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DATA REPORT

Two novel homozygous *RAB3GAP1* mutations cause Warburg micro syndrome

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Warburg micro syndrome is an autosomal recessive disease where patients present with optic, neurologic and genital symptoms. Until now, four disease genes for Warburg micro syndrome, *RAB3GAP1*, *RAB3GAP2*, *RAB18* and *TBC1D20*, have been identified. Here, we report two novel homozygous *RAB3GAP1* mutations (c.22G > T, p.Glu8* and c.1353delA, p.Pro452Hisfs*5) in two consanguineous families by whole-exome sequencing.

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Warburg micro syndrome (WARBM) is a rare, genetically heterogeneous, autosomal recessive syndrome. Patients with WARBM present with severe mental retardation, brain anomalies (polymicrogyria and corpus callosum hypoplasia), craniofacial features (microcephaly, hairy forehead, large anteverted ear, broad nasal root and micrognathia), ocular defects (congenital cataract, microphthalmia and microcornea), spasticity leading to contracture, congenital hypotonia and hypogonadism.[1–4] The four WARBM subtypes (1, 2, 3 and 4) identified to date are caused by mutations in *RAB3GAP1* (NM_001172435), *RAB3GAP2* (NM_012414), *RAB18* (NM_001256410) and *TBC1D20* (NM_144628), respectively and are clinically indistinguishable.[5–10] Here we analyze two unrelated WARBM patients to determine the underlying genetic abnormality.

Patient II-1 in family 1 (patient 1) is a 4-year-old Iranian girl born to consanguineous parents. She was born at 33 weeks of gestation after premature rupture of membranes without asphyxia (Apgar scores were 9 and 10 at 1 and 5 min, respectively). Her birth weight, length and head circumference were 1,850 g (7th centile), 49 cm (< 5th centile) and 31 cm (7th centile), respectively. At birth, she showed characteristic craniofacial features (microcephaly, bitemporal narrowing, soft cleft palate, small mouth, micrognathia and large ears), ocular symptoms (bilateral cataract, microphthalmia and microcornea) and pectus carinatum. At 1 year of age, severe mental retardation, cerebral palsy, axial hypotonia and peripheral spasticity were recognized, with no seizures. At 17 months, a brain FLAIR-image revealed dysmyelination of the white matter implying severe hypoxic-ischemic encephalopathy, and the dysgenesis of corpus callosum. At 19 months, she showed mild bilateral conductive hearing loss detected by auditory brainstem response test. Currently at 4 years, she cannot sit independently. Neither parents have hearing impairments, pectus carinatum or soft cleft palates. Chromosome analysis of the patient was normal.

Patient II-3 in family 2 (patient 2) is an Indian girl, who is the third child of two healthy first cousins (Figure 1a). She had two unaffected siblings. She was born at 38 weeks of gestation by repeat cesarean section after an uneventful pregnancy. Her birth weight was 2,000 g (< 5th centile). At 16 months of age, her weight was 6.2 kg (< 5th centile), length 72 cm (< 5th centile) and head circumference was 40 cm (< 5th centile). She showed severe mental retardation, spoke only 'mama', 'papa' and 'dada' with hypotonia, exaggerated deep tendon reflexes in both legs, and hearing impairment. She had trigonocephaly, bilateral low-set prominent ears with anteriorly angulated, broad nasal root, micrognathia and a high-arched palate, and bilateral proximal placement of the thumbs and fifth toes. Microphthalmia, unresponsive pupils, microcornea and bilateral cataracts with very small vitreous cavities, leading to complete blindness were also noted. Hematological examination, thyroid, liver and renal function, serum calcium, ammonia, lactate, pyruvate and TORCH (toxoplasmosis, rubella, cytomegalovirus and herpes virus) were all normal at 16 months of age. Echocardiography, abdominal ultrasonography and skeletal survey were all normal, chromosomes were normal, and no hearing impairments were present in other family members.

The affected individuals from families 1 and 2 and their parents were analyzed. Peripheral blood samples were collected after obtaining written informed consent. DNA was extracted from peripheral blood leukocytes using the QIAamp DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The Institutional Review Board of Yokohama City University School of Medicine approved this study.

Whole-exome sequencing was performed for the two affected individuals (II-1 in family 1, and II-3 in family 2) and for the unaffected parents of family 2 as previously reported.[11,12] Briefly, genomic DNA (3 μg per sample) extracted from peripheral blood was sheared to 200 bp fragments using a Covaris S2 system (Covaris, Woburn, MA, USA). Genome partitioning was performed

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using a SureSelect Human All Exon Kit v5 (Agilent Technologies, Santa Clara, CA, USA). The prepared libraries were sequenced on a HiSeq2000 (Illumina, San Diego, CA, USA) with 101 bp paired-end reads with 7 bp index reads. Both reads were aligned to the human reference genome hg19 by Novoalign 3.00 (http://www.novocraft.com). The aligned reads were processed using Picard to remove polymerase chain reaction (PCR) duplicates (http://picard.sourceforge.net). The variants were called using the Genome Analysis Toolkit 2.4–4 (GATK; http://www.broadinstitute.org/gatk) with the GATK Best Practice Variant Detection v3 recommendations (http://www.broadinstitute.org/gatk/guide/topic?name=best-practices) and annotated using ANNOVAR (8 March 2012;

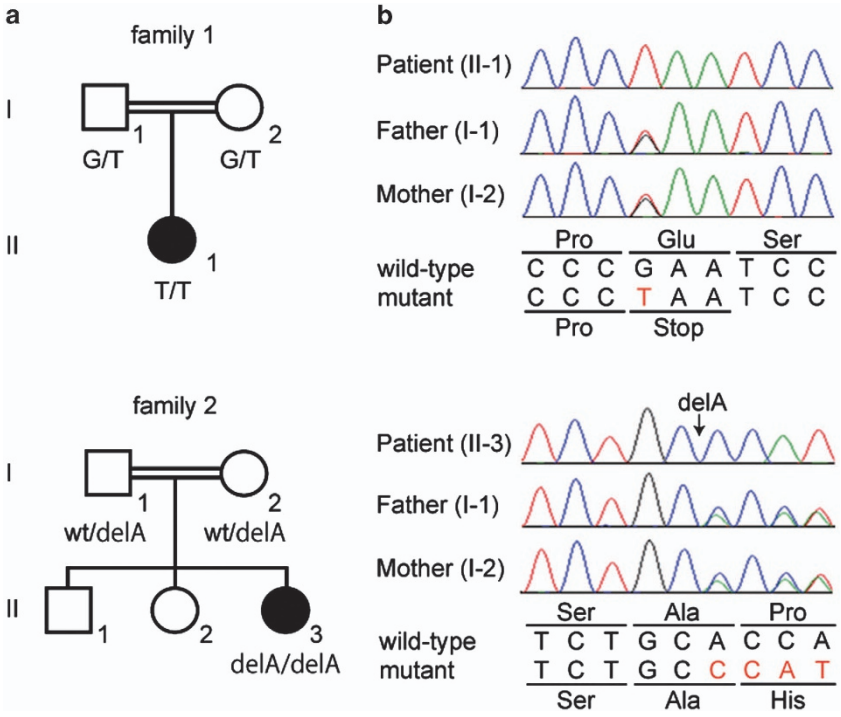

Figure 1. Familial pedigrees and mutations. (**a**) Family pedigrees with consanguinity. Black and white symbols are affected and unaffected, respectively. (**b**) Electropherograms of patients and their unaffected parents. The altered bases are shown in red characters.

Table 1. Clinical features of patients with previously reported *RAB3GAP1* mutations and of the present individuals

	WARBM1 (total 23 cases)[a]	*Family 1: II-1*	*Family 2: II-3*
Age		4 years	16 months
Sex	Male/female	Female	Female
Inheritance	AR	AR	AR
Causative genes (mutation)	*RAB3GAP1*	*RAB3GAP1* (p.E8*)	*RAB3GAP1* (p.Pro452Hisfs*5)
Consanguinity	+[b]	+	+
Common clinical phenotype			
Microcephaly	20/20 (100.0%)	+	+(trigonocephaly)
Mental retardation	18/18 (100.0%)	+	+
Congenital cataract	21/21 (100.0%)	+	+
Microphthalmia	17/19 (89.5%)	+	+
Microcornea	14/17 (82.4%)	+	+
Large anteverted ear	9/10 (90.0%)	NA	+
Truncal or axial hypotonia	18/20 (90.0%)	+	+
Spasticity	18/18 (100.0%)	+	–
Polymicrogyria	14/14 (100.0%)	–	NA
Corpus callosum hypoplasia	17/17 (100.0%)	+	NA
Genital abnormalities	12/17 (70.6%)	NA	–
Uncommon clinical phenotype			
Hearing impairment	1/2 (50.0%)	+	+
Pectus carinatum	NA	+	–
Soft cleft palate	NA	+	–

Abbreviation: NA, not assessed. [a]Only patients who had clinical details available were counted. [b]All counted patients showed homozygous *RAB3GAP1* mutations.

http://www.openbioinformatics.org/annovar). Using these criteria, only variants located in the coding region and the adjacent 2 bp were identified. Common variants registered in dbSNP build 137 (minor allele frequency $\geqslant 0.01$; http://genome.ucsc.edu/cgi-bin/hgTrackUi?hgsid=316787363&g=snp137Common&hgTracksConfigPage=configure) were excluded. On the basis of the consanguinity in both families where autosomal recessive inheritance was considered, and homozygous variants were focused and validated by Sanger method (Supplementary Tables 1 and 2). PCR products were sequenced on an ABI3500xL sequencer (Applied Biosystems, Foster City, CA, USA) and analyzed using Sequencher 5.0 (Gene Codes Corporation, Ann Arbor, MI, USA).

The average-read depths for RefSeq coding DNA sequences were 88.5–99.1x in whole-exome sequencing. After *in silico* analysis, we focused on 16 and 13 homozygous candidate variants in patient 1 and patient 2, respectively (Supplementary Tables 3 and 4). Among these variants, homozygous truncating mutations were identified in *RAB3GAP1* (NM_001143905): c.22G>T (p.Glu8*) in patient 1, and c.1353delA (p.Pro452Hisfs*5) in patient 2. Both mutations are novel. Each parents had a heterozygous mutation (Figure 1b). These mutations were absent in the NHLBI Exome Sequencing Project, the 1000 Genomes database and in our in-house exome database ($n = 575$).

The *RAB3GAP1* gene encodes RAB3 GTPase-activating protein, which has a role in converting the active GTP-bound form to the inactive GDP-bound form of RAB3 proteins, which in turn regulate hormone and neurotransmitter exocytosis.[13,14] Among the four genes involved, *RAB3GAP1* mutations are present in ~40% of WARBM patients, making it the most frequent cause. More than 50 mutations in *RAB3GAP1* have been reported to date in the Human Genome Mutation Database (https://portal.biobase-international.com). Interestingly, they are mostly truncating mutations (nonsense, frameshift and splice-site) or microdeletions.[5,6,9]

Here we report two novel truncating *RAB3GAP1* mutations (c.22G>T, p.Glu8* and c.1353delA, p.Pro452Hisfs*5) in two independent families. Both patients showed typical features of WARBM (Table 1). Of note, hearing impairment has been reported only in one patient,[6] but was recognized in both patients described here, though we could not find any mutations that would cause hearing loss. Pectus carinatum and soft cleft palate, found in patient 1, have never been reported (Supplementary Table 5). As WARBM patients show variable skeletal abnormalities like pectus excavatum, kyphoscoliosis, hip dislocation and limb anomalies, pectus carinatum appears to be one of the skeletal phenotypes in WARBM.

In conclusion, we report two WARBM patients with novel *RAB3GAP1* mutations. The hearing impairment, pectus carinatum and soft cleft palates seen in these patients have rarely or never been noted in WARBM.

HGV DATABASE

The relevant data from this Data Report are hosted at the Human Genome Variation Database at http://dx.doi.org/10.6084/m9.figshare.hgv.696, http://dx.doi.org/10.6084/m9.figshare.hgv.699.

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COMPETING INTERESTS

The authors declare no conflict of interest.

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Supplementary Information for this article can be found on the *Human Genome Variation* website (http://www.nature.com/hgv).