Further Delineation of the Microcephaly-Micromelia Syndrome Associated with Loss-of-Function Variants in *DONSON*

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Established Facts

- A severe form of microcephalic dwarfism, microcephaly-micromelia syndrome (MIMIS), has been recently associated with *DONSON* biallelic loss of function.
- Affected fetuses suffer severe growth restriction, microcephaly, and variable limb malformations which result in intrauterine or perinatal death.
- All previously described fetuses carried a homozygous founder mutation (c.1047–9A>G), a splice-altering variant that leads to transcript degradation.

Novel Insights

- Here, we describe the striking, extremely severe clinical features of 2 additional cases extending the phenotype of MIMIS to include anophthalmia, atretic meatus/auditory canal, and absent external genitalia.
- We report the novel homozygous null variant c.763C>T (p.Gln255*) as the second causative variant linked to this lethal syndrome.

Keywords

Intrauterine growth restriction · Microcephaly-micromelia syndrome · Replication fork stability

Abstract

The *DONSON* gene encodes the downstream neighbor of SON, a replisome component that stabilizes the replication fork during replication. A severe form of microcephalic dwarfism, microcephaly-micromelia syndrome (MIMIS), has been recently associated with *DONSON* biallelic loss of func-

tion. Affected fetuses suffer severe growth restriction, microcephaly, and variable limb malformations which result in intrauterine or perinatal death. All described fetuses carried a homozygous founder mutation (c.1047–9A>G), a splice-altering variant that leads to transcript degradation. We evaluated 2 newborns from a consanguineous Emirati family with severe microcephaly, micromelia, craniofacial dysmorphism, and skeletal abnormalities; both died shortly after birth. Here, we report the second homozygous loss-of-function variant (c.763C>T) in *DONSON* causing MIMIS, and we provide detailed clinical description of this very rare disorder. In

addition, we review all MIMIS cases in the literature and summarize the striking features of this phenotype. This manuscript is aimed to increase the clinical understanding of this rare, extremely severe disorder and encourage clinical and molecular geneticists to consider screening for *DONSON* loss-of-function variants in families with recurrent pregnancy loss and/or perinatal deaths.

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For proper cell proliferation and survival, maintenance of genome integrity and stability during DNA replication are essential processes. Protection against DNA damage during replication stress requires efficient activation of DNA repair mechanisms for single-stranded DNA breaks. When a replication fork is stalled, for instance in genomic regions that are difficult to replicate or are undergoing transcription, it must be stabilized and safeguarded from collapse. *DONSON* (downstream neighbor of SON) is a recently identified protein as part of the replication fork proteins that stabilizes and protects stalled replication forks and further promotes activation of cell cycle checkpoints to ensure proper DNA repair and thus preventing genomic mutations and chromosomal breakage.

Loss-of-function variants in *DONSON* (OMIM 611428) are associated with 2 main phenotypic presentations which vary in severity and outcome. MIMIS (OMIM 251230) is linked to 1 variant so far, *DONSON* [NM_017613.3]:c.1047-9A>G; Chr21 [NC_000021.8]:g.33582064T>C [GRCh38; hg38] [Evrony et al., 2017; Reynolds et al., 2017]. This variant has been shown to cause complete loss of function of this gene due to the altered splicing and the shift generated in the reading frame leading to the transcript degradation by nonsense-mediated decay [Evrony et al., 2017]. Another milder phenotype linked to variants in this gene is the microcephaly, short stature, and limb abnormality syndrome (MISSLA; MIM 617604), which is associated with the partial loss of function of *DONSON*. In this syndrome, patients have short stature, microcephaly, variable hand or feet deformities, and intellectual or speech delay may occur. Overall, 21 disease-causing variants have been reported in this gene so far, inherited as homozygous or compound heterozygous variants/haplotypes [Evrony et al., 2017; Reynolds et al., 2017; Schulz et al., 2018]. In this report, we present another family with 2 affected siblings born with MIMIS, carrying another homozygous loss-of-function variant in the *DONSON* gene and review the clinical phenotype of previously reported cases.

Case Reports

Clinical evaluation of the affected newborns was carried out at Tawam Hospital in Al Ain, UAE. DNA of the newborns and both parents were extracted from peripheral blood samples collected in EDTA tubes, and preserved at -80°C . Sanger sequencing of the *DONSON* gene was done on the ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) with primers designed with Primer3 and provided on a supplementary file. Alignments and variant in silico analysis was done as previously described [Abdelrahman et al., 2018].

The parents are first cousins of Emirati origin. They had a total of 5 children, 2 of them were affected (III-2 and III-5) (Fig. 1a). There was no history of similar presentation in the extended family.

The first affected child (III-2) was born after a normal pregnancy and normal vaginal delivery at term. There was no history of medication intake during pregnancy. Prenatal ultrasound detected complex malformations. At birth, the baby was gasping and died after a few minutes. The birth weight was 465 g (-5 SD), length 23 cm (-10.1 SD), and head circumference was 21 cm (-6.5 SD). All the limbs were deformed and short, particularly the forearms (Fig. 1c, left). The hands were rudimentary with 3 fingers only which were in syndactyly. The feet were absent and replaced by conical stumps below the knee. The baby had dysmorphic features with frontal bossing, absent eyes, a large beaked nose with very narrow nostrils, and an extremely small mouth (opening <1 cm). There was severe micrognathia and rudimentary low-set ears with atretic or nonexistent meatus. The external genitalia were absent and replaced by a skin appendage. Meconium had been passed through a tiny anteriorly positioned anal opening. Postmortem skeletal survey showed increased bone density with hypoplastic and abnormal clavicles and scapulae. All long bones were hypoplastic but particularly the radius, ulna, and tibia. The fibula was absent. The pelvic bones were hypoplastic. There was a rudimentary first rib. Postmortem MRI of the brain showed a normal brain stem, upper cervical cord, cerebellar hemispheres, and thalami. Beyond the thalami, the brain appeared grossly abnormal with no differentiation into individual lobes. Even germinal layers were not seen. There was a large interhemispheric cyst, and the midline structures were not seen. MRI of thorax and abdomen was normal apart from a slightly large liver and small spleen.

The second affected child (III-5) was born at term by normal vaginal delivery. Prenatal ultrasound at 10 weeks of gestation showed a cystic hygroma, reverse ductus venous flow, smaller fetal measurements by 1 week, and rudimentary hands and feet with short bent limbs. Ultrasound at 31 weeks of gestation revealed severe symmetrical growth restriction with a fetal weight of 200 g. The fetal face showed micrognathia and a large beaked nose. Brain anatomy revealed a grossly abnormal brain, mainly supratentorial, with absent cavum septum pellucidum and corpus callosum, a large cystic cavity within the brain matter, and no gyration. The baby was born at 40 weeks with a weight of 585 g (-4.8 SD). The baby had very similar features to the first one and lived for 10 min only (Fig. 1c, right; Table 1).

Fig. 1. a Family pedigree showing affected siblings. **b** Sequencing chromatogram showing the described variant in II.1, III.2, and an unrelated donor control. **c** Photos of the affected individuals left (III.2) and right (III.5).

Results

G-banding did not reveal any clear chromosomal defect. The Y chromosome was detected in both siblings; therefore their gender was determined as male. Sanger sequencing of the *DONSON* gene revealed a novel homozygous variant, *DONSON* [NM_017613.3]:c.763C>T; Chr21 [NC_000021.8]:g.33584613G>A [GRCh38; hg38] (Fig. 1b). This variant leads to a nonsense substitution and is predicted to be pathogenic in several in silico tools, such as MutationTaster (disease causing), Mutation As-

essor (high functional impact), and MutPred (pathogenic), as it generates a premature termination codon (p.Gln255*) in the reading sequence leading to the loss of more than half of the protein sequence (255 out of 567 amino acids). The variant was found in the newborns' genomes in a homozygous state and carried in their parents' genomes in a heterozygous state. This has also not been reported before in any public database. We, therefore, report this variant as pathogenic as per the ACMGG classification (PVS1 + PM2 + PP3, PP4) [Richards et al., 2015].

Table 1. Clinical presentation of current and previously reported microcephaly-micromelia syndrome cases

	Current (III.5)	Current (III.2)	Ives and Houston, 1980	Evrony et al., 2017	Reynolds et al., 2017
Variant, exon-intron	c.763C>T, p.Gln255*	c.763C>T, p.Gln255*	c.1047-9A>G	c.1047-9A>G	c.1047-9A>G
Variant effect	Nonsense	Nonsense	Intron retention leading to NMD	Intron retention leading to NMD	Intron retention leading to NMD
Fetuses or patients reported	1	1	14	12	2
Ethnic origin	Emirati	Emirati	Cree Indian	Cree Indian	Saudi Arabia
Consanguinity	+	+	+	+	+
IUGR	+	+	+	+	+
<i>Survival</i>					
Miscarriage	-	-	-	3 in 3 families	2 in 1 family
Stillbirth	-	-	4/14	-	2 (20 and 30 weeks' gestation)
Perinatal death	10 min after birth	A few min after birth	10/14: 7 died 20 min after birth, 3 died 2.5 and 7 days after birth	All, time not provided	-
Cause of death	Apnea, respiratory failure	Apnea, respiratory failure	Apnea, respiratory failure	Apnea, respiratory failure	-
<i>Growth parameters at birth, average</i>					
Weight	585 g (-4.8 SD)	465 g (-5 SD)	NA	1.2 kg (-3.8 SD)	800 g (-4.4 SD)
Length	NA	23 cm (-10.1 SD)	NA	34 cm (-6 SD)	NA
Head circumference	NA	21 cm (-6.5 SD)	26.5 cm (-4.1 SD)	24 cm (-5.2 SD)	13.6 cm (-9.8 SD)
<i>Facial dysmorphism</i>					
Frontal bossing	+	+	-	-	-
Anophthalmia	+	+	-	-	-
Microphthalmia	-	-	+	+	+
Atretic auditory meatus	+	+	-	-	-
Low-set ears	+	+	+	+	+
Beaked, broad nose	+	+	+	+	+
Narrow nostrils	+	+	-	-	-
Microstomia	+	+	+	+	+
Cleft palate	-	-	+	+	-
Short neck/cystic hygroma	+/-	+/-	+/+	+/-	+/+
<i>Skeletal abnormalities</i>					
Upper limbs					
Short forearms	+	+	+	+	+
Absent/underdeveloped radius and/or ulna	+	+	+	+	+
Humeroradial synostosis	+	+	+	+	-
Oligodactyly	+	+	+	+	+
Syndactyly	+	+	-	-	-
Absent thumbs	+	+	+	+	+
Lower limbs					
Short	+	+	+	+	+
Underdeveloped/absent fibula	+	+	+	+	+
Clubfeet	+	+	+	+	+
Syndactyly	-	-	+	+	-
Oligodactyly/absent toes	+	+	+	+	+
Other skeletal abnormalities					
Craniosynostosis	+	+	+	+	-
Absence of 1 or more ribs	+	+	+	+	+
Hypoplastic pelvis, clavicle, and scapula	+	+	-	-	-
Narrow chest	+	+	+	-	+
<i>Brain</i>					
Microcephaly	+	+	+	+	+
Abnormal/absent sulci and gyri	+	+	+	+	NA
Absent/diminished cerebrum	+	+	+	+	+
Dilated ventricles	+	+	+	+	+
Absent/hypoplastic corpus callosum	+	+	+	+	+
<i>Other malformations</i>					
Cardiopulmonary	-	-	-	+	-
Gastrointestinal	+	+	-	+	NA
Genitourinary	+	+	+	+	NA

IUGR, intrauterine growth restriction; NA, not available; NMD, nonsense-mediated decay; +, present; -, absent.

Discussion and Conclusion

In 1980, Ives and Houston described 14 malformed newborns born to 8 different mothers of a highly consanguineous Cree Indian family in Canada [Ives and Houston, 1980]. All 14 babies have similar striking manifestations of intrauterine growth restriction, perinatal death as well as cranial and skeletal deformities, summarized in Table 1. No molecular analysis was done at the time. Evrony et al. [2017] were able to identify a deep intronic variant in the *DONSON* gene, using integrated genomic and transcriptomic analysis for the abovementioned family and 8 other families with babies born with a similar phenotype (Table 1). Whole transcriptome analysis revealed a significant decrease in the highly conserved *DONSON* gene. The intronic variant detected, *DONSON* [NM_017613.3]:c.1047-9A>G; Chr21 [NC_000021.8]:g.33582064T>C [GRCh38; hg38], was found to cause intron 6 retention which eventually leads to transcript degradation by nonsense-mediated decay. At the same time, another family from Saudi Arabia with the same homozygous variant and severe phenotype was reported by Reynolds et al. [2017] in 2 stillbirths after 20 and 30 weeks of gestation (Table 1). The phenotype of this very rare disorder is quite striking and is characterized by severe intrauterine growth restriction with severe microcephaly and distinctive craniofacial features, limb malformation, and perinatal lethality. Affected neonates have characteristic facial features with short palpebral fissures, small eyes, a beaked and broad nose, macrosomia, and micrognathia. Malformations of the limbs affect the upper limbs more severely than the lower limbs and include oligodactyly, absent thumbs, short forearms with absent or underdeveloped radius and/or ulna, and sometimes humeroradial synostosis. Lower limbs are short with underdeveloped or absent fibula, and usually clubfeet with variable degree of syndactyly. Other skeletal abnormalities include craniosynostosis and absence of 1 or 2 pairs of ribs. Less constant malformations include a cleft palate and cardiac, gastrointestinal and genitourinary defects. The affected neonates in this report had most of the features typical of the disorder but in addition had more severe malformations not previously described in the reported cases. These included absent eyes, atretic auditory canal, and absent genitalia. Moreover, the lower limbs were more severely affected than in previously reported cases. The pelvis, femur, and tibia were hypoplastic, and the fibula was absent as well as the toes. All the reported cases with this phenotype had the same homozygous loss-of-function variant in *DONSON*. The same variant was also reported as compound heterozygous with another missense variant in a

Turkish patient who had the less severe phenotype described with mutations in this gene. No other homozygous complete loss-of-function variants have been described in the literature. This is the second homozygous loss-of-function variant in this gene to be reported. Patients' clinical profiles have been reviewed and summarized in Table 1. It is important to note that in consanguineous families, it is possible to have more than one variant cosegregating with the disease, and in this family, it was not feasible to exclude other variants which should have been screened for via whole-exome/genome approach.

Another 20 pathogenic variants were reported in *DONSON* by Reynolds et al. [2017] in 20 families with 27 patients with MISSLA. These patients presented with a significantly less severe phenotype which includes microcephaly, short stature, minor skeletal abnormalities mostly limited to upper limb deformities, and absence of facial malformations or recurrent organ dysfunction. Mild intellectual disability was detected in some patients. They also exhibited variable skeletal deformities particularly in the upper limbs. This phenotype variation appears to be due to the effect of these variants, as most of them were found to be hypomorphic. Functional analysis of these hypomorphic variants showed that they either have an impact on the protein trafficking but have preserved function or have affected the level of expression of the transcript or the protein [Reynolds et al., 2017]. Some patients with MISSLA carried heterozygous null variants; however, they were all inherited in a compound heterozygous state, where the other allele harbors a hypomorphic variant. This suggested that these biallelic mutations may cause partial loss of function.

The *DONSON* gene encodes the downstream neighbor of SON, a protein that has been recently identified as a replisome component, interacting with key proteins, such as MCM helicases and GINS complex, stabilizing replication forks during S phase [Reynolds et al., 2017]. It is assumed to act as a stability molecule of replication forks allowing symmetric and efficient movement of the replisome, particularly in stalled forks where single stranded DNA might be more exposed to damage by nucleases. It also ensures efficient activation of intra-S-phase and G/2M cell cycle checkpoints under endogenous replication stress via interaction with the ATR-dependent replication stress response signaling. In the absence of these 2 essential functions, DNA replication would be extensively dysregulated with higher chances of genomic instability and chromosomal breakage. Homozygous knockout of *DONSON* orthologs in murine models resulted in early fetal deaths and is incompatible with life in *Drosophila* models. Thus, similar to *PCNT* and *ATR* genes, which have been previous-

ly linked to a microcephalic micromelia-like phenotype [Casper et al., 2004; Rauch et al., 2004], *DONSON* provides further evidence that genes encoding either components of the DNA replication machinery (replisome) or proteins involved in genome stability are a frequent cause of microcephalic dwarfism.

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Statement of Ethics

Written informed consent was obtained from the parents for research and publication. This study has been approved by the Al-Ain Medical Human Research Ethics Committee (Approval Nr. ERH 2015-3241 15-115) according to the national regulations.

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Disclosure Statement

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Author Contributions

H.A. conducted the lab and in silico analysis, the segregation study and drafted the manuscript. A.J. assisted in the sequencing of the samples. B.A. critically reviewed the manuscript and co-supervised the project. L.A. supervised the project, clinically evaluated the described cases and critically reviewed the manuscript.