CASE REPORT

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# Novel *OBSL1* Variant in a Chinese Patient with 3M Syndrome: The c.458dupG Mutation May Be a Potential Hotspot Mutation in the Chinese Population

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### What is already known on this topic?

3M syndrome is a rare autosomal recessive disorder. It is characterized by short stature, intrauterine growth retardation, low birth weight, dysmorphic facial features, and skeletal dysplasia. 3M syndrome includes three subtypes: type 1 with *CUL7* gene mutations, type 2 with *OBSL1* gene mutations, and type 3 with *CCDC8* gene mutations, accounting for approximately 77.5%, 16%, and 5%, respectively. There is no specific therapy for the disease. The effectiveness of growth hormone therapy for 3M syndrome is controversial.

### What this study adds?

The c.427dupG mutation in the presented patient is a novel *OBSL1* variant. The c.458dupG mutation has been documented only in Chinese individuals, suggesting ethnic specificity. The phenotype and variant information of the five Chinese patients with c.458dupG mutation in the *OBSL1* gene are summarized. We suggest the c.458dupG mutation may be a hotspot mutation in the Chinese population.

## Abstract

3M syndrome is an autosomal recessive disorder characterized by short stature and skeletal developmental abnormalities. A Chinese girl with 3M syndrome and a novel *OBSL1* (obscurin-like 1 gene) variant is presented. The patient is a 2-year-old girl who presented with short stature and had intrauterine growth retardation and low birth weight. Gene analysis revealed compound heterozygote mutations in the *OBSL1* gene: c.458dupG (p.L154Pfs*100) and c.427dupG (p.A143Gfs*111). The c.427dupG mutation is novel. The c.458dupG mutation has been documented in five cases, occurring only in Chinese individuals, suggesting ethnic specificity. In cases of children with short stature presenting with intrauterine growth retardation, low birth weight, and skeletal developmental abnormalities, 3M syndrome should be considered. The c.458dupG mutation may be a hotspot mutation in the Chinese population.

**Keywords:** Short stature, 3M syndrome, *OBSL1* gene, intrauterine growth retardation

## Introduction

3M syndrome (MIM #273750, 612921, 614205) is a rare autosomal recessive disorder, which was first reported by Miller et al. (1). 3M syndrome is characterized by short stature, dysmorphic facial features, and skeletal dysplasia. 3M syndrome exhibits genetic heterogeneity. Based on different causative genes, it can be categorized into three subtypes: type 1 with *CUL7* gene mutations, type 2 with *OBSL1* gene mutations, and type 3 with *CCDC8* gene mutations, accounting for approximately 77.5%, 16%, and

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5%, respectively. However, about 1.5% of 3M cases have not yet been related to a definitive causative gene, suggesting a complex pathogenic mechanism (2).

The *OBSL1* gene consists of 22 exons with three splice variants designated as OBSL1A, B, and C. The encoded obscurin-like protein 1 (OBSL1), which is distributed in the cell membrane and encircling the nucleus, functions as a cytoskeletal adaptor protein that connects the cell interior to the cell membrane, contributing to the stability of the cellular cytoskeletal network. Approximately 200 cases of 3M syndrome have been reported to date, with only 24 cases in China. Among them, type 2 has been reported in over 50 patients worldwide, with only nine cases in China.

In this study, we report and analyze the clinical and molecular manifestations of a Chinese patient with 3M syndrome type 2 (OMIM 610991) caused by *OBSL1* gene mutations. We also review the relevant literature and summarize the other five Chinese patients with the same mutation in the *OBSL1* gene.

## Case Report

The patient was a 2-year and 11-month-old girl of non-consanguineous parents. She was referred to our hospital due to her short stature. She was born at 38 weeks of gestation. Her birth weight, length, and head circumference were 2.0 kg [-3.1 standard deviation (SD)], 42 cm (-3.8 SD), and 34 cm. Prenatal examinations at five months of gestation indicated intrauterine growth retardation, with shorter-than-expected femur length for the gestational age. The mother had undergone an artificial abortion during her first pregnancy (G1P0) due to a diagnosis of short femur length.

At presentation, the patient's body weight was 9.6 kg (-3.6 SD), and her height was 79 cm (-3.8 SD). Serum basal insulin-like growth factor-1 (IGF-1) and IGF binding protein-3 levels were 186 ng/mL (reference range: 51-303 ng/mL) and 4.86 µg/mL (reference range: 0.8-3.9 µg/mL). Growth hormone (GH) stimulation test was normal, with a peak GH level of 8.4 ng/mL. Radiographic examinations showed normal bone age, tubular bones, and vertebral bodies.

Recombinant human GH (rhGH) therapy was subsequently given over nearly five years. The patient's height increased by approximately 4 cm per year. Currently, when the patient was 7 years 6 months old, her weight was 19.0 kg (-1.7 SD), and her height was 108 cm (-3.2 SD), and she maintains normal intellectual development.

### Genetic Analysis

Next-generation sequencing (NGS) was performed during the 7-year follow-up of the patient. Based on NGS analysis, two mutations, c.458dupG and c.427dupG, were detected in the *OBSL1* gene. The c. 458dupG variant is located in exon 1, resulting in a change in the p. L154Pfs*100 amino acid residue. This is a frameshift mutation that causes premature protein translation termination. According to the standards of the American College of Medical Genetics (ACMG) criteria, this mutation is considered pathogenic (PVS1 + PM3_Strong) and has been reported in clinical cases. The c. 427dupG variant is also located in exon 1, causing a change in the p. A143Gfs*111 amino acid residue. This is also a frameshift mutation that leads to premature protein translation termination. According to the ACMG standards, this mutation is also considered pathogenic (PVS1 + PM2 + PM3(Trans)). It is a newly identified mutation with a very low population frequency of 0.0000097. The patient carries compound heterozygous mutations in two pathogenic genes, resulting in premature protein translation termination, altered protein function, and associated clinical syndrome. Sanger validation of the variant gene is shown in Figure 1. Moreover, the amino acids in positions 154 and 143 are highly conserved among different species (Figure 2).

## Discussion

We reported the clinical and genetic features of a Chinese girl with type 2 3M syndrome. The clinical manifestations of 3M syndrome lack specificity and predominantly involve short stature without accompanying intellectual impairment. In 2009, Hanson et al. (3) identified 10 cases with 3M syndrome who did not carry mutations in the *CUL7* gene. These ten individuals showed no discernible clinical distinctions compared to patients with *CUL7* gene mutations. Through high-density genome-wide SNP mapping, a second gene at 2q35-q36.1 was identified. This study reported seven mutations in *OBSL1* gene for the first time, including c.690insC (p. E231RfsX23), c.1149C→A (p.C383X), c.1273insA (p. T425NfsX40), c.1256_1265delGCACCGTGGC (p. R419PfsX10), c.1359insA (p. E454RfsX11), c.1463C→T (p.R489X), and c.2034_2035 delinsA (p.H679TfsX40). All these mutations were found within the first six exons of the gene (3).

3M syndrome patients manifest intrauterine growth retardation and short stature. In this study, the patient's primary presentation was short stature, along with a history of intrauterine growth retardation, low birth weight, and reduced birth length. In contrast, head circumference at birth remained within the normal range. These observations align with the established clinical characteristics of 3M syndrome. Most 3M syndrome patients demonstrate normal GH levels, and exhibit expected responses in GH stimulation

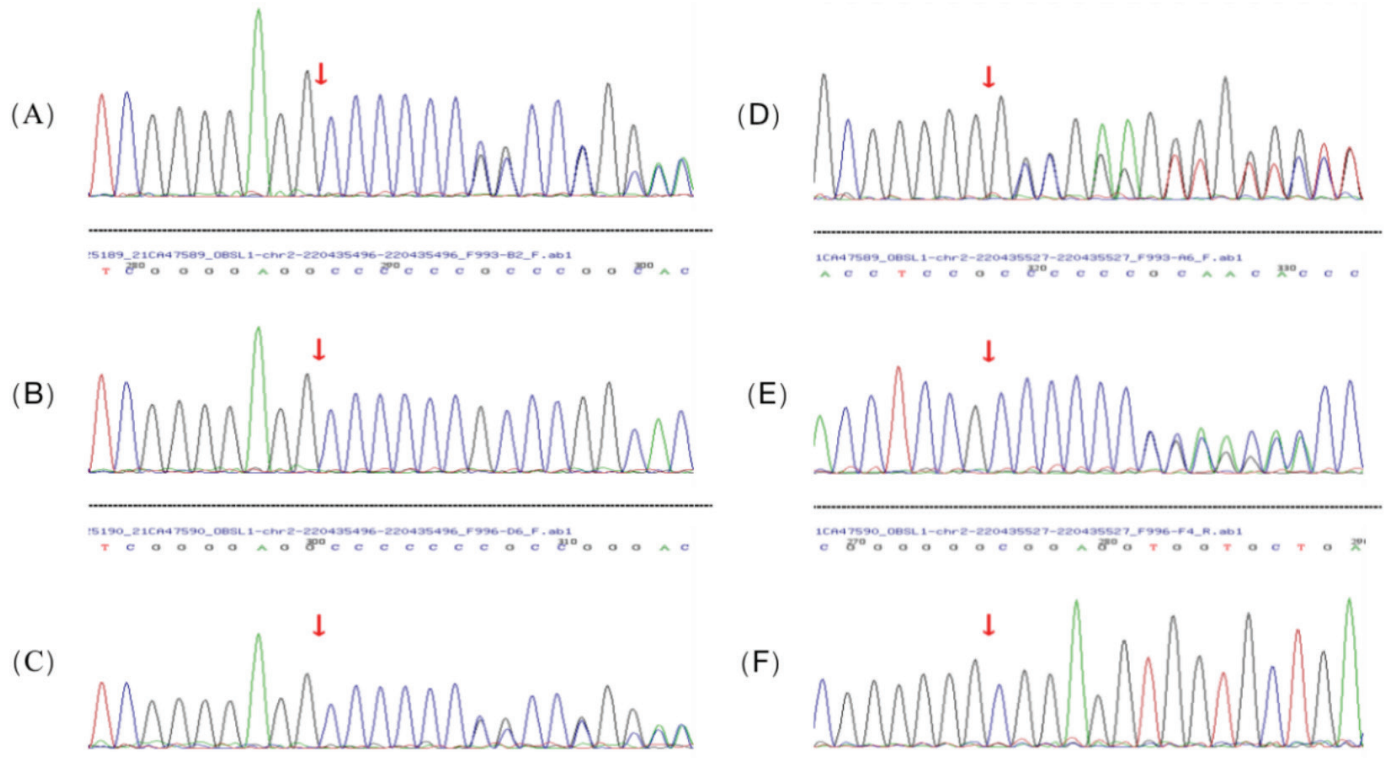


**Figure 1.** Sanger sequence showing the heterozygous mutations c.458dupG and c.427dupG. A) The patient bears the heterozygote c.458dupG frameshift mutation. B) The site is wild type in his father. C) The mother carries the heterozygote c.458dupG frameshift mutation. D) The child has the heterozygote c.427dupG frameshift mutation. E) The father carries the heterozygote c.427dupG frameshift mutation. F) The site is wild type in his mother

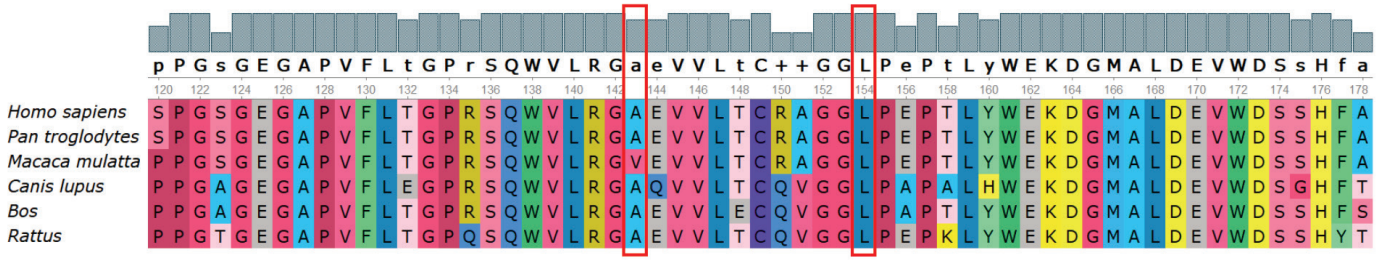


**Figure 2.** Conservation of the amino acid at positions 154 and 143 of the amino acid sequence among different species

tests. A minority have been reported to display inadequate stimulation test results (4,5). The peak GH response for the presented case was 8.4 ng/mL, and the IGF-1 level was average. According to the latest guidelines (6), this outcome rules out GH deficiency, indicating normal GH secretion.

All three subtypes of 3M syndrome may have a characteristic face, with minimal disparities among the subtypes. These features include a triangular face, pronounced forehead, flat nasal bridge, a round nasal tip, anteriorly tilted nostrils, elongated philtrum, thick lips, and prominent chin. Patients' dysmorphic facial features tend to be less noticeable when they grow up (7). In the current study, the patient exhibited a pronounced forehead, flat nasal bridge, and full round nasal tip at ten months of age (Figure 3A). By the age of five years, the pronounced forehead and depressed nasal bridge were less noticeable (Figure 3C). These findings emphasize the importance of clinicians keenly observing a child's facial appearance during their early years, which can aid in the prompt identification of this condition and subsequently facilitate timely genetic testing for a definitive diagnosis.

3M syndrome may exhibit skeletal developmental abnormalities, such as clinodactyly of the fifth finger, prominent heels, calf muscle protrusion, square shoulders, short neck, shortened chest cavity, reduced

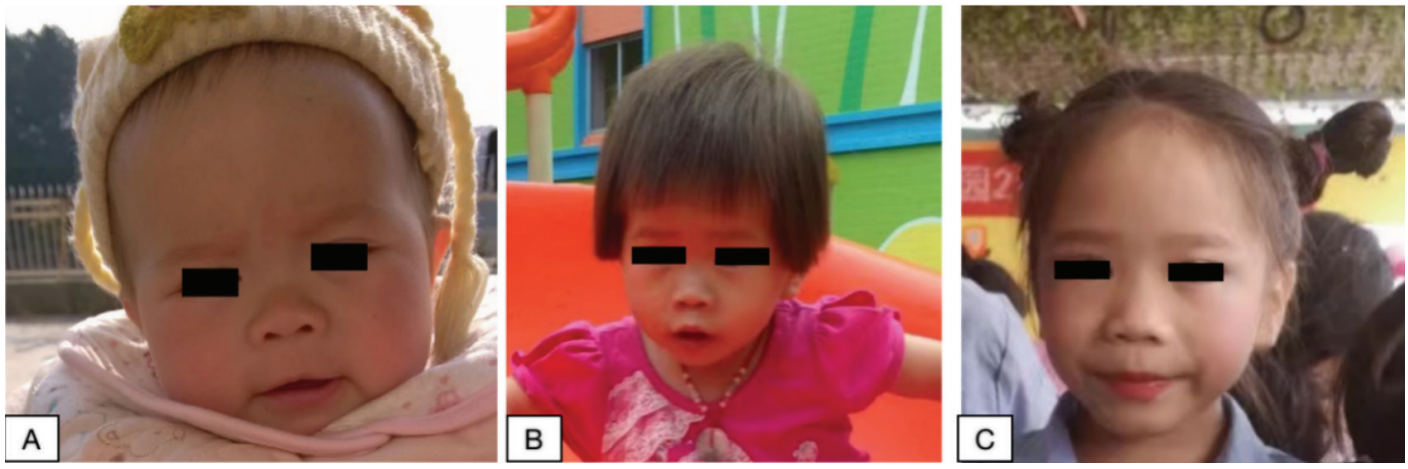


**Figure 3.** Facial appearance of our patient. A) 10 months old. A pronounced forehead, flat nasal bridge, and full round nasal tips were observed. B) Two years old. C) Five years old. A pronounced forehead and flat nasal bridge were less prominent

chest circumference, winged scapula, and anterior spinal protrusion. However, the patient in this study displayed milder clinical symptoms without any of these features.

In most 3M syndrome patients, skeletal X-ray assessments commonly reveal elongated tubular bones and tall vertebral bodies. Approximately 90% of patients display distinct characteristic alterations, including an elevated vertebral body height and a reduced anterior-posterior diameter. These alterations are especially noticeable in the lumbar vertebrae. Tüysüz et al. (8) analyzed 19 patients with 3M syndrome and found that tall vertebral bodies are more pronounced in children aged six years and older, as well as in adults. The patient in the present study did not manifest the characteristic vertebral changes, possibly due to her young age.

The *OBSL1* gene is located at 2q35 and consists of 22 exons with three splice variants designated as OBSL1A, B, and C. Its encoded product, obscurin-like protein 1 (OBSL1), comprises 1896 amino acid residues and is expressed in various cell types, including myocardium, skeletal muscle, brain tissue, and intervertebral discs. OBSL1 is distributed in the cell membrane and encircles the nucleus, functioning as a cytoskeletal adaptor protein that connects the cell interior to the cell membrane, contributing to the stability of the cellular cytoskeletal network.

More than 45 identified mutations in the *OBSL1* gene have been documented in the HGMD database. The reported mutation types include missense, nonsense, frameshift, deletion, and insertion mutations. Nine frameshift mutations have been reported. (c.35dupC, c.458dupG, c.690dupC, c.1039dupC, c.1125dupT, c.1260dupC, c.1273dupA, c.1359dupA, and c.2086_2088dupGGC). The presented case carries two mutations, c.458dupG (p. L154Pfs*100) and c.427dupG (p. A143Gfs*111). The two mutations are both frameshift mutations in exon 1, which lead to premature termination of protein translation. The c.458dupG frameshift mutation has been documented in only five cases to date (Table 1). All have occurred in Chinese individuals (9,10,11,12), which may be an indication of ethnic specificity and that this may be a hotspot mutation in the Chinese population.

Little is known about the mechanism underlying the short stature of 3M syndrome. The GH-IGF-1 axis in 3M syndrome appears normal, so the possibility of alternative pathways exists to induce abnormalities in growth plate chondrocyte development. Research into 3M syndrome pathogenesis has been multifaceted. In 2009, Huber et al. (13) revealed histological alterations in the growth plate of embryonic tibia in individuals with 3M syndrome. The researchers identified enlarged chondrocyte volume and increased density in both the resting and proliferative zones of the growth plate, accompanied by impaired extracellular matrix synthesis compared to normal cells. A separate investigation in 2013 proposed that the absence of autocrine IGF-2 functionality within the growth plates of children with 3M syndrome might contribute to their reduced stature (14). Subsequently, in 2014, Yan et al. (15) described the collaborative interaction among the proteins CUL7, OBSL1, and CCDC8, which form the 3M complex. OBSL1 acts as a bridging element between CUL7 and CCDC8. The function of the 3M complex is to uphold the integrity of microtubules - an essential aspect of mitosis and cytokinesis, crucial for normal cellular development. Notably, the study demonstrated that individual knockout of *CUL7*, *OBSL1*, and *CCDC8* genes didn't exacerbate conditions like mitotic delay, emphasizing their coordinated role within the same pathway. That may be why the three subtypes of

**Table 1. Phenotype and variants information of five patients with c.458dupG mutation in *OBSL1* gene**

| Patient ID | Our case | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| Gender | Female | Female | Male | Female | Male | Female |
| Age | 2 y 11 m | 2 y | 5 y 6 m | 4 y | 11 y 3 m | 10 y 8 m |
| cDNA change | c.427dupG<br>c.458dupG | c.458dupG<br>(homo) | c.458dupG<br>(homo) | c.1365-1387dup<br>c.458dupG | c.1118G > A<br>c.458dupG | c.690dupC<br>c.458dupG |
| Birth length/cm | 42 | NA | 45 | NA | 41 | NA |
| Birth weight/g | 2000 | NA | 2550 | NA | 2700 | 2300 |
| Current height/cm | 79 (-3.8 SD) | 74 (-3.8 SD) | 93 (-4.1 SD) | 85 (-5.8 SD) | 116.1 (-4.3 SD) | 132.4 (-2.5 SD) |
| Current weight/kg | 9.6 | 8.5 | 13.5 | 11.5 | 24 | 40.5 |
| Growth retardation | + | + | + | NA | + | + |
| Triangular face | + | + | - | NA | + | + |
| Low nasal bridge | + | + | - | NA | + | + |
| Frontal bossing | + | + | - | NA | + | + |
| Normal intelligence | + | + | + | NA | - | + |
| Delayed bone age | - | - | + | NA | + | - |
| Bone change | - | - | - | NA | Smaller pelvic<br>Long slender bones | Smaller pelvic<br>Long slender bones |

SD: standard deviation, NA: not applicable, y: year, m: month

3M syndrome exhibit closely similar clinical presentations. In a subsequent study by Wang et al. (16) in 2019, it was discovered that phosphorylated CCDC8 protein facilitated the formation of the 3M complex, prompting its relocation to the cell membrane. Once formed, the 3M complex initiated the ubiquitination-mediated degradation of LL5β. Disturbance in this process may give rise to altered microtubule dynamics, compromising cell migration and differentiation.

There is no specific therapy for the 3M syndrome. The effectiveness of GH therapy for 3M syndrome type 2 is controversial. Some researchers suggest that despite normal GH levels, some children with 3M syndrome exhibit inadequate GH stimulation, warranting GH treatment. Keskin et al. (5) reported a case of 3M syndrome type 2 treated with rhGH for six months at a dose of 7.5 IU/kg per week, resulting in a growth increment of 7 cm and a satisfactory growth rate. Clayton et al. (17) investigated the response to rhGH treatment in six individuals (including four patients who carried the causative mutation in the *OBSL1* gene). They found a small but significant increase in growth rate and height growth compared to the control group. However, some case reports indicate an ineffectiveness of GH treatment. Demir et al. (4) reported a child with homozygous *OBSL1* gene mutation (c.457_458delinsT) who underwent one year of GH treatment (dosage not mentioned), resulting in a mere 3 cm height increase. The presented patient received approximately five years of GH treatment, achieving a height increase of 4 cm per year with moderate effectiveness.

Due to the patient's average intelligence, the prenatal diagnosis of 3M syndrome remains debatable. For those with a family history or parents who are carriers of confirmed pathogenic genes and wish to have an unaffected child, a preimplantation genetic diagnosis could be considered, following ethical principles and informed consent. Regular prenatal ultrasound examinations are beneficial to early diagnosis. The growth rate of all long bones was observed to decrease. Two- and three-dimensional sonography can reveal shortened long bones and help detect mid-facial underdevelopment, aiding in prenatal diagnosis of 3M syndrome (18). Therefore, clinical prenatal examinations should focus on early identification and prompt genetic testing.

## Conclusion

In summary, 3M syndrome is a rare disease primarily presenting with short stature. It should be considered when accompanied with intrauterine growth retardation, low birth weight, facial abnormalities in infancy, average head circumference, and skeletal developmental issues. Molecular analysis is needed to confirm the diagnosis. We found a novel mutation in the *OBSL1* gene in a Chinese patient. Moreover, the c.458dupG mutation in the *OBSL1* gene may be a hotspot mutation in the Chinese population.

### Ethics

**Informed Consent:** Informed consent was obtained from the families of the study participants.

## Footnotes

### Authorship Contributions

Surgical and Medical Practices: Yurong Piao, Rongmin Li, Yingjie Wang, Congli Chen, Yanmei Sang, Concept: Yanmei Sang, Design: Yanmei Sang, Data Collection or Processing: Yurong Piao, Rongmin Li, Yingjie Wang, Analysis or Interpretation: Yurong Piao, Congli Chen, Yanmei Sang, Literature Search: Yurong Piao, Congli Chen, Writing: Yurong Piao.

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