DATA REPORT

Two novel homozygous *RAB3GAP1* mutations cause Warburg micro syndrome

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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_00172435), *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, 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 using SeqCap EZ Human Exome kit (Roche NimbleGen, Madison, WI, USA) and PCR amplified with specific primer pairs. Paired-end 100 bp reads were generated by Illumina HiSeq 2000. When the read depth was < 8, the target area was divided into a fragmented area (7–10 Mb each) and normalized sequencing was performed.

Two novel homozygous *RAB3GAP1* mutations (c.22G>T, p.Glu8* and c.1353delA, p.Pro452Hisfs*5) in two consanguineous families by whole-exome sequencing.

Human Genome Variation (2015) 2, 15034; doi:10.1038/hgv.2015.34; published online 17 September 2015
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)* | 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    | +                        | +              | +             |
| 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 antverted 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

|                  | WARBM1 (total 23 cases)* | Family 1: II-1 | Family 2: II-3 |
|------------------|--------------------------|----------------|---------------|
| Hearing impairment| 1/2 (50.0%)              | +              | +             |
| Pectus carinatum  | NA                       | +              | −             |
| Soft cleft palate | NA                       | −              | −             |

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

ACKNOWLEDGEMENTS

We thank the patients and their families for participating in this study. We also thank Ms. S Sugimoto and K Takabe for their technical assistance. This work was supported by grants from the Ministry of Health, Labour and Welfare (H Saito, N Miyake and N Matsutomo), a Grant-in-Aid for Scientific Research (A) (NM), a Grant-in-Aid for Scientific Research (B) (HS and NMII), a Grant-in-Aid for Scientific Research (C) (SM), a Grant-in-Aid for challenging Exploratory Research (HS) from the Japan Society for the Promotion of Science, the fund for Creation of Innovation Centers for Advanced Interdisciplinary Research Areas Program in the Project for Developing Innovation Systems from the Japan Science and Technology Agency (NMII), a Grant-in-Aid for Scientific Research on Innovative Areas (Transcription Cycle) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (NMII and NMIII), and the Takeda Science Foundation (HS, NMII and NMIII) and the Strategic Research Program for Brain Science (SRPBS) from Japan Agency for Medical Research and Development (AMED) (NMII).

COMPETING INTERESTS

The authors declare no conflict of interest.

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