CLINICAL REPORT

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Microcephaly-Capillary Malformation Syndrome: Brothers with a Homozygous *STAMBP* Mutation, Uncovered by Exome Sequencing

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We describe two brothers from a consanguineous family of Egyptian ancestry, presenting with microcephaly, apparent global developmental delay, seizures, spasticity, congenital blindness, and multiple cutaneous capillary malformations. Through exome sequencing, we uncovered a homozygous missense variant in *STAMBP* (p.K303R) in the two siblings, inherited from heterozygous carrier parents. Mutations in *STAMBP* are known to cause microcephaly-capillary malformation syndrome (MIC-CAP) and the phenotype in this family is consistent with this diagnosis. We compared the findings in the present brothers with those of earlier reported patients.

Key words: microcephaly-capillary malformation syndrome; MIC-CAP; *STAMBP*; exome sequencing

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INTRODUCTION

Microcephaly-capillary malformation syndrome (MIC-CAP; OMIM: 614261; ORPHA:294016) is a rare neurological and vascular disorder characterized by microcephaly, global developmental delay, seizures, and multiple cutaneous capillary malformations [Carter et al., 2011, 2013; McDonell et al., 2013]. It is caused by homozygous or compound heterozygous mutations in the STAM-binding protein gene (*STAMBP*) [McDonell et al., 2013] and was recently described as a new syndrome [Carter and Boycott, 2011; Carter et al., 2011].

Here, we report two brothers with previously unexplained microcephaly and capillary malformations who were found by exome sequencing to have a homozygous mutation in *STAMPB*, consistent with a diagnosis of MIC-CAP.

CLINICAL REPORT

Patients IV-1 and IV-2 (Fig. 1A) are brothers born to first cousin parents of Egyptian origin. Both boys presented in infancy with

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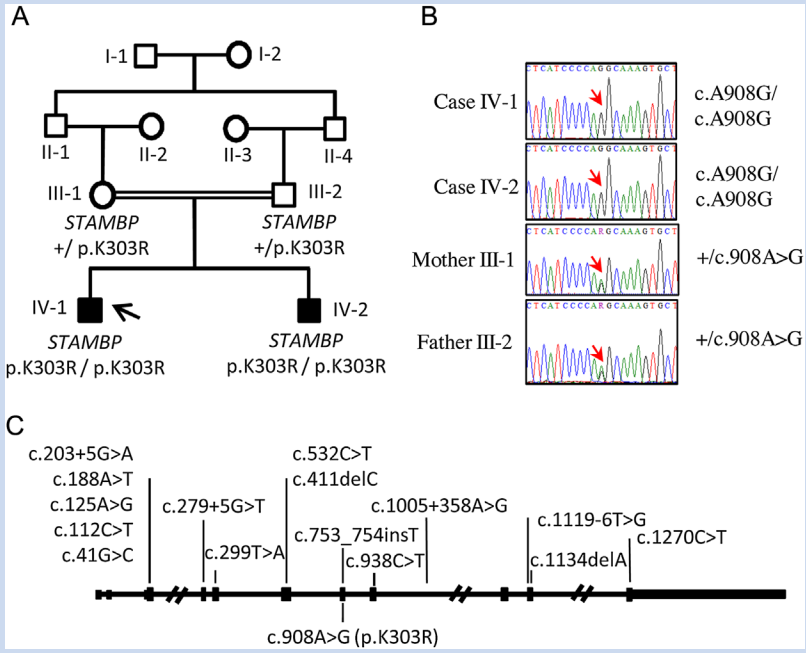

FIG. 1. (A) Pedigree; filled shape: MIC-CAP syndrome. Double line denotes a consanguineous relationship. Relevant genotypes are shown below the pedigree symbols for individuals tested (+denote wildtype allele). (B) Sequence chromatogram and the position of the mutation in *STAMBP*. The red arrow indicates the position of the mutation. (C) Mutations reported in *STAMBP* (chromosome 2, hg19, 74,056,114–74,094,295, RefSeq NM_006463) as causal for MIC-CAP. Those above the gene diagram have been previously reported, and the mutation from the two patients in this study is indicated below. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmga].

microcephaly (head circumferences from −3 to −4 standard deviations below the mean for age and sex), apparent global developmental delay, seizures, spasticity, and congenital blindness. They have a similar dysmorphic appearance including sloping forehead, long palpebral fissures, telecanthus, long philtrum, micrognathia, and underdeveloped toes (Fig. 2). They both have multiple capillary malformations of the skin. Abdominal ultrasound for both children was apparently normal. Both had brain magnetic resonance imaging scans, which showed simplified gyral patterns with increased extra-axial space and diffuse cerebral atrophy (Fig. 2). Electroencephalographies were strikingly abnormal, with generalized sharp wave epileptogenic activity for IV-1and burst suppression pattern for IV–2. Eye exam showed optic atrophy. Basic metabolic screening was normal.

METHODS

Exome Sequencing

Parent–child relationships were confirmed using the AmpFlSTR Identifiler PCR Amplification Kit (Life Technologies) according to manufacturer's instructions. We performed exome sequencing on patient IV-1 and both parents (III-1 and III-2; Fig. 1A), with total genomic DNA from whole blood, following standard protocols. We sequenced using Illumina HiSeq2500, following exome capture with the Agilent SureSelect Human All Exon V5 target enrichment kit. We aligned sequences to the hg19 reference sequence using Burrows–Wheeler Aligner (BWA) v0.5.9 [Li and Durbin, 2009], and used the Genome Analysis Toolkit (GATK) v1.1-28 [McKenna et al., 2010] to detect single nucleotide and small insertion and deletion variants. We focused our analysis on rare variants, defined as those with a frequency of <0.01 in 1000 Genomes [Genomes Project et al., 2012], National Heart, Lung, and Blood Institute (NHLBI) Exome Variant Server and Exome Aggregation Consortium (ExAc datasets; http://exac.broadinstitute.org/).

Copy Number Analysis

We genotyped patient IV-1 using the Affymetrix CytoScan HD array platform (Affymetrix Inc.) to look for clinically relevant copy number variants (CNVs). We detected CNVs using Affymetrix Chromosome Analysis Suite (ChAS), iPattern, Nexus, and Partek algorithms. We defined a stringent set of variants wherein each variant was detected by at least by two algorithms [Pinto et al., 2011]. We defined rare variants against a pool of CNVs detected from more than 10,000 individuals [Oskoui et al., 2015] and further limited the variants to those from regions that are at least 75% copy number stable in the human genome [Zarrei et al., 2015].

RESULTS

Through exome sequencing of patient IV-1 and his parents, we identified a missense variant (chr2:74077543:A>G; c.908A>G;

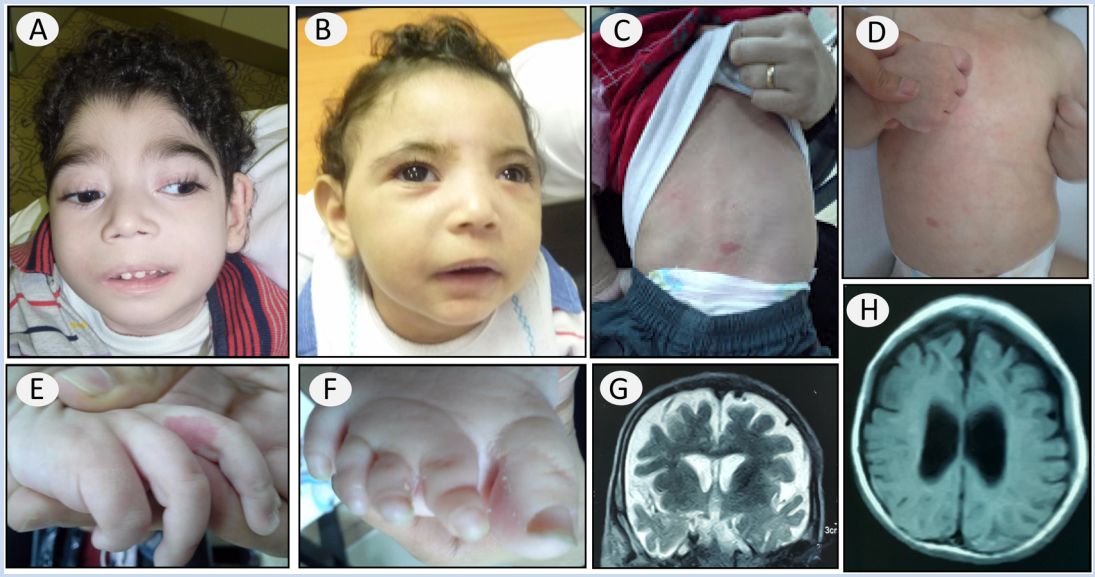

FIG. 2. (A) Patient IV-I facial appearance. Note bitemporal narrowing, long palpebral fissures with ptosis, telecanthus, long and smooth philtrum, and micrognathia. (B) Patient IV-2 facial appearance. Note bitemporal narrowing, hooded lids, telecanthus, long and smooth philtrum, and micrognathia. (C) Patient IV-I's back, showing capillary malformations. (D) Patient IV-2's torso showing capillary malformations in the right lower quadrant, and just above the umbilicus. (E) Patient IV-II's left hand, showing capillary malformations. (F) Patient IV-2's right foot, showing characteristic underdeveloped toes 2–4. (G) Coronal brain MRI for Patient IV-1, and (H) axial brain MRI for Patient IV-2, both showing simplified gyral patterns with increased extra-axial space and diffuse cerebral atrophy.

hg19) in exon seven of *STAMBP*, for which IV-1 is homozygous and both parents are heterozygous carriers (Fig. 1B and C). The variant was confirmed by targeted Sanger sequencing in all four family members, which showed that affected sibling (IV-2) is also homozygous for the variant (Fig. 1B and C). The single nucleotide change results in an arginine to lysine substitution at amino acid 303 (p.K303R) in the corresponding protein. This particular variant is not documented in dbSNP, 1000 Genomes, or NHLBI datasets, but one heterozygous carrier from the East Asian population is reported in the ExAc database (giving an allele frequency of 8×10^{-6}).

We also investigated the potential contribution of rare CNVs to the pathogenicity for IV-1, but found nothing of note.

DISCUSSION

The clinical presentations of brothers IV-1 and IV-2 are in keeping with previously reported MIC-CAP patients (Table I). Both have congenital microcephaly, generalized capillary malformations of skin, spasticity with seizures, and optic atrophy. Patients exhibit toe and nail anomalies, and both have been blind since birth.

STAMBP encodes deubiquitinating isopeptidase, which has a key role in cell surface receptor-mediated endocytosis and sorting [McDonell et al., 2013]. Patients with MIC-CAP have reduced STAMBP protein expression, associated with accumulation of ubiquitin-conjugated protein aggregate and elevated apoptosis, and these factors may contribute to the progressive neuronal loss underlying MIC-CAP syndrome [McDonell et al., 2013]. Mutations in *STAMBP* result in increased activity (insensitive to inhibition) of the RAS-MAPK pathway, which relays signals from several cell surface receptors to transcriptional regulation in the nucleus [Hrustanovic et al., 2015], and might contribute to disrupted angiogenesis and vascularization [McDonell et al., 2013]. Moreover, this gene's protein product contributes to pathways involving neuronal and growth regulation, and genetic variants contribute to the microcephaly and various brain anomalies in MIC-CAP patients [McDonell et al., 2013].

To date, 12 patients (including both males and females) have been reported with MIC-CAP, from families with consanguineous relationships and from unrelated parents [Carter and Boycott, 2011; Carter et al., 2011, 2013; Isidor et al., 2011; Mirzaa et al., 2011; McDonell et al., 2013; Pavlovic et al., 2014; Faqeih et al., 2015]. All patients reported to date have inherited defective alleles from two carrier parents, except one patient with a report of uniparental disomy [Carter et al., 2013; McDonell et al., 2013].

The syndrome is not limited to a specific ethnic background; the reported patients are European, African–American, Polynesian, and Arab. Here, we report two additional male siblings, both homozygous for an exonic mutation in *STAMBP*, born to first-cousin Egyptian parents (Fig. 1A). The variant (chr2:74077543:A:G; hg19; Fig. 1B and C), is very rare (frequency <0.01%) and was not previously known to be pathogenic. This amino acid protein change in the proximal ubiquitin binding site, within the JAMM domain, probably decreases ubiquitin binding to STAMBP [Bueno et al., 2015].

TABLE I. Comparison of Clinical Features of the Patients Reported Herein With Those of Previously Reported Individuals With MIC-CAP Due to *STAMBP* Mutations

Clinical feature	This paper (n = 2)	Previously-reported individuals with *STAMBP* mutations (n = 12)
Sex	2 males	8 males, 4 females
Dysmorphic features		
Widely spaced eyes	–	7 (58%)
Long palpebral fissures	+	9 (75%)
Underdeveloped distal phalanges	+	11 (92%)
Progressive microcephaly	+	9 (75%)
Seizures		
Infantile onset	+	11 (92%)
Intractable	+	11 (92%)
Minimal developmental progress	+	11 (92%)
Abnormal neurological exam		
Spastic quadriparesis	+	10 (83%)
Myoclonus	NA	6 (50%)
Dyskinesia	NA	5 (42%)
Multiple generalized capillary malformations	+	12 (100%)
Vision		
Optic atrophy +/– congenital blindness	+	10 (83%)
Brain imaging		
Simplified gyral pattern	+	11 (92%)
Cerebral atrophy/increased extra-axial space	+	11 (92%)
Hippocampal hypoplasia	–	6 (50%)
Other		
Aplasia cutis congenita	–	2 (17%)
Small for gestational age	–	11 (92%)
Congenital hypothyroidism	–	2 (17%)
Cardiac malformation	–	4 (34%)
Genitourinary malformation	–	2 (17%)
Umbilical or inguinal hernia	–	2 (17%)
Sensorineural deafness	–	1 (8%)
Cleft palate	–	1 (8%)

NA, Not Available.

Mutations in *RASA1* and *KRITI*, which encode p120-RasGAP and RAS-related protein 1A interactant, respectively, are found in patients with capillary malformation [Eerola et al., 2000, 2003; Revencu et al., 2013]. Patients with MIC-CAP have not shown any exonic sequence variation in *RASA1* [Carter et al., 2011; McDonell et al., 2013; Faqeih et al., 2015], nor did we find such mutation (single nucleotide variant (SNV) or CNV) in these genes in our patient IV-1.

One published patient with MIC-CAP has a 307 kb deletion on the long arm of chromosome 13 impacting *GJB6* and *CRYL1*, in addition to a homozygous mutation in *STAMBP* [Carter et al., 2011; McDonell et al., 2013] but we found no CNV or rare SNV impacting either of these genes in patient IV-1.

In summary, our report of two additional related MIC-CAP patients from the Arab background with a mutation in *STAMBP* affirms the role of STAMBP protein in the normal development of the central nervous system and in angiogenesis. These bring to six, the number of patients of Arab origin with this rare syndrome; however the mutations in *STAMBP* are unique in each family. We have also demonstrated the power of exome sequencing to facilitate diagnosis in patients with rare genetic disorders [Abu-Elmagd et al., 2015].

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AUTHORS' CONTRIBUTION

M.I.N, M.Z., and S.W.S. conceived and designed the project. M.Z. oversaw experiments confirming these results. M.Z., S.W., and D.M. analyzed and interpreted the microarray findings and the exome data. M.I.N., S.S., A.G.C., Y.A.A., M.R., M.T.C., and M.H.A. provided and interpreted phenotypic details for the patients. C.R.M. advised on the study design and writing of the manuscript. M.Z., M.T.C., S.W., and S.W.S. wrote the manuscript.

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