Two Siblings with a Mutation in *CCDC8* Presenting with Mild Short Stature: A Mild Case of 3-M Syndrome

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Short Title: Mutation in CCDC8 Causing Mild Short Stature

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- **Established Facts:** 3-M syndrome is a growth disorder presenting with severe pre- and post-natal growth retardation and is caused by mutations in three genes (*CUL7*,

OBSL1, and *CCDC8*).

- **Novel Insights:** We describe two siblings with quite mild short stature who carry a homozygous pathogenic mutation in *CCDC8*causative of 3-M syndrome. This is the mildest case of 3-M syndrome reported to date and demonstrates the utility of genomic screening in children with undiagnosed growth disorders, especially from consanguineous families.

Abstract

Background: Short stature can be caused by mutations in a multitude of different genes. 3-M syndrome is a rare growth disorder marked by severe pre- and post-natal growth retardation along with subtle dysmorphic features. There have only been two prior reports of mutations in *CCDC8* causing 3-M syndrome.

Methods: Two patients presenting with mild short stature underwent whole exome sequencing. The mutation was confirmed via Sanger sequencing. We compare the clinical characteristics of our two patients to patients previously reported with mutations in the same gene.

Results: Exome sequencing identified a homozygous frameshift mutation in *CCDC8* in both patients. They presented with a much milder phenotype than previously described patients with the same mutation.

Conclusion: In this study, we report a case of two sisters with relatively mild short stature who were found via exome sequencing to carry a previously reported homozygous mutation in *CCDC8*. These patients expand the anthropometric phenotype of 3-M syndrome and demonstrate the power of exome sequencing in the diagnosis of children with short stature. 3-M syndrome should be considered in children with mild skeletal abnormalities, normal/high GH-IGF axis parameters and normal intelligence.

Introduction

3-M syndrome (MIM 273750, 612921 and 614205) was first recognized by Miller, McMusick and Malvaux in 1975, who described a rare autosomal recessive primordial dwarfism characterized by severe prenatal and postnatal growth retardation but with normal mental development [1]. Approximately 100 cases have been reported in the literature since 1975 [2]. Children are characteristically born extremely small for gestational age (SGA) and achieve a final height 5-6 SDS below the mean. Clinical characteristics include distinctive facial dysmorphism such as a triangular face, frontal bossing, midfacial hypoplasia, fleshy tipped nose, and full fleshy lips. Characteristic radiologic findings include slender long bones with diaphyseal constriction and flared metaphyses, tall vertebral bodies, anterior wedging of thoracic vertebral bodies, irregular upper and lower endplates, thoracic kyphoscoliosis, spina bifida occulta, small pelvic bones, small iliac wings, broad thorax with slender and horizontal ribs, and slightly delayed bone age. Prominent heels are most easily recognized in affected younger children. While the gonadal status of female patients with 3-M syndrome appears to be normal, male patients can develop gonadal failure with small testicular volumes, elevated gonadotropin concentrations, and sub- or infertility [3-4]. From an endocrine perspective, serum GH concentrations are usually normal and IGF-I concentrations are normal or low while response to growth hormone therapy is variable but typically poor [5].

3-M syndrome is a genetically heterogeneous condition with three causal genes currently identified. In 2005, autozygosity mapping revealed a 3-M syndrome locus on chromosome 6p21.1, and pathogenic mutations in CULLIN7 (*CUL7* [MIM609577]) were subsequently identified as the primary cause of 3-M syndrome [6]. A previous study showed that *CUL7* accounts for approximately 84% of cases of 3-M syndrome (52/62) [7]. CUL7 is a major structural component of E3 ubiquitin ligase, which is involved in the proteasomal degradation pathway targeting p53 and the IGF-1/insulin signaling molecule IRS-1[8]. In 2009, mutations in Obscurin-like 1 (*OBSL1* [MIM 610991]) were found as the second most common cause of 3-M syndrome [9]. *OBSL1* encodes a cytoskeletal adaptor protein which has been shown to

physically interact with *CUL7*, and loss-of-function mutations in *OBSL1* lead to decreased levels of *CUL7* [9]. A third 3-M causal gene was identified in 2011, in which 6 patients from 5 families carried truncating mutations in *CCDC8*, four of whom carried the same frameshift mutation p.K205EfsX59 [10]. In co-expression studies, CCDC8 was shown to directly interact with OBSL1 but not CUL7 [10]. However, similar to CUL7, CCDC8 acts as a co-factor for p53-induced apoptotic processes [8]. To date, there has only been one additional report of a single family with a mutation in *CCDC8*[11], and thus, the full clinical spectrum of patients with mutations in this gene is yet to be elucidated.

There are a multitude of genetic causes of short stature [12], many of which can be difficult to diagnose due to the subtlety of the associated clinical features. Next generation sequencing provides the opportunity to rapidly assess many genes and even the entire exome, facilitating the diagnosis of rare genetic conditions [13]. Herein, we report a case of two sisters who lacked an obvious syndromic cause for their relatively mild short stature, and, via exome sequencing, were found to carry the previously reported homozygous p.K205EfsX59 mutation in *CCDC8*. These patients have the mildest degree of short stature associated with 3-M syndrome to date and demonstrate the power of exome sequencing in the diagnosis of children with short stature.

Case Report:

Subject 1:

Subject 1 was born at 37 weeks' gestation with a pregnancy complicated by intrauterine growth restriction, with a birthweight of 2.72 kg (-1.4 SDS). Her antenatal and postnatal course was otherwise unremarkable and she was completely weaned from breastfeeding at one year of age. Her growth remained below the 3rd percentile throughout childhood (Figure 1A). Further investigations performed indicated a normal karyotype, coeliac screen, thyroid function tests, plasma amino acids, immunoglobulins, and faecal elastase. Her appetite appeared normal throughout this period, and there were no developmental concerns.

The subject is of Pakistani descent. Family history was significant for parental consanguinity.

Her maternal great-grandfather and paternal grandfather were brothers. Her younger sister was also referred to the paediatric endocrinology team for concerns regarding her growth, but she has two older brothers of normal height (188 cm and 175 cm). Her father is 180 cm and mother is 170 cm giving her a mid-parental target height of 168cm (75^{th}-90^{th} percentile).

Examination revealed proportionate short stature, mild clinodactyly, mild frontal bossing and depression of her nasal bridge. After the molecular diagnosis was made via exome sequencing (see below), a closer examination indicated a triangular face, exaggerated lumbar lordosis, upturned nares, fleshy nose and lips, and prominent heels, which, although mild in presentation, could, together with her skeletal survey findings and family history, be suggestive of 3-M syndrome. IGF-1 and IGFBP-3 concentrations were normal [119 ng/ml, (-0.8 SDS, normal range 49-283) and 2.3 mg/l (-0.6 SDS, normal range 1.0-4.7) respectively]. Height velocity remained normal throughout childhood. A skeletal survey revealed gracile long bones and ribs, pectus excavatum and spina bifida occulta with a vertebral ossification defect in L4-5 but normal vertebral height (Figure 2).

At the age of 11 years, Subject 1 started puberty with Tanner stage 2 breast development. A glucagon stimulation test was performed at this point due to her slightly suboptimal height velocity of 4.2 cm/year, but this revealed a normal GH peak of 15.2 ng/ml and a normal cortisol peak of 1070 nmol/l. The rest of her pituitary function screen was normal (free T_4 15.3 pmol/l (normal range 10.8-19.0), TSH 1.4 mU/l (normal range <6.0), LH <0.2 U/l, FSH 0.3 U/l, oestradiol <44 pmol/l, PRL 115 mU/l). Height at last follow-up (11.9 years) was 134.6 cm (-2.2 SDS), with a weight of 34.5 kg (-1.0 SDS), BMI 19.0 kg/m^2 (+0.4 SDS), and head circumference of 53.2 cm (-0.35 SDS).

Subject 2:

Subject 2, Subject 1's younger sister was born at 36 weeks' gestation with a pregnancy complicated by intrauterine growth restriction, with a birthweight of 2.27 kg (-2.6 SDS). She was noted to feed poorly and exhibited poor weight gain, only reaching 6.6 kg (-4.9 SDS) at

1.3 years of life when she was referred to paediatric endocrinology. Early clinical examination revealed frontal bossing and midfacial hypoplasia. Investigations indicated a normal karyotype, celiac screen, and thyroid function tests. IGF-1 and IGFBP-3 concentrations were normal [111 ng/ml (-1.0 SDS, normal range 51-303) and 2.6 mg/l (+0.3 SDS, normal range 0.8-3.9) respectively]. Height velocity remained normal throughout childhood (Figure 1B).

Further examination performed at 5.7 years of age revealed similar features to her sister, with upturned nares, fleshy nose and lips, long palpebral fissures and prominent heels. Repeat endocrine biochemistry was performed at 9.1 years due to a deceleration in height velocity but these investigations were all normal (IGF-1 111 ng/ml (-1.7 SDS, normal range 88-452), IGFBP-3 3.9 mg/l (-0.7 SDS, normal range 2.1-7.7), free T_4 15.6 pmol/l (normal range 10.8-19.0), TSH 2.6 mU/l (normal <6.0), LH <0.2 U/l, FSH 0.3 U/l, PRL 92 mU/l). A glucagon stimulation test demonstrated a normal GH peak of 29 ng/ml and a cortisol peak of 1286 nmol/l. Height at last follow-up (10.2 years) was 121.8 cm (-2.7 SDS), weight 21.3 kg (-2.9 SDS), BMI 14.4 kg/m^2 (-1.5 SDS), and head circumference 51.2 cm (-1.0 SDS).

Molecular analysis

Ethical approval for the protocol was granted by the Cincinnati Children's Hospital Medical Center Institutional Review Board. Written informed consent was obtained from the subjects and their parents. Both subjects underwent whole exome sequencing at Cincinnati Children's Hospital Medical Center using previously described methods [14]. Given the known consanguinity and two affected siblings with unaffected parents, we hypothesized that the causal variant would be a rare homozygous non-synonymous variant present in both subjects. We excluded all variants present with a minor allele frequency greater than 0.001 in the 1000 Genomes database (www.1000genomes.org), the Exome Aggregation Consortium browser (http://exac.broadinstitute.org), or our internal variant database. Only a single variant met these criteria, the homozygous p.K205EfsX59 mutation in *CCDC8*. We then confirmed this mutation via Sanger sequencing (Figure 3). PCR primers are available on request.

Discussion

To date, there have only been two reports describing a total of nine patients in six families carrying *CCDC8* mutations causing 3-M syndrome, and only 3 mutations were identified[10,11]. The typical description of 3-M syndrome includes severe prenatal (usual birth weight SD scores < -3SD) and postnatal growth retardation (adult height in the range of -8 to -4 SD) although the birth weight can fall within the lower part of the normal range [8]. Interestingly, patients with mutations in CCDC8 (i.e. 3-M syndrome type 3, MIM 614205) tend to have a milder phenotype with a mean height at presentation of -4.4 SD (Table 1). In this report, we describe two patients with a previously described homozygous frameshift mutation in *CCDC8* who present with a considerably milder phenotype than previously described (Table 1). Their current height SDS scores of -2.2 and -2.7 fall within the relatively mild-moderate short stature range and, notably, Subject 1 had a normal birth weight, thus lacking prenatal growth retardation. Unfortunately, birth length data were unavailable for these patients so it is possible that Subject 1 was small for gestational age based on length criteria. Notably, their parents were rather tall with a mid-parental target height of +1 SD, thus, their actual degree of growth failure is more significant when this is taken into account. This highlights the importance in considering the deviation from target height when assessing a patient's degree of short stature. Both subjects had normal head circumferences as is observed in 3-M syndrome. In retrospect, both subjects do exhibit mild features seen in 3-M syndrome including a slightly exaggerated lumbar lordosis, pectus excavatum and hypermobile finger joints, but these are relatively non-specific and did not raise suspicion for this rare diagnosis. The fleshy heels, a distinct finding in 3-M syndrome, became less prominent with time. Similarly, the radiological features were quite subtle and were not fully appreciated until after the molecular diagnosis was obtained.

It is interesting to note that both subjects had elevated peak growth hormone and normal cortisol concentrations during a glucagon stimulation test. Additionally, they had normal IGF-1 levels. Taken together, the relatively robust growth hormone levels with normal IGF-1

levels may suggest a degree of growth hormone and possibly IGF-1 resistance, as has been previously described in 3-M syndrome [5]. In other cases reported in the literature, two patients with 3-M syndrome have been shown to have complete or partial growth hormone deficiency, although the majority have a normal growth hormone axis on testing [5, 15-17]. The rest of their pituitary function (TSH, LH, FSH, and PRL) screening was normal.

These two girls are older than the *CCDC8* deficient patients reported in the previous study, and the elder sister has started puberty with no evidence of gonadal failure. Gonadal status of female patients with 3-M syndrome has previously been reported to be normal, while males may have hypergonadotropic hypogonadism. It is not clear whether mutations in *CCDC8* affect gonadal status or if this is only the case in individuals with *CUL7* mutations. Further follow-up of these patients is needed.

In conclusion, we report a case of two siblings carrying a previously reported homozygous frameshift mutation in *CCDC8* with mild short stature, one of whom had a normal birth weight, thus representing the mildest presentation of 3-M syndrome to date. An explanation for the same mutation causing highly variable clinical presentations remains to be fully elucidated, although it is important to consider the distance from the mid-parental target height when quantifying the degree of growth failure. 3-M syndrome should be considered in children with mild skeletal abnormalities, normal/high GH-IGF axis parameters and normal intelligence. We highlight the power of exome sequencing in children with short stature, which provides comprehensive genetic evaluation for patients who lack obvious specific molecular aetiologies.

Table 1. Clinical characteristics of the two affected subjects compared to patients previously reported with *CCDC8* mutations

Characteristic	Previous reported patients with *CCDC8* mutation (n=6) [10-11]	Subject 1	Subject 2
Birth weight	Range -2.2 to -6.3 SDS Mean -3.4 SDS	2720 grams (-1.4 SDS)	2270 grams (-2.6 SDS)
Postnatal Growth (height)	Range -3 to -6.7 SDS Mean -4.4 SDS	134.6 cm (-2.2 SDS, 11.9 years)	121.8 cm (-2.7 SDS, 10.2 years)
Postnatal Growth (weight)	Range -3 to -5.2 SDS Mean -3.8 SDS	34.5 kg (-1.0 SDS, 11.9 years)	21.3kg (-2.9 SDS, 10.2 years)
Head Circumference	4 out of 6 subjects with recorded HC were normal	53.2 cm (-0.35 SDS, 11.9 years)	51.2 cm (-1.0 SDS, 10.2 years)
Skeletal Survey	Tall vertebral bodies (5/9), slender long bones (5/9), short thorax (6/6)	Gracile long bones and ribs, pectus excavatum and spina bifida occulta with a vertebral ossification defect in L4-5 but normal vertebral height	Slightly exaggerated lumbar lordosis, pectus excavatum
Facial Features	Triangular face (7/9), fleshy tipped nose (9/9), frontal bossing (8/9), pointed chin (7/9), midface hypoplasia (6/9), long philtrum (1/6)	Triangular face, mild clinodactyly, upturned nares, fleshy nose and lips, mild frontal bossing and depression of her nasal bridge	Frontal bossing and midfacial hypoplasia, upturned nares, fleshy nose and lips, long palpebral fissures
Other Distinguishing Features	Prominent fleshy heels (6/9) and hypermobility of joints (2/9)	Fleshy heels and hypermobile finger joints	Fleshy heels and hypermobile finger joints
Puberty	NA	Tanner stage 2 breast development (11.9 years)	Pre-pubertal (10.2 years)

Figure 1: Growth charts of the two subjects. A. Subject 1. B. Subject 2.

Figure 2: Skeletal survey images of Subject 1 demonstrating (a) gracile ribs with evidence of pectus excavatum (oblique anterior rib angles and relatively horizontal posterior ribs), and (b-c) gracile long bones in the upper limb. The left humerus (b) is also bowed with slight expansion of the diaphysis. Similar long bone appearances were observed in the lower limbs (not shown).

Figure 3: Chromatograms showing a homozygous insertion of a guanine nucleotide in the two subjects.

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