

DATA REPORT

A patient with autosomal recessive Alport syndrome due to segmental maternal isodisomy

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We report the case of a 22-year-old male with autosomal recessive Alport syndrome. Molecular analysis showed that this patient has a homozygous missense (NM_000091.4:c.3266G > A) Gly1089Asp mutation in the *COL4A3* gene. The proband inherited the mutation from his heterozygous carrier mother, whereas the father carried only wild-type alleles. We performed comparative genome hybridization and single-nucleotide polymorphism microarray analyses and confirmed that there was partial maternal isodisomy.

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Alport syndrome (AS), the major cause of hereditary nephritis, is associated with sensorineural hearing loss, ocular abnormalities and ultrastructural abnormalities of the glomerular basement membrane, which causes persistent hematuria, proteinuria and end-stage renal failure. AS is due to defects in type IV collagen $\alpha 3$ ($\alpha 3$ [IV]), $\alpha 4$ ($\alpha 4$ [IV]) or $\alpha 5$ ($\alpha 5$ [IV]) chains, which are encoded by the *COL4A3* (2q36-q37), *COL4A4* (2q35-q37) and *COL4A5* (Xq22) genes, respectively. The majority of AS (>80%) is inherited in an X-linked manner with mutations found in the *COL4A5* gene,¹ and autosomal recessive AS (ARAS) is caused by mutations in the *COL4A3* or *COL4A4* gene.²

Uniparental disomy (UPD) arises when a diploid individual carries either both homologs of a chromosomal pair from a single parent (uniparental heterodisomy) or two copies of a single parental chromosome (uniparental isodisomy).³ Isodisomy causes several autosomal recessive conditions, but there are few reports of isodisomy of the telomeric end of chromosome 2.⁴ Furthermore, there is no report of AS due to segmental UPD. This study sought to describe segmental maternal isodisomy of the telomeric end of chromosome 2, including the *COL4A3* gene region, resulting in an ARAS phenotype in the patient.

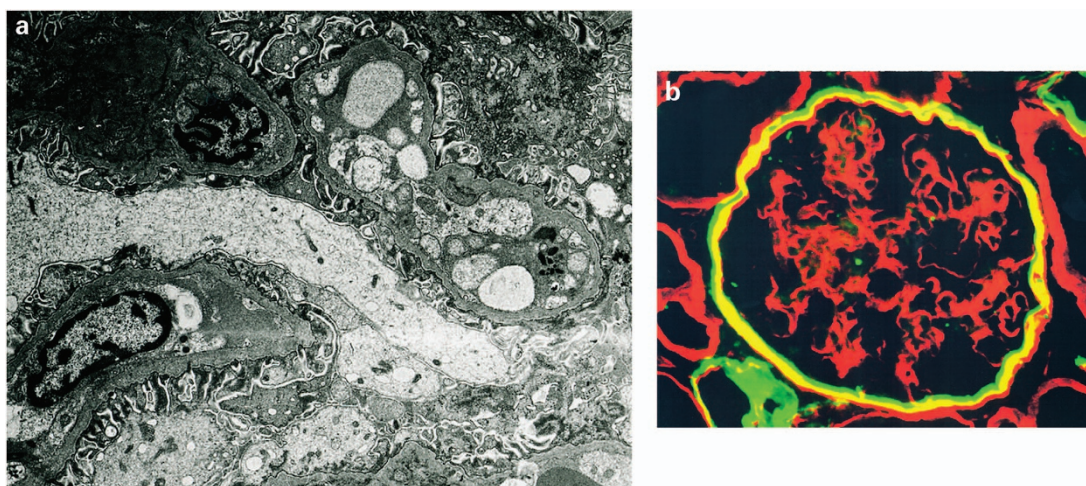


Figure 1. Electron microscopy findings show thinning of the glomerular basement membrane (GBM; **a**). Immunohistochemical staining for the $\alpha 2$ (red) and $\alpha 5$ (green) chains of type IV collagen shows the expression of the $\alpha 2$ chain in the GBM and the $\alpha 5$ chain only in Bowman's capsule (**b**).

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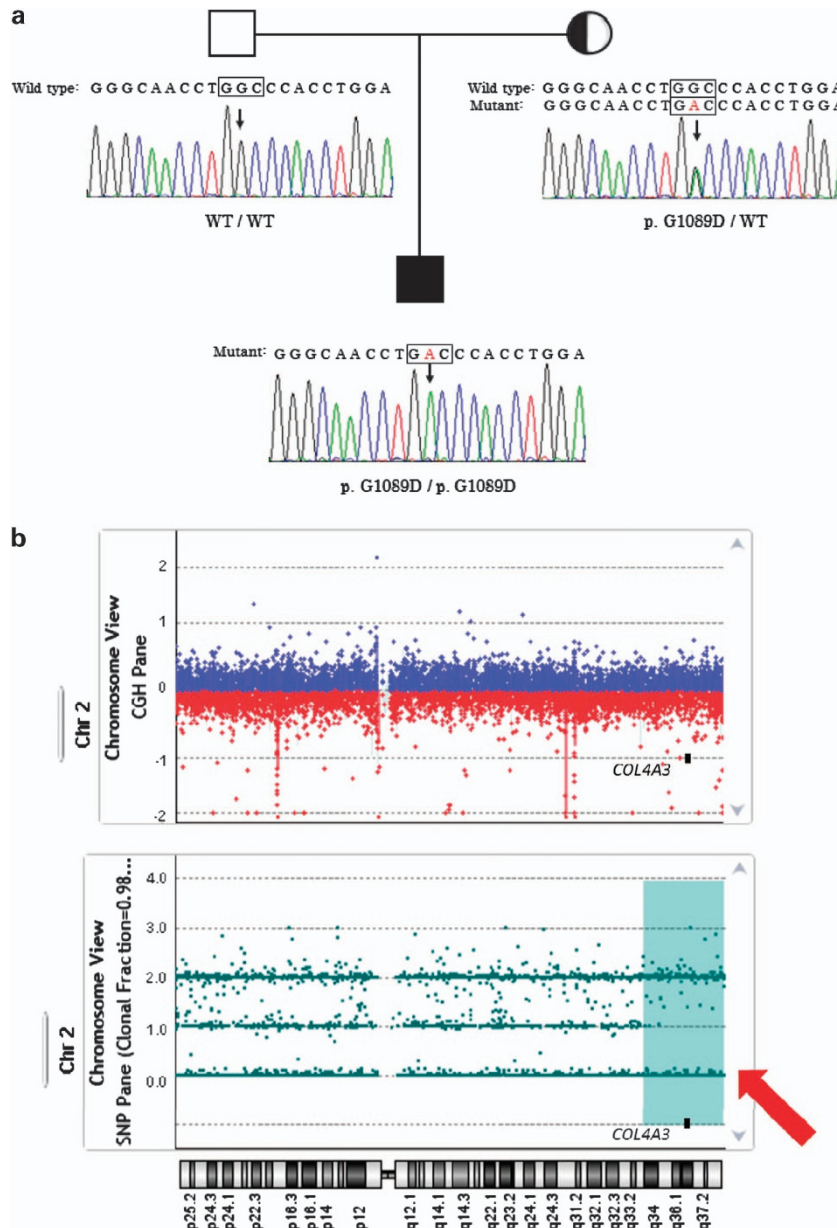


Figure 2. The family pedigree and sequencing of the *COL4A3* gene show a homozygous missense Gly1089Asp mutation in the patient. His mother is a carrier, whereas his father does not carry the mutation (a). Agilent CytoGenomics Analysis Software shows that there is no 2q deletion, and LOH is detected in the telomeric end of chromosome 2 (chr2: 207,541,513–243,014,630; b). CGH, comparative genome hybridization; SNP, single-nucleotide polymorphism; WT, wild type.

The patient is a 22-year-old Japanese male who was born from nonconsanguineous parents. He presented with persistent proteinuria and microhematuria since the age of 2 years and a history of renal biopsy performed at age 7 years. Renal electron microscopic findings showed thinning of the glomerular basement membrane (Figure 1a). Immunohistochemical staining for type IV collagen $\alpha 2$ [IV] expression was observed in the glomerular basement membrane, and staining for $\alpha 5$ [IV] was observed only in Bowman's capsule (Figure 1b). These findings were compatible with typical ARAS findings. At the age of 17 years, he was diagnosed with progressive bilateral sensorineural hearing loss, and at 22 years, he underwent kidney transplantation. He has no other disorders, including physiological developmental delay. The patient's mother had mild proteinuria but normal renal function. She had a normal audiogram and no ocular abnormalities. No other family member suffered from renal disease.

We performed genetic analysis of the patient to confirm the diagnosis. The sequences of the *COL4A3*, *COL4A4* and *COL4A5* genes were examined by direct DNA sequencing and showed a novel homozygous missense NM_000091.4:c.3266G>A, Gly1089Asp mutation in exon 39 of the *COL4A3* gene. Genetic analysis of the mother showed that she is a heterozygous carrier of the mutation, whereas genetic analysis of the father showed only the wild-type sequence (Figure 2a). No mutations were found in the *COL4A4* or *COL4A5* genes. To rule out a hemizygotic mutation of this allele, we performed semiquantitative PCR analysis, which showed that the patient has two copies of the mutant *COL4A3* gene. From these results, we considered the possibility of maternal isodisomy of chromosome 2, including the *COL4A3* gene. To confirm the precise gene copy number and single-nucleotide polymorphism (SNP) haplotype of the region of the *COL4A3* gene, microarray analysis using comparative genome

hybridization (CGH) and SNP microarray (SurePrint G3 Human CGH +SNP Microarray 4 × 180 K, Agilent Technologies, Santa Clara, CA, USA) analysis were performed for the patient. SNP microarray data analysis for the patient revealed loss of heterozygosity (LOH) located in the chromosome region 2q33.3–2q37.2. The *COL4A3* gene is located in this ~35 Mb LOH region (Figure 2b). The region of LOH was determined to be copy number neutral, with no gain or loss of genetic material. Comparative analysis of SNP genotyping data in the region of LOH confirmed the occurrence of maternal isodisomy. This finding strongly suggests a segmental uniparental isodisomy of maternal origin in this region that includes the *COL4A3* gene. To confirm UPD, we tested polymorphisms using 16 single-nucleotide microsatellite markers spanning the entire length of chromosome 2. Nine markers were uninformative because they could have been inherited from either parent. Four other markers showed a heterozygous pattern compatible with a biparental mode of inheritance. Three markers showed a homozygous maternal inheritance, suggesting that reduction to homoallelism for the mutant *COL4A3* gene allele was due to segmental maternal isodisomy of the telomeric end of chromosome 2. These results confirmed that the patient has ARAS due to maternal isodisomy. Finally, we determined that a homozygous mutation in the *COL4A3* gene was caused by nonMendelian inheritance with segmental maternal isodisomy of the telomeric end of chromosome 2.

The present case is the first reported case of AS due to partial segmental UPD and is the second reported case of segmental maternal isodisomy of the telomeric end of chromosome 2q. The first report described reduction to homoallelism for a primary hyperoxaluria type 1 mutation in the case of a patient with complete liver alanine:glyoxylate aminotransferase deficiency with no symptoms, but liver and kidney dysfunction.⁵ Although many cases of UPD—such as Beckwith–Wiedemann syndrome (UPD11) or Prader–Willi syndrome (UPD15)—are related to genomic imprinting and show various phenotypes, including intellectual disability, the present case shows typical ARAS. This outcome may stem from the fact that there are no imprinting regions in chromosome 2.⁶ Because the recurrence risk after the birth of a child with segmental UPD seems to be negligible,⁷ our result is useful for genetic counseling of the family.

We confirmed that there is a homozygous missense Gly1089Asp mutation of the *COL4A3* gene in the patient. Although this mutation has not been reported in ARAS patients, the PolyPhen-2 score (<http://genetics.bwh.harvard.edu/pph2/>) is 1.000, which indicates 'probably damaging,' and glycine substitutions in the collagenous domain of the *COL4A3* gene lead to the crucial constitutional changes resulting in the development of renal abnormalities. Although various genetic abnormalities of AS, including missense mutation, nonsense mutation, splicing error, nucleotide deletion and/or insertion, have been reported,⁸ to the best of our knowledge, the present case is the first case of a patient with AS due to UPD. We have also reported the cases of 30 Japanese ARAS patients,⁹ and among this group, there were no UPD patients other than the present patient (who is Patient number 114 in the literature).

The mechanisms of monosomy or trisomy rescue result in complete UPD, whereas segmental isodisomy with a normal status on the rest of chromosome as observed in this study indicates a fusion of maternal and paternal chromosomes, and was therefore most likely due to a postzygotic event.³ Somatic recombination may be the possible mechanism for the segmental isodisomy. In this patient, homologous recombination between paternal and maternal chromatids may have occurred in the very early postzygotic period, as the vast majority of the patient's cells were

found to carry the homozygous mutation based on the sequence data. Homologous recombination is one of the mechanisms for the repair of double-strand breaks. In this patient, the region of segmental isodisomy extends to the end of the long arm of chromosome 2, suggesting that break-induced replication, one of the double-strand break repair pathways similar to homologous recombination, was likely to lead to the generation of the large segmental isodisomy.¹⁰

In conclusion, this is the first reported case of a patient with AS due to UPD. Our observations may lead to an improved understanding of the genetic polymorphism of AS.

HGV DATABASE

The relevant data from this Data Report are hosted at the *Human Genome Variation Database* at http://hgv.figshare.com/genome_variation/13.

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COMPETING INTERESTS

The authors declare no conflict of interest.

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