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# Natural history of facial and skeletal features from neonatal period to adulthood in a 3M syndrome cohort with biallelic *CUL7* or *OBSL1* variants

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ABSTRACT

3M syndrome is characterized by severe pre- and post-natal growth restriction, typical face, slender tubular bones, tall vertebral bodies, prominent heels and normal intelligence. It is caused by biallelic variants of *CUL7*, *OBSL1* and, more rarely, *CCDC8*. The aim of this study is to evaluate facial and skeletal findings in 3M patients from neonatal period to adulthood. A total of 19 patients with a median age of diagnosis of 9.2 months were included in this study and were followed for two to 20 years. *CUL7* and *OBSL1* variants were found in 57.9% and 42.1% of patients, respectively, five of which are novel. Most of patients had triangular face, frontal bossing, short fleshy nose, full fleshy lower lip, transverse groove of rib cage, hyperlordosis and prominent heels. Three new early-diagnostic signs were observed in infants; two were infraorbital swelling of the lower lid and facial infantile hemangioma, both of which became less pronounced with aging. The third was the central tubercle of the upper lip that became more prominent with in time. While slender long bones did not change with aging, the tall vertebral bodies became more prominent radiologically. The mean birth length in patients was −4.3 SDS. Eight patients reached a mean final height of −4.9 SDS. Despite described growth hormone (GH) insensitivity in 3M syndrome, 12 patients either with GH deficiency or with normal GH levels were treated with GH; seven patients responded with an increase in height SDS. This study not only provided early diagnostic signs of the syndrome, but also presented important follow-up findings.

## 1. Introduction

3M syndrome (MIM# 273750,612921,614205) is a rare autosomal recessively inherited dwarfism, characterized by pre- and post-natal growth retardation, distinctive face, prominent heels, and tall vertebral bodies. Biallelic loss of function mutations in three genes *CUL7*, *OBSL1* and less commonly *CCDC8* leads to 3M syndrome. Cullin 7 encoded by *CUL7* is an important structural component of an E3 ligase in the ubiquitin proteasomal degradation pathway targeting p53, cyclin D1, and the IGFI/insulin signaling molecule IRS-1 (Huber et al., 2005, 2009). Obscurin-like 1, encoded by *OBSL1*, is a cytoskeletal adapter protein that connects the inner cytoskeleton of cells to the cell membrane and is required for maintenance of normal CUL7 levels (Hanson et al., 2009; Huber et al., 2011). The function of CCDC8 protein is unknown but it was suggested that it binds to OBSL1 (3) and is required for p53-mediated apoptosis following DNA damage, and localization of CUL7 to the centrosome (Hanson et al., 2012; Yan et al., 2014). 3M syndrome is a ubiquitination disorder and abnormal mRNA splicing is responsible for p53 dysfunction, growth hormone (GH) and/or IGF1 resistance, and cellular IGF2 deficiency (Hanson et al., 2011, 2014); but, the mechanism is not clearly known. Although increased H19 gene expression profiles and decreased IGF2, similar to Silver Russell syndrome (SRS), were shown in 3M Syndrome, hypomethylation of 11p15 could not be detected and it was claimed that both syndromes use common but unidentified epigenetic pathways to regulate IGF2 and H19 expression (Murray et al., 2013).

Characteristic craniofacial features of 3M patients are relative macrocephaly, dolichocephaly, triangular face, frontal bossing, short, fleshy

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**Table 1**
*CUL7/OBSL1* variants identified in 14 families with 3M syndrome.

| Family no | Patient no | Parental consanguinity | Gene Transcript | Biallelic Mutation | Novel/ known | Protein change | Location | Mutation type | ACMG classification | Minor Allele Frequency[a] (ALFA) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | First cousin | *CUL7* | c.2064-1G>A | Novel | – | Intron 8 | Splicing | Pathogenic (PVS1, PM2, PP3) | Variant was not found in ExAC, 1000G, or gnomAD |
| 2 | 2 | Same town | *CUL7* | c.1938dupG | Known | p.Thr647AspfsTer33 | Exon 8 | Frameshift | Likely pathogenic (PVS1, PM2) | Variant was not found in ExAC, 1000G, or gnomAD. |
| 3 | 3 | First cousin | *CUL7* | c.616C>T | Novel | p.Gln206Ter | Exon 3 | Nonsense | Pathogenic (PVS1, PM2, PP3) | Variant was not found in ExAC, 1000G, or gnomAD. |
| 4 | 4 | First cousin once removed | *CUL7* | c.3076delG | Novel | p.Asp1026ThrfsTer115 | Exon 16 | Frameshift | Likely pathogenic (PVS1, PM2) | Variant was not found in ExAC, 1000G, or gnomAD. |
| 5 | 5 | First cousin | *CUL7* | c.2706_2707dupGG | Known | p.Ala903GlyfsTer22 | Exon 13 | Frameshift | Pathogenic (PVS1, PM2, PP3) | Variant was not found in ExAC, 1000G, or gnomAD. |
| 6 | 6 | Same town | *CUL7* | c.3136delC /c.4333C>T (Compound) | Known Known | p. [Leu1046TrpfsTer95]; [Arg1445Ter] | Exon 16 Exon 23 | Frameshift/ Nonsense | Pathogenic (PVS1, PM2, PP3)/ Pathogenic (PVS1, PS3, PM2,PP3) | 0.000/0.000 |
| 7 | 7 | Second cousin | *CUL7* | c.1938dupG | Known | p.Thr647AspfsTer33 | Exon 8 | Frameshift | Likely pathogenic (PVS1, PM2) | Variant was not found in ExAC, 1000G, or gnomAD. |
| 8 | 8–10 | First Cousin | *CUL7* | c.4814delG | Novel | p.Gly1605ValfsTer30 | Exon 26 | Frameshift | Likely pathogenic (PVS1, PM2) | Variant was not found in ExAC, 1000G, or gnomAD. |
| 9 | 11 | First Cousin | *CUL7* | c.1938dupG | Known | p.Thr647AspfsTer33 | Exon 8 | Frameshift | Likely pathogenic (PVS1, PM2) | Variant was not found in ExAC, 1000G, or gnomAD. |
| 10 | 12–14 | First cousin | *OBSL1* | c.1273dupA | Known | p.Thr425AsnfsTer40 | Exon 2 | Frameshift | Pathogenic (PVS1, PP5, PM2, PP3) | AA = 0.000 (as) AA = 0.00025 (european) |
| 11 | 15, 16 | First cousin | *OBSL1* | c.1273dupA | Known | p.Thr425AsnfsTer40 | Exon 2 | Frameshift | Pathogenic (PVS1, PP5, PM2, PP3) | AA = 0.000 AA = 0.00025 (european) |
| 12 | 17 | First cousin | *OBSL1* | c.1273dupA | Known | p.Thr425AsnfsTer40 | Exon 2 | Frameshift | Pathogenic (PVS1, PP5, PM2, PP3) | AA = 0.000 (asian) AA = 0.00025 (european) |
| 13 | 18 | First cousin | *OBSL1* | c.1277_1282+5del TCAAAGGTCAG | Known | – | Exon and intron 2 | Splice loss | Pathogenic (PVS1,PM2, PP3, PP5) | CTG = 0.0000 (eurepan) CTG = 0.00 (as) (Del CTGACCTTTGA) |
| 14 | 19 | No consanguinity | *OBSL1* | c.3670G>A | Novel | p.Glu1224Lys | Exon 11 | Missense | Uncertain significance (PM2, PP3, BP1) | A = 0.0000 (EUR) A = 0.0000 (AS) |

[a] Phan et al.,2020. http://www.ncbi.nlm.nih.gov/snp/docs/gsr/alfa/.

nose, anteverted nares, full fleshy lower lip and pointed chin (Clayton et al., 2012; Holder-Espinasse et al., 2014). Although there are typical facial features of 3M syndrome, some patients are initially diagnosed as SRS, the better known of the two, because of common findings like intrauterine onset growth retardation, triangular face and wide forehead (Akawi et al., 2011; Al-Dosari et al., 2012). However, in patients with 3M syndrome, there is no asymmetry as found in SRS, whereas most patients have pathognomonic skeletal findings such as fleshy protruding heels and tall vertebral bodies. SRS is sporadic; whereas, 3M is inherited autosomal recessively. Al-Dosari et al. (2012) suggested that children thought to have autosomal recessive SRS actually have 3M syndrome and it may be common in highly consanguineous populations for the patients with 3M syndrome are not sufficiently recognized. It is also estimated that the disease may actually be more common than known in China and Japan (Hu et al., 2017; Takizaki et al., 2020). A study published in recent years also suggested that skeletal abnormalities become less prominent at the age of 2 years compared to the neonatal period (Takizaki et al., 2020).

The aim of this study is to evaluate the facial and skeletal findings beginning from the neonatal period up to adulthood in a Turkish 3M cohort, to determine the pivotal findings that will help in the early diagnosis of the disease and to present novel variants in *CUL7* and

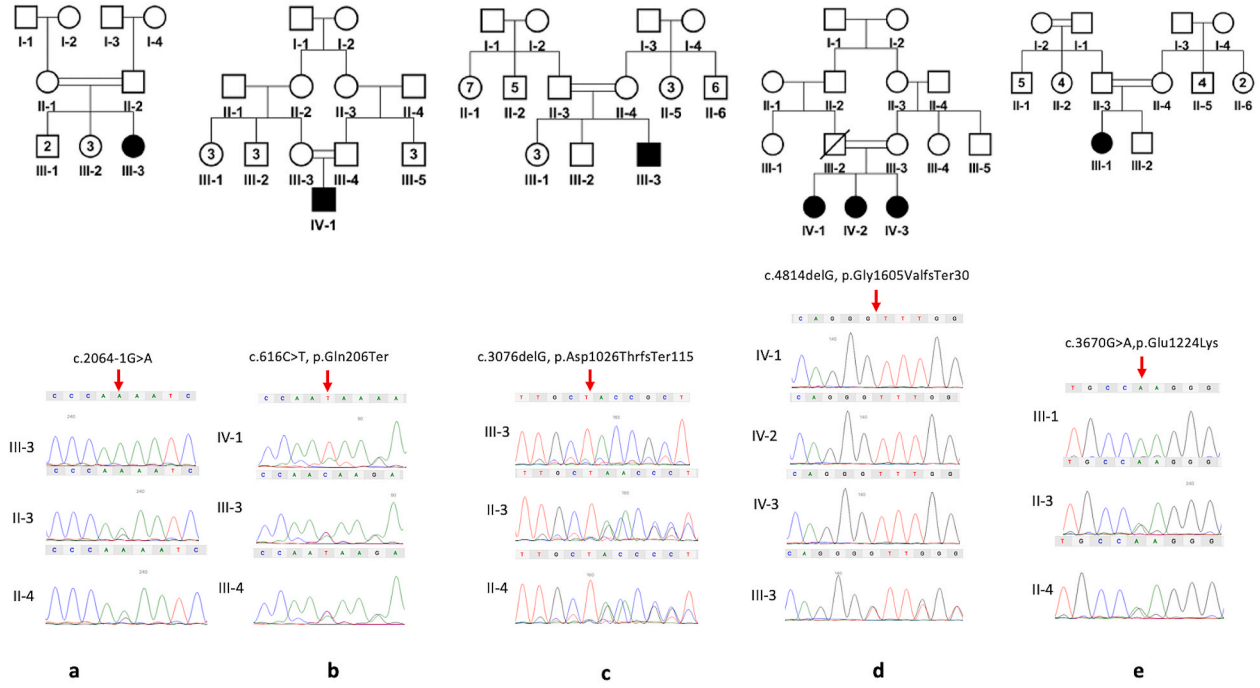


**Fig. 1.** The pedigrees of five families, and electropherograms representing Sanger sequencing of five novel variants (four in *CUL7*, one in *OBSL1*). Nucleotide sequence of *CUL7* or *OBSL1* showing biallelic variants in affected cases, while it is heterozygous in carrier parents. Note c.2064-1G>A variant in Family 1 (a), c.616C>T in Family 3 (b), c.3076delG in Family 4 (c), c.4814delG in affected three sisters in Family 8 (d) in *CUL7*. Biallelic missense variant (c.3670G>A) was shown in *OBSL1* in Family 13 (e). Variants are indicated by arrows.

*OBSL1*.

## 2. Patients and methods

A total of 19 patients (10 boys, 9 girls) from 14 families with a median age of diagnosis of 9.2 months were included in this study and were followed for 2–20 years. Out of 14 families, eleven were consanguineous, three unrelated. Signed informed consent and also the permission for the publication of clinical information and the photos of children were obtained from the parents. The Ethics Committee of Cerrahpaşa Medical Faculty at Istanbul University-Cerrahpaşa approved this study (April 3rd, 2018/Decision number: A-30). The clinical diagnosis of 3M syndrome was made according to clinical and radiographic features. Height, weight, head circumference and sitting height were measured by a pediatrician and standard deviation scores (SDS) of all anthropometric measurements were obtained. SDS was calculated by using a national pediatric calculator loaded with national standards (https://www.ceddcozum.com). Serum basal levels of IGF1 and IGFBP3 were determined using immunoradiometric assays. GH stimulation tests were performed with levodopa and/or clonidine, and the peak GH level>10 ng/mL was considered normal GH response, values between 5 and 10 were accepted as the partial deficiency, <5 were accepted as severe GH deficiency (Grimberg et al., 2016). Three families did not accept the GH stimulation test. GH therapy was given to twelve patients who underwent GH stimulation testing.

After the patients were diagnosed with 3M syndrome with clinical and radiological findings, pathogenic variants were searched in three genes by targeted gene sequencing based on next-generation sequencing (NGS) in all of the patients, firstly. Then, the confirmation of the detected pathogenic variants in the proband and segregation analysis in the family were performed by Sanger sequencing.

Genomic DNA was extracted from peripheral blood cells of the patient and both parents using standard techniques after obtaining informed consents from the parents of the patients. The exonic regions covering exon intron boundaries of the *CUL7, OBSL* and *CCDC8* were amplified by PCR using customized primers. Libraries covering targeted regions were prepared according to the Nextera XT DNA Library Prep protocol and sequenced in MiSeq® NGS system (Illumina Inc., USA). Analyses of NGS data were performed using Alamut® Visual mutation interpretation and The Integrative Genomics Viewer (IGV) softwares. Variant interpretation was made according to the American College of Medical Genetics and Genomics (ACMG) practice guidelines (Richards et al., 2015). Bioinformatics tools (PolyPhen2, SIFT, Mutation Taster, DANN) and data collected from electronic databases (dbSNP, ExAC, 1000G, ClinVar, Varsome, HGMD Professional version) were used to identify the deleterious amino acid changes. The segregation study and candidate variants were confirmed by Sanger sequencing. Since minor allele frequencies of Turkish population data is not available for detected variants; we made a search in ALFA (Allele Frequency Aggregator) database for European and Asian population frequency data (Phan et al., 2020).

For statistical analysis Microsoft Excel® and IBM corp. SPSS Statistics® version 21.0 were used.

Results were presented as mean ± standard deviation (SD) or median (min-max value). Comparison of the means was performed with Student's *T*-test for paired samples if the data were normally distributed. Categorical values were compared with chi-square, mean was compared with Mann Whitney U test.

## 3. Results

### 3.1. Molecular studies

Biallelic eleven different variants in *CUL7* and *OBSL1* were detected in 14 families (Table 1). *CUL7* and *OBSL1* variants were found in 57.9% and 42.1% of patients, respectively, five of which are novel. None of the patients had a variant in *CCDC8*.

Eight different variants in the *CUL7* were identified in nine families; One of them was in the compound heterozygous state and four were novel. The pedigree of five families with novel variants and presence of

**Table 2**
Clinical and Radiological Features of the Patients with *CUL7* variants.

| Family number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | | | 9 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8–10 | | | 11 | |
| **Gender (F/M)** | F | M | M | M | M | M | M | F | F | F | M | 7M/4F |
| **Lenght SDS at birth** | −3.01 | −5 | −5 | −5.48 | NA | −2.7 | −4.57 | NA | NA | NA | −4.82 | −4,3 ± 1,07 |
| **Weight SDS at birth** | −2.77 | −3,03 | −3.8 | −3.14 | −3.5 | −2 | −1.83 | NA | NA | NA | −3.86 | −2.9 ± 0.7 |
| **Head circumference SDS at birth** | +1.2 | 0.43 | −1.9 | NA | NA | 0.07 | +1.2 | NA | NA | NA | NA | −0.2 ± 1.2 |
| **Age at diagnosis Median (range)** | 9.5mo | 13 days | 1 day | 16 mo | 3.5 mo | 3mo | 4.5 mo | 19y | 17.5y | 13y, 3mo | 1 y,4mo | 9.5mo (1 day-17.5y ) |
| **Height SDS at diagnosis** | −4.7 | −4.86 | −5 | −5.07 | −3.7 | −4.2 | −4.99 | −5.96 | −4.95 | −6.1 | **−6.04** | −5.4 ± 1.16 |
| **Weight SDS at diagnosis** | −4.8 | −3.16 | −3.8 | −5.38 | −3.3 | −2.2 | −3.42 | −4.8 | −5.5 | −1.96 | −5,46 | −3.8 ± 1.25 |
| **Head circumference SDS at diagnosis** | −0.5 | 0.01 | −1.9 | −0.94 | −0.35 | −0.12 | −0.41 | −2.8 | −2.15 | −3.4 | −2.3 | −1.35 ± 1.19 |
| **Age at last examination, years Median (Range)** | 14y | 11y | 9y | 3y | 20.5y | 4y,2mo | 13 mo | 27y | 25y | 20.5y | 18 y | 13.9 (1.2–27) |
| **Height SDS at last examination** | −4.2 | −3.7 | −5.68 | −4.99 | −6.2 | −3.4 | −6.09 | −5.96 | −4.95 | −5.61 | −5,7 | −5.04 ± 1.01 |
| **Weight SDS at last examination** | +0.27 | −2.2 | −6.2 | −4.5 | −3 | −2.6 | −4.21 | −2.19 | −2.39 | −3.27 | −1,3 | −3.02 ± 1.71 |
| **Sitting Height/Height** | 0.53 | 0.60 | 0.57 | 0.61 | 0.49 | 0.52 | 0.56 | 0.56 | 0.52 | 0.54 | 0.59 | 0.55 ± 0.3 |
| **Facial features** | | | | | | | | | | | | |
| Dolicocephaly | – | + | + | + | + | + | + | – | – | – | – | 6/11 |
| Triangular face | + | + | + | + | + | + | + | + | + | + | – | 10/11 |
| Frontal bossing | + | + | + | + | + | + | + | + | + | + | – | 10/11 |
| Midline facial hemangioma | – | + | + | + | – | + | + | – | – | – | – | 5/11 |
| Infraorbital swelling | – | + | + | – | – | – | + | – | – | – | – | 3/11 |
| Thick eyebrows | + | + | + | + | + | + | – | – | + | + | + | 9/11 |
| Midface retrusion | + | + | + | + | + | + | + | – | – | – | + | 8/11 |
| Short, upturned, fleshy nose | + | + | + | + | + | + | + | + | + | + | + | 11/11 |
| Prominent central tubercle of upper lip | + | + | + | – | + | + | + | + | + | – | – | 8/11 |
| Long philtrum | – | + | + | + | + | + | + | + | + | + | + | 10/11 |
| Full fleshy lip | + | + | + | + | + | + | – | + | + | + | + | 10/11 |
| Pointed chin | + | + | + | + | + | + | + | + | + | + | + | 11/11 |
| **Skeletal features** | | | | | | | | | | | | |
| Short neck | + | - | - | - | + | + | + | + | + | + | + | 8/11 |
| Short and broad thorax | + | + | + | + | + | + | + | + | + | + | + | 11/11 |
| Transvers groove of lower rib cage | - | + | + | - | + | + | - | - | + | + | - | 6/11 |
| Hyperlordosis | + | + | + | - | - | - | - | + | - | - | + | 5/11 |
| Fleshy prominent heels | + | + | + | + | + | + | + | + | + | + | + | 11/11 |
| Pes planus | + | + | + | + | + | + | + | + | + | + | + | 11/11 |
| 5th finger clinodactyly | - | + | + | + | + | + | - | + | + | - | - | 7/11 |
| Simian line | - | - | - | + | + | + | - | + | + | + | - | 6/11 |
| **Radiological findings** | | | | | | | | | | | | |
| Delayed bone age | – | + | + | – | + | – | + | – | – | – | – | 4/11 |
| Tall vertebral bodies | + | + | + | + | + | + | + | + | + | + | – | 10/11 |
| Slender long bones | + | + | + | + | + | + | + | + | + | + | + | 11/11 |
| Diaphyseal constriction | + | + | + | + | + | + | + | + | + | + | + | 11/11 |
| Flared metaphyses | + | + | + | + | + | + | + | + | + | + | + | 11/11 |
| Scoliosis | – | – | – | – | + | – | – | – | – | – | – | 1/11 |
| **GH stimulation tests** | | | | | | | | NA | NA | | | |
| Basal IGF-1 (ng/mL)/SDS | 451/3 | 57.6/-1.9 | 24.3/-2.6 | 97.4/0.1 | – | 63.1/-0.7 | 16.1/-3.05 | | | 550/1.2 | 80/-1.6 | |
| Basal IGFBP-3 (µg/mL)/SDS | 6.11/1.2 | – | – | – | 4.3/-2.1 | – | 1.31/-2.8 | | | – | 6.2/-3.1 | |
| Basal GH (ng/mL) | 5.3 | 0.26 | 7.2 | 11.05 | 0.4 | 1.21 | 0.74 | | | 0.17 | 0.6 | |
| Peak GH level (ng/mL) (L-Dopa and/or clonidine) | – | 3.3/12.7 (No GHD) | 4.7/1.5 (GHD detected) | – | 3.2/10.8 (No GHD) | 7.2/11.2 (No GHD) | – | | | 17.2 (No GHD) | 3.2/5.4 (GHD detected) | |
| **GH therapy** | – | + | + | – | + | + | – | – | – | + | + | 6/11 |
| **GH treatment outcome** | | Response | Nonresponse | | Nonresponse | Response | | | | Nonresponse | Nonresponse | 2/6 response 4/6 nonresponse |
| **Other Features** | | | | | | ASD | HD | | | | | |

F:Female; M:Male; NA: data not available; mo: month; y: year; SDS: Standard deviation score; GH: Growth hormone; GHD: Growth hormone deficiency; ASD: Atrial septal defect; HD; Hip dislocation.

**Table 3**
Clinical and radiological Features of the Patients with *OBSL1* variants.

| Family number | 10 | | | 11 | | 12 | 13 | 14 | Total |
|---|---|---|---|---|---|---|---|---|---|
| Patient number | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | |
| **Gender (F/M)** | F | F | F | M | M | F | M | F | 5 F, 3 M |
| **Lenght SDS at birth** | −5.04 | −5.37 | −4.9 | −5.91 | NA | −3.95 | NA | −1.6 | −4.4 ± 1.54 |
| **Weight SDS at birth** | −3.64 | −3.2 | −3.38 | −2.95 | −2.8 | −3.46 | −3.08 | −1.6 | −3.3 ± 0.64 |
| **Head circumference SDS at birth** | −1.3 | −1.81 | 0 | NA | NA | −0.36 | NA | 0.36 | −0.62 ± 0.9 |
| **Age at diagnosis Median (range)** | 21 days | 2.5 y | 1 day | 8 mo | 7 y | 5 mo | 1 y | 14.5 mo | 9mo (1 day-7y) |
| **Height SDS at diagnosis** | −4.95 | −4.76 | −4.9 | −4.6 | −4.8 | −4.54 | −4.73 | −3.97 | −4.6 ± 0.32 |
| **Weight SDS at diagnosis** | −2.53 | −3.4 | −3.38 | −4.3 | −3.5 | −4.01 | −3.42 | −2.64 | −3.3 ± 0.6 |
| **Head circumference SDS at diagnosis** | −1.87 | −0.27 | 0 | −1.12 | −1.2 | −2.3 | −0.82 | −0.4 | −0.96 ± 0.85 |
| **Age at last examination Median (range)** | 10.1 y | 5 y | 13 mo | 14y 3mo | 16 y | 6.6 y | 12 y 3 mo | 8.5 y | 9.3y 13mo-16 y |
| **Height SDS at last examination** | −3.6 | −4.8 | −5.3 | −3.5 | −4.1 | −3.5 | −3.83 | −2.1 | −3.8 ± 1.03 |
| **Weight SDS at last examination** | −1.8 | -.2.5 | −4.97 | −4.9 | −3.8 | −2.1 | −3.26 | 2.08 | −2.4 ± 2.3 |
| **Sitting Height/Height** | 0.52 | 0.60 | NA | 0.54 | 0.53 | 0.55 | 0.50 | 0.57 | 0.55 ± 0.02 |
| **Facial features** | | | | | | | | | |
| Dolicocephaly | + | + | + | – | – | – | + | + | 5/8 |
| Triangular face | + | + | + | + | + | + | + | + | 8/8 |
| Frontal bossing | + | + | – | + | + | + | + | + | 7/8 |
| Midline facial hemangioma | + | + | – | – | – | – | – | + | 3/8 |
| Infraorbital swelling in lower lip | + | – | – | – | – | – | – | – | 1/8 |
| Thick eyebrows | + | + | + | + | + | + | + | + | 8/8 |
| Midface retrusion | + | + | + | + | + | + | + | + | 8/8 |
| Short,upturned, fleshy nose | + | + | + | + | + | + | + | + | 8/8 |
| Prominent central tubercle of upper lip | + | + | + | + | + | + | + | + | 8/8 |
| Long philtrum | + | + | + | + | + | + | + | + | 8/8 |
| Full fleshy lower lip | – | – | + | + | + | – | + | + | 5/8 |
| Pointed chin | + | + | + | + | + | + | + | + | 8/8 |
| **Skeletal features** | | | | | | | | | |
| Short neck | + | + | + | + | + | + | + | + | 8/8 |
| Short and broad thorax | + | + | + | + | + | + | + | + | 8/8 |
| Transvers groove of lower rib cage | + | + | – | + | + | + | + | + | 7/8 |
| Hyperlordosis | + | + | + | + | + | – | – | + | 6/8 |
| Fleshy prominent heels | + | + | + | + | + | + | + | + | 8/8 |
| Pes planus | + | + | + | + | + | + | + | + | 8/8 |
| 5th finger clinodactyly | + | + | – | – | – | – | - | – | 2/8 |
| Simian line | – | – | + | – | – | – | – | – | 1/8 |
| **Radiological findings** | | | | | | | | | |
| Delayed bone age | – | + | – | + | + | + | + | – | 5/8 |
| Tall vertebral bodies | + | + | + | + | + | + | – | + | 7/8 |
| Slender long bones | + | + | + | + | + | + | + | + | 8/8 |
| Diaphyseal constriction | + | + | + | + | + | + | + | + | 8/8 |
| Flared metaphyses | + | + | + | + | + | + | + | + | 8/8 |
| Scoliosis | – | – | + | + | + | – | – | – | 3/8 |
| **GH stimulation tests** | | | NA | | NA | | | | |
| Basal IGF-1 (ng/mL)/SDS | 132,5/1.1 | 71.1/-1.6 | | 25/-2.9 | | 24.5/-3.2 | 187/-0.87 | 203/0.6 | |
| Basal IGFBP-3 (μg/mL)/SDS | – | 2.2/-0.4 | | – | | – | 4.85/-3.8 | – | |
| Basal GH (ng/mL) | 1.7 | 0.38 | | 1.06 | | 1.02 | 1.1 | 0.04 | |
| Peak GH response (L-Dopa and/or Clonidine, ng/mL) | 2.2/6.8 (Partial GHD detected) | 4.7/11.5 (No GHD) | | 3.3/15.4 (No GHD | | 5.5/4.2 (No GHD) | 5.6/0.68 (No GHD) | 0.1/4.8 (GHD detected) | |
| **GH therapy** | + | + | – | + | – | + | + | + | 6/8 |
| **Treatment outcome** | Response | Response | – | Non-response | – | Response | Response | Response | 5/6 response 1/6 nonresponse |
| **Other Features** | ASD | – | – | ASD | – | ASD, PS | – | – | |

F:Female; M:Male; NA: data not available; mo: month; y: year; SDS: Standard deviation score; GH: Growth hormone; GHD: Growth hormone deficiency. ASD:Atrial septal defect; PS:Pulmonary stenosis.

biallelic variants in affected patients and heterozygous state in parents are seen in Fig. 1. Novel pathogenic variants are c.2064-1G>A, c.616C>T (p.Gln206Ter), 3076delG (p.Asp1026ThrfsTer115) and c.4814delG (p.Gly1605ValfsTer30) in *CUL7*. Three different variants of the *OBSL1* were shown in five families, two of which are known, one novel (c.3670G>A, p.Glu1224Lys). The two known variants: c.1938 dupA was detected in three *CUL7* families and c.1273 dupA was detected in three *OBSL1* families.

Novel variants were not present in dbSNP, 1000 genome project or Exome Aggregation Consortium (ExAC) database. The pathogenicity of the variants found was assessed as PVS1 (very strong), PM2 (moderate), and PP3 (promoting) according to the ACMG classification (Richards et al., 2015). Minor allele frequencies both European and Asian population were quite low for all the variants, where most of them have not been observed (Table 1).


**Fig. 2.** Photographs of patients with 3M syndrome at different ages. Top row (patients with *CUL7* variants): Patient 3 at the age of 2 days (a), 15 months (b) and 4.5 years (c). Note the infraorbital swelling of the lower eyelid, (a) and the midline hemangioma (b) and they reduced and disappeared as the child grows (c). Patient 6, at the age 5 months and of 2 years (ç,d), patient 4, at the age 16 months and 3.5 years (e,f) and patient 2, at 4 and 6 years (g,h) of age. Triangular face with frontal bossing, midline hemangioma (ç,e), fleshy nose, prominent central tubercle of upper lip (ç,e) and full fleshy lower lip, pectus deformities with transverse grooves of the lower rib cage especially prominent on the left side (d,f,g), hyperlordosis, fleshy protruding heels and pes planus (h) were observed. Second row (patients with *OBSL1* variants). Patient 12, at the age 28 days, and 2 and 5 years (ı-j). The infraorbital swelling in the lower eyelid that was present in the neonatal period, decreased and disappeared as the infant grew older. Patient 18, at the age of 18 months and 6 years (k,l), Patient 19, at the age of 3 (m) and 10 years (n,o). Characteristic facial features (j,l,n), short neck (i), pectus deformities (l,m) and hyperlordosis (l–o) with growth became more pronounced. Bottom row: Patient 7 at the age of 32 days, 5 and 13 months (ö-r). Note infraorbital swelling in the lower eyelid at neonatal period. Patient 19 at the age of 1 and 9.5 years (s,ş). Patient 11, at the age of 17.5 years (t-ü), patient 1, at the age of 11 years (v) and patient 5, at the age of 17 years (y). Note triangular face, frontal bossing, fleshy nose, prominent central tubercle of upper lip and full fleshy lower lip becoming more evident from the neonatal to the adult period (a–h).

### 3.2. Clinical reports

Ten patients with *CUL7* mutation, whose age at diagnosis was between 1 day and 19 years (median 9.5 months), and nine patients with *OBSL1* mutation, who were between 1 day and 7 years (median 9 months), were followed for 2–20 years. Clinical, radiological and biochemical findings of the eleven patients with *CUL7* and the eight patients with *OBSL1* variants were summarized in Tables 2 and 3, respectively. All of the patients were born uneventfully at term. Their intellectual and motor developments were normal. The mean SDSs of birth length, weight and head circumference of the patients with *CUL7* mutations were $-4{,}3 \pm 1{,}07$, $-2.9 \pm 0.7$, $-0.2 \pm 1.2$, respectively while these measurements were −4.46, −3.3 and −0.6 in those with *OBSL1* mutation.

The facial photographs of children with 3M syndrome from newborn to adulthood are exhibited in Fig. 2. The triangular face, frontal bossing, thick eyebrows, short, upturned, fleshy nose, mid-face retrusion, long philtrum, full fleshy lower lip and pointed chin were the facial findings that became more prominent from the newborn to adulthood in all patients. Infraorbital swelling on the lower eyelid was noticed in four newborns (Fig. 2 a,ı,ö) and midline facial hemangiomas were observed in eight infants (Fig. 2 a,ç,k,p). These two crucial findings disappeared in early childhood. In addition, we observed that the central tubercle, which is the fleshy prominence in the middle of the upper lip, becomes prominent in most patients as the child grows. Clinical and laboratory findings of patients with *CUL7* and *OBSL1* mutations are compared in Table 4; facial findings were similar in both groups of patients.

Musculoskeletal features such as short neck, short and broad thorax, hyperlordosis, fleshy protruding heels, pes planus, 5th finger clinodactyly and simian line, were present in most of the patients (Figs. 2 and 3, Tables 2 and 3). In most of the patients, due to hyperlordosis that became evident after 5–6 years of age, the abdomen seemed enlarged as it was pushed forward. We also noticed transverse grooves of the lower rib cage, particularly prominent on the left side, in five patients (Fig. 2 e, g, l,m,ş). While other musculoskeletal findings were similar in both groups, clinodactyly and simian line was more common in patients with the *CUL7* variant (Table 4). We evaluated changes in skeletal radiological findings beginning from neonatal period till adulthood (Fig. 3). All patients had slender long bones with diaphyseal narrowing, flared metaphyses and tall vertebral bodies at lumbar region which became more prominent with aging. Scoliosis developed in four patients. Bone age was slightly delayed in half of the patients, while it corresponded to chronological age in other patients. These findings were at the same frequency in patients with both *CUL7* and *OBSL1* mutations (Table 4).

Height SDS of patients with *CUL7* and *OBSL*1 variants; at the time of diagnosis, the SDSs were −5.4 (median at 9.5 months) and −4.6 (median at 9 months), while at the final visit the SDSs were −5.04 (median 13.9 years) and −3.85 (median 9.3 years) respectively. The patients with the *OBSL1* variant were statistically significantly taller than those with the *CUL7* variant at the last visit (Table 4). Eight patients reached a final mean height of −4.9 SDS. At the last examination, the sitting height to height ratio of all patients was 0.56.

The GH axis was evaluated in six patients with *CUL7* and six of the patients with *OBSL1* variants who accepted the stimulation test (Tables 2 and 3). GH deficiency was detected in two of the six patients with *CUL7*. GH treatment was started in a total of six patients, either

**Table 4**
Comparison of Clinical and Radiological Features of the Patients with *CUL7* and *OBSL1* variants.

| | *CUL7* N:11 | *OBSL1* N:8 | Overall N:19 | P value |
|---|---|---|---|---|
| **Lenght (cm) SDS at birth (mean)** | −4,3 ± 1,07 | −4.46 ± 1.5 | −4.3 ± 1.3 | 0.900 |
| **Weight (kg) SDS at birth (mean)** | −2.9 ± 0.7 | −3.3 ± 0.64 | −3.1 ± 0.7 | 0.307 |
| **HC (cm)SDS at birth (mean)** | −0.2 ± 1.2 | −0.62 ± 0.90 | −0.21 ± 1.12 | 0.273 |
| **Age at diagnosis Median (range)** | 9.5mo (1 day-17.5 years) | 9 mo (1 day-14.5 months) | 9.2mo (1 day- 17.5 years) | 0.475 |
| **Height SDS at diagnosis (mean** | −5.4 ± 1.16 | −4,6 ± 0,32 | −5.0 ± 0.99 | 0.108 |
| **HC (cm) SDS at diagnosis (mean)** | −1.35 ± 1.19 | −0.96 ± 0.85 | −1.1 ± 1.06 | 0.518 |
| **Age (year) at last examination (median)** | 13,9 (1.2–27) | 8.5 (1.1–14.2) | 10.1 (1.1–27) | 0.128 |
| **Height (cm) SDS at last examination** | −5.04 ± 1.01 | −3.85 ± 1.03 | −4.5 ± 1.16 | **0.031** |
| **Sitting Height/ Height** | 0.55 ± 0.3 | 0.55 ± 0.02 | 0.55 ± 0.033 | 0.953 |
| **Facial features** | | | | |
| Dolicocephaly | 6/11 | 5/8 | 11/19 | 0.729 |
| Triangular face | 10/11 | 8/8 | 18/19 | – |
| Frontal bossing | 10/11 | 7/8 | 17/19 | 0.250 |
| Midline facial hemangioma | 5/11 | 3/8 | 8/19 | 0.729 |
| Infraorbital swelling | 3/11 | 1/8 | 4/19 | 0.435 |
| Thick eyebrows | 9/11 | 8/8 | 17/19 | 0.202 |
| Midface retrusion | 8/11 | 8/8 | 16/19 | 0.107 |
| Short, upturned, fleshy nose | 11/11 | 8/8 | 19/19 | – |
| Long philtrum | 8/11 | 8/8 | 16/19 | 0.107 |
| Prominent central tubercle of upper lip | 10/11 | 8/8 | 18/19 | 0.381 |
| Full fleshy lip | 10/11 | 5/8 | 15/19 | 0.134 |
| Pointed chin | 11/11 | 8/8 | 19/19 | – |
| **Skeletal features** | | | | |
| Short neck | 8/11 | 8/8 | 16/19 | 0.107 |
| Short and broad thorax | 11/11 | 8/8 | 19/19 | – |
| Transvers groove of lower rib cage | 6/11 | 7/8 | 13/19 | 0.127 |
| Hyperlordosis | 5/11 | 6/8 | 10/19 | 0.198 |
| Fleshy prominent heels | 11/11 | 8/8 | 19/19 | – |
| Pes planus | 7/11 | 8/8 | 19/19 | 0.055 |
| 5th finger clinodactyly | 7/11 | 2/8 | 9/19 | **0.026** |
| Simian line | 6/11 | 1/8 | 7/19 | **0.05** |
| **Radiological findings** | | | | |
| Delayed bone age | 4/11 | 5/8 | 9/19 | 0.260 |
| Tall vertebral bodies | 10/11 | 7/8 | 17/19 | 0.811 |
| Silender long bones | 11/11 | 8/8 | 19/19 | – |
| Diaphyseal constriction | 11/11 | 8/8 | 19/19 | – |
| Flared metaphyses | 11/11 | 8/8 | 19/19 | – |
| Scoliosis | 1/11 | 3/8 | 4/19 | 0.134 |
| **Growth hormone** | | | | |
| Basal IGF-1 (ng/mL)/ | 204 ± 232 | 91.3 ± 76 | 160,2 ± 189 | 0.32 |
| IGF 1 SDS | −0.38 ± 2.3 | −1.2 ± 1 | −0.7 ± 2.2 | 0.56 |
| Basal IGFBP-3 (μg/mL)/ | 4.3 ± 2.5 | 3.5 ± 1.8 | 3.63 ± 2.1 | 0.71 |
| IGFBP3 SDS | −0.23 ± 2.3 | 0.05 ± 0.6 | 0.60 ± 0.73 | 0.88 |
| Basal GH (ng/mL) | 1.71 ± 2.5 | 0.84 ± 0.6 | 1.40 ± 2.02 | 0.46 |
| Peak GH level (L-Dopa and/or Clonidine, ng/mL) | 6.8 ± 5.2 | 3.1 ± 2.1 | 5.3 ± 4.6 | 0.18 |
| | 7.7 ± 5.1 | 8.5 ± 4.7 | 8.5 ± 4.8 | 0.80 |

HC: Head circumference; SDS: Standard deviation score

responding or not responding to the stimulus test. The percentile improved in two patients, the treatment was discontinued because four patients did not respond (Table 2). In six patients with the *OBSL1* variants, while GH deficiency was not detected in four, partial GH deficiency was found in one and complete GH deficiency in another. Of the six patients who were started on GH treatment, five responded (Table 3). In total, four out of twelve patients had GH deficiency, but all were treated. Altogether, seven patients, two with the *CUL7* and five with the *OBSL1* variant, responded to GH therapy (Tables 2 and 3). Five of our seven patients who responded to treatment and received GH therapy for an average of 3.6 ± 1.7 years were still in the prepubertal period, only two were pubertal. While the mean height SDS of patients who responded to treatment (n = 7) was −4.57 ± 0.36 before treatment, mean height SDS increased significantly to −3.6 ± 0.82 at the last examination (p = 0.006). The mean gain in height SDS of these patients was 1.03 ± 0.05 SDS.

## 4. Discussion

The frequency of *CUL7* variants in patients with 3M syndrome has been reported to be 77.5%, *OBSL1* 16.3% and *CCDC8* variants <5% (Hanson et al., 2009; Huber et al., 2011). We found *CUL7* variants in 57.9% of patients and *OBSL1* variants in 42.1%. There were no patients with variant *CCDC8*. While *OBSL1* variant frequency was higher than the literature, it was similar to a study conducted in our country (Simsek-Kiper et al., 2019). We also described four novel variants in the *CUL7* and one in *OBSL1*. To date, 88 *CUL7* and 36 different *OBSL*1 variants in HGMD that cause 3M syndrome have been published in human gene mutation database (http://www.hgmd.cf.ac.uk). The majority of these variants are frameshift, nonsense and missense. Eight of our study's 11 variants were frameshift, two were splicing and one was nonsense; and, five of these were novel. We had three families each with the same *CUL7* variant (c.1938 dupA) and *OBSL1* variant (c.1273dupA); these variants were also reported in studies involving Turkish patients (Huber et al., 2011; Simsek-Kiper et al., 2019).

Characteristic facial features of children with 3M syndrome whom we followed from the neonatal period to adulthood were triangular face, frontal bossing, thick eyebrows, short and fleshy nose, midfacial retrusion, long philtrum, full lower lip and pointed chin, all of which had become more obvious as they got older (Fig. 2). However most of the patients had a round face in infancy, the typical triangular face became prominent towards early childhood. We also noticed that two distinct findings which have not been reported in 3M syndrome until today are observed in the neonatal period and disappear towards the end of early childhood as the child grows up. The first of these, which is prominent infraorbital swelling in lower lid (presence of lower eyelid fat pads) was present in four patients. In fact, these finding was present in infants with 3M syndrome reported by Hu et al. (2017); however, they were not specified as features belonging to this syndrome. The second finding was midface hemangioma seen in eight of our patients in the neonatal and infantile periods. Another facial finding we also observed in 3M patients was the prominent central tubercle of upper lip which was not reported until today, as well as the full lips that become more pronounced with increasing age. We noticed that both midline facial hemangioma and the prominent central tubercle of upper lip, were present in the photograph of the patient reported in literatüre (Simsek-Kiper et al., 2019; Lugli et al., 2016; Al-Dosari et al., 2012).

The head circumference is usually normal, but since the height is short, it gives the appearance of a disproportionately large head in children with 3M syndrome (Clayton et al., 2012). While all of our patients had relative macrocephaly, a short and broad thorax and prominent fleshy heel, the majority had other skeletal findings of the syndrome, such as pes planus, hyperlordosis, thoracic kyphoscoliosis, and clinodactyly of the 5th finger and simian line. Interestingly, the transverse groove of lower rib cage was present as a specific finding in most of our patients, especially on the left side in our patients. Although

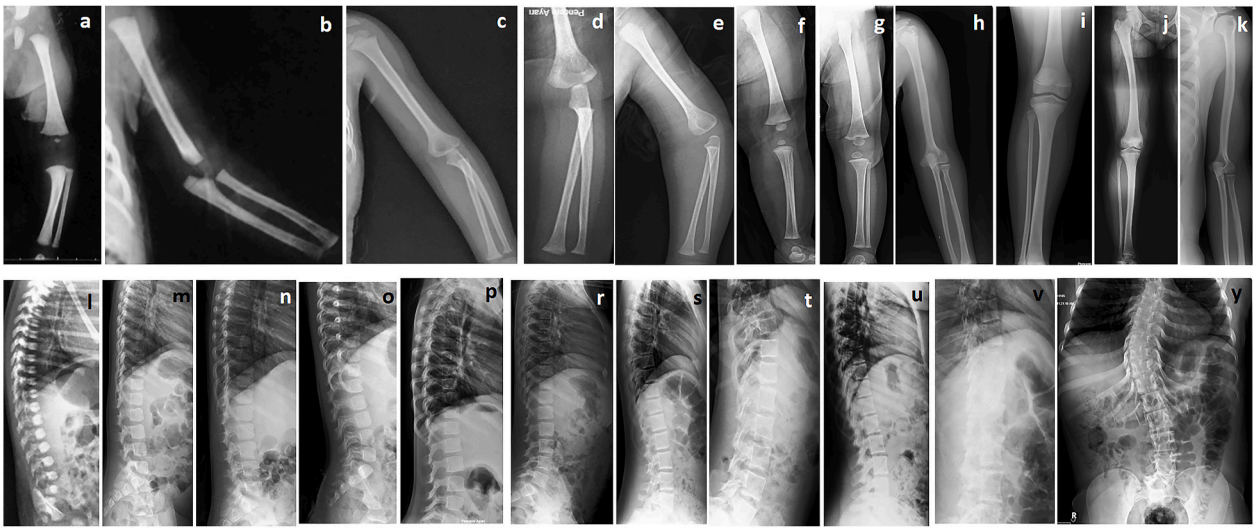


**Fig. 3.** Skeletal radiographs of the patients. Top row (long bone radiographs). Patient 13 at 1 day (a,b) and 6 years (c) of age, Patient 18 at 5 months (d,e), Patient 4 at 13 months (f–h), Patient 1 at 10.5 years (h,i) and 12 years (j) of age, Patient 5 at 19 years of age (k). Slender long bones with diaphyseal narrowing and enlarged metaphysis are observed in long bone radiographs taken from different patients from 1 day to 19 years of age (a–k) and in the same patients at different ages (a-c and h-j). There was no change in these findings with age. Bottom row (spine radiographies): Patient 3 at 20 days (l), 3 years and 8 months (m) and 8 years (n) of age, Patient 4 at 13 months (o) of age, Patient 2 at 3 and 8 years of age (p,r), Patient 1 at 11 years of age (s), Patient 6 at 16 years of age (t), Patient 9 at 15 years of age (u), Patient 5 at 19 years of age (v). Note varying degrees of tall vertebral bodies increasing in height from 20 days to 19 years of age (p–u). Antero-posterior plane spinal radiographs of Patient 5 at 19 years (v) of age showing dorsolumbosacral scoliosis.

this finding was reported previously, its specificity for the disease was not emphasized enough (van der Wal et al., 2001).

Typical radiological findings of the patients with 3M syndrome that can be useful in differential diagnosis are slender long bones with diaphysial constriction and flared metaphyses, and tall vertebral bodies particularly in the lumbar region (Huber et al., 2005; Clayton et al., 2012; Holder-Espinasse et al., 2014). Huber et al. (2009 and 2011) suggested that skeletal features were generally normal at birth except for the broad thorax, typical features such as long bones with diaphyseal narrowing, enlarged metaphysis and long vertebral bodies emerged over time. However, Takizaki et al. (2020), proposed that while 3M patients displayed cardinal skeletal features of 3M syndrome in both prenatal and neonatal periods, by the age of two, slender long tubular bones and tall vertebral bodies are less pronounced. We observed that typical long bone findings did not change with aging when extremity radiographs of different ages from newborn to adult were evaluated. However, as the child grew older, the tall vertebral bodies became more prominent. Other skeletal features were seen in some patients with 3M syndrome such as progressive thoracic scoliosis developed in four of and delayed bone age in half of our patients. Hip dysplasia detected in 11% of 3M syndrome was present in only one of our patients (Huber et al., 2009; Badina et al., 2011; Holder-Espinasse et al., 2014; Lugli et al., 2016). Skeletal and radiological findings were similar in both groups in our cohort as also reported by Huber et al. (2011). Interestingly, atrial septal defect which has not been reported before with this syndrome, was found in four patients presented here.

It has been reported that the mean SDS of height in children with 3M syndrome is −5 SDs at birth and no catch-up growth occurs in the postnatal period and final height is 5–6 SDs below the mean (Lugli et al., 2016; van der Wal et al., 2001; Shapiro et al., 2017). The mean height SDS at birth was −4.3 in our patients. Different from what has been reported before, the mean height at birth was not different between *CUL7* and *OBSL1* patients (Simsek-Kiper et al., 2019). The mean height SDS of patients with *CUL7* and *OBSL1* variants was −5.04 and −3.85 at the last examination, respectively and the patients with the *OBSL1* variant were significantly shorter than those with the *CUL7* variant. The mean age of the patients in this group was 2 years younger than the others, and they were in the prepubertal period, the patients may not be able to do the growth spurt in puberty and may remain short finally. In eight of our patients who reached final height, the mean height SDS was −4.97. Although it is known that there is proportional short stature (Irving and Holder-Espinasse, 2019) in patients with 3M syndrome, the ratio of sitting height to height was higher more than normal in our patients (0.56). While the thorax is short, the high sitting height can be explained by the characteristic tall lumbar vertebra.

The patients with 3M syndrome usually have normal GH levels and severe IGF-1 deficiency, while there is no response to the GH treatment. They show a growth potential similar to that seen in untreated GH deficiency or GH insensitivity syndromes (Huber et al., 2011; Meazza et al., 2013, Lee et al., 2020; Andrews et al., 2021). There are conflicting results in the literature regarding efficacy of GH in children with 3M syndrome. Nevertheless, in view of individual differences in growth response, a trial of rhGH treatment is recommended (Clayton et al., 2012; Deeb et al., 2015). Despite the fact that the mean gain in height SDS was 1.03 ± 0.05 SDS in seven of our patients who responded to treatment, we could not evaluate the effect of treatment on final height since five of them were still in the prepubertal period. However, the fact that some our patients had a normal GH response to the stimulation tests despite their severe short stature and that the patients who reached final height did not benefit from GH treatment support the argument that they may have GH resistance.

It has been reported that there is intrafamilial variability of clinical findings in patients with 3M syndrome (Marik et al., 2002; Al-Dosari et al., 2012; Deeb et al., 2015; Lugli et al., 2016). However, we observed that in our cohort all siblings from the same family had similar clinical features.

**In conclusion,** we detected *CUL7* variants in 57.4% of patients and *OBSL1* in 42.1% of patients. We also identified five novel variants of *CUL7* and *OBSL1*. We observed three pivotal early diagnostic signs in patients. Two of these findings were prominent infraorbital swelling of the lower lid and facial infantile hemangioma. These features became less pronounced as child grow. The third was the prominent central tubercle of the upper lip, which became evident as the child grew. Skeletal radiographs revealed no manifestations with progressively less-distinct features of the long bones; however, height of vertebral bodies became more pronounced as patients aged from the neonatal period to

adulthood. Clinical and radiological findings showed similarity in *CUL7* and *OBSL1* variant patients. The data presented in this study will raise awareness for early clinical diagnosis of the syndrome and will be very useful in avoiding costly and unnecessary diagnostic tests in infants with short stature.

## Author contribution

**B.T.**: Project administration, Conception and design of the study, Writing-Review&Editing, Supervision; **Z.A.U:** Writing, Data Curation; **H.T.:** Writing, Statistical analysis, Data Curation; **G.Y.:** Software, Validation, Formal analysis, **A.G., DUA:** Acquisition of data, **B.K.:** Software, Validation, Formal analysis, **M.V.:** Acquisition of data **O.E.:** Data Curation, interpretation of data, Final approval: all authors.

## Declaration of competing interest

The authors report no conflicts of interest.

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