Further Expansion of the Mutational Spectrum of 3MC Syndrome: A Novel *MASP1* Pathogenic Variant in a Male Patient

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Established Facts

- 3MC syndrome is a rare autosomal recessive syndrome characterized by facial dysmorphism, developmental and growth delay, and additional multisystemic findings.
- Biallelic pathogenic variants in *MASP1*, *COLEC11*, and *COLEC10* are responsible for 3MC syndrome. To date, only 16 homozygous/compound heterozygous pathogenic variations (12 missense, 3 splice site, 1 deletion) in 27 patients from 22 families have been reported in *MASP1*-associated 3MC syndrome.
- Identifying new patients and reporting their clinical and molecular findings will enable delineating this rare syndrome's phenotypic and genotypic spectrum.

Novel Insights

- We report on a patient with a novel homozygous splice site mutation in *MASP1*.
- Pyloric stenosis and relative macrocephaly are findings previously described in only 2 patients, and their association with the syndrome had been reported controversial. The presence of these findings in our patient supports considering the abovementioned as components of 3MC syndrome.

Keywords

3MC syndrome · *MASP1* · Dysmorphism · Novel variant · Macrocephaly · Pyloric stenosis · Prenatal finding

Abstract

The 3MC syndrome is a rare autosomal recessive syndrome characterized by facial dysmorphism, multiple congenital abnormalities, and postnatal growth deficiency. Hyper-telorism, blepharophimosis, blepharoptosis, high-arched

eyebrows, and cleft lip/palate compose the facial gestalt, which is the key component for diagnosing the syndrome. Biallelic pathogenic variants in *MASP1*, *COLEC11*, and *COLEC10* are responsible for 3MC syndrome in which both genotypic and phenotypic heterogeneity is described. To date, 16 homozygous/compound heterozygous pathogenic variations in 27 patients from 22 families have been reported in the *MASP1* gene associated with 3MC syndrome. Here, we report a male patient with a novel homozygous pathogenic variant in *MASP1* in whom macrocephaly, pyloric stenosis,

and prenatal findings including polyhydramnios, aortic dilatation, and intracranial cysts beside the distinctive facial features were detected. Reporting detailed clinical and molecular findings in patients is pivotal in terms of enabling the phenotypic and genotypic spectrum of this rare syndrome to be delineated.

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Introduction

The 3MC syndrome is a rare autosomal recessive syndrome characterized by facial dysmorphism, multiple congenital abnormalities, and postnatal growth deficiency. It is an umbrella diagnosis based on the initials of the Michels, Malpuech, Carnevale, and Mingarelli syndromes, which share overlapping phenotypic features, especially in the facial gestalt [Michels et al., 1978; Carnevale et al., 1989; Malpuech et al., 1983]. Hypertelorism, blepharophimosis, blepharoptosis, high-arched eyebrows, and cleft lip/palate are the main components of the dysmorphic features. Skeletal anomalies including craniosynostosis and radioulnar synostosis, anterior chamber defects of the eye, hearing loss, caudal appendage, umbilical hernia, and cardiac and urogenital anomalies are the other reported features of the syndrome [Sirmaci et al., 2010; Atik et al., 2015; Urquhart et al., 2016; Gardner et al., 2017; Munye et al., 2017; Basdemirci et al., 2019].

Biallelic pathogenic variants (PV) in *MASPI*, *COLEC11*, and *COLEC10* are responsible for 3MC syndrome [Sirmaci et al., 2010; Graul-Neumann et al., 2018], and all are involved in the lectin complement pathway [Degn et al., 2011]. Among these, *MASPI* has been shown to play an essential role in both craniofacial development and complement activation [Rooryck et al., 2011]. To date, 16 homozygous/compound heterozygous PV (12 missense, 3 splice-site, 1 deletion) in 27 patients from 22 families have been reported in the *MASPI*-associated 3MC syndrome [Sirmaci et al., 2010; Atik et al., 2015; Urquhart et al., 2016; Gardner et al., 2017; Munye et al., 2017; Graul-Neumann et al., 2018; Basdemirci et al., 2019].

Herein, we report on a patient with a novel homozygous splice-site pathogenic variant in *MASPI* to further delineate both the phenotypic and genotypic spectrum of this rare syndrome.

Case Report

The proband is the fifth child of healthy, consanguineous (first-cousin marriage) Syrian parents. He was born via cesarean delivery at 35 weeks, with a birth weight of 2,420 g (<3rd centile). Birth length was 46 cm (3rd centile), and head circumference was 34.5 cm (25th–50th centile). In the prenatal period, polyhydramnios, intracranial cystic lesions, and dilatations in the descending aorta were detected in fetal ultrasonography; however, fetal MRI was not performed. During the neonatal period, he was hospitalized in the intensive care unit due to feeding difficulty, frequent vomiting, and pyloric stenosis. On the postnatal fourth day, surgical intervention was performed for gastric rupture. The patient was referred to the genetics department at the age of 8 months because of dysmorphic facial features. At admission, his weight was 6,000 g (<3rd centile), length was 64 cm (<3rd centile), and his head circumference was 45 cm (15th centile). Physical examination revealed a high and wide forehead, large frontal fontanel, hypertelorism, blepharophimosis, blepharoptosis, epicanthus inversus, high-arched eyebrows, low-hanging columella, broad nasal bridge, retrognathia, skin tag at xiphoid process, umbilical hernia, clinodactyly of 5th fingers in both hands, hypoplastic scrotum, cryptorchidism, and a sacral dimple (Fig 1). The developmental milestones were compatible with his peers. Parents mentioned a sibling with similar facial features and cleft palate who died at the age of 3 months due to congenital heart disease.

The audiometric evaluation and eye examination were normal. Echocardiography revealed a small muscular ventricular septal defect, patent ductus arteriosus, and patent foramen ovale. Transfontanel ultrasonography was normal. Abdominal ultrasonography revealed lobulated contoured kidneys fused at the base at the midline pelvic region (horseshoe kidney). With the clinical findings and similarly affected sibling history, we considered 3MC syndrome in the patient. Clinical exome sequencing was performed since the genetic heterogeneity defined in the syndrome.

Method and Results

Automatic DNA isolation was performed by the standard protocols of the QIAamp DNA Mini (Qiagen) kit from peripheral blood samples. The sequencing was done on Illumina NextSeq 500 platform within the test scope with SOPHIA Clinical Exome Solution using Illumina V2 chemicals. Sequence analysis covered each gene's coding region, including all coding exons $\pm$ 10 bp of adjacent intronic sequences, and each nucleotide is read at a depth of at least 50 $\times$. Any variants that fall outside these regions and exonic variants with a minor allele frequency of less than 10% were considered as false positives and not analyzed. The DNA sequences were aligned to the NCBI Build36 (hg18) version of the human genome. The Sophia-DDM-V5.2 bioinformatics analysis software performed variant calling and data analysis. The interpretation of the variants was performed according to the 2015

Fig. 1. Clinical picture of the patient at the age of 8 months (a) and 3 years and 6 months (b). Note the high and wide forehead, hypertelorism, blepharophimosis, blepharoptosis, epicanthus inversus, high-arched eyebrows, low-hanging columella, broad nasal bridge, retrognathia (a, b), skin tag at xiphoid process, and umbilical her-

nia (a, c). Pedigree of the family (d). Sanger sequencing image of the *MASPI* c.744+1G>A variant in the father (e) and Integrative Genomics Viewer (IGV) screenshot of the *MASPI* c.744+1G>A variant in the mother (f). IGV screenshot and Sanger sequencing images of the *MASPI* c.744+1G>A variant in the patient (g, h).

ACMG standards and guidelines [Richards et al., 2015]. GnomAD, 1000 genome projects, dbSNP data were used as the control population. In silico prediction programs such as SIFT, PolyPhen, EIGEN, FATHMM-MKL, MutationTaster, and GERP were used for variant pathogenicity predictions.

In clinical exome sequencing analysis, *MASPI* (NM_001031849), c.744+1G>A, homozygous splice donor variation was detected, which is a null variant affecting donor splice site of *MASPI* (PVS1). In silico prediction algorithms (EIGEN, FATHMM-MKL, and Muta-

tionTaster) indicate that the variant has a deleterious effect at protein level (PP3). The allele frequency of the variant is 2/251.232 (0.00000796 %), and it is not listed in a homozygous state in the current genome variant database gnomAD (PM2). Also, this variant has not been reported in ClinVar or in the Human Genome Mutation Database (HGMD). Considering the evidence described above (PVS1, PM2, PP3) and the clinical findings of the patient, the *MASPI* (NM_001031849) c.744+1G>A variant has been classified as pathogenic. The variant was confirmed by Sanger sequencing. Primer sequences and

Table 1. Summary of clinical findings in reported patients with 3 MC syndrome and the present patient

	Published cases	Present case
Mutation type, <i>n</i>	20 SNV, 1 intergenic deletion	SNV
Number of patients	27	+
Number of families	22	+
Origin, <i>n</i>	9 Turkey, 3 Sri Lanka 3 Brazil, 2 Italy 2 Pakistan, 1 Israel 1 Greece, 2 India 1 German, 1 Syria	1 Syria
Gender	13 F/12 M/1 NA	M
Consanguinity	14/25	+
Short stature(<3rd centile)	11/24	+
Cognitive impairment	13/24	–
Abnormal skull shape	9/21	+
High-arched eyebrows	25/25	+
Telecanthus/hypertelorism	23/26	+
Blepharoptosis	25/26	+
Cleft palate/lip	16/27	–
Abnormal umbilicus/diastasis	11/20	+
Renal anomalies	11/26*	Horseshoe kidney
Caudal appendage	20/27	–
Hearing loss	15/24	–
Genital anomaly	10/23	Cryptorchidism
Congenital cardiac abnormality	16/25**	VSD, PDA, PFO
Structural anomaly in cranial/spinal MRI	9/22	NA
Others	Duplicated concha nasalis Hyperpigmented retina Vascularization of lenses Corneal clouding Polar cataracts Skin tag Neonatal teeth Hypopigmented retina Radioulnar synostosis (2 patients) Dysmorphic ears Flattened nasal root Neonatal tooth Left clubfoot Accessory nipple on the left Bilateral fifth finger camptoclinodactyly Solid lesion in liver Bilobed right lung Posterior rotation of the left ear Overriding fifth toe of the right foot	Pyloric stenosis Macrocephaly Polyhydramnios Aortic dilatation (prenatal) Intracranial cysts (prenatal)

VSD, ventricular septal defect; PDA, patent ductus arteriosus; PFO, patent foramen ovale; NA, not available; +, present; –, absent.* Renal anomalies detected in previously reported patients: horseshoe kidney in 3 patients, cross renal ectopy in 2 patients, bilateral duplicated kidney, solitary ectopic kidney, left renal agenesis, and hydronephrosis in 1 patient each. ** Congenital cardiac abnormalities detected in previously reported patients: patent ductus arteriosus in 9 patients, atrial septal defect in 7 patients, ventricular septal defect in 1 patient. In 5 patients, detailed information about cardiac abnormality could not be obtained.

Fig. 2. Protein domains of *MASPI* and distribution of reported pathogenic variants. The variant detected in the present case is indicated with *.

reaction conditions are available on request. In segregation analysis, the parents were found to be heterozygous for the same variation.

Discussion and Conclusion

The 3MC syndrome constitutes a highly heterogeneous group (both phenotype and genotype). So far, approximately 22 families have been reported with variants in *MASPI* associated with 3 MC syndrome [Basdemirci et al., 2019] (Table 1). All of the cases shared dysmorphic facial findings that were considered as pathognomonic, along with the rarer findings identified in other organ systems [Sirmaci et al., 2010; Atik et al., 2015; Urquhart et al., 2016; Gardner et al., 2017; Munye et al., 2017; Basdemirci et al., 2019; Garfield and Sergent, 2021]. Given both genotypic and phenotypic heterogeneity, describing new patients with novel clinical and molecular findings is essential to delineate phenotype-genotype correlation.

Herewith, we report on a male patient with a novel splice site variation in *MASPI* with additional findings. Characteristic facial features, postnatal growth retardation, cardiac anomalies, horseshoe kidney, clinodactyly, skin tag at xiphoid process, and sacral dimple are among the previously reported findings which are also present in our patient. Among the facial features, cleft palate/lip is present in approximately 50% of the reported patients with 3MC syndrome, especially those with PV in *MASPI* [Atik et al., 2015]. Cleft lip/palate was not present in our patient; however, there is a history of a sibling with similar dysmorphic features and cleft palate resembling 3MC syndrome. Although segregation analysis could not be performed on the deceased sibling, this finding may suggest variable expressivity in *MASPI*, even in the same

family. Intellectual disability/developmental delay (ID/DD) is considered as a component of the syndrome; however, the incidence is variable according to the underlying molecular etiology. ID/DD was reported slightly lower (28.5%) among patients with *MASPI* PV [Atik et al., 2015]. As compatible with this observation, our patient's developmental milestones were also consistent with his peers, but given the patient's early age, long-term follow-up is required to make a clear psychometric assessment.

Macrocephaly and pyloric stenosis reported in our patient are extremely rare findings among patients with 3MC syndrome. Macrocephaly was previously described in a patient by Graul-Neumann et al. [2018]; however, it was assessed as a familial finding, not a feature of the syndrome. In our patient, the parents' head circumference was within normal limits. Considering the patient reported by Graul-Neumann et al. [2018], we suggest that the relative macrocephaly is one of the rare findings of 3MC syndrome. Pyloric stenosis was previously reported in a patient with *COLEC11* PV, and our patient is the first individual to have pyloric stenosis reported in *MASPI* PV [Urquhart et al., 2016]. On the other hand, pyloric stenosis is considered a birth defect with multifactorial inheritance, especially when it does not accompany another anomaly. The incidence is 2.4 per 1,000 live births in Caucasians, and the male gender is frequently affected [Garfield and Sergent, 2021]. Therefore, it should be kept in mind that pyloric stenosis may be a secondary accompanying finding in the reported patients. Additional patients are needed to enroll this finding as a component of the syndrome.

Prenatal findings, including polyhydramnios, aortic dilatation, and intracranial cysts, are the novel features in our patient. Despite the approximately 22 reported families with *MASPI*-related 3MC syndrome so far, informa-

tion on prenatal findings of the syndrome is scant. Recently, Lawson et al. [2020] reported an affected fetus with cleft palate, cardiac anomalies, and caudal appendage. In our patient, intracranial cysts were regressed after delivery; also, findings related to aortic dilatation were not detected in echocardiography at the age of 1 month. Since polyhydramnios may have been seen secondary to pyloric stenosis, and aortic dilatation or intracranial cysts could not be detected in the neonatal period, it is controversial to suggest that these findings are related to the syndrome [Pappalardo et al., 2009]. New patients are required to determine the association of these findings with the syndrome.

The *MASP1* gene encodes 3 isoforms, MASP1, MASP3, and MASP4, which differ in their carboxyl terminals [Sirmaci et al., 2010; Atik et al., 2015]. MASP1 and MASP3 isoforms consist of 6 protein domains, while MASP4 has 4 protein domains [Sirmaci et al., 2010; Atik et al., 2015] (Fig. 2). Among these, the serine protease domain stands out as the most frequently affected domain in patients with 3MC syndrome and is present only in MASP1 and MASP3 isoforms [Sirmaci et al., 2010; Atik et al., 2015]. To date, there were 16 PV in *MASP1* associated with 3MC syndrome in the HGMD, including missense, nonsense variants, large deletion, and intronic variants that affect splice sites as in our patient. All reported so far in *MASP1* are shown in Figure 2. The present *MASP1* (NM_001031849), c.744+1G>A variant lies in the donor splice site affecting exon 5 and shared by all 3 isoforms. It is also found to be in a highly conserved region with PhyloP and GERP scores of 4.88 and 5.53, respectively. This variant is predicted to affect the CUB2 domain, the second most common protein domain in which PV are reported in 3MC syndrome (Fig 2). Among the patients with PV affecting the CUB2 domain, we figured out that in all but one patient had renal anomalies in addition to the characteristic features of the syndrome [Sirmaci et al., 2010; Urquhart et al., 2016] whereas, only one patient had renal anomaly among patients with PV in the serine protease domain [Sirmaci et al., 2010; Atik et al., 2015; Urquhart et al., 2016; Graul-Neumann et al., 2018; Basdemirci et al., 2019]. Our patient also had a horseshoe kidney, consistent with these patients' findings. However, the number of patients was insufficient to establish a reliable correlation between the renal features and affected protein domains.

In conclusion, we report on a male patient with a novel homozygous variant in *MASP1* to expand the mutational spectrum of *MASP1*-related 3MC syndrome. Since there is a limited number of reported patients, identifying

new patients and elaborating their clinical findings will increase clinicians' awareness and enable establishing phenotype-genotype correlations.

Statement of Ethics

This study was performed in accordance with the Declaration of Helsinki Principles. Written informed consent was obtained from the patients' parents for publication of this case report and any accompanying images.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

The authors contributed to the conception and design of the work, drafted and revised it critically, and approved the final version of the manuscript. The authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are properly investigated and resolved.

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