

LETTER TO THE EDITOR

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Large homozygous *RAB3GAP1* gene microdeletion causes Warburg Micro Syndrome 1

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Abstract

Warburg micro syndrome (WARBM) is a genetic heterogeneous disease characterized by microcephaly, intellectual disability, brain, ocular, and endocrine anomalies. WARBM1-4 can be caused by biallelic mutations of the *RAB3GAP1* (*RAB3* GTPase-activating protein 1), *RAB3GAP2*, *RAB18* (*RAS*-associated protein *RAB18*), or *TBC1D20* (*TBC1* domain protein, member 20) gene, respectively. Here, we delineate the so far largest intragenic homozygous *RAB3GAP1* microdeletion. Despite the size of the *RAB3GAP1* gene deletion, the patient phenotype is mainly consistent with that of other WARBM1 patients, supporting strongly the theory that WARBM1 is caused by a loss of *RAB3GAP1* function. We further highlight osteopenia as a feature of WARBM1.

Keywords: *RAB3GAP1*, WARBM, Warburg micro syndrome, Microcephaly, Intellectual disability, Congenital cataract, Array CGH

Letter to the editor

Warburg micro syndrome (WARBM) is a rare autosomal recessive disorder characterized by neurodevelopmental abnormalities such as congenital or postnatal microcephaly, severe intellectual disability, pachy- or polymicrogyria, and hypoplasia/agenesis of the corpus callosum as well as ocular manifestations including congenital cataract, microcornea, microphthalmia, and optic atrophy [1-3]. Further features of WARBM comprise hypothalamic hypogonadism, epilepsy, limb spasticity, and joint contractures. WARBM1 (MIM#600118) is caused by biallelic mutations of the *RAB3* GTPase-activating protein 1 gene *RAB3GAP1* (2q31; MIM#602536), WARBM2 (MIM#614225) by mutations of the *RAB3* GTPase-activating protein 2 gene *RAB3GAP2* (1q41; MIM#609275), WARBM3 (MIM#614222) by mutations of the *RAS*-associated protein *RAB18* gene *RAB18* (10p12.1; MIM#602207), and WARBM4 (MIM#615663) by mutations in the *TBC1* domain protein, member 20, gene *TBC1D20* (MIM#611663). Most mutations were predicted to result in nonsense-mediated mRNA decay and/or loss-of-

protein-function [1,2,4-7], putatively explaining the lack of a genotype-phenotype correlation. We here report the largest *RAB3GAP1* gene microdeletion to date in patients with WARBM1 and compare their phenotype with that of other WARBM1 patients. The two index patients were born at term without complications as the first and second child of healthy, consanguineous parents of Kurdish-Armenian descent (Figure 1). Pregnancies were uneventful, and anthropometric data in the first months of life were reported to be normal by the parents. Both patients were diagnosed with bilateral cataracts in the first months of life, and cataract surgery was performed in patient IV.2. The parents noted progressive hypotonia with loss of head control and finally developmental delay when their child did not attempt to roll within the first year of life. At first presentation at 6 (IV.1) and 5 (IV.2) years-of-age, the patients were not able to roll over, sit, stand, or speak, exhibited a short stature, dystrophy, and microcephaly (IV.1: height 90 cm, 16 cm <3. centile, -5.2 SD; weight 11 kg, 4 kg <3. centile, -3.4 SD; head circumference 47 cm, 1.5 cm <3. centile, -2.6 SD; IV.2: height 95 cm, 6 cm <3. centile, -3.3 SD; weight 10.3 kg, 5 kg <3. centile, -3.7 SD; head circumference 45 cm, 4 cm <3. centile, -3.8 SD), and had bilateral cataracts (unilateral iatrogenic aphakia in IV.2), microcornea, and microphthalmia. Bilateral cryptorchidism was present in IV.2. In both patients, poor head

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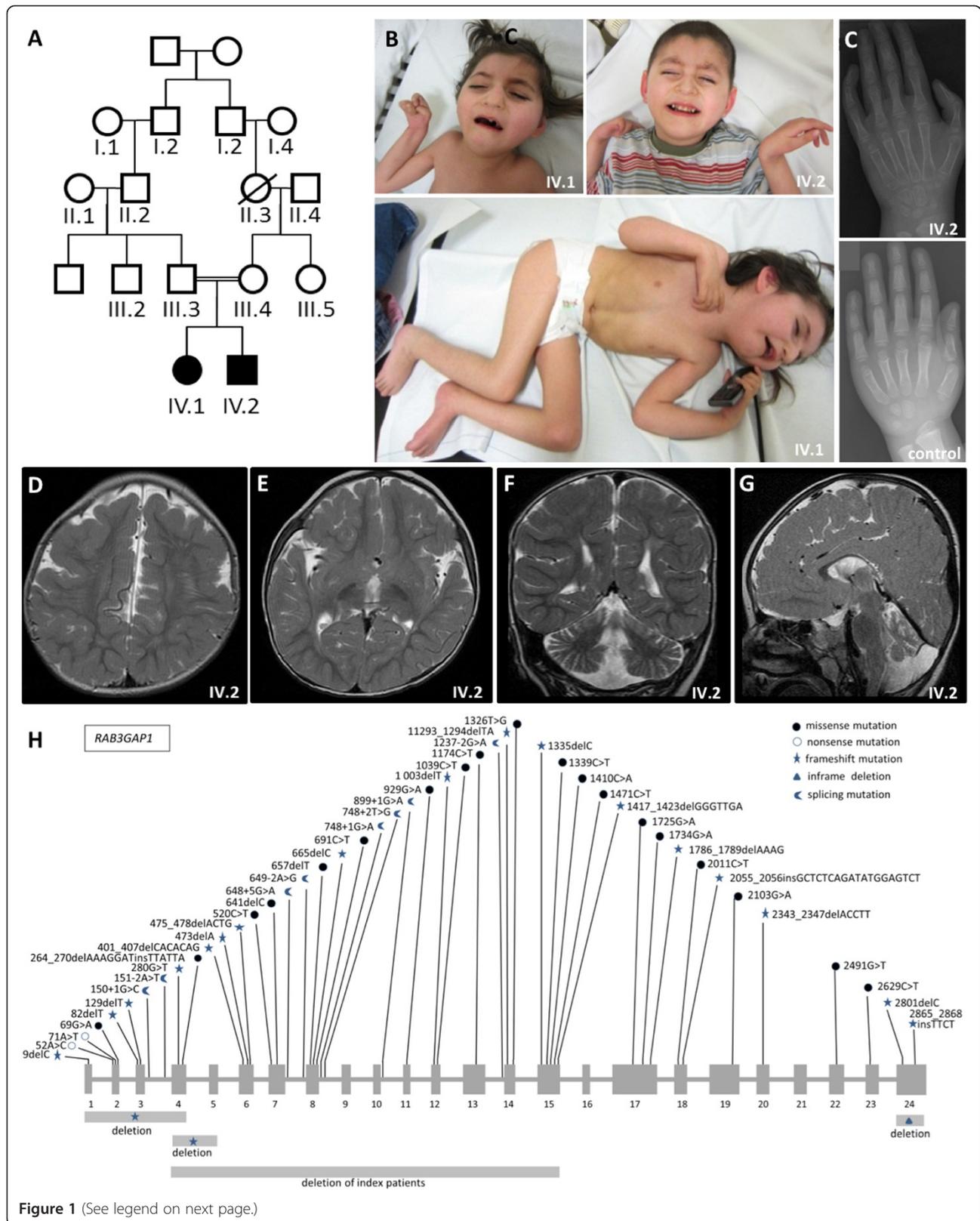


Figure 1 (See legend on next page.)

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Figure 1 Phenotype of the index patients with WARBM1. (A) Pedigree. **(B)** Pictures of the index patients illustrating severe dystrophy, microcephaly, and distal contractures. Facial features include a prominent nasal root, relatively short nose, large ears, and a mild facial hypertrichosis. **(C)** Appropriate skeletal age but severe osteopenia on conventional X-rays of left hand of patient IV.2 when compared to an age- and sex-matched control. **(D-G)** Cranial MRI of patient IV.2 revealed parietal pachygyria **(D, axial T2)**, widened sylvian fissure **(E, axial T2)**, cerebellar atrophy **(F, coronal T2)**, and corpus callosum dysmorphism with agenesis of the splenium corpori **(G, sagittal T2)**. **(H)** Scheme depicts all previously reported mutations in the *RAB3GAP1* gene in patients with WARBM1 and the novel deletion in our index patients.

Table 1 Comparison of phenotypic features of the index patients with those described in other patients with Warburg micro syndrome 1–3 and Martsof syndrome

Characteristics and symptoms	HPO ID	Patient II.1	Patient II.2	WARBM1	WARBM2	WARBM3	MS
Pedigree ID		II.1	II.2				
Gender		Female	Male				
Age at last assessment (years)		5.9	4.5				
Growth							
Short stature	0004322	+	+	+	+	+	+
Postnatal failure to thrive	0001508	+	+	+	+	+	+
Growth hormone deficiency	0000824	+	+	NR	NR	NR	+
Head and neck							
Postnatal microcephaly	0000252	+	+	+	+	+	(+)
Micrognathia	0000347	+	-	+	-	-	+
Large ears	0000400	-	+	+	+	-	-
Microphthalmia	0000568	+	+	+	+	+	+
Microcornea	0000482	+	+	+	+	+	+
Congenital cataract	0000519	+	+	+	+	+	+
Ptosis	0000508	-	-	(+)	-	-	-
Nystagmus	0000639	-	-	(+)	-	-	-
Epicanthal folds	0000286	-	-	-	-	-	+
Genitourinary							
Cryptorchism	0086889	NA	+	+	+	+	+
Hypogenitalism	0003241	-	+	+	+	+	+
Skeletal							
Osteoporosis	0000939	++	++	NR	NR	NR	NR
Kyphoscoliosis	0002751	+	+	+	NR	+	+
Joint hypermobility	0001382	-	-	(+)	-	-	-
Joint contractures	0002803	+	+	+	+	+	-
Foot deformities	0001760	-	-	+	+	-	+
Hair							
Facial hypertrichosis	0002219	+	+	+	-	-	-
Neurologic							
Intellectual deficit	0001249	++	++	++	++	++	+
Optic atrophy	0000658	+	+	+	+	+	-
Hyperreflexia	0007034	+	+	+	+	+	+
Muscular hypotonia	0001290	+	+	+	+	+	+
Spastic diplegia	0001264	+	+	+	+	+	(+)

Table 1 Comparison of phenotypic features of the index patients with those described in other patients with Warburg micro syndrome 1–3 and Martsolf syndrome (Continued)

Seizures	0001250	-	-	+	-	+	-
Inability to walk	0002540	+	+	+	+	+	(+)
Absent speech	0001344	+	+	+	(+)	+	(+)
Cranial MRI							
Abnormal corpus callosum	0001273	+	+	+	+	+	(+)
Cerebral atrophy	0002059	-	-	+	(+)	+	(+)
Cerebral malformations	0007319	-	-	+	-	-	-
Polymicrogyria	0002126	-	-	+	(+)	+	(+)
Pachygyria	0001302	+	+	+	-	-	-
Enlarged sylvian fissures	0100952	+	+	+	-	+	(+)
Cerebellar hypoplasia	0001321	+	+	+	(+)	+	-
Dysmyelination	0007266	-	-	+	(+)	+	-
Cardiovascular							
Cardiomyopathy	0001638	-	-	NR	NR	NR	+
Cardiac failure	0001635	-	-	NR	NR	NR	+
Respiratory							
Recurrent infections	0002205	-	-	NR	NR	NR	+

All symptoms are listed according to the nomenclature and the systematics of the OMIM "Clinical Synopsis" and the Human Phenotype Ontology (HPO [14]). Abbreviations: +, present; -, not present; (+) mild or rare; ++, severe; NA, not applicable; NR, not reported; HPO, human phenotype ontology; WARBM1-3, Warburg micro syndrome 1–3; MS, Martsolf syndrome.

control, sparse voluntary movements, axial hypotonia, thoracolumbar scoliosis, lower-limb-spasticity and contractures, and unilateral hip dislocation were apparent. Cranial MRI revealed bilateral parietal pachygyria, dysgenesis of the corpus callosum with agenesis of the splenium, prominent fissura sylvii, mild cerebellar atrophy, and hypotrophic optic chiasma in both patients (Figure 1, Additional file 1: Figure S1). Their short stature was associated with severe osteopenia, mild growth hormone deficiency (levels -2.2 to -3 SDS), but appropriate bone age and normal calcium, phosphate, alkaline phosphatase serum levels (Figure 1). Vitamin D supplementation over 8 months did not improve osteopenia.

We identified the largest intragenic *RAB3GAP1* micro-deletion published to date in the index patients through combined Sanger sequencing and array CGH (arr[hg19] 2q21.3(135,837,294 × 2,135,857,789 - 135,872,940 × 0,135,896,068 × 2) and arr[hg19]4p16.3(68,185×2,72,477-156,130×1,165,852×2)) and further characterized the deletion breakpoints using multiple PCR amplicons (Figure 1, Additional file 2: Supplemental Data). The additional small 4p16.3-deletion, containing parts of the genes *ZNF718* and *ZNF595* is likely not relevant for the phenotype of the patients. The deletion is very small, encompassing only 84 kb, and overlaps with deletions documented in the normal population in the database of genomic variants (DGV). All deletions listed in decipher affecting the respective chromosome are vastly larger. Moreover, both corresponding genes have not been associated

with clinical phenotypes (search in: OMIM, PubMed, Uniprot, Genecards). However, we confirmed a homozygous 2q21.3-deletion of 50.4 kb encompassing exons 4–15 of *RAB3GAP1* corresponding to about 45% of the coding gene sequence: 135,840,320-135,891,847 (hg 19) in both patients, which is heterozygous in the parents as shown by qPCR. The extensive size of the patients' deletion, their phenotypes resemble previous descriptions of WARBM1 (Table 1), thereby firmly supporting the theory that all WARBM1 phenotypes are caused by a loss of *RAB3GAP1* function and/or by nonsense-mediated mRNA decay. Surprisingly, some WARBM1-associated neuro-ophthalmological anomalies were absent in our patients, such as ptosis and nystagmus, delayed myelination, cerebral atrophy, and seizures [1,2,8-11]. Such mild phenotypic variability of WARBM1 is not well understood.

Osteopenia present in our patients has not been highlighted in WARBM so far. While osteopenia can result from vitamin D deficiency, it may also be caused by *RAB3GAP1* dysfunction itself as *RAB3GAP1* arrests the activity of the osteoclastic bone resorption promoter *RAB3D* [12]. Uncontrolled activity of the latter is associated with bone structure defects in humans [12]. Osteopenia through *RAB3GAP1* deficiency is supported by (i) the serum findings in our patients arguing against a severe rachitis secondary to vitamin D deficiency and (ii) the ineffectiveness of vitamin D supplementation with respect to osteopenia in the patients.

In summary, we report that even the largest micro-deletion of 45% of *RAB3GAP1* provokes a rather typical WARBM1 phenotype. We thereby strongly support the theory that all truncating *RAB3GAP1* mutations generate a loss-of-protein-function and/or nonsense-mediated-mRNA-decay and therefore result in a similar phenotype. Only hypomorphic *RAB3GAP1* mutations induce the milder Martsof syndrome phenotype [5,9,13]. Severe osteopenia needs to be considered as a feature of WARBM, and future insight into the role of RAB3D in WARBM may help to understand skeletal abnormalities and assist in establishing a therapeutic approach.

Consent statement

Written informed consent was obtained from the patients' legal guardian for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Additional files

Additional file 1: Figure S1. Cranial MRI of index patient IV.1 with WARBM1.

Additional file 2: Supplemental Data.

Abbreviations

RAB3GAP1: RAB3 GTPase-activating protein 1; *RAB3GAP2*: RAB3 GTPase-activating protein 2; *RAB18*: RAS-associated protein RAB18; *TBC1D20*: TBC1 domain protein, member 20; WARBM: Warburg micro syndrome; SDS: Standard deviation score.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

CH, AMK, and SPM recruited subjects, gathered patient history as well as clinical information and contributed clinical samples. BS analyzed radiological images. EK and DH performed array CGH and further genetic analysis. AB and BH analyzed the breakpoint boundaries of the patient's deletion. SPM and AMK wrote the manuscript, which was read, corrected and approved by all coauthors.

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