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RESEARCH NOTE

Novel mutation in *Cul7* gene in a family diagnosed with 3M syndrome

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Abstract. This study evaluates a family with two siblings having severe growth retardation and facial dysmorphism, born to consanguineous normal healthy parents. Affymetrix CytoScan 750K microarray showed a 34-Mb pericentric homozygous region on chromosome 6 for both siblings. *CUL7* was one of the 141 genes present in this region. Sanger sequencing of *CUL7* gene detected a 2-bp novel deletion in the 15th exon (c.2943_2944delCT of the cDNA). This deletion leads to a frameshift and a premature termination signal much upstream of the wild-type termination signal, leading to a nonsense mediated decay of the mRNA. CUL7 protein plays an important role in formation of 3M complex, ubiquitination, microtubule dynamics and cell cycle regulation. Mutations in *CUL7* gene is known to cause a rare 3M syndrome. Information about the novel mutation has been accepted in the ClinVar database with rs1064792895.

Keywords. 3M syndrome; ubiquitination; proteasomal degradation; skeletal dysplasia; consanguinity.

3M syndrome (OMIM: 273750) is a rare autosomal recessive disorder characterized by prenatal and postnatal growth retardation, facial dysmorphism, skeletal dysplasia and normal intelligence. Mutations in any one of three genes, Cullin 7 (*CUL7*), obscurin-like 1 (*OBSL1*) and coiled-coil domain-containing protein 8 (*CCDC8*) are known to cause 3M syndrome (Hanson *et al.* 2009, 2011a, b, 2012; Meazza *et al.* 2013; Deeb *et al.* 2015; Lugli *et al.* 2016). The 77.5% of the 3M syndrome cases are due to *CUL7* gene (Lugli *et al.* 2016).

The current study involves a family with two affected sibs, 12 year-old male and 5 year-old female, having severe growth retardation, facial dysmorphism with height and weight less than 3rd percentile. Table 1 in electronic supplementary material at http://www.ias.ac.in/jgenet/ shows anthropometric values of the affected sibs. These children are born to consanguineous healthy parents with mean parental height of 160.5 cm. Figure 1 in electronic supplementary material shows pedigree of the family. The family also reported a short statured maternal aunt, who was not available for analyses.

In view of the clinical features, a differential diagnosis was done for the elder sib on his initial visit to the hospital. The child was reported to have intrauterine growth retardation and showed characteristic dysmorphic features in the form of moderately enlarged head with prominent forehead, depressed nasal bridge, triangular face, nonprominent heels, clinodactyly and disproportionate short stature. CT scan of the brain showed mild fullness of lateral ventricles. He was very lean and parents failed to report consanguinity or presence of a close relative with short stature (<3 feet). The patient was referred to a panel endocrinologist and follow up investigation was conducted for achondroplasia and Silver–Russell syndrome (SRS) using molecular methods and karyotyping. Karyotyping revealed a normal chromosomal complement. Genomic DNA was tested in another institute for fibroblast growth factor receptor 3 gene mutation for evaluating achondroplasia

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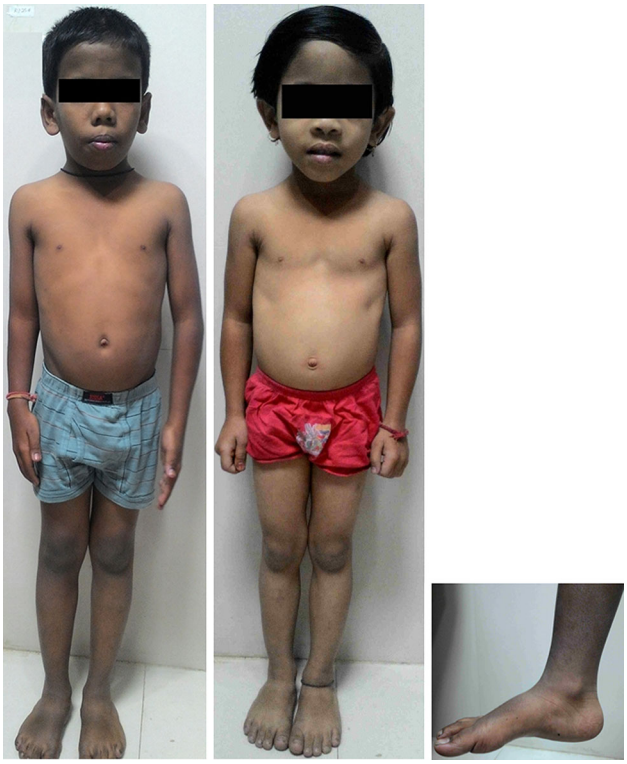

Figure 1. Images of the affected sibs, male and female sibling, showing the typical 3M features of facial dysmorphology broad thorax, backward heel protrusion.

and the result was negative for mutation (1138G>M). Molecular diagnosis of UPD7 to evaluate for SRS was also done in another institute by amplification of sodium bisulphite modified genomic DNA. It tested negative for SRS. Growth hormone and IGF1 levels were normal and radiological evaluation revealed delayed bone age.

Subsequently he was lost for follow-up for 7 years. The parents then visited the hospital with the second child (age 5 years) who also showed features similar to the elder sibling. Figure 1 shows the images of the affected sibs.

Parents and the affected sibs were referred to us for genetic evaluation. Karyotyping was done by 72 h phytohemagglutinin stimulated whole blood culture method and evaluated using Zeiss metasystems. Normal chromosomal complement was reported for parents and affected sibs. Parents and both sibs were assessed using Affymetrix CytoScan 750K, CGH-SNP microarray. Cychip files from the array results were evaluated by us using Affymetrix CHAS 3.0 software. Data analyses revealed that a 34 Mb pericentric loss of heterozygosity (LOH) region on chromosome 6 common in both affected sibs, figure 2 in electronic supplementary material. Information about consanguinity and presence of an affected relative was revealed only after CGH revealed a large LOH in both sibs.

Of the 141 OMIM genes present in this LOH region, mutations in *CUL7* gene was known to cause 3M syndrome, phenotype of which matched the phenotype of our affected sibs, table 2 in electronic supplementary material.

Affected sibs were later radio-diagnosed and also evaluated for backward protrusion of heels. Radio-diagnosis of the affected sibs matched with the features of 3M syndrome, figure 3, a–c in electronic supplementary material. Their heels were found to be less prominent. It is known that these features often become less noticeable with age (Maksimova *et al.* 2007; Clayton *et al.* 2012). Thirteen overlapping primers were designed using ExonPrimer software from UCSC browser, to cover the CDS (25 of 26 exons) of *CUL7* gene and excluding the first exon which is the 5′ UTR region, figure 4 in electronic supplementary material. These primers were confirmed for uniqueness using Primer-BLAST (Ye *et al.* 2012). PCR conditions were standardized using HotStart *Taq* DNA polymerase from MERCK specialties to get a single clean product. PCR products were sequenced using BigDye Terminator v3.1 cycle sequencing method. Variations detected were confirmed with Ensembl variation database.

Analyses revealed a novel 2-bp homozygous deletion in the 15th exon at position *NM_014780.4* (*CUL7*):c.2943_2944delCT of the cDNA in both the affected sibs, table 1. The 2-bp deletion leads to a frameshift and finally to

Table 1. Clustal W alignment output from UGENE v1.25.0 (Okonechnikov *et al.* 2012) showing 2-bp homozygous deletion in affected sibs (M P8F/R and A P8F/R) in the *CUL7* gene.

Sequence ID	Sequences	Position
NG_016205.1†	CTGGCCAGTGTTCCGGGAGCAGCTCTGTCGTCACACACGCCTCTTCTACA	13650
M_P8F_	CTGGCCAGTGTTCCGGGAGCAG--CTGTCGTCACACACGCCTCTTCTACA	13650
M_P8R_rev_compl	CTGGCCAGTGTTCCGGGAGCAG--CTGTCGTCACACACGCCTCTTCTACA	13650
A_P8F	CTGGCCAGTGTTCCGGGAGCAG--CTGTCGTCACACACGCCTCTTCTACA	13650
A_P8R_rev_compl	CTGGCCAGTGTTCCGGGAGCAG--CTGTCGTCACACACGCCTCTTCTACA	13650
CUL7_ensembl_seq#	CTGGCCMGYGTTCYRGGAGCAGCTCTGTYRTCACAYRYRCCTCTYCTACR	13650
	****** * **** ******* **** ***** ***** ****	

† NG_016205.1, Homo sapiens cullin 7 (*CUL7*), RefSeqGene on chromosome 6.
# ENSG00000044090 -May 2016 version from ensembl.org. Position refers to nucleotide position in NG_016205.1.

a premature termination signal after insertion of 31 incorrect amino acids.

Since the premature termination codon occurs upstream of the wild-type termination codon, this mRNA would likely be targeted for nonsense mediated decay (Brogna and Wen 2009; Lykke-Andersen and Jensen 2015; Hug *et al.* 2016). Information about this novel mutation has been published in ClinVar database with variation ID- 425545 (dbSNP - rs1064792895).

One of the 3M reports identified LOH region to be isodisomy/heterodisomy (Sasaki *et al.* 2011). Hence we assessed genotype calls of parents and affected sibs from microarray data. The 34-Mb LOH region, did not show any long and continuous stretch of genotype calls that were same in parents and affected sibs. At various positions, genotype calls of affected sibs were heterozygous whereas parents were homozygous for that location or affected sibs were homozygous and parents were heterozygous for that location. The allelic discordance at the LOH region confirms that the 34 Mb region on chromosome 6 is due to consanguinity and not UPD/isodisomy.

To further confirm, percentage homozygosity was studied. Homozygosity, 57–66% was seen among parents in all the chromosomes and 72% homozygosity was seen in the 34-Mb LOH region. Similarly affected sibs showed 63–91% homozygosity among themselves in all the chromosomes and 97% homozygosity in the 34-Mb LOH region, table 3 in electronic supplementary material.

This indicated two things: (i) homozygosity percentage in LOH region is higher than the nonLOH regions. (ii) Homozygosity percentage (LOH and nonLOH regions) among affected sibs is higher than among parents. Kirin *et al.* (2010) in their study stated that long runs of homozygosity are observed in recent inbreeding and the extent of homozygosity in individuals born from consanguineous unions is higher than expected in consanguineous individuals (parents) (Kirin *et al.* 2010). Homozygosity pattern in our study is in accordance with the Kirin *et al*. study.

CUL7 gene is located at 6p21.1 and belongs to the Cullin family. It plays an important role in the formation of Cullin-RING E3 ubiquitin ligases of ubiquitin–proteasome system. This system essentially functions in timely degradation of a variety of regulatory proteins through 26S mediated proteasome. CUL7 protein plays a role in microtubule dynamics, cell cycle regulation and formation of 3M complex.

How exactly the *CUL7* disruption results in the 3M phenotype is still unclear and needs further studies. 3M is a rare syndrome and is underdiagnosed, especially in India. Although there are many communities with consanguineous marriages, and many children are born SGA, yet less than 10 reports of *CUL7* mutation are available from India (Nampoothiri *et al.* 2014). Hence 3M syndrome should be considered in cases of dysmorphic features and short stature born to consanguineous parents.

In conclusion, awareness about such rare autosomal recessive mutations, role of consanguineous marriages and good phenotypic evaluation is utmost essential.

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References

Brogna S. and Wen J. 2009 Nonsense-mediated mRNA decay (NMD) mechanisms. *Nat. Struct. Mol. Biol*. **16**, 107–113.

Clayton P. E., Hanson D., Magee L., Murray P. G., Saunders E., Abu-Amero S. N. *et al*. 2012 Exploring the spectrum of 3-M syndrome, a primordial short stature disorder of disrupted ubiquitination. *Clin. Endocrinol. (Oxf.)* **77**, 335–342.

Deeb A., Afandi O., Attia S. and El Fatih A. 2015 3-M syndrome: a novel CUL7 mutation associated with respiratory distress and a good response to GH therapy. *Endocrinol. Diabetes Metab. Case Rep*. **2015**, 150012.

Hanson D., Murray P. G., Sud A., Temtamy S. A., Aglan M., Superti-Furga A. *et al*. 2009 The primordial growth disorder 3-M syndrome connects ubiquitination to the cytoskeletal adaptor OBSL1. *Am. J. Hum. Genet*. **84**, 801–806.

Hanson D., Murray P. G., Black G. C. M. and Clayton P. E. 2011a The genetics of 3-M syndrome: unravelling a potential new regulatory growth pathway. *Horm. Res. Paediatr*. **76**, 369–378.

Hanson D., Murray P. G., O'Sullivan J., Urquhart J., Daly S., Bhaskar S. S. *et al*. 2011b Exome sequencing identifies CCDC8 mutations in 3-M syndrome, suggesting that CCDC8 contributes in a pathway with CUL7 and OBSL1 to control human growth. *Am. J. Hum. Genet*. **89**, 148–153.

Hanson D., Murray P. G., Coulson T., Sud A., Omokanye A., Stratta E. *et al.* 2012 Mutations in CUL7, OBSL1 and CCDC8 in 3-M syndrome lead to disordered growth factor signalling. *J. Mol. Endocrinol*. **49**, 267–275.

Hug N., Longman D. and Caceres J. F. 2016 Mechanism and regulation of the nonsense-mediated decay pathway. *Nucleic Acids Res.* **44**, 1483–1495.

Kirin M., McQuillan R., Franklin C. S., Campbell H., McKeigue P. M. and Wilson J. F. 2010 Genomic runs of homozygosity record population history and consanguinity. *PLoS One* **5**, e13996.

Lugli L., Bertucci E., Mazza V., Elmakky A., Ferrari F., Neuhaus C. *et al*. 2016 Pre- and post-natal growth in two sisters with 3-M syndrome. *Eur. J. Med. Genet*. **59**, 232–236.

Lykke-Andersen S. and Jensen T. H. 2015 Nonsense-mediated mRNA decay: an intricate machinery that shapes transcriptomes. *Nat. Rev. Mol. Cell Biol*. **16**, 665–677.

Maksimova N., Hara K., Miyashia A., Nikolaeva I., Shiga A., Nogovicina A. *et al*. 2007 Clinical, molecular and histopathological features of short stature syndrome with novel CUL7 mutation in Yakuts: new population isolate in Asia. *J. Med. Genet*. **44**, 772–778.

Meazza C., Lausch E., Pagani S., Bozzola E., Calcaterra V., Superti-Furga A. *et al*. 2013 3-M syndrome associated with growth hormone deficiency: 18 year follow-up of a patient. *Ital. J. Pediatr*. **39**, 21.

Nampoothiri S., Yesodharan D., Sainulabdin G., Narayanan D., Padmanabhan L., Girisha K. M. *et al*. 2014 Eight years experience from a skeletal dysplasia referral center in a tertiary hospital in Southern India: a model for the diagnosis and treatment of rare diseases in a developing country. *Am. J. Med. Genet. A* **164A**, 2317–2323.

Okonechnikov K., Golosova O. and Fursov M. 2012 Unipro UGENE: a unified bioinformatics toolkit. *Bioinformatics* **28**, 1166–1167.

Sasaki K., Okamoto N., Kosaki K.,Yorifuji T., Shimokawa O., Mishima H. *et al*. 2011 Maternal uniparental isodisomy and heterodisomy on chromosome 6 encompassing a *CUL7* gene mutation causing 3M syndrome. *Clin. Genet*. **80**, 478–483.

Ye J., Coulouris G., Zaretskaya I., Cutcutache I., Rozen S. and Madden T. L. 2012 Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. *BMC Bioinform*. **13**, 134.

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