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Further delineation of *CDC45*-related Meier-Gorlin syndrome with craniosynostosis and review of literature

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ABSTRACT

Meier-Gorlin syndrome (MGS) is a rare autosomal recessive disorder characterized by the triad of short stature, microtia and absent or small patellae. We report on a patient with MGS secondary to biallelic mutations in *CDC45* detected on whole exome sequencing (WES). Patients with MGS caused by mutations in *CDC45* display a distinct phenotype characterized by craniosynostosis and anorectal malformation. Our patient had craniosynostosis, anorectal malformation and short stature, but did not have the microtia or patella hypoplasia. Our report also highlights the value of WES in aiding diagnosis of patients with rare genetic diseases. In conclusion, our case report and review of the literature illustrates the unique features of *CDC45*-related MGS as well as the benefits of WES in reducing the diagnostic odyssey for patients with rare genetic disorders.

1. Introduction

Meier-Gorlin syndrome (MGS) (MIM 224690, 613800, 613803, 613804, 613805, 617063, 617564, 616835), also known as the "ear-patella-short stature syndrome", is a rare autosomal recessive disorder causing primordial dwarfism and is characterized by the triad of short stature, microtia and patellar aplasia/hypoplasia (de Munnik et al., 2015). This syndrome was first described by Meier and Rothschild in 1959 (Meier et al., 1959), and Gorlin further delineated it by describing a second patient with a similar phenotype in 1979 (Gorlin et al., 1975). Additional clinical features include pulmonary emphysema, feeding issues during infancy, abnormal genitalia and musculoskeletal abnormalities (de Munnik et al., 2012). To date, approximately 80 patients have been reported in the literature.

Currently, there are eight forms of Meier-Gorlin syndrome, each of them with a slightly different phenotype and are caused by mutations of different genes that are involved in DNA replication. Biallelic hypomorphic mutations in genes arising from various components of the pre-replication complex *ORC1* (MIM 601902)(Bicknell et al., 2011a; Bicknell et al., 2011b), *ORC4* (MIM 603056)(Bicknell et al., 2011; Guernsey et al., 2011), *ORC6* (MIM 607213)(Bicknell et al., 2011), *CDT1* (MIM 605525)(Bicknell et al., 2011), and *CDC6* (MIM 602627) (Bicknell et al., 2011), the helicase complex *MCM5* (MIM 602696) (Vetro et al., 2017) as well as *de novo* stabilizing mutations in the replication inhibitor *GMNN* (MIM 602842)(Burrage et al., 2015) have been implicated in MGS. More recently, biallelic mutations in the *CDC45* (MIM 603465), encoding a component of both the pre-initiation and helicase complex have also been found to cause MGS (Fenwick et al., 2016).

We report on a patient who presented in the neonatal period with

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List of abbreviations

ACMG	American College of Medical Genetics and Genomics
DNA	Deoxyribonucleic acid
HGVS	Human Genome Variation Society
MGS	Meier-Gorlin syndrome
OFC	Occipital frontal circumference
PSARP	Posterior sagittal anorectoplasty
SD	Standard deviation
WES	Whole exome sequencing

craniosynostosis, anorectal malformation and short stature. She was initially given a clinical diagnosis of Antley-Bixler syndrome (MIM 201750, 207410), however, genetic testing for Antley-Bixler syndrome was negative. With the aid of WES, after a prolonged diagnostic odyssey of 16 years, she was diagnosed with MGS caused by biallelic mutations in *CDC45*. Our patient did not present with the classical triad of clinical features seen in MGS - she did not have microtia or patella hypoplasia. Upon review of existing reports on MGS, we note that patients with *CDC45*-related MGS have a distinct phenotype, and in this report, we summarize these clinical features. This report also highlights the role of WES in reducing the diagnostic odyssey for patients with rare disorders (Monroe et al., 2016).

2. Case presentation

Our patient is the third female child of non-consanguineous Singaporean Chinese parents and was born well at 39 weeks of gestation with a birthweight of 2.29 kg (3rd centile) and length of 46 cm (10th centile) to a 29-year-old mother and 31-year-old father. Family history was unremarkable, and her siblings were healthy. Her mother had one previous miscarriage. Antenatal history was significant for vaginal thrush for which her mother was treated with topical

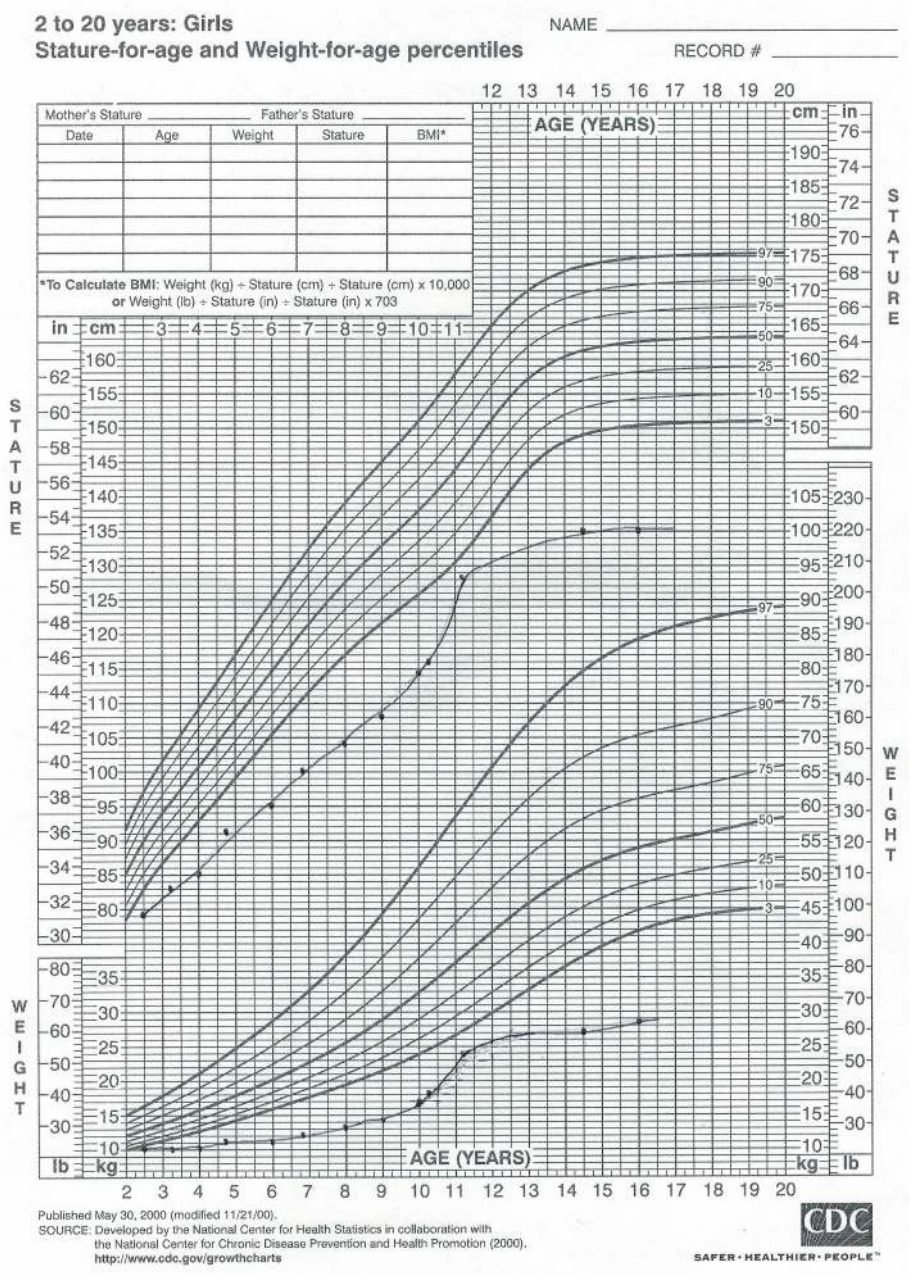

Fig. 1. Growth chart showing progressive growth failure.

fluconazole on two separate occasions. Screening ultrasound scan at 19 weeks was normal, however, a subsequent ultrasound scan done at 32 weeks gestation raised concerns about an abnormal head shape. Postnatally, she was found to have turricephaly secondary to bicoronal and lambdoidal sutures craniosynostosis. In addition, she had anorectal malformation with rectovesitibular fistula.

In view of her craniosynostosis, she underwent cranioplasty and fronto-orbital advancement at two months of age. She subsequently required three further cranio-facial surgeries within the first two years of life. Her anorectal malformation required a colostomy creation on day three of life, followed by posterior sagittal anorectoplasty (PSARP) at three months of life. Her colostomy was closed at seven months of age. In view of the abovementioned craniosynostosis and anorectal malformation, she was referred to a paediatric geneticist and based on her clinical phenotype, she was given a diagnosis of possible Antley-Bixler syndrome.

Her infancy was complicated by problems with oral feeding and she required tube feeding. In view of recurrent chest infections related to gastroesophageal reflux disease and nasogastric tube feeding, she underwent operative gastrostomy creation with gastrojejunostomy tube placement at five months of age. She was gradually transitioned to full oral feeds by two years of age. After removal of gastrostomy device, she had a persistent fistula that required surgical closure at ten years of age.

Developmental milestones were slightly delayed; she stood with support at fourteen months and walked a few steps unsupported at seventeen months. At two years, she was able to speak two-syllable words with a vocabulary of about 20 words. At two and a half years, she could speak in short phrases, but pronunciation was not clear. She was enrolled in a special school at two years where she received speech and physical therapy. At the age of four, she entered nursery and her development was on par with her peers. During her primary school years, she had some difficulty with English and Mathematics, and was found to have dyslexia. Otherwise, she was coping relatively well in a mainstream school curriculum though she did require learning support from school, and faced some challenges academically especially in English and Arts.

She was noted to be short since birth and was referred to the Endocrinology team for evaluation (Fig. 1). A glucagon stimulation test at four years of age showed a normal growth hormone response. She also underwent a short synacthen test at eight years of age, which did not show any evidence of adrenal insufficiency (in view of possibility of disordered steroidogenesis in children with Antley-Bixler syndrome). She started puberty at 12 years with a height of 133 cm. With a concern of short final height, there was an attempt to block puberty with Lucrin depot injection. However, the bone age advanced and she got her first menstruation at 13 years and reached final adult height of 135 cm with no height acceleration in puberty. This shows that short stature is an inherent feature and not related to growth hormone or estrogen deficiency.

At 16 years of age, her height was 135 cm (−4.2 SD) and weight was 28.6 kg (−3.7 SD). On examination, she was microcephalic with occipital frontal circumference (OFC) of 46 cm and had turribrachycephaly with healed surgical scars. Examination of her facial features revealed thin eyebrows, narrow palpebral fissures of her eyes, strabismus and high arched palate, but there was no microtia. There was bilateral clinodactyly of the fifth finger, syndactyly of the second and third toes as well as digital clubbing. She had bilateral pes planus and joint laxity, but no scoliosis and her patellae were normal. She also had breast hypoplasia and clitoromegaly. Cardiovascular, respiratory and neurological examinations were unremarkable.

3. Methodology

She was recruited for whole exome sequencing under an IRB approved research protocol (CIRB, 2014/571/F). DNA was extracted from peripheral leucocytes using Qiagen Gentra Puregene DNA extraction blood kit. Trio whole exome sequencing (WES) using Agilent SureSelect Human All Exon V5 on proband's and parent's DNA. Sequencing was performed on HiSeq 2500 as per manufacturer's protocol. The raw NGS sequencing reads from HiSeq machine were mapped onto the human reference genome, GRCh37 using BWA version 0.75a. Joint variant calling was performed on the trio samples using GATK version 3.7 in accordance with GATK Best Practices (Van der Auwera et al., 2013). The output VCF was then separated to SNP and indels for different filtering criteria for both types of variants. Lastly, this refined set of variants were annotated using ANNOVAR version dated June 3rd' 2018 with information such as coding or non-coding variant (Wang et al., 2010), known population allele frequencies (ExAC, 1000G, Singapore Exome Consortium) and *in-silico* predictions from variant consequence predicting tools (such as SIFT, Polyphen, MutationTaster, among others). Filtered variants were then assessed for concordance with the phenotype to identify candidate variants. Candidate variants were then confirmed by Sanger sequencing. All variants were reported according to Human Genome Variation Society (HGVS) nomenclature and classified based on American College of Medical Genetics and Genomics (ACMG) criteria for variant classification (Richards et al., 2015).

4. Results

Karyotype analysis done during the neonatal period was normal (46,XX). Chromosomal microarray analysis did not detect any clinically significant variants. A multi-gene panel for craniosynostosis syndromes and sequencing and deletion/duplication analysis for *POR* gene (Antley-Bixler syndrome) were negative.

WES identified biallelic variants (c.326_329dupTATA; p.V109fs (paternally inherited) and c.1117C > T; p.R373W (maternally inherited))in *CDC45* [NM_001178010.2]. These variants were at very low allele frequency (< 0.001%) in control databases (GNOMAD (Lek et al., 2016), 1000G (Genomes Project et al., 2015)) as well as our internal control database (http://beacon.prism-genomics.org/) (Bylstra et al., 2019) and had not been reported in disease mutation databases either (Clinvar (Landrum et al., 2014), HGMD (professional) (Stenson et al., 2014)). p.V109fs was predicted to lead to a frameshift leading to a premature termination 13 codons downstream, which would be predicted to lead to nonsense mediated decay. p.R373W alters a highly conserved residue across all vertebrates and was predicted to be deleterious by multiple *in silico* algorithms (Polyphen-2 (Adzhubei et al., 2010), MutationTaster (Schwarz et al., 2010) and PROVEAN (Choi et al., 2012)) (Table 1). Based on the ACMG variant classification criteria [10], the p.V109fs variant was classified as "pathogenic" - frameshift variant in a gene where LOF is a known mechanism of disease (category PVS1), absent or low frequency in controls (category PM2) and patient's phenotype is specific for a disease with a single genetic aetiology (category PP4); while the p.R373W variant was classified as "likely pathogenic" - absent or low frequency in controls (category PM2), detected in *trans* with a pathogenic variant (category PM3), multiple lines of computational evidence support a deleterious effect on the gene or gene product (category PP3), and patient's phenotype is specific for a disease with a single genetic aetiology (category PP4). Sanger validation confirmed the presence of these variants (Fig. 2). Of note, there were also no mutations in the common craniosynostosis

Table 1
Scores and conclusions from the *in silico* algorithms.

In silico algorithms	Score	Interpretation
Polyphen-2	0.971	Probably damaging
MutationTaster	NA	Disease causing
Provean	−3.58	Deleterious
LRT	–	Damaging
GERP	3.12	–
SIFT	0.14	Tolerated

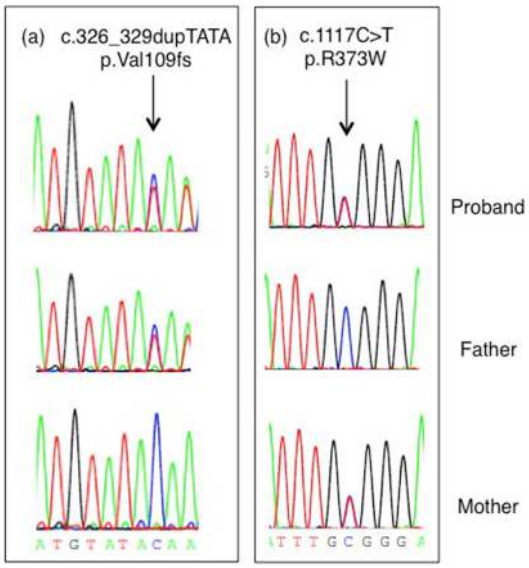

Fig. 2. Sanger chromatogram showing the paternally inherited c.326_329dupTATA and maternally inherited c.1117C > T variants in *CDC45* [NM_001178010.2].

genes (*FGFR2, FGFR3, TWIST1, FGFR1, ERF, GLI3, MEGF8, MSX2* and *RAB23)*.

5. Discussion

In this report, we present our patient, a 16-year-old Singaporean Chinese girl, with features consistent with *CDC45*-related MGS. The prevalence of MGS is estimated to be less than 1–9/1,000,000, although this might be an under-estimation due to under-diagnosis (de Munnik et al., 2015). To our knowledge, she is the first patient to be reported from South-East Asia.

Biallelic hypomorphic mutations in five genes from the pre-replication complex, namely *ORC1, ORC4, ORC6, CDT1* and *CDC6*, have been implicated in MGS (de Munnik et al., 2015). Recently, mutations in *GMNN, MCM5* and *CDC45* genes have also been found to cause MGS. The pre-replication complex is formed at the origins of DNA replication and is essential in the initiation of genome replication (de Munnik et al., 2012). The complex is comprised of the origin recognition complex (encompassing the subunits *ORC1* to *ORC6*), regulatory proteins (*CDC6* and *CDT1*) and the helicase complex (*MCM* proteins) (de Munnik et al., 2012). *GMNN* is an inhibitor of *CDT1* (Fenwick et al., 2016). The impaired function of the pre-replication complex limits DNA replication during the G1-S phase of the cell cycle, thus affecting cellular proliferation. This results in a reduction in total cell number, thereby resulting in decreased growth. *CDC45* encodes a component of both the pre-initiation and helicase complexes, which is involved in initiating DNA replication origin firing and ongoing DNA synthesis during the S-phase of the cell cycle (Fenwick et al., 2016). Although, impairment in DNA replication and subsequent impairment in cellular proliferation can be hypothesized as the likely mechanism of growth failure in *CDC45*-related MGS, this has not been demonstrated experimentally in the literature. In addition, other pathomechanisms, including abnormalities in cilia signaling and resulting ciliary dysfunction may underlie the developmental issues such as craniosynostosis, anal malformations and growth failure (Stiff et al., 2013).

Patients with MGS may present with variable features, and the classical triad of features (short stature, microtia and patella aplasia/hypoplasia) was found to be present in 82% of patients with MGS (Shawky et al., 2014). In fact, our patient only had short stature. Table 2 illustrates and compares the clinical features of the various subtypes of MGS. Individuals with *CDC45*-related MGS have a slightly different phenotype as compared to MGS caused by the other genetic mutations. Two of the clinical features that are distinctively associated with *CDC45*-related MGS are craniosynostosis and anorectal malformations, both of which were present in our patient. In addition, patients with *CDC45*-related MGS may present with pure craniosynostosis syndrome (Fenwick et al., 2016). Table 3 illustrates the features of MGS described in some of the available literature and compares these with the features present in our patient. Our patient has majority of the features present in other patients with *CDC45*-related MGS such as strabismus, high arched palate, syndactyly of second and third toes. However, she does not have any cardiac abnormalities that is seen in 40% of patients with *CDC45*-related MGS (Fenwick et al., 2016). She also had feeding problems requiring enteral feeding, which has not been previously described in patients with *CDC45*-related MGS, but was described in other patients with MGS (de Munnik et al., 2012).

Individuals with *CDC45*-related MGS develop craniosynostosis due to disruption of cranial suture homeostasis. Our patient was initially given the diagnosis of Antley-Bixler syndrome in light of her extensive craniosynostosis. As illustrated in Table 3, twelve out of fifteen (80%) of individuals with *CDC45*-related MGS had craniosynostosis, in contrast to only 4% of individuals with MGS caused by the mutations in other genes. Types of craniosynostosis which have been described include lambdoid, unicoronal or bicoronal and sagittal craniosynostosis (Fenwick et al., 2016).

Neurologically, individuals with MGS tend to have normal intellect, despite their microcephaly. Previous case studies on individuals with MGS have found that 95–97% of them have normal intellect, and delayed motor development and/or speech development without intellectual disability were present in 19% and 16% of those individuals respectively (de Munnik et al., 2012). Our patient attends a mainstream secondary school, although she had some academic difficulties due to dyslexia.

Anorectal malformation is also unique to *CDC45*-related MGS. The malformations can include anterior anus, anal stenosis and imperforate anus (Fenwick et al., 2016). In individuals with MGS caused by other genetic mutations, gastrointestinal manifestations include mainly gastroesophageal reflux and feeding problems in early infancy, and anorectal malformations have not been described in any of those patients. Our patient had imperforate anus with rectovestibular fistula that required surgical management as well as gastroesophageal reflux and feeding problems in infancy requiring tube feeding.

For MGS, growth failure tends to be progressive. A study on 45 patients with MGS showed that postnatal growth was delayed during the first year of life, and growth velocity was normal thereafter, hence these patients do not have significant catch-up growth (de Munnik et al., 2015). *ORC1* mutations were found to cause the most severe growth failure and microcephaly, followed by *ORC4* mutations (Shawky et al., 2014). Our patient's growth trend is illustrated in Fig. 1. The role of growth hormone in patients with MGS is equivocal. Patients with MGS can have suboptimal or even elevated growth hormone levels, thus suggesting that the short stature is due to the underlying molecular defect rather than growth hormone deficiency (Shawky et al., 2014). As such, growth hormone treatment may not always be useful, especially in those patients with a known mutation. Growth hormone therapy was started in eight patients with genetic mutations and two of them showed positive response (de Munnik et al., 2012). Our patient had undergone a glucagon stimulation test and was found to have a normal growth hormone response, thus was not given growth hormone.

In terms of secondary sexual characteristics and pubertal development, it was found that all post pubertal females who were diagnosed with MGS had mammary hypoplasia (de Munnik et al., 2015). Treatment with exogenous oestrogen helped in breast development in two siblings with *ORC4* mutations. Sparse or absent axillary hair was also reported in 75% of post-pubertal individuals with MGS (de Munnik et al., 2012). Our patient also has mammary hypoplasia, though her

Table 2
Table comparing clinical features of different phenotypes of MGS (Bicknell et al., 2011a; Bicknell et al., 2011b; Guernsey et al., 2011; Vetro et al., 2017; Burrage et al., 2015; Fenwick et al., 2016).

Phenotype	MGS 1	MGS 2	MGS 3	MGS 4	MGS 5	MGS 6	MGS 7	MGS 8
OMIM number	224690	613800	616803	613804	613805	613835	617063	617564
Gene involved	ORC1	ORC4	ORC6	CDT1	CDC6	GMNN	CDC45L	MCM5
Inheritance	AR	AR	AR	AR	AR	AD	AR	AR
Growth								
Short stature	✓	✓	✓	✓	✓	✓	✓	✓
Failure to thrive	✓	✓	✓	✓	✓	✓	✓	✓
Head								
Microcephaly	✓	✓	✓	✓	✓	✓	✓	✓
Triangular face			✓		✓			
Craniosynostosis							✓	
Micrognathia	✓	✓			✓	✓		✓
Mandibular hypopolasia	✓	✓	✓	✓	✓			
Microtia	✓	✓	✓	✓	✓	✓	✓	✓
Hearing loss	✓					✓	✓	
Strabismus	✓					✓	✓	
Short palpebral fissures	✓							
Entropion						✓		
High nasal bridge			✓					
Long philtrum					✓			
Small mouth	✓	✓	✓	✓			✓	
Cleft palate	✓					✓	✓	
High arched palate	✓							
Respiratory								
Broncho/ tracheomalacia		✓	✓			✓		
Emphysema (congenital)	✓			✓				
Chest								
Pectus carinatum	✓							
Hooked clavicles	✓			✓				
Short ribs	✓		✓					
Breast hypoplasia	✓	✓	✓	✓			✓	
ASD/ VSD							✓	
Abdomen								
Feeding problems in infancy	✓	✓	✓	✓	✓	✓		
Gastroesophageal reflux	✓	✓	✓		✓	✓		
Anorectal malformation							✓	
Genitourinary								
Micropenis	✓		✓		✓		✓	
Cryptochidism	✓		✓		✓	✓	✓	✓
Clitoromegaly	✓	✓	✓				✓	
Hypoplastic labia minora and majora	✓	✓	✓			✓		
Renal hypoplasia								✓
Skeletal								
Joint laxity	✓	✓			✓			
Genu varum	✓		✓					
Aplastic or hypoplastic patellae	✓	✓	✓	✓	✓	✓	✓	✓
Camptodactyly	✓	✓						
Fifth finger clinodactyly	✓		✓		✓			
Club feet			✓					
Slender long bones	✓	✓	✓	✓	✓			
Excessive lumbar lordosis						✓		
Scoliosis							✓	
Hip dysplasia						✓		
Others								
Developmental delay	✓		✓			✓	✓	
High pitched voice		✓						
Absent or sparse axillary/ pubic hair			✓					
Hyperconvex nails	✓							

Key: ASD – Atrial septal defect, VSD – Ventricular septal defect

Shaded rows indicate clinical features that are found in all phenotypes of MGS
✓ in bold indicates clinical features that are found in that particular phenotype of MGS only

Table 3
Clinical features of Meier-Gorlin syndrome described in the literature as compared to our patient A: Genotype-phenotype study in 45 individuals with MGS (de Munnick et al., 2012) B: Clinical evaluation of 15 patients with *CDC45*-related MGS (Fenwick et al., 2016).

Clinical Features	A: de Munnick et al., 2012	B: Fenwick et al., (2016)	Our patient
Classical triad of clinical features			
Short stature (height for age < −2 SD)	40/45 (89%)	10/13 (77%)	+
Microtia	44/45 (98%)	13/15 (87%)	–
Patella hypoplasia/aplasia	39/42 (93%)	8/11 (73%)	–
Facial features			
Microcephaly (OFC < -2 SD)	16/39 (41%)	12/13 (92%)	+
Craniosynostosis	2/45 (4%)	12/15 (80%)	+
Thin eyebrows	NA	15/15 (100%)	+
Strabismus	NA	1/15 (7%)	+
Low set ears	24/38 (63%)	2/15 (13%)	–
Posteriorly rotated ears	13/27 (48%)	NA	–
Convex nasal profile	13/23 (57%)	NA	–
Choanal atresia	1/45 (2%)	1/15 (7%)	Choanal stenosis
Full lips	30/40 (75%)	NA	–
High arched palate	NA	4/15 (27%)	+
Micro/retrognathia	35/39 (90%)	NA	–
Neurological			
Intellectual disability	2/41 (5%)	NA	–
Delayed motor development	10/42 (24%)	NA	+
Delayed speech development	8/42 (19%)	NA	+
Respiratory			
Respiratory problems during infancy	16/33 (48%)	NA	–
Pulmonary emphysema	13/38 (34%)	NA	–
Pulmonary hypoplasia	NA	1/15 (7%)	–
Tracheomalacia	11/34 (32%)	NA	–
Cardiac anomalies	2/40 (5%)	6/15 (40%)	-
Gastrointestinal			
Feeding problems during infancy	36/42 (86%)	NA	+
Nasogastric feeding/gastrostomy	17/41 (42%)	NA	+
Failure to thrive	13/41 (32%)	9/12 (75%)	+
Gastroesophageal reflux	15/41 (37%)	NA	+
Anorectal malformation	NA	7/15 (47%)	+
Urogenital anomalies			
Micropenis/hypospadias/cryptorchidism/small testes	12/17 (71%)	3/7 (43%)	NA
Clitoromegaly	3/28 (11%)	1/8 (13%)	+
Renal anomalies	3/45 (7%)	2/15 (13%)	–
Secondary sexual characteristics			
Mammary hypoplasia	43/43 (100%)	1/8 (13%)	+
Absent or sparse axillary/pubic hair	9/12 (75%)	NA	–
Musculoskeletal anomalies			
Genu recurvatum	9/35 (26%)	NA	–
Scoliosis	NA	1/15 (7%)	–
Syndactyly of second, third and fourth fingers	NA	1/15 (7%)	–
Syndactyly of second and third toes	NA	1/15 (7%)	+
Digital clubbing	NA	1/15 (7%)	+
Others	4 dislocated joints, 2 bifid uvula, 1 cleft palate	1 meconium peritonitis, 1 cleft palate, 1 bowed legs, 1 polydactyly	Clinodactyly, bilateral pes planus

axillary hair growth was normal. Individuals with MGS were also known to have minor genital anomalies including micropenis, cryptorchidism, hypospadias in males and clitoromegaly, hypoplastic labia minora in females. Our patient was noted to have clitoromegaly on examination.

Absent or hypoplastic patella is one of the key features of MGS, but this was not seen in our patient. In addition, there are also musculoskeletal anomalies including syndactyly of the second and third fingers and/or toes, polydactyly, scoliosis and joint dislocation (de Munnik et al., 2012; Fenwick et al., 2016). Respiratory features described in individuals with MGS include pulmonary emphysema, tracheomalacia, laryngomalacia and bronchomalacia. Specifically, for *CDC45*-related MGS, pulmonary hypoplasia has been described (Fenwick et al., 2016). Cardiovascular abnormalities that have been described in MGS include atrial septal defect, ventricular septal defect, atrioventricular canal and atrioventricular conduction block (Shawky et al., 2014; Fenwick et al., 2016). These were not seen in our patient.

WES has proven to be very useful in aiding the diagnosis of rare genetic conditions that otherwise might remain undiagnosed using traditional single-gene or panel testing or microarray analysis. WES has a particular diagnostic advantage in situations where there is genetic heterogeneity, incomplete or atypical clinical presentation or as-yet undiscovered causal gene. There is also a large cost advantage from avoiding numerous investigations and hospital admissions which arise from a delay in diagnosis. A study investigating 17 patients' trio-WES yield with the retrospective costs of diagnostic procedures showed that WES resulted in diagnostically useful outcomes in 29.4% of patients, and also resulted in average cost savings of $1727 for genetic investigations in undiagnosed patients (Monroe et al., 2016). Just like our patient, who waited for 16 years and underwent multiple investigations with multiple genetic tests, WES finally provided the answer and ended her prolonged search and diagnostic odyssey. This would also be useful in genetic counselling with regards to future pregnancies.

6. Conclusion

In conclusion, we report a patient who presents with multiple clinical features described in *CDC45*-related MGS. In this case, the use of WES was integral in reaching the diagnosis given the non-specific nature of her presentation, under-recognition of this rare genetic

syndrome as well as the fact that *CDC45* mutation causing MGS was only first described in the literature in 2016.

Conflicts of interest

The authors declare that they have no conflict of interest.

Ethical approval

Patient and her family were recruited for whole exome sequencing under an IRB approved research protocol (CIRB, 2014/571/F)

Author contributions

All authors were involved in the management of this patient. CY Ting reviewed the literature and drafted the manuscript. SS Jamuar revised the manuscript and supervised the process. The other authors reviewed and revised the manuscript. All authors approved the final manuscript as submitted.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmg.2019.04.009.

References

Adzhubei, I.A., Schmidt, S., Peshkin, L., Ramensky, V.E., Gerasimova, A., Bork, P., Kondrashov, A.S., Sunyaev, S.R., 2010. A method and server for predicting damaging missense mutations. Nat. Methods 7 (4), 248–249.

Bicknell, L.S., Bongers, E.M., Leitch, A., Brown, S., Schoots, J., Harley, M.E., Aftimos, S., Al-Aama, J.Y., Bober, M., Brown, P.A., van Bokhoven, H., Dean, J., Edrees, A.Y., Feingold, M., Fryer, A., Hoefsloot, L.H., Kau, N., Knoers, N.V., Mackenzie, J., Opitz, J.M., Sarda, P., Ross, A., Temple, I.K., Toutain, A., Wise, C.A., Wright, M., Jackson, A.P., 2011a. Mutations in the pre-replication complex cause Meier-Gorlin syndrome. Nat. Genet. 43, 356–359.

Bicknell, L.S., Walker, S., Klingseisen, A., Stiff, T., Leitch, A., Kerzendorfer, C., Martin, C.A., Yeyati, P., Al Sanna, N., Bober, M., Johnson, D., Wise, C., Jackson, A.P., O'Driscoll, M., Jeggo, P.A., 2011b. Mutations in ORC1, encoding the largest subunit of the origin recognition complex, cause microcephalic primordial dwarfism resembling Meier-Gorlin syndrome. Nat. Genet. 43, 350–355.

Burrage, L.C., Charng, W.L., Eldomery, M.K., Willer, J.R., Davis, E.E., Lugtenberg, D., Zhu, W., Leduc, M.S., Akdemir, Z.C., Azamian, M., Zapata, G., Hernandez, P.P., Schoots, J., de Munnik, S.A., Roepman, R., Pearring, J.N., Jhangiani, S., Katsanis, N., Vissers, L.E., Brunner, H.G., Beaudet, A.L., Rosenfeld, J.A., Muzny, D.M., Gibbs, R.A., Eng, C.M., Xia, F., Lalani, S.R., Lupski, J.R., Bongers, E.M., Yang, Y., 2015. De novo GMNN mutations cause autosomal-dominant primordial dwarfism associated with meier-gorlin syndrome. Am. J. Hum. Genet. 97, 904–913.

Bylstra, Y., Kuan, J.L., Lim, W.K., Bhalshankar, J.D., Teo, J.X., Davila, S., Teh, B.T., Rozen, S., Tan, E.C., Liew, W.K.M., Yeo, K.K., Tan, P., group SinGapore Incidental Finding study,Saw, S.M., Cheng, C.Y., Cook, S., Foo, R., Jamuar, S.S., 2019. Population genomics in South East Asia captures unexpectedly high carrier frequency for treatable inherited disorders. Genet. Med. 21, 207–212.

Choi, Y., Sims, G.E., Murphy, S., Miller, J.R., Chan, A.P., 2012. Predicting the functional effect of amino acid substitutions and indels. PLoS One 7, e46688.

de Munnik, S.A., Hoefsloot, E.H., Roukema, J., Schoots, J., Knoers, N.V., Brunner, H.G., Jackson, A.P., Bongers, E.M., 2015. Meier-Gorlin syndrome. Orphanet J. Rare Dis. 10, 114.

de Munnik Sonja A., Louise S. Bicknell, Salim Aftimos, Jumana Y. Al-Aama, Yolande van Bever, Michael B. Bober, Jill Clayton-Smith, Alaa Y. Edrees, Murray Feingold, Alan Fryer, Johanna M. van Hagen, Raoul C. Hennekam, Maaike C. E. Jansweijer, Diana Johnson, Sarina G. Kant, John M. Opitz, A. Radha Ramadevi, Willie Reardon, Alison Ross, Pierre Sarda, Constance T. R. M. Schrander-Stumpel, Jeroen Schoots, I. Karen Temple, Paulien A. Terhal, Annick Toutain, Carol A. Wise, Michael Wright, David L. Skidmore, Mark E. Samuels, Lies H. Hoefsloot, Nine V. A. M. Knoers, Han G. Brunner, Andrew P. Jackson, and Ernie M. H. F. Bongers. 2012. 'Meier–Gorlin syndrome genotype–phenotype studies: 35 individuals with pre-replication complex gene mutations and 10 without molecular diagnosis', Eur. J. Hum. Genet., 20: 598.

Fenwick, A.L., Kliszczak, M., Cooper, F., Murray, J., Sanchez-Pulido, L., Twigg, S.R., Goriely, A., McGowan, S.J., Miller, K.A., Taylor, I.B., Logan, C., Bozdogan, S., Danda, S., Dixon, J., Elsayed, S.M., Elsobky, E., Gardham, A., Hoffer, M.J., Koopmans, M., McDonald-McGinn, D.M., Santen, G.W., Savarirayan, R., de Silva, D., Vanakker, O., Wall, S.A., Wilson, L.C., Yuregir, O.O., Zackai, E.H., Ponting, C.P., Jackson, A.P., Wilkie, A.O., Niedzwiedz, W., Bicknell, L.S., 2016. Mutations in CDC45, encoding an essential component of the pre-initiation complex, cause meier-gorlin syndrome and craniosynostosis. Am. J. Hum. Genet. 99, 125–138.

Genomes Project, Consortium, Auton, A., Brooks, L.D., Durbin, R.M., Garrison, E.P., Kang, H.M., Korbel, J.O., Marchini, J.L., McCarthy, S., McVean, G.A., Abecasis, G.R., 2015. A global reference for human genetic variation. Nature 526, 68–74.

Gorlin, R.J., Cervenka, J., Moller, K., Horrobin, M., Witkop Jr., C.J., 1975. Malformation syndromes. A selected miscellany. Birth Defects Orig. Artic. Ser. 11, 39–50.

Guernsey, D.L., Matsuoka, M., Jiang, H., Evans, S., Macgillivray, C., Nightingale, M., Perry, S., Ferguson, M., LeBlanc, M., Paquette, J., Patry, L., Rideout, A.L., Thomas, A., Orr, A., McMaster, C.R., Michaud, J.L., Deal, C., Langlois, S., Superneau, D.W., Parkash, S., Ludman, M., Skidmore, D.L., Samuels, M.E., 2011. Mutations in origin recognition complex gene ORC4 cause Meier-Gorlin syndrome. Nat. Genet. 43, 360–364.

Landrum, M.J., Lee, J.M., Riley, G.R., Jang, W., Rubinstein, W.S., Church, D.M., Maglott, D.R., 2014. ClinVar: public archive of relationships among sequence variation and human phenotype. Nucleic Acids Res. 42, D980–D985.

Lek, M., Karczewski, K.J., Minikel, E.V., Samocha, K.E., Banks, E., Fennell, T., O'Donnell-Luria, A.H., Ware, J.S., Hill, A.J., Cummings, B.B., Tukiainen, T., Birnbaum, D.P., Kosmicki, J.A., Duncan, L.E., Estrada, K., Zhao, F., Zou, J., Pierce-Hoffman, E., Berghout, J., Cooper, D.N., Deflaux, N., DePristo, M., Do, R., Flannick, J., Fromer, M., Gauthier, L., Goldstein, J., Gupta, N., Howrigan, D., Kiezun, A., Kurki, M.I., Moonshine, A.L., Natarajan, P., Orozco, L., Peloso, G.M., Poplin, R., Rivas, M.A., Ruano-Rubio, V., Rose, S.A., Ruderfer, D.M., Shakir, K., Stenson, P.D., Stevens, C., Thomas, B.P., Tiao, G., Tusie-Luna, M.T., Weisburd, B., Won, H.H., Yu, D., Altshuler, D.M., Ardissino, D., Boehnke, M., Danesh, J., Donnelly, S., Elosua, R., Florez, J.C., Gabriel, S.B., Getz, G., Glatt, S.J., Hultman, C.M., Kathiresan, S., Laakso, M., McCarroll, S., McCarthy, M.I., McGovern, D., McPherson, R., Neale, B.M., Palotie, A., Purcell, S.M., Saleheen, D., Scharf, J.M., Sklar, P., Sullivan, P.F., Tuomilehto, J., Tsuang, M.T., Watkins, H.C., Wilson, J.G., Daly, M.J., MacArthur, D.G., Consortium Exome Aggregation, 2016. Analysis of protein-coding genetic variation in 60,706 humans. Nature 536, 285–291.

Meier, Z., Poschiavo, Rothschild, M., 1959. [Case of arthrogryposis multiplex congenita with mandibulofacial dysostosis (Franceschetti syndrome)]. Helv. Paediatr. Acta 14, 213–216.

Monroe, G.R., Frederix, G.W., Savelberg, S.M., de Vries, T.I., Duran, K.J., van der Smagt, J.J., Terhal, P.A., van Hasselt, P.M., Kroes, H.Y., Verhoeven-Duif, N.M., Nijman, I.J., Carbo, E.C., van Gassen, K.L., Knoers, N.V., Hovels, A.M., van Haelst, M.M., Visser, G., van Haaften, G., 2016. Effectiveness of whole-exome sequencing and costs of the traditional diagnostic trajectory in children with intellectual disability. Genet. Med. 18, 949–956.

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W.W., Hegde, M., Lyon, E., Spector, E., Voelkerding, K., Rehm, H.L., Acmg Laboratory Quality Assurance Committee, 2015. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of medical genetics and genomics and the association for molecular pathology. Genet. Med. 17, 405–424.

Schwarz, J.M., Rodelsperger, C., Schuelke, M., Seelow, D., 2010. MutationTaster evaluates disease-causing potential of sequence alterations. Nat. Methods 7, 575–576.

Shawky, Rabah M., Heba Salah, Abd-Elkhalek Elabd, Gamal, Radwa, Abdelsattar Mohammad, Shaimaa, Gad, Shaimaa, 2014. Meier-Gorlin syndrome: report of an additional patient with congenital heart disease. Egyptian Journal of Medical Human Genetics 15, 393–398.

Stenson, P.D., Mort, M., Ball, E.V., Shaw, K., Phillips, A., Cooper, D.N., 2014. The Human Gene Mutation Database: building a comprehensive mutation repository for clinical and molecular genetics, diagnostic testing and personalized genomic medicine. Hum. Genet. 133, 1–9.

Stiff, T., Alagoz, M., Alcantara, D., Outwin, E., Brunner, H.G., Bongers, E.M., O'Driscoll, M., Jeggo, P.A., 2013. Deficiency in origin licensing proteins impairs cilia formation: implications for the aetiology of Meier-Gorlin syndrome. PLoS Genet. 9, e1003360.

Van der Auwera, G.A., Carneiro, M.O., Hartl, C., Poplin, R., Del Angel, G., Levy-Moonshine, A., Jordan, T., Shakir, K., Roazen, D., Thibault, J., Banks, E., Garimella, K.V., Altshuler, D., Gabriel, S., DePristo, M.A., 2013. From FastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. Curr Protoc Bioinformatics 43 11 10 1-33.

Vetro, A., Savasta, S., Russo Raucci, A., Cerqua, C., Sartori, G., Limongelli, I., Forlino, A., Maruelli, S., Perucca, P., Vergani, D., Mazzini, G., Mattevi, A., Stivala, L.A., Salviati, L., Zuffardi, O., 2017. MCM5: a new actor in the link between DNA replication and Meier-Gorlin syndrome. Eur. J. Hum. Genet. 25, 646–650.

Wang, K., Li, M., Hakonarson, H., 2010. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. 38, e164.