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Whole exome sequencing reveals a novel mutation in *CUL7* in a patient with an undiagnosed growth disorder

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Abstract

We present a 19 year old male with a growth disorder, which was undefined, despite extensive evaluation. Whole exome sequencing demonstrated a novel homozygous frameshift mutation in *CUL7*, one of the causative genes of 3M syndrome. We discuss the utility of exome sequencing in diagnosing rare disorders.

Keywords

3M Syndrome; Dwarfism; Genomics

The proband was the second child born to Irish parents. He was born at 39 weeks gestation with a birth weight of 1850 grams (−3.7 SDS) and length of 33 cm (−6.8 SDS). He had difficulty feeding and subsequent poor growth, resulting in gastrostomy tube placement at 17 months, which was removed at 3 years. His head circumference was always large for his age (range +1.9 to +2.4 SDS), but cognitive development was normal. Given his severe short stature and dysmorphic features, he was assumed to have a syndromic growth disorder, although his clinical features did not suggest a specific diagnosis. He had normal IGF-1 and IGFBP-3 levels as well as an extensive genetic work up all of which was unrevealing (Table I). Multiple skeletal surveys were performed throughout his life but did not yield a distinct diagnosis despite evaluation by international experts. During one evaluation, he was noted to have gracile-appearing long bones at age 15 months. He was treated with growth hormone for a period of approximately 5 years with transient mild increase in growth velocity. His history is also notable for significant scoliosis requiring surgical repair at age 14 years, delayed dentition, as well as tonsillectomy and tympanostomy tube placement as a toddler

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for frequent otitis media. Additionally, he had testicular maldescent and inguinal hernias requiring surgical repair. There were no other affected family members. Physical and radiographic features are depicted in the Figure and Table II (available at www.jpeds.com).

This study was approved by the Children's Hospital Boston Institutional Review Board and written informed consent was obtained from all participants. Whole exome sequencing of the proband, his brother, and both parents was performed at the Broad Institute. Hybrid selection was performed using Agilent's SureSelect Human All Exon Kit v2 (Agilent Techonologies, Santa Clara, CA). We sequenced the 2 samples using the Illumina HiSeq 2000 platform (Illumina Inc., San Diego, CA), aligned the resulting reads to the hg19 reference genome with BWA(1), applied GATK(2) base quality score recalibration, indel realignment, and performed SNP and indel discovery and genotyping across all samples simultaneously using variant quality score recalibration (3). Variants were annotated for functional effect using SnpEff 2.0.5 (http://snpeff.sourceforge.net/). As individuals with phenotypes of this severity are extremely uncommon, we assumed that if there were underlying causal genetic variants, they would also be quite rare. We filtered out all variants with minor allele frequency greater than 1% in the 1000 Genomes project (February 2012 release) (4), the NHLBI exome variant server(5) or in the 50 HapMap control exomes whose variants were called in conjunction with our subject's variants.

A priori, we did not know whether our subject's condition was due to a de novo dominant or an inherited recessive disorder. To assess for the possibility of a dominant variant, we searched for rare de novo nonsynonymous variants and found one such missense variant in *FAM134A*. To investigate the recessive model, we searched for autosomal genes containing either a homozygous or two heterozygous rare nonsynonymous variants. We found nine genes meeting these criteria. Using the parental exome data, we were able to exclude seven of these genes as both rare variants were present in one of the two parents indicating that the proband is not a compound heterozygote. In addition to *FAM134A*, this left two candidate genes, *CUL7* and *EPG5*, with *CUL7* clearly being the causal gene as it is known to cause the rare primordial growth disorder 3M syndrome (MIM #273750), which fits our subject. We identified a novel homozygous frameshift mutation in *CUL7* (NM_014780.4 c. 2836_2839dupATAG) resulting in loss of the terminal 740 amino acids (NP_055595.2 p.Arg948Aspfs*12). The variant was confirmed by Sanger sequencing and was present in the heterozygous state in both parents and the brother.

As noted above, *CUL7* is one of three genes (*CUL7*, *OBSL1*, and *CCDC8*) responsible for causing 3M syndrome (6), a very rare primordial growth disorder presenting with severe pre and post-natal growth retardation, relative macrocephaly, normal intelligence, characteristic facial features, and X-ray findings of long, slender bones, tall vertebral bodies and small pelvic bones (7) (Table II). 3M syndrome was first described in 1975 (8) and a recent article stressed the need for increased recognition of this disorder as a cause of severe short stature (9). 3M syndrome shares many features with Russell-Silver syndrome including pre- and post-natal growth retardation with normal head size and intelligence (10). Despite 3M's description over 30 years ago, there have been fewer than 100 families reported in the medical literature (11). Despite evaluation by multiple experienced geneticists, endocrinologists, radiologists and the review of his radiographs by international experts, our subject's diagnosis remained elusive. This case demonstrates the potential clinical utility of whole exome sequencing for the diagnosis of rare Mendelian conditions. Whole exome sequencing facilitated a rapid presumptive diagnosis in this case, with the causal variant being identified within an hour after the receipt of the variant calls, by systematic filtering and interpretation of the few remaining candidate variants in light of the patient's clinical picture. This diagnosis has profound clinical ramifications for our subject. First, he is now aware that his condition is due to a recessive condition, and thus, the likelihood of passing it

on to his children is negligible. Second, the diagnosis led to a re-examination of his testicular volume (5 cc and 8 cc as adult) and the realization that he has hypergonadotrophic hypogonadism (LH 12.99 IU/L, normal 1.4–11.1, and FSH 21.96 IU/L, normal 1.3–12.7). Referral was made to urology and further discussions regarding fertility preservation options are ongoing. There was one prior report of decreased testicular volume and elevated FSH in three males with 3M syndrome but the molecular etiology was not known (12). This is the first documented case of hypergonadotrophic hypogonadism in a subject with a *CUL7* defect.

Whole exome sequencing has the potential to be cost-saving and more effective compared with performing multiple targeted genetic tests when faced with a puzzling clinical presentation that is suggestive of a monogenic disorder (13). For our patient, targeted genetic testing alone cost almost $8000 and did not reveal the diagnosis. In addition, the patient had multiple physician visits for diagnostic evaluation, including multiple radiological studies. Clinical exome sequencing for a similar cost to his genetic testing would have revealed the diagnosis in a much more expeditious fashion. We believe that this case is an excellent example of how exomic and potentially genomic sequencing technology can revolutionize the way genetic diagnoses are made, leading to more rapid and less expensive evaluations. Of course, exome sequencing will not resolve every undiagnosed condition. Many phenotypes are not caused by single gene defects in known or likely pathogenic genes. Even where a single gene defect is responsible, the mutations may escape detection, or may lie in genes that have not yet been connected to the clinical phenotype. There are many other issues surrounding the interpretation of exome sequence results, including the discovery of incidental genetic findings that may have implications for a patient's health (14). Despite these challenges, it is increasingly clear that exome sequencing will be of substantial benefit when applied to the right patients. Our case demonstrates the power of this new technology to dramatically shorten the diagnostic odyssey.

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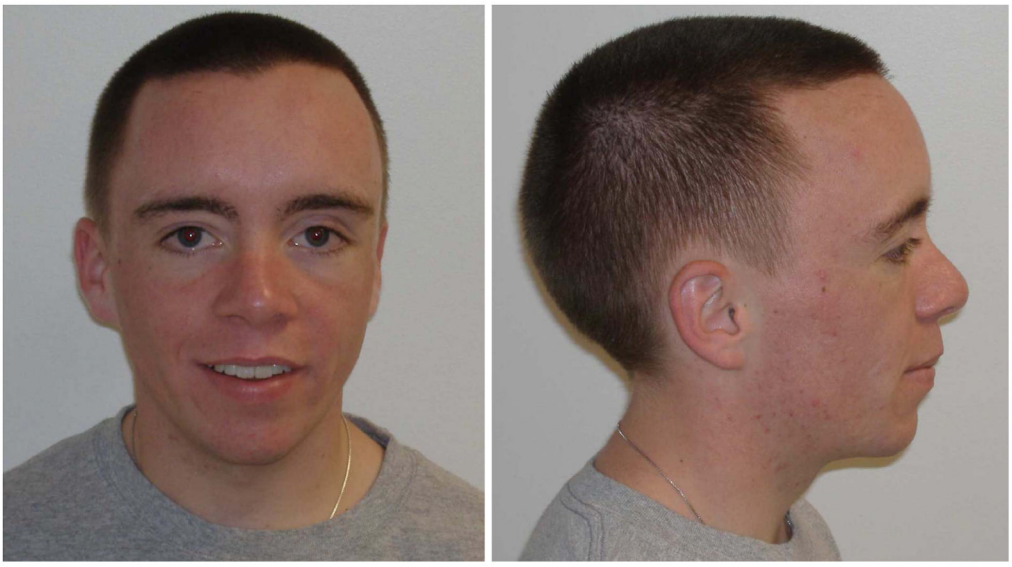

Figure.
Subject at 19 years of age. Many of the typical facial features of 3M syndrome, such as triangular face, prominent mouth and lips, pointed chin, and mid-face hypoplasia, are more prominent in younger children making the syndrome more difficult to diagnose in adulthood.

Table 1

Proband's Diagnostic Genetic Testing

Genetic Test	Cost
Karyotype	$315
Chromosomal Microarray	$1595
PTPN11 Gene Sequencing (Noonan Syndrome)	$1400
SOS1 Gene Sequencing (Noonan Syndrome)	$2400
FGFR3 Mutational Analysis (Achondroplasia/Hypochondroplasia)	$310
SHOX Gene Sequencing	$990
Russell-Silver Testing (H19 Methylation and Uniparental Disomy of Chromosome 7)	$900
Total Cost of Genetic Testing Performed	$7910
Clinical Exome Sequencing	$7000–7900

Costs are based on current pricing at various commercial laboratories in the United States. Research exome sequencing costs ~$1000.

Table II

Clinical and Radiographic Features of 3M Syndrome

	Typical 3M syndrome	**Our Subject**
Physical Exam Finding		
Birth Weight	Low	1850 grams (−3.7 SDS)
Birth Length	Severely low	33 cm (−6.8 SDS)
Postnatal Growth	Severely retarded, Final height typical −5 to −6 SDS	Adult height 146.6 cm (−4.17 SDS), weight 51.4 kg (−2.27 SDS)
Body Proportions	Normal	Upper to lower segment ratio normal at 0.93.
Head Size	Relatively large compared to body size	Large (Adult head circumference +2.4 SDS)
Intelligence	Normal	Normal
Characteristic Facies	Triangular face, hypoplastic midface, full eyebrows, fleshy nose tip, long philtrum, prominent mouth and lips, and pointed chin.	Mildly depressed midface, long philtrum, asymmetric forehead, and mild retrognathia. Triangular face more prominent as a child.
Feet	Prominent Heels	Prominent Heels and Pes Cavus
Joint Mobility	Increased joint laxity	Hyperextensible thumb but no increased laxity at wrists, elbows or knees.
Hypogonadism	Small testicular size	Small testicular size (5 cc and 8 cc as adult) with normal phallus and Tanner 5 pubic hair.
Radiographic findings		
Vertebral Bodies	Tall with reduced anterior-posterior and transverse diameter	Normal
Long Bones	Slender with widened metaphyses	Long “gracile” bones without widened metaphyses
Pelvic Bones	Small	Normal

For more extensive description see review by Muriel Holder-Espinasse (Reference 7 in main text).