference charts: birth to 21 years. J. Pediatr. 156, 907−913.e2 (2010).

sister 303 (60 years old) and cousin 312 (42 years old), had disproportionate short stature; limbs were of normal lengths for age. In addition, round face, short neck, barrel chest, and thoracic kyphoscoliosis were apparent (Figure 1C). IQ was normal.

Features not in common with cousin 406 in the radiographic evaluation of individual 409 are deformed middle phalange of index fingers and proximal phalange of right 5th finger, thoracic kyphoscoliosis, and irregular upper and lower endplates (Figure 2; Table 2).

Genetic Findings

After homozygosity mapping, investigation of the SNP data and rare homozygous exome variants in the >1Mb candidate homozygosity regions revealed only one candidate locus, a 1.60-Mb region at 2q35 flanked by rs934026 and rs673951 (nucleotides 218869388 and 220473355) [21]. After the exome filtration strategy was applied, in the region only two rare homozygous variants common to all three exome files remained (Figures 3, 4; Appendix B: Supplemental Table 2): *OBSL1* c.848delG (p.Gly283AlafsTer54; rs773698181; NM_015311.3) is the only variant found in the shared homozygous SNP interval. Segregation of the variant with disease was confirmed with Sanger sequencing (Figure 1B, Appendix A: Supplementary Figure 1). The variant is categorized as pathogenic when evaluated according to American College of Medical Genetics (ACMG) 2015 guidelines [22]. It is submitted to ClinVar with submission ID SUB7319171. The other variant common to all three exomes was *CCDC80* c.G982A (p.V328M; rs200258226) is homozygous in one South Asian at gnomAD and functionally not relevant. Such rare homozygous variants in other regions of the genome were assessed to not contribute to the condition (Appendix B: Supplementary Table 2).

As one of the affected individuals (401) had low IQ which is not a characteristic of 3M2, we launched a search in his exome file for a homozygous candidate variant which could underlie the trait (Appendix A: Supplementary Material). Having not found any good candidate, we hypothesized that low IQ could be a rare, variable finding in 3M2, considering that another case has been reported with mild ID [4].

As affected siblings 407 and 409 and father's sister 303 and cousin 312 had features unusual to 3M2, including disproportionate dwarfism without facial dysmorphism, we also investigated the exome data of individual 409 for a possible causative modifier variant. We found six homozygous candidate variants which passed our candidate variant criteria and were not in exomes files of affected relatives 401 and 406. However, none of the genes of those variants has been associated with a disease with skeletal anomalies or dwarfism phenotypes (Appendix B: Supplementary Table 2).

DISCUSSION

We report a large Pakistani kinship afflicted with a disorder where the affected members shared the features of short stature, short and broad thorax, prominent forehead, and thick eyebrows. We assessed that rare homozygous *OBSL1* frameshift variant c.848delG that we detected underlies the disorder, because the gene is responsible for 3M2, a syndrome with overlapping features with the disorder afflicting the family. In all affected family members, features characteristics of 3M2 include prenatal or postnatal low weight, short stature, reduced sitting height, prominent forehead, and short and broad thorax. All affected individuals but 312 also have long philtrum and prominent ears. Additional clinical features common to individuals 303, 312, 407, and 409 are round face, short and broad neck, barrel chest and thoracic kyphoscoliosis. Additional features siblings 401 and 406 have in common are growth retardation, characteristic orofacial dysmorphisms such as triangular face, frontal bossing, fleshy nasal tip, bulbous nose, full lips, prominent and pointed chin, deformed and protruding sternum, joint hypermobility, clinodactyly of the fifth finger, pes planus, hypogonadism, and hypospadias. Of those features, round face, thick eyebrows, disproportionate short stature, barrel chest, hypogonadism, and hypospadias are not reported for 3M2. There is considerable deviation from 3M2 considering that in the kinship some typical characteristics of the syndrome were not observed: dolichocephaly, midface hypoplasia, delayed eruption of teeth, malocclusion and enamel hypocalcification, anteverted nares, low nasal bridge, pectus excavatum, sacral hyperlordosis, spina bifida occulta, anterior wedging of thoracic vertebrae, prominent heels, or prominent talus. Due to the unusual combination of variable features as well as the inter-familial variability in this kinship, clinicians had been unable to reach a conclusive clinical diagnosis. The SNP-based homozygosity mapping elucidated the candidate locus at chromosome 2q35, and in subsequent WES of three affected individuals we detected the causative *OBSL1* variant and facilitated the genetic diagnosis of 3M2.

Most of the computational prediction algorithms provide pathogenicity scores for only single nucleotide variations and not insertions or deletions. We applied the two algorithms that do provide prediction scores for deletions to the frameshift variant we detected, *OBSL1* c.848delG (p.Gly283AlafsTer54). MutationTaster2 predicts the variant as disease-causing. CADD score for the variant is 33, which is much above the recommended threshold (>20) and means that the variant is in the top 0.05% of the deleterious variants classified by CADD. This variant

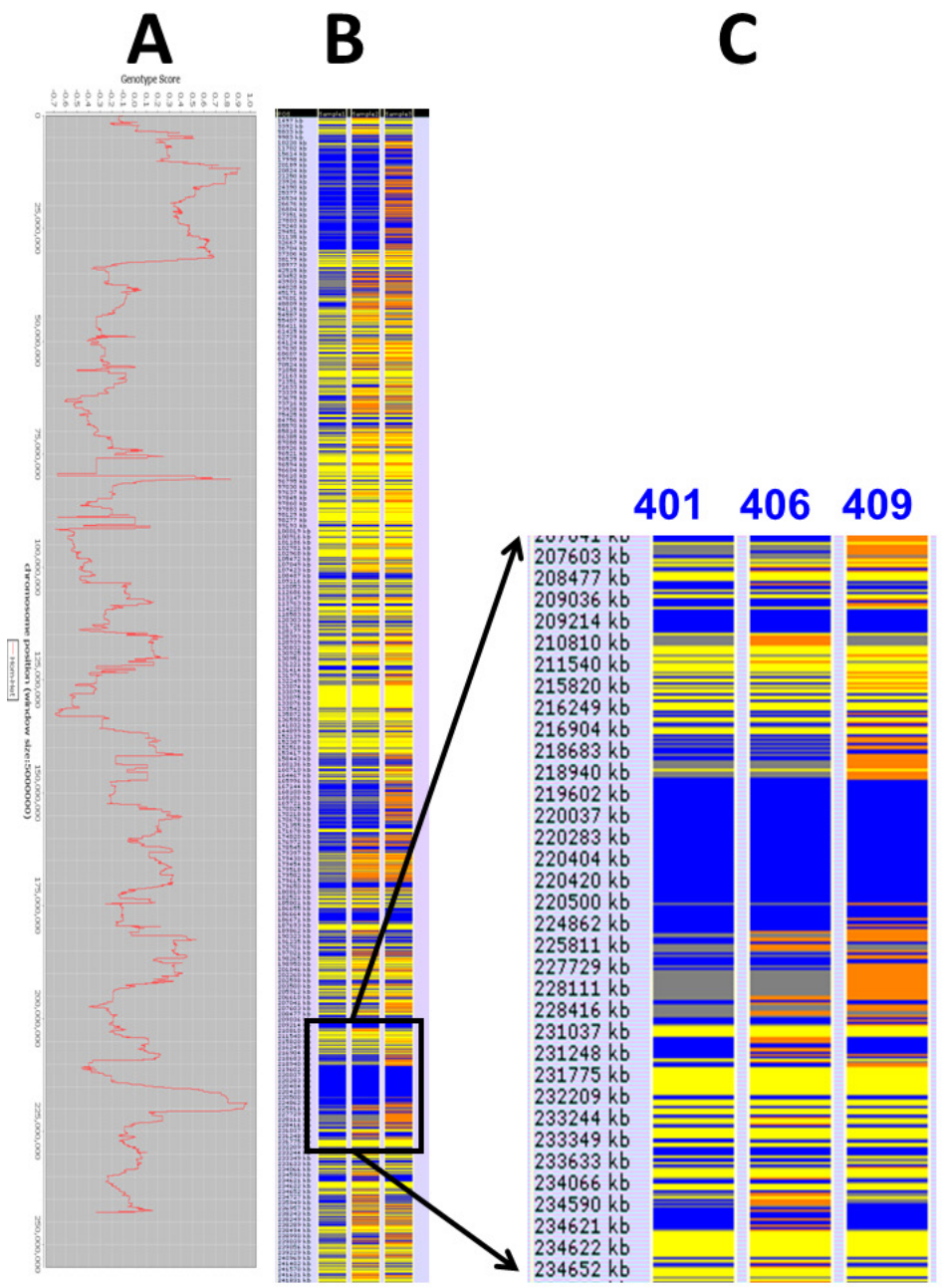

Figure 3. **Homozygosity at chromosome 2 around the interval harboring *OBSL1* drawn with HomSi** [21]. **A**. Line chart depicting genotype score of three affected individuals along the chromosome axis. **B-C**. Homozygosity map with exome data of three patients; shared homozygous region is framed with a black rectangle; Blue, homozygous interval; Orange or yellow, heterozygous.

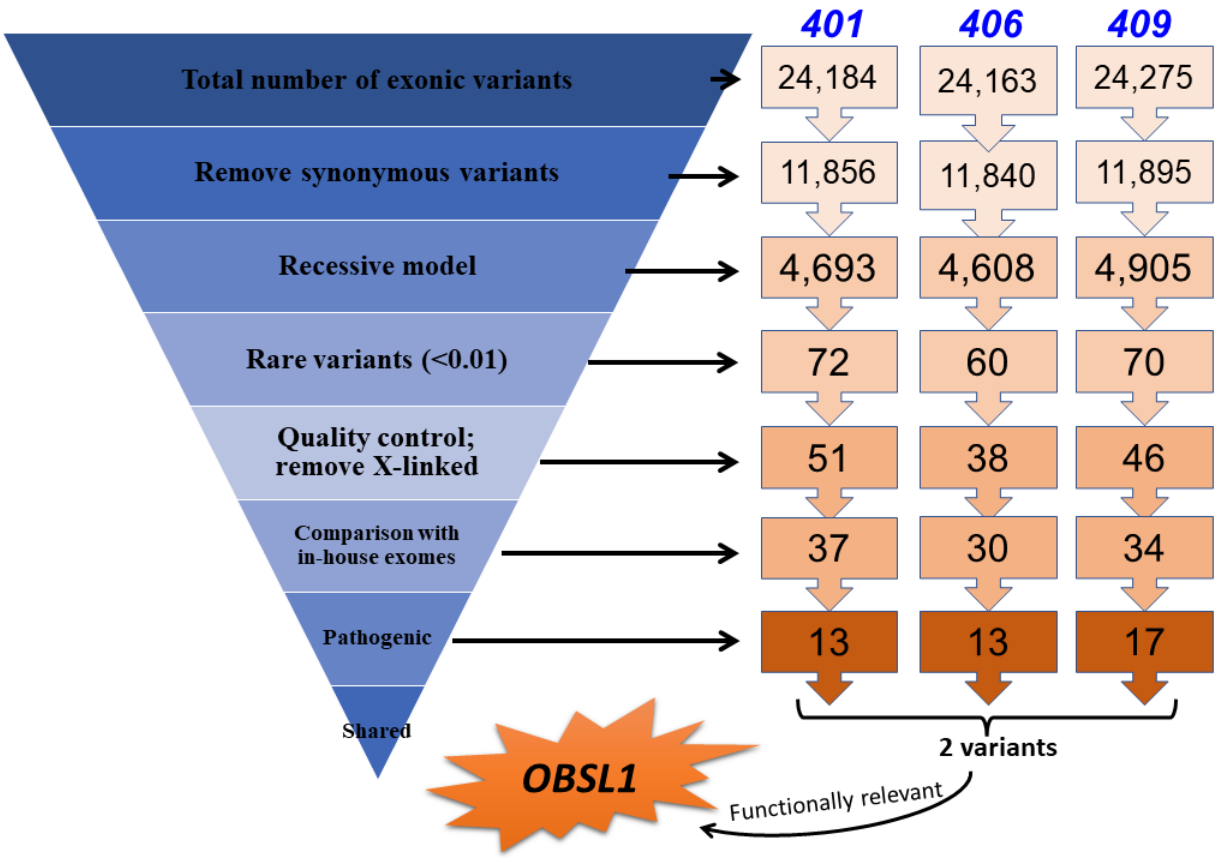

Figure 4. **Exome filtration strategy and the detection of the causal rare variant**.

is extremely rare; it is absent from 1000Genomes and in gnomAD is present in only one South Asian individual, in heterozygous state (1 in 30580 South Asian alleles). There are two homozygous loss of function variants p.Gln1578Ter and p.Glu1651Ter reported for *OBSL1* in gnomAD, both in single individuals only. However, these variants are towards the C terminus of the protein, and thus, if those individuals are not 3MS, then it could be because the variants do not alter much the protein's function whereas the variant we detected results in a severe truncation, deleting 1614 of the 1896 native amino acids and adding 53 non-native residues. Each of the reported 25 truncating variants also delete at least 1083 native residues (Appendix A: Supplementary Table 1). This observation reiterates that the sites of the homozygous damaging variants reported in gnomAD should be taken into consideration in the evaluation of candidate variants.

CONCLUSION

This study adds to the clinical variation of *OBSL1*-related 3MS and demonstrates that unbiased molecular analysis such as SNP-based genotyping and whole exome sequencing can provide a definite diagnosis for individuals with rare conditions or unusual symptoms, particularly in the background of high parental consanguinity as in the Pakistani population [23]. As the cost of exome sequencing is decreasing, the analysis should be integrated more into healthcare services in countries with high rates of parental consanguinity as those technologies can considerably improve prevention, diagnosis, and management options for afflicted families.

Accession Number: *OBSL1* c.848delG (p.Gly283AlafsTer54) is submitted to ClinVar (Accession ID VCV000870250.1).

Data Availability: Data will be made available upon request.

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Conflict of Interest Statement: Authors have no conflict of interest.

Online Data Sources utilized:
Agilent.com: https://www.agilent.com/cs/library/usermanuals/public/G9702-90000.pdf
ClinVar: https://www.ncbi.nlm.nih.gov/clinvar/
GeneCards: https://www.genecards.org/
GeneDistiller: https://www.genedistiller.org/
MutationTaster: https://www.mutationtaster.org/
Online Mendelian Inheritance in Man (OMIM): https://www.omim.org/
Orphanet: https://www.orpha.net/consor/cgi-bin/Disease.php?lng=EN
Phenolyzer: https://phenolyzer.wglab.org/cgi-bin/loh.cgi
Polyphen2: http://genetics.bwh.harvard.edu/pph2/
REVEL: https://sites.google.com/site/revelgenomics/
SIFT: https://sift.bii.a-star.edu.sg/
UniProt: https://www.uniprot.org/

REFERENCES

1. Nguengang Wakap S, Lambert DM, Olry A, Rodwell C, Gueydan C, Lanneau V, et al. Estimating cumulative point prevalence of rare diseases: analysis of the Orphanet database. Eur J Hum Genet. 2020 Feb;28(2):165–73.
2. Stevenson DA, Carey JC. Contribution of malformations and genetic disorders to mortality in a children's hospital. Am J Med Genet A. 2004 May;126A(4):393–7.
3. Gulati A, Somlo S. Whole exome sequencing: a state-of-the-art approach for defining (and exploring!) genetic landscapes in pediatric nephrology. Pediatr Nephrol. 2018 May;33(5):745–61.
4. HabibUllah H, Al-Baradie R, Bashir S; HabibUllah H. Al-Baradie R, Bashir S. 3-M Syndrome: A Local Case Report. Am J Case Rep. 2019;20:36–8.
5. Miller JD, McKusick VA, Malvaux P, Temtamy S, Salinas C. The 3-M syndrome: a heritable low birthweight dwarfism. Birth Defects Orig Artic Ser. 1975;11(5):39–47.
6. Fuhrmann W, Nägele E, Gugler R, Adili E. [Familial dwarfism with disproportionately high vertebral bodies]. Humangenetik. 1972;16(4):271–82.
7. Huber C, Munnich A, Cormier-Daire V. The 3M syndrome. Best Pract Res Clin Endocrinol Metab. 2011 Feb;25(1):143–51.
8. Yan J, Yan F, Li Z, Sinnott B, Cappell KM, Yu Y, et al. The 3M complex maintains microtubule and genome integrity. Mol Cell. 2014 Jun;54(5):791–804.
9. Hanson D, Murray PG, Coulson T, Sud A, Omokanye A, Stratta E, et al. Mutations in CUL7, OBSL1 and CCDC8 in 3-M syndrome lead to disordered growth factor signalling. J Mol Endocrinol. 2012 Oct;49(3):267–75.
10. Simsek-Kiper PO, Taskiran E, Kosukcu C, Arslan UE, Cormier-Daire V, Gonc N, et al. Further expanding the mutational spectrum and investigation of genotype-phenotype correlation in 3M syndrome. Am J Med Genet A. 2019 Jul;179(7):1157–72.
11. Hanson D, Murray PG, Sud A, Temtamy SA, Aglan M, Superti-Furga A, et al. The primordial growth disorder 3-M syndrome connects ubiquitination to the cytoskeletal adaptor OBSL1. Am J Hum Genet. 2009 Jun;84(6):801–6.
12. Demir K, Altıncık A, Böber E. Severe short stature due to 3-M syndrome with a novel OBSL1 gene mutation. J Pediatr Endocrinol Metab. 2013;26(1-2):147–50.
13. Al-Dosari MS, Al-Shammari M, Shaheen R, Faqeih E, Alghofely MA, Boukai A, et al. 3M syndrome: an easily recognizable yet underdiagnosed cause of proportionate short stature. J Pediatr. 2012 Jul;161(1):139–45.e1.
14. Marshall CR, Farrell SA, Cushing D, Paton T, Stockley TL, Stavropoulos DJ, et al. Whole-exome analysis of foetal autopsy tissue reveals a frameshift mutation in OBSL1, consistent with a diagnosis of 3-M Syndrome. BMC Genomics. 2015;16(Suppl 1 Suppl 1):S12.
15. Shapiro L, Chatterjee S, Ramadan DG, Davies KM, Savage MO, Metherell LA, et al. Whole-exome sequencing gives additional benefits compared to candidate gene sequencing in the molecular diagnosis of children with growth hormone or IGF-1 insensitivity. Eur J Endocrinol. 2017 Dec;177(6):485–501.
16. Hu X, Li H, Gui B, Xu Y, Wang J, Li N, et al. Prenatal and early diagnosis of Chinese 3-M syndrome patients with novel pathogenic variants. Clin Chim Acta. 2017 Nov;474:159–64.
17. Keskin M, Muratoğlu Şahin N, Kurnaz E, Bayramoğlu E, Savaş Erdeve Ş, Aycan Z, et al. A Rare Cause of Short Stature: 3M Syndrome in a Patient with Novel Mutation in OBSL1 Gene. J Clin Res Pediatr Endocrinol. 2017 Mar;9(1):91–4.
18. Meazza C, Lausch E, Pagani S, Bozzola E, Calcaterra V, Superti-Furga A, et al. 3-M syndrome associated with growth hormone deficiency: 18 year follow-up of a patient. Ital J Pediatr. 2013 Mar;39(1):21.
19. Koprulu M, Kumare A, Bibi A, Malik S, Tolun A. The first adolescent case of Fraser syndrome 3, with a novel nonsense variant in GRIP1. Am J Med Genet A. 2021 Jun;185(6):1858–63.
20. Shabbir RM, Nalbant G, Ahmad N, Malik S, Tolun A. Homozygous CHST11 mutation in chondrodysplasia, brachydactyly, overriding digits, clino-symphalangism and synpolydactyly. J Med Genet. 2018 Jul;55(7):489–96.
21. Görmez Z, Bakir-Gungor B, Sağıroğlu MS. HomSI: a homozygous stretch identifier from next-generation sequencing data. Bioinformatics. 2014 Feb;30(3):445–7.
22. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al.; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015 May;17(5):405–24.
23. Nawaz A, Zaman M, Malik S. Consanguinity, inbreeding coefficient, fertility and birth-outcome in population of Okara district, Pakistan. Pak J Med Sci. 2021;37(3):770–5.

Appendix A: Supplementary Material

Candidate Gene Search Underlying Low IQ in Affected Individual 401

One of the affected individuals (401) had low IQ which is not a characteristic of 3M2. We launched a search for a candidate variant which can underlie the trait. In patient 401 exome file, we detected two hemizygous variants in *GRIA3* (c.-16C>T and c.1181G>A), a gene associated with Mental Retardation, X-linked 94 (OMIM 300699). However, both variants were present also in the exome file of brother 406 who has normal IQ. The first variant is in 5' UTR and was reported in 37 individuals in hemizygous state in gnomAD and the other in only one hemizygous individual. The latter variant is classified as "benign" or "likely benign" in ClinVar by four independent submissions. Interestingly, neither of the variants was found in South Asian samples in gnomAD, which contains at least 10,000 Pakistani exomes. Because both brothers have the variants, we hypothesized that none of the two variants or the variants together in synergy could underlie the low IQ in brother 401. Therefore, we hypothesized that low IQ could be a rare, variable finding in 3M2, considering that another case has been reported with mild ID (1).

Reference

1. HabibUllah H, Al-Baradie R, Bashir S. 3-M Syndrome: A Local Case Report. Am J Case Rep. 2019;20:36-8.

Supplementary Table 1. Reported 3M2 Syndrome cases and the *OBSL1* variants they carry

Variant	State of the variant	Number of families	Reference
c.848delG (p.Gly283AlafsTer54)	Homozygous	1	This study
c.35dup p.(Cys13Valfs*241) c.1273dup p.(Thr425Asnfs*40)	Compound heterozygous	1	Jacob and Girisha, 2021
c.82G > A (p.Glu28Lys) & c.3337C > T (p.Arg1113Cys)	Compound heterozygous	1	Yang and Liang, 2021
c.457_458delinsT (p.Gly153SerfsTer105)	Homozygous	1	Demir et al., 2013
c.458dupG (p.Leu154fs) & c.1365-1387dup (p.Arg463fs)	Compound heterozygous	1	Yang and Liang, 2021
c.458dupG (p.Leu154ProfsTer100)	Homozygous	1	Hu et al., 2017
c.690insC (p.Glu231ArgfsTer23)	Homozygous	1	Hanson et al.,2009
c.928C>T (p.Gln310Ter)	Homozygous	1	Hanson et al., 2012
c.947_966del20bases (p.Leu316GlnfsTer32)	Homozygous	1	Huber et al., 2011
c.1118G>A (p.W373*), c.458dupG (p.L154Pfs*1002) & c.690dupC (p.E231Rfs*23),	Compound heterozygous	2	Xu and Zhong, 2023
c.1036dupC (p.Leu347ProfsTer8)	Homozygous	1	Huber et al., 2011
c.1036del (p.Leu347CysfsTer56)	Homozygous	2	Al-Dosari et al., 2012
c.1125dupT (p.Glu376Ter)	Homozygous	1	Simsek-Kiper et al., 2019
c.1187G>A (p.Arg396His)	Homozygous	1	Simsek-Kiper et al. 2019
c.1273dupA (p.Thr425AsnfsTer40)	Homozygous	16	4 by Hanson et al.,2009 5 by Huber et al., 2011 1 by Hanson et al., 2012 1 by Keskin et al., 2017 1 by Marshall et al., 2015 1 by Simsek-Kiper et al., 2019 3 Tüysüz et al. 2021

c.1273insA & c.1149C>A (p.Thr425AsnfsTer40) & (p.Cys383Ter)	Compound heterozygous	1	Hanson et al.,2009
c.1273insA & c.836G>A (p.Thr425AsnfsTer40) & (p.Trp279Ter)	Compound heterozygous	1	Hu et al., 2017
c.1273insA & c.1256_1265del (p.Thr425AsnfsTer40) & (p.Arg419ProfsTer10)	Compound heterozygous	1	Hanson et al.,2009
c.1273insA & c.2032C>T (p.Thr425AsnfsTer40) & (p.Gln678Ter)	Compound heterozygous	1	Huber et al., 2011
c.1277_1282+5del TCAAAGGTCAG (splice junction lost)	Homozygous	2	1 Simsek-Kiper et al., 2019 1 Tüysüz et al. 2021
c.1359dupA (p.Glu454ArgfsTer11)	Homozygous	6	1 by Hanson et al.,2009 1 by Huber et al., 2011 4 by Shapiro et al., 2017*
c.1463C>T (p.Thr488Ile)	Homozygous	3	1 by Hanson et al.,2009 2 by Shapiro et al., 2017*
c.1534+5G > C	Homozygous	1	Jacob and Girisha, 2021
c.1534+2T>C (splicing)	Homozygous	1	Hanson et al., 2012
c.1857delG (p.Ala620GlnfsTer53)	Homozygous	1	Huber et al., 2011
c.2034_2035delinsA (p.His679ThrfsTer40)	Homozygous	1	Hanson et al.,2009
c.2086_2088dupGGC (p.Gly696dup)	Homozygous	1	Huber et al., 2011
c.2134+1G>A	Homozygous	1	Guo et al. 2014
c.2135-3_2135-2del & c.3341G>A (p.Trp1114*)	Compound heterozygous	1	Lee et al. 2020
c.2434C>T (p.Arg812Ter)	Homozygous	1	Huber et al., 2011
c.2441_2442delAT (p.His814ArgfsTer16)	Homozygous	1	Huber et al., 2011
c.3670G>A (p.Glu1224Lys)	Homozygous	1	Tüysüz et al. 2021

*Shapiro et al., 2017: Patients have short stature but diseases are not defined as 3MS

References

1. Jacob P, Girisha KM. Three M syndrome 2 in two Indian patients. Am J Med Genet A. 2021 Feb;185(2):614-616.

2. Lee IK, Lim HH, Kim YM. The Effect of Combined Growth Hormone and a Gonadotropin-Releasing Hormone Agonist Therapy on Height in Korean 3-M Syndrome Siblings. Yonsei Med J. 2020 Nov;61(11):981-985.

3. Tüysüz B, Alp Ünkar Z, Turan H, Gezdirici A, Uludağ Alkaya D, Kasap B, Yeşil G, Vural M, Ercan O. Natural history of facial and skeletal features from neonatal period to adulthood in a 3M syndrome cohort with biallelic CUL7 or OBSL1 variants. Eur J Med Genet. 2021 Dec;64(12):104346

4. Xu N, Liu K, Yang Y, Li X, Zhong Y. Chinese patients with 3M syndrome: clinical manifestations and two novel pathogenic variants. Front Genet. 2023:14.

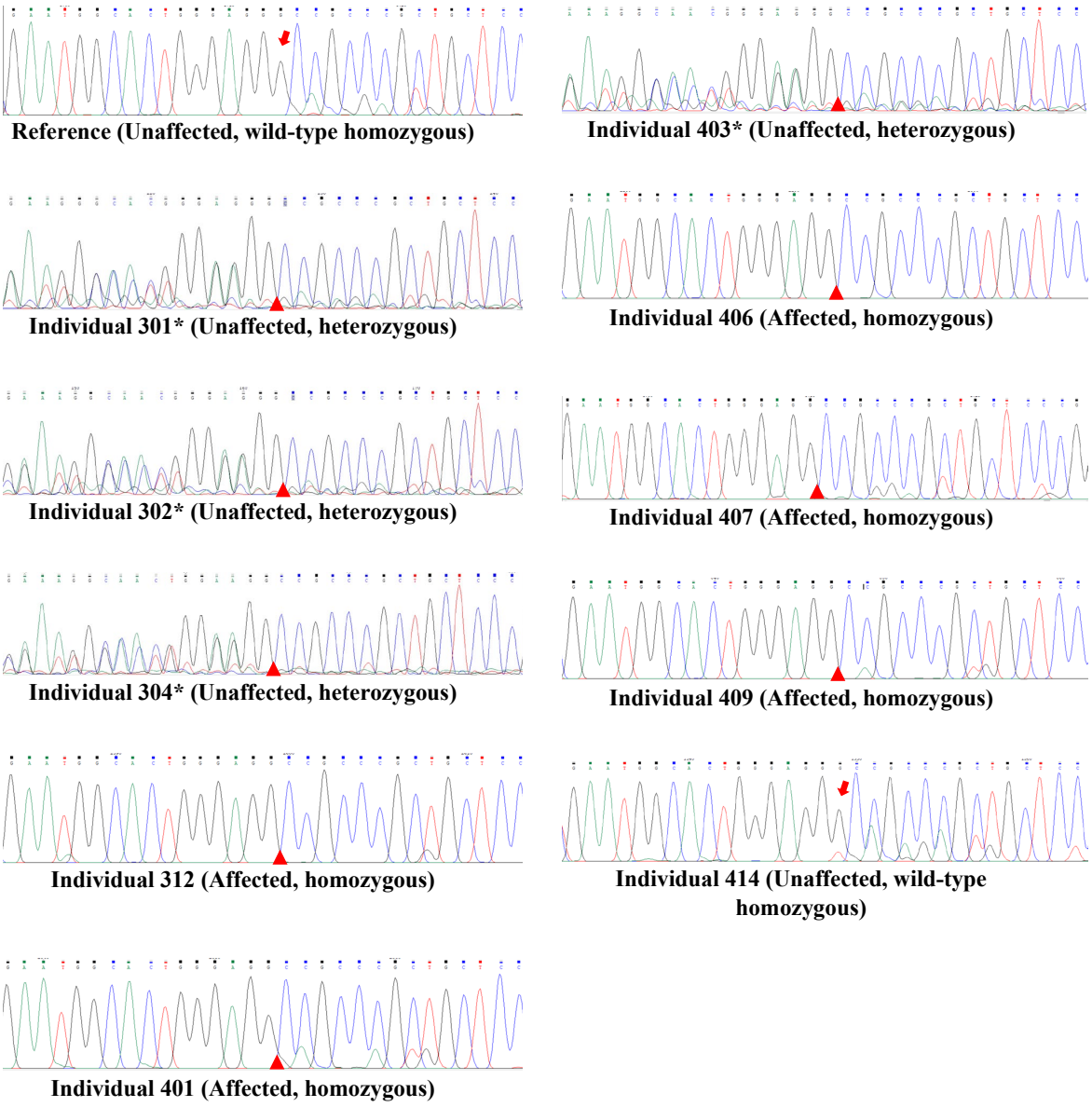
5. Yang LL, Liang SS. Study on pathogenic genes of dwarfism disease by next-generation sequencing. World J Clin Cases. 2021 Mar 6;9(7):1600-1609.

Supplementary Table 2. Rare homozygous variants in each exome file.

Only two variants were shared among the three exomes (highlighted): *OBSL1* (c.848delG) and *CCDC80* (c.G982A). The variant in *OBSL1* falls in the homozygous interval and was functionally relevant, whereas the variant in *CCDC80* is homozygous for one South Asian at gnomAD and functionally not relevant.

Supp. Table 2 in Excel file.

Supplementary Figure 1. Electropherograms showing the segregation of *OBSL1* c.848delG in the kinship. The site of the deleted nucleotide is marked with a triangle and the wild-type nucleotide is shown with an arrow.



*_OBSL1_ gene is encoded on the reverse strand, and Sanger-sequencing was performed using the forward primer. The shift caused by c.848delG in the heterozygotes is apparent, yet in a reverse direction due opposite orientations of the primer and gene sequence.