

## SHORT COMMUNICATION

# Characterization of contiguous gene deletions in *COL4A6* and *COL4A5* in Alport syndrome-diffuse leiomyomatosis

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Alport syndrome-diffuse leiomyomatosis (AS-DL, OMIM: 308940) is a rare variant of the X-linked Alport syndrome that shows overgrowth of visceral smooth muscles in the gastrointestinal, respiratory and female reproductive tracts in addition to renal symptoms. AS-DL results from deletions that encompass the 5' ends of the *COL4A5* and *COL4A6* genes, but deletion breakpoints between *COL4A5* and *COL4A6* have been determined in only four cases. Here, we characterize deletion breakpoints in five AS-DL patients and show a contiguous *COL4A6/COL4A5* deletion in each case. We also demonstrate that eight out of nine deletion alleles involved sequences homologous between *COL4A5* and *COL4A6*. Most breakpoints took place in recognizable transposed elements, including long and short interspersed repeats, DNA transposons and long-terminal repeat retrotransposons. Because deletions involved the bidirectional promoter region in each case, we suggest that the occurrence of leiomyomatosis in AS-DL requires inactivation of both genes. Altogether, our study highlights the importance of homologous recombination involving multiple transposed elements for the development of this continuous gene syndrome and other atypical loss-of-function phenotypes.

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## INTRODUCTION

Alport syndrome (AS) is the most common hereditary nephropathy, characterized by a progressive renal failure, sensorineural deafness and ocular abnormalities. The most common X-linked form of this disease (XLAS, OMIM: 301050) results from mutations in the *COL4A5* gene, which encodes the  $\alpha 5$  chain of type IV collagen.<sup>1</sup> Alport syndrome-diffuse leiomyomatosis (AS-DL, OMIM: 308940) occurs as a rare variant of XLAS that shows overgrowth of visceral smooth muscles in the gastrointestinal, respiratory and female reproductive tracts, in addition to renal symptoms.<sup>1–3</sup> *COL4A5* is located on the long arm of the X chromosome (Xq22) and is head-to-head with the *COL4A6* gene. The *COL4A6* gene encodes the  $\alpha 6$  chain of type IV collagen, which is mainly expressed in heart, human esophagus, aorta and bladder smooth muscle basement membrane.<sup>3–5</sup>

AS-DL patients exhibit contiguous gene deletions at the *COL4A5–COL4A6* locus.<sup>5</sup> Sixteen AS-DL patients reported so far have been found to have a deletion that encompassed the 5' end of the *COL4A5*

and *COL4A6* genes, and included the bidirectional promoter (the Human Genome Mutation Database). The *COL4A6* deletion breakpoints have been consistently found within intron 2, whereas the *COL4A5* breakpoints usually occur in intron 1.<sup>6</sup> However, the breakpoints were characterized at a single-nucleotide level only in four cases.<sup>6–9</sup>

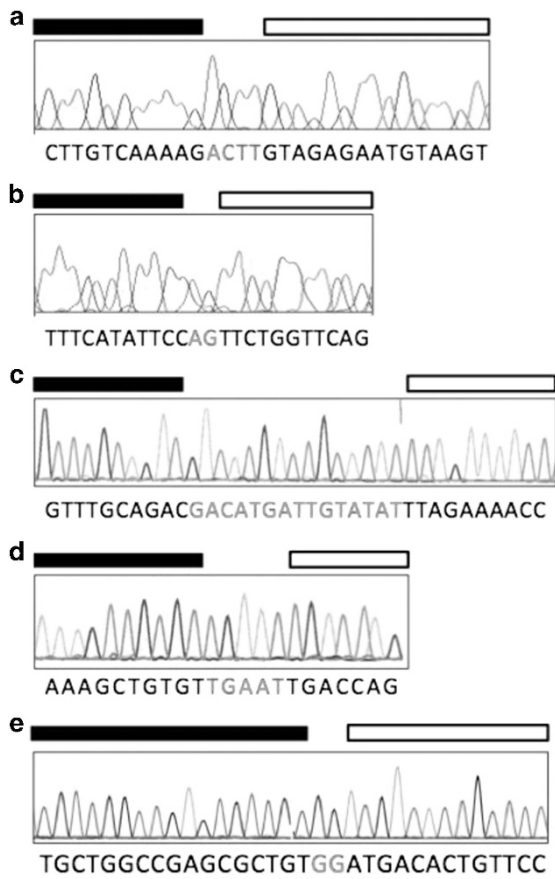
## PATIENTS AND METHODS

In our laboratory, we conduct a comprehensive molecular diagnostics of inherited kidney diseases, including AS. So far, 415 suspected AS patients underwent molecular genetic tests, five of them with clinical signs of leiomyomatosis. The pedigrees of the five cases are shown in Supplementary Figure 1.

Genomic DNA was isolated from peripheral blood leukocytes. Screening of contiguous gene deletions was performed with the multiplex ligation probe amplification (MLPA) using the SALSA P191/192 Alport MLPA assay (MRC-Holland, Amsterdam, the Netherlands).<sup>2,10,11</sup> Long-range PCR amplification and direct sequencing of *COL4A5* and *COL4A6* was

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**Figure 1** Sequence chromatograms of contiguous gene deletion breakpoints in five AS-DL cases. (a) Case 1 (c.66+5840 of *COL4A6* and c.81+8068 of *COL4A5*). (b) Case 2 (c.66+25107 of *COL4A6* and c.81+18040 of *COL4A5*). (c) Case 3 (c.66+85676 of *COL4A6* and c.276+3257 of *COL4A5*). (d) Case 4 (c.66+119476 of *COL4A6* and c.3246+6706 of *COL4A5*). (e) Case 5 (c.66+84055 of *COL4A6* and c.3246+66915 of *COL4A5*). *COL4A6* and *COL4A5* sequences are shown as black and open rectangles, respectively. Homologous sequence in each case is shown in red. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

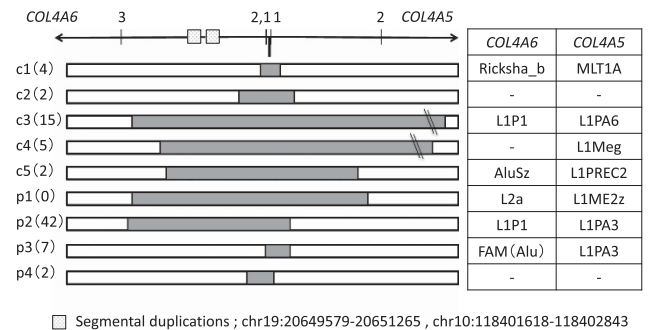
conducted to characterize the deletion breakpoints. The human reference sequence of NG\_011977.1 and NG\_012059.2 for *COL4A5* and *COL4A6* was used, respectively.

To detect a large heterozygous deletion in Case 2, we conducted a semi-quantitative PCR amplification using capillary electrophoresis.<sup>12,13</sup>

## RESULTS

The MLPA analysis of five patients with AS-DL revealed hetero- or hemizygous deletions in each case (Supplementary Figure 2). This analysis was followed by long-range PCRs and direct sequencing to identify each breakpoint (Figures 1a–e, Supplementary Figure 3 and Supplementary Data 1). For a female patient (Case 2, Supplementary Figure 2B), we also conducted a semi-quantitative PCR assay (Supplementary Figure 4).

Deletions are schematically shown in Supplementary Figure 3.<sup>6–9</sup> To examine the genomic context of deletion breakpoints, we analyzed their flanking sequences using RepeatMasker (Figure 2). Their alignments with Refbase entries<sup>15</sup> revealed the presence of transposed elements (TEs) in 13/18 (72%) breakpoints in nine cases (Figure 2). Cases p2 and p3 showed *COL4A5*-side breakpoints at different positions within the same long interspersed element, family L1. Similarly, Cases c3 and p2 exhibited *COL4A6*-side breakpoints at



**Figure 2** Schematics of novel and previously reported deletions Location of both sides of the breakpoints in the in *COL4A5* and *COL4A6*. Deletions are shown as dark rectangles. Transposable elements in centromeric (*COL4A6*) and telomeric (*COL4A5*) breakpoints are shown to the right. The segmental duplication in intron 2 is marked as a vertical rectangle at the top of the figure. Exons are numbered at the top. c1–c5, our case 1–5, p1 (ref. 4), p2 (ref. 14), p3 (ref. 15), p4 (ref. 13). The number of homologous nucleotides (Figure 1 and Supplementary Data 1) is shown in parentheses.

different positions in the same L1 copy. Homologous sequences at the recombination breakpoints were apparent in eight of nine patients. The length of overlapping sequences varied from 2 to 42 bp (Supplementary Data 1). No breakpoints were located in the annotated duplicated segment (UCSC genome browser) of *COL4A6* intron 2 (Figure 2).

MLPA analysis of maternal samples of Cases 1, 3 and 4 failed to detect any deletion, suggesting that, unlike Cases 2 and 5, these are sporadic cases.

## DISCUSSION

This work has more than doubled the number of sequence-characterized *COL4A5* and *COL4A6* breakpoints in AS-DL. Although eight out of nine cases had homologous sequences at the deletion breakpoints, only two cases (c3 and p2) showed relatively long homologous sequences in the L1 family of long interspersed elements, consistent with a well-known L1-mediated recombination mechanism.<sup>16</sup> Almost a half of the human genome is occupied by recognizable TEs, with L1 occupying ~17%.<sup>17</sup> TEs have been shown to provide a source of new exons, genes and regulatory sequences, dramatically influencing evolutionary history, exon–intron structure, speciation and regulation of gene expression. TEs facilitate non-allelic homologous recombination events leading to inherited diseases,<sup>14</sup> including AS-DL (Figure 2).<sup>8</sup> As compared to c3 and p2, Cases c1, c4, c5, p1 and p3 revealed shorter homologous sequences indicative of the same mechanism. The number of breakpoints in TEs 13/18 (72%) appears to be higher than expected since TEs represent only 40% and 62% of the genomic sequences of *COL4A6* and *COL4A5*, respectively suggesting that TEs might have some roles for causing recombination in this disease.

All deletion breakpoints characterized at the single-nucleotide level in *COL4A6* took place in intron 2.<sup>6–9</sup> Recently, an AS-DL case was identified with a deletion extending into *COL4A6* beyond this intron,<sup>2</sup> but the precise breakpoint was not characterized. On the *COL4A5* side, the deletion breakpoint usually maps to intron 1; however, some cases, including two of ours (c3 and c4), showed breakpoints in intron 36 and intron 4, respectively. Recently, Sa *et al.* reported an AS-DL case with a *COL4A5*-only gene deletion, which encompassed exons 1–51 but did not include the common promoter region or exon 1 of *COL4A6*.<sup>18</sup> This report may still be compatible with the requirement for inactivation of both genes in AS-DL, as first proposed by

Zhou *et al.*,<sup>5</sup> because the authors did not analyze *COL4A6* expression nor did they exclude inactivation of this gene by other mechanisms. Recent studies in yeasts revealed that deletion of many genes were associated with altered mRNA levels of the neighboring genes.<sup>19</sup> These studies are particularly relevant for bidirectional promoters which generate products of two adjacent, often related genes in stoichiometric quantities, ensuring their co-expression in the same or similar biological pathway.

In conclusion, we have more than doubled the number of large contiguous deletions in AS-DL characterized at the single-nucleotide level. Our results show that most deletions were mediated by transposons via homologous recombination events and support the original proposal<sup>5</sup> that inactivation of both genes is required for the development of leiomyomas in AS-DL.

### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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