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Biallelic and *De Novo* Variants in *DONSON* Reveal a Clinical Spectrum of Cell Cycle-opathies with Microcephaly, Dwarfism and Skeletal Abnormalities

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Abstract

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Conflicts of Interest

J.R.L. has stock ownership in 23andMe, is a paid consultant for Regeneron, and a co-inventor on multiple United States and European patents related to molecular diagnostics for inherited neuropathies, eye diseases and bacterial genomic fingerprinting. The other authors declare no conflict of interest.

Co-occurrence of primordial dwarfism and microcephaly together with particular skeletal findings are seen in a wide range of Mendelian syndromes including microcephaly micromelia syndrome (MMS, OMIM 251230), microcephaly, short stature, and limb abnormalities (MISSLA, OMIM 617604), and microcephalic primordial dwarfisms (MPDs). Genes associated with these syndromes encode proteins that have crucial roles in DNA replication or in other critical steps of the cell cycle that link DNA replication to cell division. We identified four unrelated families with 5 affected individuals having biallelic or *de novo* variants in *DONSON* presenting with a core phenotype of severe short stature (z score < −3 SD), additional skeletal abnormalities, and microcephaly. Two apparently unrelated families with identical homozygous c.631C>T p.(Arg211Cys) variant had clinical features typical of Meier-Gorlin syndrome (MGS), while two siblings with compound heterozygous c.346delG p.(Asp116Ile*62) and c.1349A>G p.(Lys450Arg)variants presented with Seckel-like phenotype. We also identified a *de novo* c.683G>T p.(Trp228Leu) variant in *DONSON* in a patient with prominent micrognathia, short stature and hypoplastic femur and tibia, clinically diagnosed with Femoral-Facial syndrome (FFS, OMIM 134780). Biallelic variants in *DONSON* have been recently described in individuals with microcephalic dwarfism. These studies also demonstrated that DONSON has an essential conserved role in the cell cycle. Here we describe novel biallelic and *de novo* variants that are associated with MGS, Seckel-like phenotype and FFS, the last of which has not been associated with any disease gene to date.

INTRODUCTION

The cell cycle involves a highly orchestrated series of events that occur during an individual cell's lifespan, encompassing genome replication and ending in the generation of two daughter cells each comprising the same finite genomic variants as their mother cell. Maintaining the fidelity of this process is essential during prenatal and postnatal life and is important to both developmental processes and differentiated tissue and organ system maintenance. DNA replication and damage checkpoint pathways guard genomic integrity throughout each critical phase of the cell cycle (Elledge 1996). Aberrations in the regulation and processing of the cell cycle of somatic cells have been shown to be associated with familial cancer syndromes, such as Li-Fraumeni syndrome (OMIM 151623) caused by pathogenic variants in *TP53*. Perturbations of DNA replication or the cell cycle also play a role in a wide range of Mendelian diseases, including "microcephalic primordial dwarfisms" (MPDs), with a core phenotype of pre- and postnatal growth restriction and microcephaly, with or without skeletal abnormalities. Microcephalic osteodysplastic primordial dwarfism (MOPD, OMIM 210710), Meier Gorlin syndrome (MGS, OMIM 224690), Seckel syndrome (SS), microcephaly-micromelia syndrome (MMS; MIM: 251230), and microcephaly, short stature and limb abnormalities (MISSLA; MIM: 617604) are all forms of MPD (Evrony et al. 2017) (Figure 1).

To date, biallelic variants in components of the pre-replication complex *ORC1*, *ORC4*, *ORC6*, *CDT1*, *CDC6*, *CDC45*, and more recently *MCM5*, which is a component of the helicase complex, have been associated with MGS; in addition, *de novo* mutations in *GMNN* were identified in patients with MGS providing the first description of dominantly inherited MGS (Burrage et al. 2015; Vetro et al. 2017). Similarly, MOPD type I (MOPD1,

OMIM 210710) is associated with *RNU4ATAC* encoding a protein that is involved in the splicing of many mRNAs that encode DNA replication related proteins (Edery et al. 2011; He et al. 2011), while MOPD type II (MOPD2, OMIM 210720) is caused by mutations in *PCTN* which encodes a centrosomal protein essential for formation of the mitotic spindle and proper chromosome segregation (Rauch et al. 2008). Seckel syndrome is caused by mutations in TRAIP, CEP63, ATR, NSMCE2, DNA2, CENPJ, NIN, CEP152, and RBBP8, encoding proteins that have crucial roles in DNA repair, chromosome segregation, and genome stability (Harley et al. 2016). Most recently, biallelic mutations in *DONSON* have been identified in families with MPD phenotypes of varied severity and clinical description, including Microcephaly micromelia syndrome (MMS, OMIM 251230), Microcephaly, short stature, and limb abnormalities (MISSLA, OMIM 617604), and microcephalic primordial dwarfisms (MPD) (Evrony et al. 2017; Reynolds et al. 2017; Schulz et al. 2018). These studies demonstrated that DONSON is a critical replication fork protein which is associated with DNA replication and genome stability (Evrony et al. 2017; Lesly et al. 2017; Reynolds et al. 2017). Here we present biallelic novel likely damaging variants in *DONSON* in families with MGS and Seckel-like phenotype; we also describe the first *de novo* variant of *DONSON* in a patient with Femoral-Facial syndrome (FFS [OMIM 134780]) for which an etiologic gene had not been previously identified despite the broader availability of next-generation sequencing techniques.

MATERIAL AND METHODS

Written informed consent was obtained in accordance with protocols approved by the appropriate human subjects' ethics committees at Baylor College of Medicine (IRB protocol number: H-29697, Patients 1, 2 and 5) and Medical Chamber of Hamburg (No. PV3802, Patients 3, 4). Written consent to use the photographs in this report was also obtained from the parents of all patients. Exome sequencing (ES) was performed through the Baylor Hopkins Center for Mendelian Genomics (BHCMG, Patient 1), and the Bezmialem Medical Genetics Diagnostic laboratory (Patient 2). Patients 3 and 4 were identified through GeneMatcher based on a shared candidate gene (Sobreira et al. 2015a; Sobreira et al. 2015b). Patient 5 was ascertained following a review of the Baylor Genetics (BG) diagnostic laboratory exome variant database for additional cases with mono- or biallelic variation in *DONSON*. Variant interpretation was performed according to current ACMG guidelines for classification of variant pathogenicity (Richards et al. 2015). Exome sequencing, Sanger variant confirmations and segregation analyses were performed in each family according to previously described protocols (Karaca et al. 2015; Shashi et al. 2016; Bayram et al. 2017).

Identified variants are listed in Table 2 with ExAC and gnomAD database minor allele frequencies (Lek et al. 2016), conservation score using phyloP (Cooper and Shendure 2011) and GERP (Davydov et al. 2010), and functional prediction algorithms that include CADD (Kircher et al. 2014), SIFT (Ng and Henikoff 2003), Polyphen2 (Adzhubei et al. 2010), and MutationTaster (Schwarz et al. 2014). *DONSON* transcript NM_017613.2 was used.

CLINICAL REPORTS

Patient 1

Patient BAB5065 is a Turkish a boy who first presented to clinic when he was 10 years old. Clinical features included mild intellectual disability, short stature, hearing impairment, and small ears (Figure 2A). He was born at term by vaginal delivery without any complication, following a pregnancy without prenatal medical care. Anthropometric measurements at birth were as follows: weight (W): 1500 g (−6 SD), length (L): 45 cm (−3 SD), occipitofrontal circumference (OFC): 32 cm (−2.5 SD) (Table 1). He had poor sucking and frequent ear infections during early infancy. Anthropometric measurements at age 10 years were: L 108cm (−4.86 z), OFC 48cm (−4.16 z) (weight not available; Table 1). Physical examination showed intellectual disability (ID) with prominent short stature, dysmorphic features including a slender body, triangular face, high forehead, down slanting palpebral fissures, microstomia with thick vermilion of upper and lower lips, micrognathia, remarkably small and dysplastic low set ears, a relatively long neck, micropenis, reduced scrotal volume, sacral dimple and multiple hypopigmented skin lesions particularly concentrated on the forearms and hands (Figure 2A, Table 1). Bone survey revealed slender long bones and ribs, a delayed bone age (bone age consistent with 4 years at 10-year chronological age) and bilateral absence of the patellae. Metabolic screening, brain MRI and ophthalmologic examination did not reveal any abnormalities, while audiology revealed bilateral mild hearing loss. Karyotype demonstrated 46,XY and molecular analysis (genomic DNA sequencing for known Meier-Gorlin syndrome genes) of *ORC1*, *ORC4*, *ORC6*, *CDT1* and *CDC6* did not identify any pathogenic variants. ES revealed a homozygous c.631C>T (p.Arg211Cys) missense variant in *DONSON* (Figure S1A).

Patient 2

BAB9258 was first seen as a 15-day-old Turkish female who was referred due to congenital dislocation of the knee. She was the first child of healthy consanguineous parents, who denied any known relationship to the parents of patient 1, and was born by C-section at 38 weeks gestation due to fetal malposition, following an uncomplicated pregnancy. The Apgar scores were 7 and 8. The infant's birth weight was 2350 g (−2.03 SD), her length was 46 cm (6.5^{th} centile; −1.52 SD), and OFC was 35 cm (54.9^{th} centile) at birth. Bilateral knee and hip dislocations were noted at birth. Additional findings included a small face, convex nasal ridge, bilateral microtia, severe microretrognathia, clitoromegaly and hypoplastic labia majora, bilateral absence of the patellae and *pes varus* (Figure 2A, Table 1). She was monitored in the intensive care unit for 12 days due to respiratory distress. The karyotype was 46,XX. She was intermittently hospitalized during the first years of life for treatment of recurrent respiratory infections. She also received high energy nutrients for management of growth restriction and low calorie intake. On admission to the hospital at the age of 19 months, the height, weight, and OFC were 69 cm (−4.37 z), 6500 g (−4.25 z), and 44 cm (−3.13 z), respectively. The developmental milestones were appropriate for her age except sitting and walking independently, which were delayed. She had difficulty swallowing solid food and had severe gastroesophageal reflux. The hearing was normal. Spinal and brain MRIs were also normal. On pelvic ultrasound, the left ovary was not visualized whereas the right ovary and uterus were appropriately sized for her age (right ovary 10×6.6 mm; uterus

19×5.1 mm). Echocardiography revealed small atrial septal and muscular ventricular septal defects. On the abdominal ultrasound, multiple 5 to 6 millimeter stones in clusters were visualized within each renal sinus. She received a 6-month course of IVIG (Intravenous Immunoglobulin Treatment) for complicated recurrent respiratory infections despite lack of any obvious dysfunction of T and B cells. ES revealed the same homozygous c.631C>T (p.Arg211Cys) *DONSON* variant that had been identified in Patient 1 (Figure S1A, Table 2).

Patient 3 and Patient 4

Patient 3 and 4 are siblings born to a German couple. Patient 3 was born by C-section after a pregnancy complicated by suspected placental insufficiency and maternal gestational diabetes at 38+2 weeks to a 21-year-old mother and a 22-year-old father. His birth weight was 1980 g (−2.35 SD), his length 44 cm (−2.22 SD), and his OFC 27.5 cm (−2.98 SD). Temperature instability and poor sucking were noted soon after birth. Facial dysmorphism included high forehead, microretrognathia, and a high arched palate. Head ultrasound showed a thin corpus callosum (Table 1). Follow-up examinations showed continued, but slow, growth. The boy suffered also from recurrent airway infections during infancy. At the age of one year, motor delay was apparent. Hearing was found to be normal. Laboratory investigations including routine metabolic studies, karyotype, and sequencing of *RNU4ATAC, RBBP8* and *ATR* were normal. A chromosomal microarray revealed a 42 kb duplication in 7p15.2 (arr[hg19] 7p15.2(26,899,505–26,940,789)x3; not found in the mother; the father was not available for further testing) and a maternally inherited 400 kb duplication in Xq27.2 (arr[hg19] Xq27.2(140,353,091–140,756,586)x2), both of which were interpreted as being of unclear clinical significance.

On physical examination at age 3 years and 1 month, his height was 81 cm (−4.73 z), weight was 9.2 kg (−3.89 z), and OFC was 39 cm (−10.66 z). His intellectual and language development were delayed, whereas his motor development was appropriate for age. Dysmorphic features included a triangular face, high forehead, narrow palpebral fissures, thick vermilion of upper and lower lips, microretrognathia, mildly ‘fleshy’ neck, and dry skin. The skin of his hands and feet maintained a reddish-livid appearance and was cool to touch. His thumbs were proximally implanted, and his 5th fingers showed only a single flexion crease (Figure 2B, Table 1). The boy’s parents were of Caucasian origin and non-consanguineous. His mother showed mild short stature (148 cm, −3.1 SD) and normal head circumference, with a history of learning disability. His father’s anthropometric measurements were all within the normal range.

Patient 4 is the younger sister of patient 3. She was born by C-section at 37+1 weeks gestation with a birth weight of 1790 g (−2.93 SD), length of 39 cm (−5.55 SD), and OFC of 27 cm (−6.45 SD) as the second child of the family. After birth, she showed similar dysmorphic face as seen in her brother, but in addition had a submucosal cleft palate. Cranial and renal ultrasounds were normal. At age 4.5 months, severe hearing loss on the left side was diagnosed and mild hearing loss on the right was suspected. Motor development was slightly delayed (Table 1). Physical examination at age 2 years 2 months showed moderately short stature, height was 72 cm (−5.61 z) and weight was 6 kg (−5.85 z) as well as severe microcephaly (OFC was 36 cm, −6.2 z). Her cognitive development was similarly delayed,

as in her older brother, and she was able to speak some single words. Similar facial dysmorphic features and discolored and cold hands and feet as well as proximally implanted thumbs as in her brother were noted (Figure 2A). ES performed in both siblings revealed compound heterozygous c.346delG (p.Asp116Ilefs*62) and c.1349A>G (p.Lys450Arg) variants in *DONSON* in both siblings (Figure S1B, Table 2).

Patient 5

Patient 5 is a 25-month-old Hispanic male first suspected to have a skeletal dysplasia when prenatal imaging at 27 weeks gestation identified shortened bilateral femurs, micrognathia and edema of the soles of the feet suspicious for FFS. The pregnancy was complicated by maternal gestational diabetes treated with insulin. Patient 5 was delivered by C-section at 30 4/7 weeks gestation, with Apgar scores of 5 (1 minute) and 9 (5 minutes). Post-natal examination revealed dysmorphic facial features including micrognathia with cleft palate, a short nose with broad tip and ante-verted notched nares, a long philtrum, narrow mouth, sparse medial eyebrows, brachydactyly and 2–3 partial syndactyly of the left foot. Birth weight was 1.429 kg (50%ile), birth length 34.5 cm (<3%ile) and FOC 28 cm (50%ile) (Table 1). Post-natal skeletal survey demonstrated prominent bilateral femoral hypoplasia with convex bowing at the mid-diaphysis. Additional skeletal abnormalities included a shortened and hypoplastic right fibula, micrognathia, hypoplastic posterior vertebral arch of C1, vertebral fusion abnormality of C2–C3, severely hypoplastic left S2 sacral wing, right forefoot valgus, and left talipes equinovarus. By 2 years of age, his height was 76.8 cm (−3.04 SD), with preserved head size (OFC −0.49 SD). The clinical impression was that the dysmorphic craniofacial features and imaging best fit the phenotypic spectrum for FFS (Figure 2B, Table 1). Trio-ES identified a *de novo* c.683G>T (p.Trp228Leu) variant in *DONSON* (Table 2) and confirmed the biological relationships of both parental samples to the proband.

DISCUSSION

Cell cycle defects account for a large number of human diseases associated with various malignancies and disorders with delayed development and growth due to aberrant and/or reduced cell proliferation, and increased apoptosis upon widespread DNA damage. Not surprisingly, several genes (e.g.. *ATR*, *XRCC4, LIG4*) encoding proteins that are involved in the DNA damage response pathway are associated with both cancer and MPD phenotypes. There are several genes that are associated with primary microcephaly, which similarly encode crucial components of the cell cycle. For instance, *CDK6* is associated with primary microcephaly 12 (OMIM 616080) and its encoded protein promotes the transition from G1 to S phase, a very critical stage in the cell cycle (Hussain et al. 2013). Moreover, biallelic mutations in *CEP152* cause both primary microcephaly 9 (OMIM 614852) and Seckel syndrome type 5 (OMIM 613823), and this gene encodes the core protein of the centrosome whose role is the formation of the mitotic spindle and the accurate segregation of the replicated genome to the daughter cells (Andersen et al. 2003). Although disorders associated with each of these cell cycle-related genes are classified under different entities such as MOPDs (OMIM 210710 and 210720), MGS (OMIM 224690), SS (OMIM 210600), MMS (OMIM 251230), and MISSLA (OMIM 617604), they involve common clinical

features which may challenge precise diagnosis on the basis of clinical evaluation alone. Indeed, MPDs constitute a fraction on the wide spectrum of “cell cycle-opathies” (Figure 1), as primary microcephaly and other cancer syndromes that are consequences of defects in various cell cycle steps.

Until recently, data elucidating the function of *DONSON* were very limited. In 2005, Bandura *et al*. presented the first functional assay of the *Drosophila* homolog of *DONSON*, *humpty dumpty* (*hd*), which reveal that *hd* is essential for DNA amplification and cell proliferation (Bandura et al. 2005). In addition, they also demonstrated that *Hd*-null flies survived until metamorphosis, and displayed a small brain size, no detectable imaginal discs, and small under-replicated polytene chromosomes, all features highly suggestive of defects in DNA replication and cell-proliferation (Bandura et al. 2005). These findings are consistent with the molecular pathogenesis proposed to underlie MPDs: perturbed genome replication and cell proliferation (Klingseisen and Jackson 2011). Hd is co-expressed with components of minichromosome maintenance complexes (Mcm2–7), which are essential for DNA replication initiation and elongation (Groth et al. 2007), supporting a role for DONSON in DNA replication. Notably, maternal Hd levels in *Drosophila* have been shown to impact early embryogenesis (Lesly et al. 2017). More recent functional studies have further elucidated DONSON’s critical role in mammalian genome replication and genome stability (Evrony et al. 2017; Reynolds et al. 2017).

The present study demonstrates balletic variant alleles in *DONSON* associated with 2 forms of MPD, MGS and Seckel-like phenotype, expanding the phenotypic features described in association with variation at this locus (Figure 3). Both Turkish MGS patients have an identical homozygous missense variant c.631C>T p.(Arg211Cys) with a MAF of 0.1 % amongst over 1000 Turkish exomes from our in-house exome database. The families of these Turkish patients deny any known familial relationship; unfiltered variant call files from Patient 2 are unavailable to perform an analysis of the coefficient of relatedness between these two probands. Both patients presented with a less severe microcephaly than the Patient 3 and Patient 4, who harbor one frameshift (c.346delG p.[Asp116Ile*62]) and one missense (c.1349A>G p.[Lys450Arg]) variant. In addition, Patient 3 and 4 who display a Seckel-like phenotype also have proximally implanted thumbs, and his 5th fingers showed only a single flexion crease that is suggestive of radial ray deficiency, which represents the mild end of the same phenotypic spectrum as micromelia in *DONSON*-related MMS. The difference in the severity of phenotypes (i.e. MGS and FFS represent milder phenotypes than the Seckel-like phenotypes, MMS and MISSLA) may result from different types of mutations (frameshift vs missense), and different locations of the amino acid residue changes. In order to make an appropriate interpretation and comparison of potential pathophysiological effects of these residue changes, further elucidation of the detailed domain structure and the role of each domain in the functions and interactions of DONSON is needed. The genotype-phenotype data in Figure 3 indicate that a majority of the variants associated with severe phenotypes and prominent microcephaly are located beyond exon 4, primarily clustering within (and around) exon 5 and exon 8, whereas the variants identified in patients with a less severe phenotype (MGS and FFS) are located within exon 4. This might suggest that the domain encoded by exon 4 is less critical for the functionality of DONSON compared to the domain(s) which are associated with / encoded by exons 5–10. Given the significant clinical

overlap among these phenotypic forms of MPDs, we propose that these Mendelizing disease traits represent a continuum of the same clinical spectrum of cell cycle-opathies, rather than discreet clinical entities. Certainly, the development and phenotypic analysis of a *DONSON* allelic series will likely allow further elucidation of genotype-phenotype correlations at this locus.

Moreover, we also describe a *de novo* mutation in a patient who has manifestations of FFS (OMIM 134780). FFS was previously described in a single case report, in association with a *de novo*, complex genomic rearrangement at 2q37; however, there has not been any definitive association between FFS and any particular disease genes, despite the broader availability of NGS technologies (Spielmann et al. 2016). In the present case (Patient 5), high quality exon-by-exon sequencing coverage of *DONSON* was confirmed within the ES data, and no second, rare *DONSON* variant was identified, supporting the possibility that this case represents a condition for which dominant inheritance may be observed. There is one other known *de novo DONSON* variant reported in an individual with developmental delay (Deciphering Developmental Disorders 2017). The identified variant is a stopgain variant (c.1282C>T p.(Q428*), CADD score= 37), leading to a termination codon in exon 8 of 10 that is predicted to result in a loss of function due to nonsense-mediated decay. This same stopgain variant (c.1282C>T p.(Gln428*), has also been reported in two unrelated individuals with MISSLA (Reynolds et al. 2017). In both cases, the stopgain was present *in trans* with a haplotype defined by 3 co-segregating variants (c.82A>C:p.Ser28Arg; c.786–33A>G; c.1466A>C:p.Lys489Thr), and the authors proposed that biallelic variants in *DONSON* were etiologic for MISSLA. It is possible that distinct impacts on protein function (i.e. loss of function due to c.1282C>T p.(Gln428*) versus gain of function due to c.683G>T p.(Trp228Leu)) may result in distinct phenotypic outcomes, and distinct modes of inheritance.

FFS, also known as femoral hypoplasia-unusual facies syndrome (FHUFS), is a rare sporadic syndrome characterized by bilateral femoral hypoplasia and characteristic facial features, such as long philtrum, thin upper lip, micrognathia with or without cleft palate, upward-slanting palpebral fissures, a short nose with broad tip, and variable renal anomalies (Nowaczyk et al. 2010). FFS shows some clinical overlap with MPD syndromes, but lacks microcephaly, one of the key features observed in MPDs. Interestingly, 35% of the reported FFS cases had maternal diabetes reported in the prenatal history, which might suggest a combination of genotype and environmental effects in the etiology of this condition. Some cases diagnosed as FFS even share some common features with the MPD spectrum. This observation suggests that further testing of FFS cohorts for *DONSON* and other related cell cycle genes is needed to better understand whether FFS could be related to the broader category of MPD or cell cycle-opathies. The broader question relates to variant allele contributions to cellular phenotypes versus organismal developmental phenotypes

The vast majority of MPDs arise from biallelic mutations, except *GMNN* associated MGS (OMIM 616835) in which *de novo* truncating mutations were identified in patients with an MGS phenotype (Burrage et al. 2015). FFS syndrome has been reported in more than 100 patients to date and with the exception of 2 patients described in the literature, the syndrome was observed to be sporadic. A recent report of sporadic FFS occurring in one of two

monozygotic twins would seem to support the potential role of *de novo* mutations in this condition (Lacarrubba-Flores et al. 2018), making FFS another notable exception to the previously described recessive inheritance pattern of most MPDs. These findings suggest that defects in *DONSON* could lead to both dominant and recessive traits, similar to that reported for several loci, such as *EMC1* (OMIM 616875), *MAB21L2* (OMIM 615877), *ATAD3A* (OMIM 617183), and *LMNA* (OMIM 176670) (Verstraeten et al. 2006; Rainger et al. 2014; Harel et al. 2016a; Harel et al. 2016b).

A homozygous c.631C>T p.(Arg211Cys) variant was detected in two unrelated families with MGS (Figures 2A and S1A). In addition to a classical MGS phenotype, patient 1 (BAB5065), also had multiple hypopigmented skin lesions prominently seen on both forearms (Figure 2A), suggesting an effect from sun exposure. Although this finding has not been reported in two recent studies or in any MG syndromes to our knowledge, areas of abnormal skin pigmentation (i.e. hypopigmentation and hyperpigmentation) can be seen in MOPD2 (OMIM 210720). This clinical finding may represent an expansion of the known DONSON-associated phenotypes, and suggests further clinical evidence for DONSON's role in preservation of genome stability and activation of cell cycle check points to prevent stress-induced DNA damage (Reynolds et al. 2017). Patient BAB9258 (female), on the other hand, presented with congenital dislocation of the knee and absent left ovary, features not observed in patient BAB5065 (male) who harbors the exact same homozygous c.631C>T p.(Arg211Cys) variant, implying that a genomic modifier and/or sex-limited expression might be responsible for interfamilial phenotypic variability. External genital anomalies are relatively common in both genders in most cases of MGS, however internal genital anomalies (i.e. cryptorchidism) have only been reported in males. Ascertainment bias may contribute to this apparent discrepancy, as male internal genital anomalies such as cryptorchidism, are more readily detectable compared to female internal genital anomalies.

In conclusion, we present patients with MGS, Seckel-like phenotype and FFS who were found to harbor novel biallelic (MGS and Seckel-like) and monoallelic (*de novo*, FFS) mutations in *DONSON*. Our genomic and clinical data expand the previously reported genotype-phenotype correlations observed in *DONSON*-related cell cycle-opathy dependent MPDs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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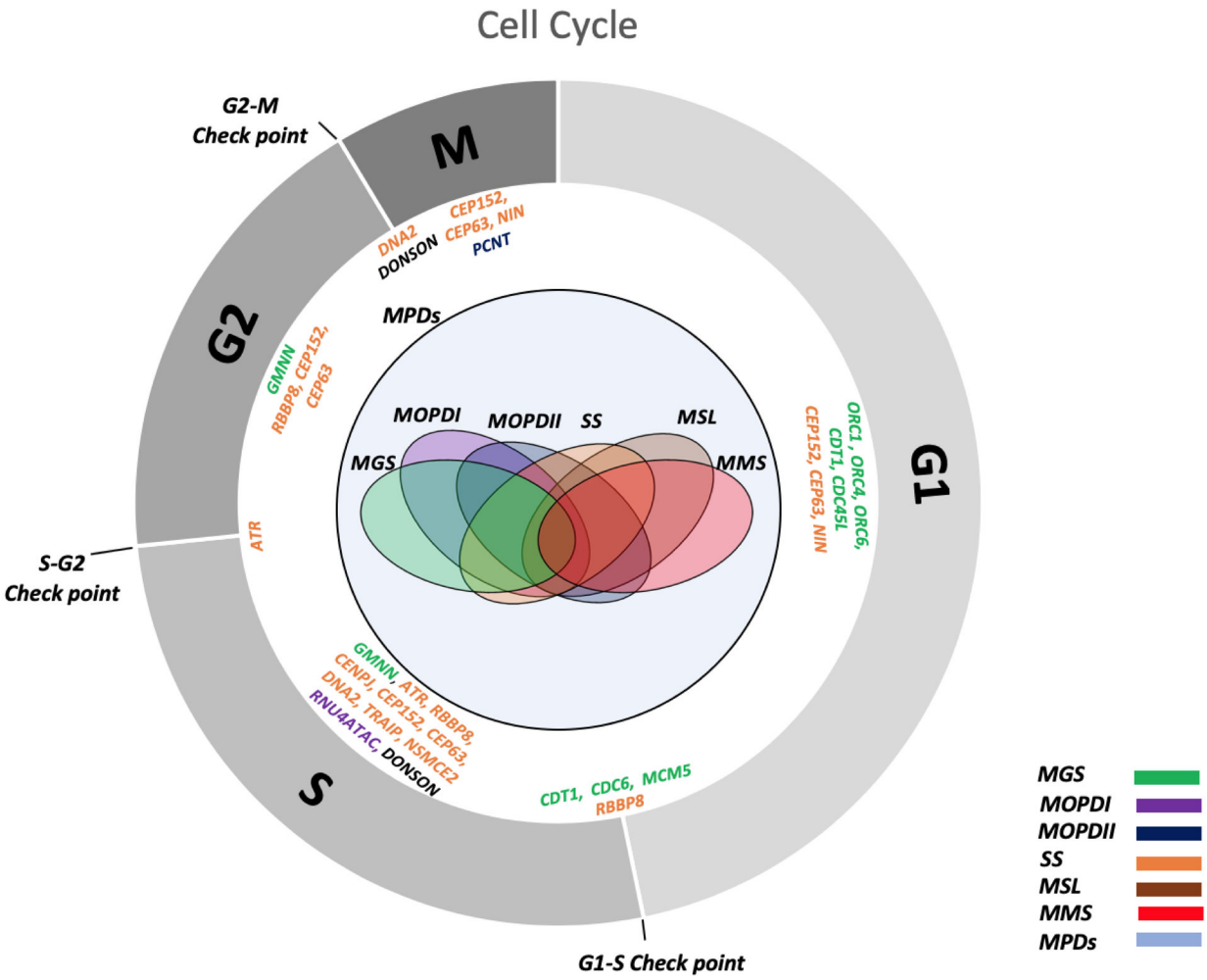

Figure 1.

Conceptualization of the subtypes of MPDs in the context of their impact on the cell cycle and shared disease traits. MPD-associated disease genes impacting each phase of the cell cycle are indicated and color-coded according to their primary associated disease trait. A Venn diagram depicts the degree of phenotypic overlap among MPD subtypes.

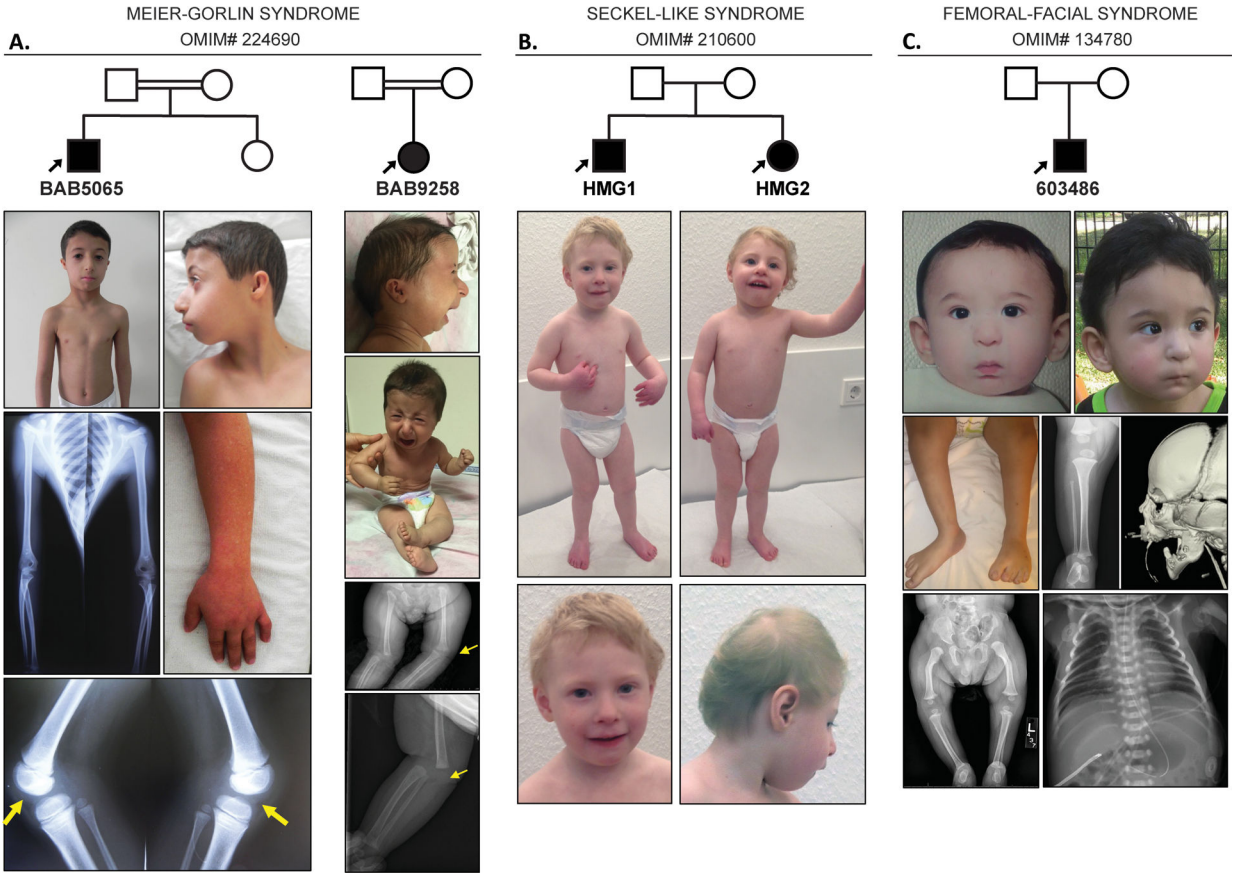

Figure 2.

Genetic pedigrees and clinical features of each proband. (**A**) Clinical evaluation of patients 1 and 2 is most consistent with a diagnosis of Meier-Gorlin syndrome. Patient 1 at 10 years of age demonstrated intellectual disability, short stature, microcephaly, a long neck and slender body, triangular facies with a high forehead, downslanting palpebral fissures, microstomia, thick vermilion of upper and lower lips, micrognathia, and small dysplastic and low-set ears. XR demonstrates slender long bones and ribs with absent patellae. Patient 2 at 19 months of age demonstrated similar features, including a prominent forehead, triangular facies, downslanting palpebral fissures, micro- and retrognathia, and absent patellae. (**B**) Clinical evaluation of patients 3 and 4 are most consistent with a diagnosis of microcephalic osteodysplastic primordial dwarfism, with shared features of triangular facies, high forehead, narrow palpebral fissures, thick vermilion of upper and lower lips, microretrognathia, and livid coloration of the hands and feet with proximally implanted thumbs. (**C**) Clinical evaluation of patient 5 at 25 months of age demonstrated features of femoral facial syndrome, including shortened femurs, microstomia, micrognathia with cleft palate, a narrow mouth, and brachydactyly with partial 2–3 syndactyly of the feet.

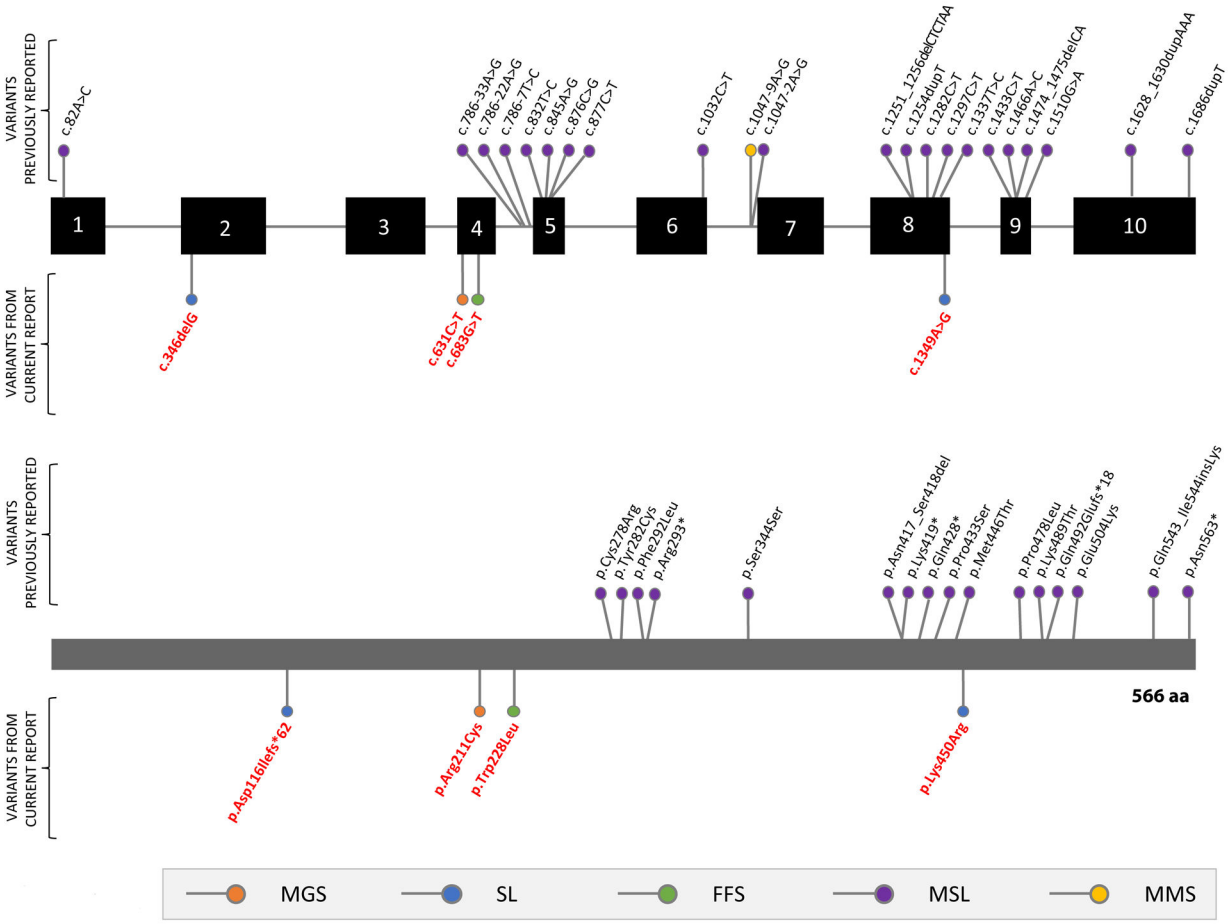

Figure 3.

Depiction of *DONSON* variants identified in association with MPDs to date mapped to exon structure (top) and protein structure (bottom, splice variants excluded). Note that there is no known protein domain structure for DONSON. Previously reported variants are indicated along the top of the figure, and presently identified variants are indicated along the bottom of the figure. Lollipop color corresponds to variant-associated phenotypes: Meier-Gorlin syndrome (MGS, orange), Seckel-like (SL, blue), femoral facial syndrome (FFS, green), microcephaly, short stature, and limb abnormalities (MISSLA, purple), and microcephaly-micromelia syndrome (MMS, yellow). The domain structure of DONSON has not yet been elucidated.

Table 1.

Phenotypic features for each of the 5 described cases.

Abbreviations: AR -- autosomal recessive; FFS -- femoral facial syndrome; MGS -- Meier-Gorlin syndrome; MOPD1 -- microcephalic osteodysplastic primordial drawfism; m -- months; NA -- not available; ND -- not done; y -- years

Patients	Patient 1 BAB5065	Patient 2 BAB9258	Patient 3 HMG-1	Patient 4 HMG-2	Patient 5 603486
Clinical diagnosis	**MGS**	**MGS**	**Seckel-like**	**Seckel-like**	**FFS**
Gender	Male	Female	Male	Female	Male
***DONSON* nucleotide alteration**	c.631C>T	C.631C>T	c.346delG, c.1349A>G	c.346delG, c.1349A>G	c.683G>T
DONSON amino acid alteration	p.(Arg211Cys)	p.(Arg211Cys)	p.(Asp116Ile*62), p.(Lys450Arg)	p.(Asp116Ile*62), p.(Lys450Arg)	p.(Trp228Leu)
Inheritance	AR	AR	AR (comp. het.)	AR (comp. het.)	*de novo*
Age	10y	19m	3y1m	2y2m	2y
GROWTH					
Short stature	+ **(108cm, −4.93 SD)**	+ **(69cm, −3.68 SD)**	+ **(81cm, −4.2 SD)**	+ **(72cm, −4.29 SD)**	+ **(76.8cm, −3.04 SD)**
Birth length less than 3rd percentile	+	+	+	+	+
Prenatal growth retardation	?	+	+	+	+
Failure to thrive	+	+	+	+	+
HEAD & NECK					
Microcephaly	+ **(48cm, −3.73 SD)**	+ **(44cm, −2.01 SD)**	+ **(39cm, −8.13 SD)**	+ **(36cm, −7.4 SD)**	− **(48.1cm, z score= −0.49)**
Small anterior fontanelle	**NA**	?	+	+	+
Micrognathia	+	+	−	−	+
Maxillary hypoplasia	+	−	−	−	+
Mandibular hypoplasia	+	+	+	+	+
Microtia	+	+	−	−	−
Low set ears	+	+	−	−	+
Hearing loss	+	+	−	+	+
Dysplastic ears	+	+	+	+	+
Short palpebral fissures	+	+	+	+	+
Small mouth	+	+	−	−	+
Full lips	+	+	−	+	+

Patients	Patient 1 BAB5065	Patient 2 BAB9258	Patient 3 HMG-1	Patient 4 HMG-2	Patient 5 603486
Clinical diagnosis	**MGS**	**MGS**	**Seckel-like**	**Seckel-like**	**FFS**
Cleft palate	−	+	−	+	+
CHEST					
Narrow chest	+	+	+	+	+
Slender ribs	+	?	**ND (no X-ray)**	**ND (no X-ray)**	+
Gastrointestinal/feeding problems	+	?	−	−	+
GENITOURINARY					
Micro penis/Clitoromegaly	+	+	−	**ND**	−
Cryptorchidism	+		−	**NA**	−
Inguinal hernia	−	−	−	−	+
Skeletal	+	+			
Vertebral anomaly	−	+	**ND (no X-ray)**	**ND (no X-ray)**	+
Joint contractures/dislocation	−	+	−	−	−
Aplastic or hypoplastic patella	+	**NA (early age)**	**ND (no X-ray)**	**ND (no X-ray)**	**NA (early age)**
Slender long bones	+	+	**ND (no X-ray)**	**ND (no X-ray)**	+
Talipes equinovarus (club foot)	−	+	−	−	+
SKIN, NAILS, HAIR					
Hypopigmentation	+	−	−	−	−
Long eyelashes	−	+	+	+	+
Sparse scalp hair	+	+	+	+	+
Sparse eyebrows	+	+	+	+	+
NEUROLOGIC					
Intellectual disability/developmental delay	+	+	+	+	+

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Table 2.

DONSON variants identified in 5 affected individuals in 4 families.
Variants are reported using transcript NM_017613.2.

Case	Hg19 coordinate	Variant	Zygosity	Inh	Mutation	ExAC MAF	gnomAD MAF	CADD Phred	SIFT	Polyphen2	MutTaster	Phylop	GERP	ACMG Classification
Patient 1	21:34957050	c.631C>T (p.Arg211Cys)	Hom	AR	Missense	0	0	34	D	P	D	4.592	5.11	VUS
Patient 2	21:34957050	c.631>T (p.Arg211Cys)	Hom	AR	Missense	0	0	34	D	P	D	4.592	5.11	VUS
Patient 3	21:34953609	c.1349A>G (p.Lys450Arg);	Comp Het	AR	Missense	0	0	33	T	P	D	3.418	5.44	Likely Pathogenic
	21:34959884	c.346delG (p.Asp116Ilefs*62)			Non-FS	0	0	NA	NA	NA	D	NA	NA	Pathogenic
Patient 4	21:34953609	c.1349A>G (p.Lys450Arg);	Comp Het	AR	Missense	0	0	33	T	P	D	3.418	5.44	Likely Pathogenic
	21:34959884	c.346delG (p.Asp116Ilefs*62)			Non-FS	0	0	NA	NA	NA	D	NA	NA	Pathogenic
Patient 5	21:34956998	c.683G>T (p.Trp228Leu)	Het	*De novo*	Missense	0	0	31	D	P	D	4.588	5.11	Likely Pathogenic

P-probably damagingP-polymorphism

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