Polymorphism

RESEARCH

Identification of a novel mutation c.1172_1181 del TGGTGCAAGC (p.Leu391fs) in the CUL7 gene in a patient with 3M syndrome

Priyanka Vishwakarma[1], Ashish Dubey[1], Deepika Kalo[1], Vishal Kumar Mishra[2]

[1]Department of Clinical Genomics, Redcliffe Lifetech Private Limited, Noida, Uttar Pradesh, India

[2]Department of Bioinformatics, Redcliffe Lifetech Private Limited, Noida, Uttar Pradesh, India

*Corresponding author e-mail: dr.priyanka@redcliffelabs.com

ABSTRACT

3-M syndrome is a disorder characterized by the skeletal abnormalities including a short stature and unusual facial features. The affected people have low birth weight and length and remain much smaller than others in their family members, growing to an adult height of approximately 4 feet to 4 feet and 6 inches. In this study, we report a novel frameshift mutation (c.1172_1181delTGGTGCAAGC; p. Leu391fs) in the *CUL7* gene in a patient of the 3M Syndrome. We performed whole exome sequencing and the likely pathogenic variant was validated by Sanger sequencing method. As per the public databases, this variant has not been reported till date. This study identified a novel frameshift mutation (c.1172_1181delTGGTGCAAGC; p. Leu391fs) in the *CUL7* gene in a patient with the 3M Syndrome. The study also highpoints the clinical utility of exome sequencing in the accurate diagnosis of the provisionally diagnosed genetic disorders whose ultimate diagnosis is important, especially for their management.

KEYWORDS: 3M syndrome, CUL7 gene, c.1172_1181del TGGTGCAAGC (p. Leu391fs)

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INTRODUCTION

3-M syndrome is a disorder that causes skeletal aberrations with a short height and abnormal facial appearance (Meazza et al., 2013). The name of this condition comes from the identities of three researchers who first acknowledged the name of the 3M syndrome (Miller, McKusick, and Malvaux) (Meazza et al., 2013). The mutations in any one of the three genes, *CUL7, OBSL1,* and *CCDC8* are responsible for the manifestation of this disorder. The inheritance pattern of this disorder is autosomal recessive (Meazza et al., 2013). The incidence of this disorder is very low, so far less than 100 cases have been identified worldwide. In 2005, the autozygosity mapping discovered a 3-M syndrome locus on chromosome 6p21.1, and pathogenic mutations in the *CULLIN7* (*CUL7* [MIM 609577]) gene were successively recognized as the primary cause of the 3-M syndrome (Miller, et al, 1975, Huber et al., 2005; Muñoz et al., 2015). Later, autozygosity mapping discovered a second 3-M syndrome locus on chromosome 2q35-q36.1 with the basic mutations identified in Obscurin-like 1 gene (*OBSL1* [MIM 610991]) (Nasiri et al., 2005).

The people with 3-M syndrome struggle from the severe prenatal growth retardation owing to the growth adjournments during the fetal development, resulting in a low birth weight. They have low birth weight and the length remains much smaller than others in their family, growing to an adult height of approximately 4 feet - 4 feet 6 inches (120 centimeters to 130 centimeters). In some affected individuals, the head is normal-sized but looks disproportionately large in the comparison with their full body. In other people with this disorder, the head has an unusually long and narrow shape (dolichocephaly). Intelligence is unaffected by 3-M syndrome, and the life expectancy is generally normal. The growth adjournment continues even after birth throughout the childhood and the adolescent stage, ultimately leading to a short stature (dwarfism). Many of the physical features associated with the disorder are congenital. The distinguishing craniofacial abnormalities typically comprise a long, narrow head that is inconsistent with the body size, a broad and prominent forehead, and a triangular-shaped face with a hypoplastic midface, long philtrum, pointed chin, depressed nasal bridge, fleshy-tipped reversed nose, protruding mouth, large ears, and full lips (Miller et al., 1975; Winter et al., 1984; Guven A et al., 2011; Meazza et al., 2013).

Other skeletal irregularities that repeatedly occur in this disorder comprise a short, broad neck and chest, prominent shoulder blades, and shoulders that slope less than usual (square shoulders). The affected individuals may have abnormal spinal curvature such as a rounded upper back that also curves to the side (kyphoscoliosis) or inflated curvature of the lower back (hyperlordosis). People with 3-M syndrome can also have the unusual curving of the fingers, also called clinodactyly, short pinky (small) fingers, prominent heels, and loose joints. The additional skeletal irregularities, such as remarkably slender long bones in the arms and the legs; tall, narrow spinal bones (vertebrae); or slightly delayed bone age may be apparent in the X-ray pictures.

A variant of 3-M syndrome called the Yakut short stature syndrome has been acknowledged in isolated Yakut population in the Russian province of Siberia (Habibullah). In addition to having most of the physical features characteristic of the 3-M syndrome, people with this form of the disorder are often born with breathing problems that can be life-threatening in infancy.

Genetic background of the 3M syndrome

Mutations in the *CUL7* gene cause 3-M syndrome in more than three-quarters of affected individuals, including those in the Yakut population (Medline plus, 2008). The mutations in the *OBSL1* gene cause around 16 percent of cases of this disorder.

Mutations in other genes, some of which have not been identified, account for the remaining cases.

The *CUL7* gene provides instructions for making a protein called cullin-7. Cullin-7 protein shows a role in the cell machinery that breaks down or degrades the unwanted proteins, called ubiquitin-proteasome system. Specifically, the cullin-7 helps in assembling a complex called E3 ubiquitin ligase, which tags the unwanted proteins for degradation. The protein produced from the *OBSL1* gene is thought to help in maintaining normal levels of cullin-7.

The ubiquitin-proteasome system helps in the regulation of proteins tangled in several critical cell activities such as cell division and growth. In particular, the proteins formed from the genes related with 3-M syndrome are thought to help to regulate the proteins involved in the body's response to growth hormones, although their specific role in this process is unknown (Muñoz et al., 2015).

Mutations in the *CUL7* or *OBSL1* gene prevent the cullin-7 protein from bringing together the components of the E3 ubiquitin ligase complex, interfering with the process of tagging proteins for degradation. The body's response to growth hormones may be impaired as a result. However, the specific relationship between the *CUL7* and *OBSL1* gene mutations and the signs and symptoms of 3-M syndrome are unknown (Huber et al., 2005).

Patient's information

A 7 months old female patient was presented to the clinician with the family history of the shortening of the limbs, the abnormal ribs and the cleft palate. Siblings the has similar condition in the family history. The proband's parents presented a consanguineous marriage history (Pedigree Fig. 2).

DNA extraction and quality analysis

The genomic DNA was extracted from 2 mL of the peripheral venous blood sample of the patient using the standard method of DNA extraction, called phenol–chloroform method (Nasiri et al., 2005). The quality of DNA was evaluated on the basis of 2% agarose gel electrophoresis, and the quantity of the DNA was measured by spectrophotometry using NanoDrop. This study has been approved by the Ethics Committee and the parents of the patient have provided informed written consent.

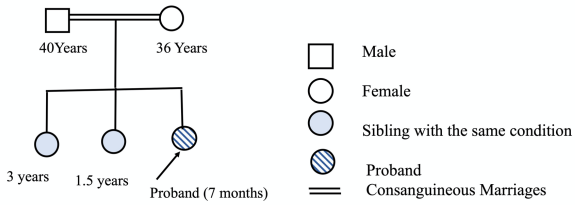

Fig. 2. Pedigree of the affected family.

Exome sequencing

The DNA extracted from the blood was used to perform the targeted gene capture using an Agilent sure select exome V6 kit (Table 1). The libraries were sequenced to mean 100X coverage on Illumina sequencing platform and 6 to 8 GB data were generated. The data generated were aligned to the human reference genome (GRCh37/hg19) and variant analysis was performed by using the set of bioinformatics pipelines. The clinically relevant mutations were annotated by using published variants and a set of diseases databases such as ClinVar, OMIM, GWAS, HGMD and the SwissVar. Common variants were filtered based on allele frequency in 1000 Genome Phase 3, ExAC, EVS, dbSNP147, 1000 Japanese Genome etc. The genetic variations from exome data were further filtered using different bioinformatics pipelines. The effect of non-synonymous variants were calculated by using multiple algorithms such as the PolyPhen-2, SIFT, Mutation Taster2, Mutation Assessor and LRT.

Only the non-synonymous and splice site variants identified in the clinical exome panel comprising of a specific set of the genes were used for further consideration. The silent variations that did not result in any changes in the amino acid sequence in the coding region were not reported.

Sanger sequencing

For the confirmation of novel likely pathogenic mutation identified by exome sequencing, we Sanger sequencing for confirmation of the mutations (Fig 1).

RESULTS

After performing the exome sequencing in the patient, we found that the individual carried two copies (homozygous) of a frameshift variant (c.1172_1181delTGGTGCAAGC; p. Leu391fs) in the *CUL7* gene, which was predicted to cause a frameshift and consequent premature truncation of the protein (Table 1). The variant has been reported in the dbSNP database with an identification number rs1160826185 and in the Genome Aggregation Database (gnomAD) with a rare allele frequency of 0.0003990%. Since, this variant is predicted to produce a truncated protein which might result in loss-of-function and other frameshift truncating variants in this gene are also known to cause similar phenotype, therefore, this variant has been labelled as likely pathogenic.

Table 1. The most promising mutation identified by the exome sequencing.

Gene	Chromosomal Coordinates	Exon	Variant	Zygosity	Condition group	Significance	Inheritance	Coverage
CUL7	chr6:43019009:GGCTTGCACCA:G NM_001168370.1	4	c.1172_1181delTGGTGCAAGC p.Leu391fs	Homozygous	3-M syndrome 1	Likely Pathogenic	Autosomal Recessive	88

The protein encoded by this gene is a component of an E3 ubiquitin-protein ligase complex. The encoded protein interacts with *TP53*, *CUL9*, and *FBXW8* proteins. Defects in this gene are a cause of 3M syndrome type 1 (3M1). Two transcript variants encoding different isoforms have been found for this gene. The Sanger sequencing confirmation of the homozygous variant in the *CUL7 gene* (c.1172_1181delTGGTGCAAGC; p. Leu391fs) was established (Fig 1).

DISCUSSION

The 3-M syndrome is a rare autosomal recessive disorder. Its categorized by a triangular-shaped face with the frontal bossing, sunken nasal bridge, mild malar hypoplasia with a plump tip of nose and full lips and upturned nares. The 3M patients typically have larger heads for their height, the dolichocephaly, and present with usual intelligence. Another clinical findings comprises a short wide thorax, brachydactyly, clinodactyly, micromelia and prominent heels. With this, both the sexes are

affected likewise. There are no hormonal insufficiencies reported. Our patient had nearly all of the typical clinical features, along with the above-mentioned bone abnormalities, but did not show radiological findings of the slender long bones with thin diaphysis and tall vertebral bodies. This finding may also give the impression future in some patients. The radiographic examination, although abnormal, is not diagnostic, as similar X-ray changes have been acknowledged in other disorders.

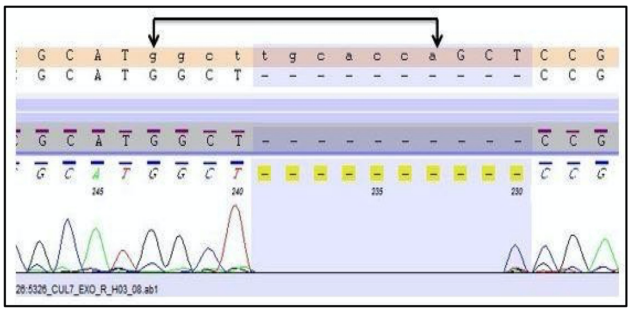

Fig. 1. Electropherogram showing the pathogenic mutation.

In another study, Maksimova et al. reported 43 patients with short stature, hydrocephaloid skull, and typical face in an isolated Yakut population. Although the clinical features were similar to the 3-M syndrome, but the slender long bones and tall vertebral bodies have not been usually observed in this short-stature syndrome in Yakuts (Le Merrer., 1991), as in Makisimova's patients (Huber et al., 2009).

Another study by Le Merrer et al. (1991) designated nine cases of children with primordial dwarfism and facial dysmorphism regarded as the 'gloomy face.' Despite very short stature, there were no radiological irregularities of the bones and there was no hormone deficiency (Maksimova et al., 2007). Le Merrer et al. (1991) recommended that this was a distinct complaint with the features of the 3-M syndrome (Le Merrer., 1991).

The physical findings of the numerous individuals such as Silver-Russell syndrome (SRS) are parallel to the 3-M syndrome. Silver-Russell syndrome has many similarities with the 3-M syndrome: intrauterine growth retardation, short stature, triangular face, relatively large skull, asymmetry of body or limbs, and clinodactyly. Mild mental retardation also can be found in patients with SRS, but abnormalities of the skeletal system have not been reported.

By using the direct sequencing method, Huber et al. (2005) reported 25 dissimilar mutations in the cullin-7 gene (CUL7; 609577) in the 29 families with the 3-M syndrome (Guven et al., 20https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6335979/ - b7-amjcaserep-20-3611).

In all 43 affected individuals with Yakut short-stature syndrome, Maksimova et al. (2007) identified homozygosity for a founder mutation in the CUL7 gene. Delayed bone age has been reported in the 3M syndrome patients; our patient was one year behind her chronological age. An early diagnosis is very significant for genetic counselling in the 3-M syndrome, particularly in countries where the consanguineous marriages are very frequent, and the autosomal recessive genetic disorders are always a risk. 3-M syndrome should always be considered in the differential diagnosis of patients with growth retardation having prenatal onset.

CONCLUSION

In this study, we reported a novel mutation c.1172_1181 del TGGTGCAAGC (p.Leu391fs) in the CUL7 gene in a patient of 3M syndrome from the Indian subcontinent, which was not previously reported. The exome sequencing approach provided a major advantage in identifying the likely pathogenic mutation in this case.

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Author's contribution

PV drafted the manuscript. AD reviewed all the study. DK gave patients details and VKM provided the bioinformatics inputs in whole exome sequencing data. All authors contributed to the article and approved the submitted version.

Conflict of interest

Authors have no conflict of interest.

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Declaration of originality

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