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DATA REPORT

Exome-first approach identified a novel gloss deletion associated with Lowe syndrome

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Lowe syndrome (LS) is an X-linked disorder affecting the eyes, nervous system and kidneys, typically caused by missense or nonsense/frameshift *OCRL* mutations. We report a 6-month-old male clinically suspected to have LS, but without the Fanconi-type renal dysfunction. Using a targeted-exome sequencing-first approach, LS was diagnosed by the identification of a deletion involving 1.7 Mb at Xq25-q26.1, encompassing the entire *OCRL* gene and neighboring loci.

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Lowe syndrome (LS, the oculocerebrorenal syndrome of Lowe, OCRL; OMIM #309000) is a rare, X-linked disorder characterized by the diagnostic triad of congenital cataracts, cognitive and behavioral impairment and renal proximal tubulopathy in almost all affected males, with an estimated prevalence of 1 in 500,000 in the general population.[1–3] At birth, ocular involvement with bilateral cataracts and severe hypotonia were usually present, but these findings may also manifest in unrelated disorders, including congenital infections, peroxisomal disorders, mitochondriopathies, congenital myotonic dystrophies or congenital myopathies.[1] Varying degrees of renal involvement (proximal renal tubular dysfunction of the Fanconi type) may not be clinically apparent in the first few months of life, making differential diagnosis to exclude alternative disorders difficult.[1]

LS is caused by mutations involving *OCRL* (OMIM #300535), which encodes a member of the inositol-5-phosphatase protein family, OCRL1. *OCRL* locates to Xq25-q26.1 (hg19), and contains 24 exons, 23 of which are for protein coding.[4] LS is typically attributed to *OCRL* protein-truncating nonsense/frameshift mutations or, less frequently, missense mutations involving the catalytic domain. Approximately 6% of LS cases result from exonic deletions, predominantly involving the 5′ region of *OCRL*.[5,6] Deletion of the entire *OCRL* gene has also been previously described in four affected males: some of these large-scale deletion events were associated with more profound clinical features.[6–9] Among them, only one case with an ~4 Mb deletion encompassing *OCRL* and several flanking genes were reported in affected males with severe features of LS.[9]

Recently, next-generation sequencing (NGS), particularly whole-exome sequencing (WES) or targeted-exome sequencing (TES), have been adopted in clinical testing for patients with undiagnosed congenital diseases suspected to be genetic in origin. Single-nucleotide variations (SNVs) and small insertions/deletions (indels), as well as gross copy-number variations (CNVs) involved in a defect of disease-causing genes can be detected using WES/TES simultaneously.[10] Here, we report a second case of LS caused by a large deletion encompassing the entire *OCRL* gene and nearby loci, successfully detected by a TES-first approach using a disease-related exome panel with subsequent chromosomal microarray (CMA)-based fine mapping.[11]

A 6-month-old male infant was the first child born to healthy, non-consanguineous Japanese parents with an unremarkable family history. After an uneventful pregnancy, he was delivered at 38 weeks and 5 days of gestation by Cesarean section at a local hospital. He had a birth weight of 2,100 g (−2.5 s.d.), a length of 44.5 cm (−2.0 s.d.) and a head circumference of 32 cm (−0.9 s.d.). Generalized hypotonia, right cryptorchidism and inguinal hernia were noted at birth. At the age of 13 days, mild corneal opacities were observed bilaterally. Because of these symptoms and being underdeveloped for his gestational age, congenital disease was suspected and the infant transferred to our hospital at age 49 days: when he weighed 3,530 g (−2.3 s.d.) and was 51.5 cm (−2.3 s.d.) in length. Cranial ultrasound was unremarkable. His clinical course was characterized by developmental delay, short stature, generalized hypotonia, and bilateral mild corneal opacity and congenital cataracts. Early remedial surgery was recommended after ophthalmologic examination to prevent form-deprivation amblyopia. Blood investigation revealed elevated alkaline phosphatase (2,187 IU/l), whereas other laboratory tests, including assays for various metabolic disorders, were unremarkable. Conventional chromosome G-banding analysis presented a normal 46,XY karyotype. At age 79 days, lensectomy was performed. At the age of 4 months, horizontal nystagmus was conspicuous. Cranial ultrasound showed obscure corpus callosum, and enlarged bilateral lateral ventricles and subarachnoid space. By 6 months, he weighed 5,580 g (−2.7 s.d.) and was 60.9 cm (−2.9 s.d.) in length. He lacked head control. Blood and urine tests were essentially normal. The patient was suspected to have LS because of his bilateral dense congenital cataracts, infantile congenital hypotonia, delayed development, short stature and mild ventriculomegaly,[1–3] although no signs of renal tubular dysfunction were observed.

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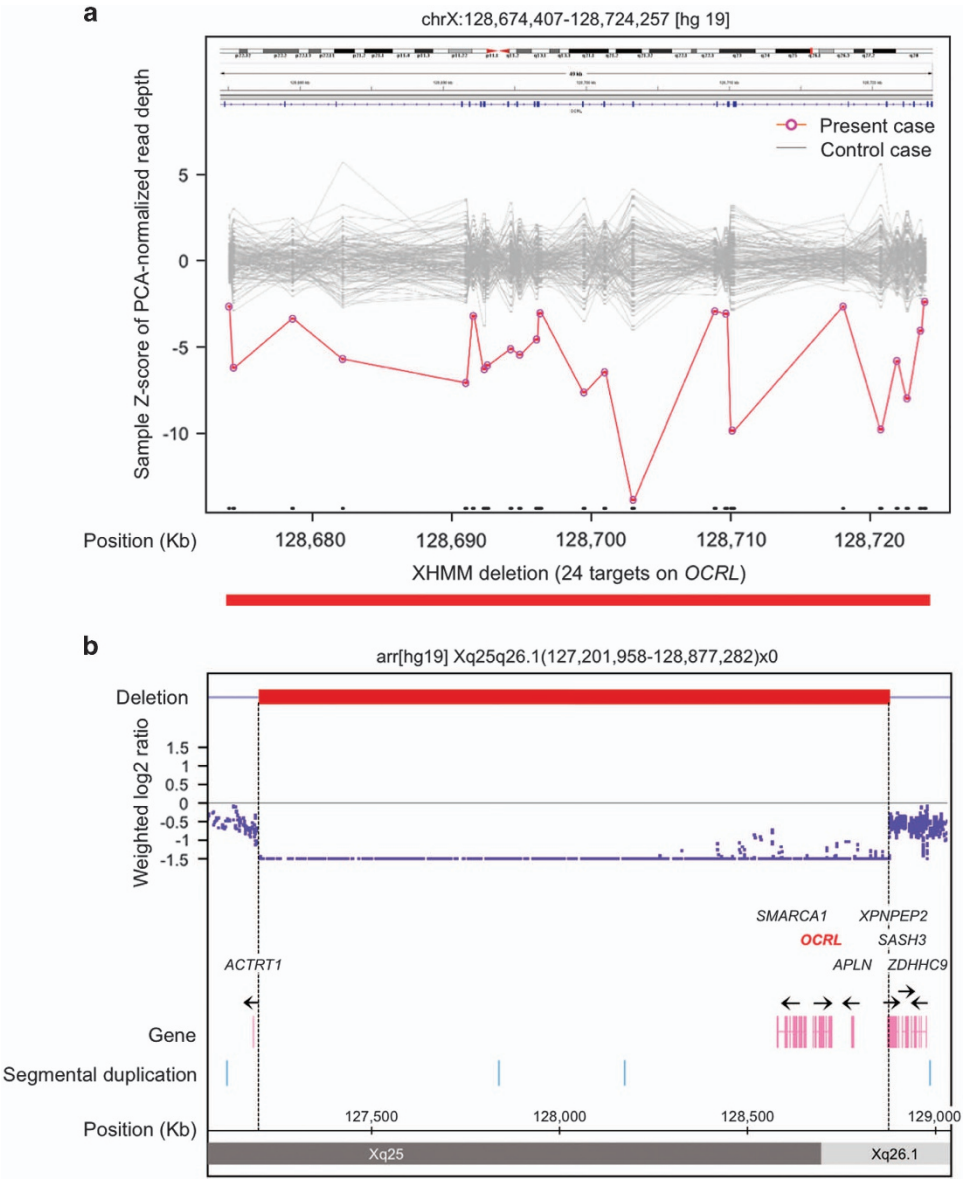

Figure 1. (**a**) An eXome-Hidden Markov Model v1.0 (XHMM, https://atgu.mgh.harvard.edu/xhmm/) analysis using TES data detected genomic copy-number loss of the entire *OCRL* gene (24 target exons) located within Xq25-q26.1, suggesting an ~50 kb deletion (red horizontal bar). The *x* axis denotes the physical position and the *y* axis indicates the *Z*-score of principal component analysis (PCA)-normalized read depth. Purple circles connected by red lines show values for the individual subjected to TES in this study. Gray dots with gray connected lines represent the results of normalized read depth obtained from in-house control data ($N = 126$). (**b**) CMA using an Affymetrix Cytoscan HD array demonstrated a 1.7 Mb deletion within Xq25-q26.1 (red horizontal line). Weighted copy-number $\log_2$ ratio, genes and segmental duplications around the deleted region are shown. Arrows indicate direction of transcription. Dashed lines indicate deletion break points. Among the four genes involved in deleted region, *OCRL* is the only locus included in the TruSight One sequencing panel for TES. Compared with TES-based CNV detection, distal and proximal break points of the deleted region were shifted to distal and proximal sides, respectively, in CMA analysis because of lacking target exons other than those of *OCRL* within the deleted region. CMA, chromosomal microarray; CNV, copy-number variation; OCRL, oculocerebrorenal syndrome of Lowe; TES, targeted-exome sequencing.

Molecular genetic analysis was performed on genomic DNA extracted from a blood sample after informed consent was obtained from the parents. The study was approved by the Ethics Committee at Tokushima University. We performed TES using a TruSight One Sequencing Panel (Illumina, San Diego, CA, USA) and a MiSeq (Illumina) with our pipeline for NGS data analysis, as described previously.[11,12] The detection of CNVs using TES data with a resolution of a single exon to several exons, depending on exon sizes, was performed as described previously.[10,11] These analyses revealed no disease-causing SNVs or indels, but did detect a deletion of all exons of the *OCRL* gene between positions 128,674,407 and 128,724,257 of chromosome X (Xq25-q26.1, hg19), indicating the complete loss of at least the entire *OCRL* gene (Figure 1a). Validation and fine mapping of the deleted region were performed by CMA using an Affymetrix CytoScan HD chromosome microarray platform (Affymetrix, Santa Clara, CA, USA), as described previously.[10,11] CMA detected a complete chromosomal deletion within Xq25–q26.1 as 46,XY.arr[hg19] Xq25q26.1(127,201,958–128,877,282) × 0 (Figure 1b). In addition to the entire *OCRL* gene, the deleted region comprises two genes, *SMARCA1* (OMIM #300012) encoding a member of the SWI/SNF family of proteins involved in chromatin remodeling and *APLN*

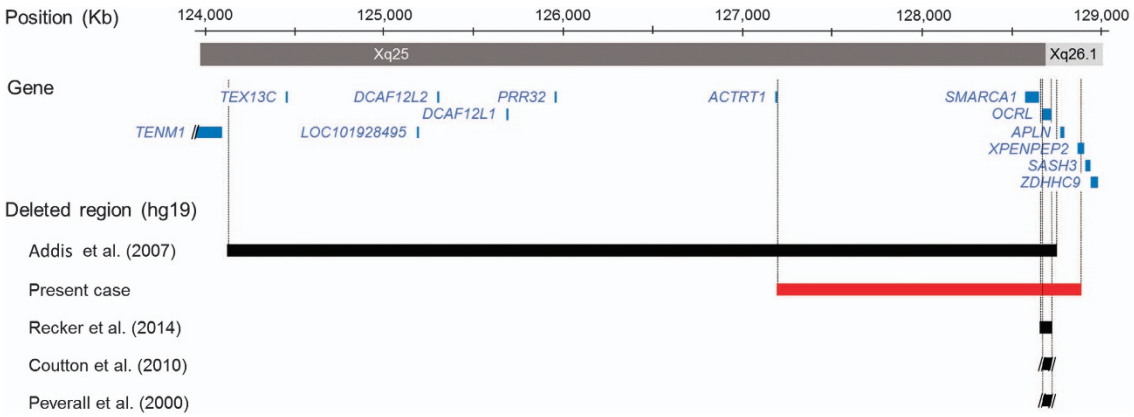

Figure 2. A map of the Xq25-q26.1 deletions observed in cases with LS. The RefSeq genes located around the deletions are indicated. The red horizontal bar represents the deleted region observed in the present case. The four black bars represent the deleted regions in four previously reported cases with LS, whose deletions include the entire *OCRL* gene. In two cases,[6,7] deletion of the *OCRL* gene was detected by multiplex ligation-dependent probe amplification[6] or fluorescence *in situ* hybridization,[7] but their extent was not shown. The deleted region determined by array-based comparative genomic hybridization is the estimated maximum extent.[9] LS, Lowe syndrome; OCRL, oculocerebrorenal syndrome of Lowe.

(OMIM #300297) specifying an endogenous ligand for the G protein-coupled apelin receptor, and the 5′ region of *XPNPEP2* (OMIM #300145) encoding aminopeptidase P (Figure 1b). Apelin is a neuropeptide expressed in the supraoptic and paraventricular nuclei.[13] The patient presented no additional clinical symptoms or dysmorphic features besides LS consequent on deletion of these *OCRL*-flanking loci.[9] This deletion is not present in the Human Gene Mutation Database professional 2016.2 (HGMD, http://www.hgmd.org/) and ClinVar (http://www.ncbi.nml.nih.org/clinvar/), suggesting it to be novel. This deletion was not confirmed as *de novo* because of the unavailability of his mother's DNA.

LS should be suspected in infant males presenting with congenital cataracts, severe hypotonia and renal tubular dysfunction of the Fanconi type during early postnatal life.[1–3] Early diagnosis and treatment of metabolic disturbances may reduce the morbidity and mortality implicated in this syndrome.[14] For the diagnosis of LS, defects involving ocular, central nervous and renal systems are requisite.[1] In early infancy, it may be difficult to exclude alternative diagnoses because of a frequent lack of renal Fanconi-type syndrome, as in the present case.[1] Therefore, multiple genes possibly responsible for clinical manifestations, including *OCRL*, should be evaluated for the differential diagnosis of LS in some cases. In addition, genetic variations such as SNV/indels, gross insertion/deletion and translocations may underlie disease-causing gene defects. Considering that TES can simultaneously detect both exonic SNVs/indels and CNVs in multiple disease-causing genes in a diagnostic setting for human genetic diseases,[10,11] this TES-first approach can facilitate differential diagnosis of LS for patients lacking distinctive manifestations/symptoms, as observed in the present case, in a cost- and time-effective manner.

Previously, Addis *et al.*[9] have reported a male patient with an ~4 Mb microdeletion encompassing *OCRL* and flanking genes, such as *DCAF12L1* (*WDR40B*), *PRR32*, *ACTRT1* and *SMARCA1*, who was diagnosed with LS at birth on the basis of typical oculocerebrorenal manifestations and renal Fanconi disease (Figure 2). As in the present case, no additional clinical symptoms or dysmorphic features besides LS consequent on deletion of these flanking genes were presented until his death from unknown causes at 5 months of age, although only *SMARCA1* is the commonly deleted flanking gene between the two cases.[9] On the basis of the phenotypes observed in two cases, therefore, it remained unclear whether patients with LS caused by gloss deletions present additional features besides LS that are able to be explained by *OCRL*-flanking genes involved in the deleted regions. A small number of cases with LS caused by microdeletions containing *OCRL* and a few nearby genes suggest that larger deletions around *OCRL* in males might be lethal (Figure 2).

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.897.

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COMPETING INTERESTS

The authors declare no conflict of interest.

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