Severe Alport syndrome in a female caused by a t(X;1)(q22.3;p36.32) balanced translocation

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Abstract

The course of renal involvement and hearing loss is much milder in most of female X-linked Alport syndrome than in male patients. We examined the molecular mechanism of development of the disease in a female patient with severe Alport syndrome. The patient showed heavy proteinuria, hematuria, nuerosensory hearing loss and primary amenorrhea. Renal biopsy findings of electron microscopy and α5 chain of type IV collagen immunostaining indicated a female X-linled Alport syndrome. G-banding chromosomal analysis showed a t(X;1)(q22.3;p36.32) balanced translocation. *COL4A5* gene analysis by genomic DNA sequencing, cDNA sequencing and multiplex ligation-dependent probe amplification assay showed no mutations and deletions/duplications of the gene. However, fluorescence in situ hybridization using the probes for exon 1 and exon 51 of *COL4A5* gene showed disruption of one copy of the gene. Replication R-banding chromosomal analysis indicated preferential inactivation of the normal X chromosome. This is the first report of severe Alport syndrome in a female carrying a balanced translocation between the chromosome X and 1 producing the disruption of one copy of *COL4A5* gene and silencing of the other copy because of preferential inactivation of the normal X chromosome. Chromosomal abnormalities should be considered in female patients with severe form of Alport syndrome.

(200 words)

Keywords: Alport syndrome, female, chromosomal abnormalities, balanced translocation, X inactivation, gonadal dysgenesis

Introduction

Alport syndrome is characterized by progressive hematuric nephritis with ultrastructural and immunohistochemical changes of the glomerular basement membrane (GBM), frequently associated with neurosensory hearing loss [1–8]. Mutations in the *COL4A5* gene are responsible for the more common X-linked dominant form of the disease (OMIM 301050). Although progression to renal failure and hearing loss is considerably frequent in male X-linked Alport syndrome, the course of renal involvement and hearing loss is much more variable in female patients, remaining mild in the majority of them.

We examined the molecular mechanism of development of the disease in a female patient with severe Alport syndrome. And, we found, for the first time, a balanced translocation between the chromosome X and 1 producing the disruption of one copy of *COL4A5* gene and silencing of the other copy because of preferential inactivation of the normal X chromosome.

Methods

All procedures were reviewed and approved by the Institutional Review Board of Kobe University School of Medicine.

Patient

A female patient suffered from proteinuria and hematuria when she was 2 years old. She underwent renal biopsies at the age of 6 and 8 years, and was diagnosed as minor glomerular abnormalities, respectively. She was diagnosed having neurosensory hearing loss at the age of 14 years.

She was referred to our hospital at 19 years of age. Her body height was 150.3 cm (-1.5 SD) and body weight 35.7 kg. Her blood pressure was 120/60 mmHg under the treatment with an angiotensin-converting enzyme inhibitor and an angiotensin receptor antagonist. Laboratory investigation showed microscopic hematuria and heavy proteinuria (1.63 g/day) and mild hypoalbuminemia (3.6 g/dl) with normal renal function (Creatinine clearance: 95.4 ml/min/1.73m^2). She also showed primary amenorrhea, hypoplastic uterus with no ovaries, and hypergonadotrophic hypogonadism. Thus, she was diagnosed having premature ovarian failure and was treated with estrogen and progesterone.

Immunohistochemical analysis of kidney biopsy specimens

The immunofluorescence methods used for immunohistochemical analysis have been described previously [9]. Rat monoclonal antibodies that recognize the 2 and 5 chains (H22 and H52; Shigei Medical Research Institute, Okayama, Japan) were used [10]. The type IV collagen α5 chain in glomerular basement membrane (GBM α5(IV)) expression ratio was analyzed using ImageJ 1.42q (National Institutes of Health, Bethesda, MD) on a Machintosh computer (Apple Japan, Tokyo Japan). The whole glomerular tufts and α5(IV) positive parts of glomerular tufts were identified using threshold levels, and the both area were measured. The GBM α5(IV) expression ratio was calculated by dividing the latter by the former.

Genomic DNA analysis

Genomic DNA was isolated from peripheral blood leukocytes of the patient with the Qiagen kit (Qiagen Inc., Chatsworth, CA, USA), according to the manufacturer's instructions. Specific exons of *COL4A5* and exon-intron boundaries were amplified by the polymerase chain reaction (PCR), and the PCR-amplified products were purified and directly subjected to sequencing using an automatic DNA sequencer (model ABI Prism 310; Perkin Elmer Applied Biosystems, Foster City, CA, USA).

RNA expression analysis

Total RNA was extracted from blood leukocytes. RNA was isolated with the aid of Isogen Kit (Nippon Gene Co., Toyama, Japan), and was then reverse-transcribed onto cDNA by using random hexamers and the Superscript III kit (Invitrogen, Carlsbad, CA, USA). cDNA was amplified by means of nested PCR using primer pairs for *COL4A5* described elsewhere [9, 11]. PCR-amplified products were purified and directly subjected to sequencing.

Multiplex ligation-dependent probe amplification analysis

To reveal the heterozygous large deletion of *COL4A5* gene, we utilized multiplex ligation-dependent probe amplification (MLPA) analysis using the SALSA P191/192 Alport MLPA assay (MRC-Holland, Amsterdam, The Netherlands).

Fluorescence in situ hybridization (FISH) analysis

FISH was performed according to standard procedures using specific DNA probes for exon 1 and exon 51 of the *COL4A5* gene. The probes were synthesized by amplification of human genomic DNA using PCR (Takara LA PCR Kit Ver. 2.1, Takara Bio Inc, Tokyo, Japan). The primers for exon1 of *COL4A5* gene were 5'-CTGCCAGCTTAAGTAGCTAGTGAAACTG-3' (forword) and 5'-TATCCCACTGACAGTGAATCAGTACAGG-3' (reverse), and those for exon 51 of

the *COL4A5* gene were 5'-ACCCCAGGAATTGAAGTTACAGTCTGTC-3' (forward) and 5'-GTGTTGGGTATGCAGTCCACTAACAGAG-3' (reverse).

Replicating R-banding chromosomal analysis

Peripheral blood lymphocytes were cultured with PHA (EY Laboratories, San Mateo, CA, USA), and thymidine (Sigma, St.Louis, MO ,USA) was added to synchronize cells at the S/G2 stage. The cells were then rinsed, treated with BrdU (Sigma), and incubated in fresh medium for an additional 6 hours. Colcemid (Gibco, Grand Island, NY ,USA) was added before the final incubation. Chromosome slides were heated, stained with Hoechst 33258 (Sigma), and rinsed in distilled water. The slides were dipped in 2 x SSC, heated, exposed to UV light, and stained with 2% Giemsa stain solution (Muto Pure Chemicals, CO.,LTD., Tokyo, Japan).

Results

She underwent renal biopsy again at 19 years of age, and was diagnosed as minor glomerular abnormalities by light microscopy (not shown). However, electron microscopy revealed a widespread basket-weave pattern of GBM (Fig. 1B). Immunohistochemical staining for α5(IV) collagen revealed mosaic patterns in the GBM (Fig. 1C & 1D). We examined 10 glomeruli, and the GBM α5(IV) expression ratio was 10-20% in all glomeruli. Therefore, she was diagnosed as female X-linked Alport syndrome. And, the normal X chromosome may have been preferentially inactivated in kidney tissues.

G-banding chromosomal analysis showed a balanced translocation, 46, X, t(X;1)(q22.3;p36.32) (Fig. 2). Her parents had no chromosomal abnormalities, indicating her chromosomal abnormality was *de novo*. *COL4A5* gene analysis by genomic DNA sequencing, cDNA sequencing (RNA expression analysis) and multiplex ligation-dependent probe amplification assay showed no mutations and deletions/duplications of the gene. However, fluorescence in situ hybridization using the probes for exon 1 and exon 51 of *COL4A5* gene showed disruption of one copy of the gene (Fig 3A & 3B).

To examine the skewed X inactivation, the polymorphic regions of the *AR* gene, the *PGK* gene and the *FMR1* gene were analyzed as previously described [12-14]. However, they were not informative because the patient showed homozygous status at the three loci. However, replicating R-banding chromosomal analysis revealed that the normal X chromosome was late replicating in all the 10 examined cells, indicating that the normal X chromosome was preferentially inactivated, whereas the der(X)t(X;1), bearing the disrupted *COL4A5* gene, was active in lymphocytes. (Fig. 3C).

Discussion

This is the first report of severe Alport syndrome in a female carrying a balanced translocation between the chromosome X and autosome. In female Alport syndrome, there was not the genotype-phenotype correlation but the large intrafamilial phenotypic heterogeneity, and risk factors for developing renal failure have been identified: the occurrence and progressive increase in proteinuria, and the development of a hearing defect [15]. Our patient showed heavy proteinuria and neurosensory hearing loss. In addition, her renal biopsy findings revealed a widespread basket-weave pattern of GBM and the low expression rate of the GBM α5(IV). These findings suggest that she was suffering from severe Alport syndrome with high risk for the development of renal failure in the future.

In the case of balanced X-autosome translocations arising in females, selective inactivation of the entire X chromosome often occurs. This phenomenon can be the consequence of cell death of the clones inactivating the derivative X-autosome chromosome. In fact, cells carrying derivative X-autosome chromosome would have partial monosomy of the autosome involved and partial disomy of the chromosome X, with gene unbalance. The efficacy of this process depends on the position of the X breakpoint and the size of the noninactivated region and of the autosome genomic segment [16]. In the case of breakage of a gene located on the chromosome X, due to translocation of the X-autosome, the predominance of the clones carrying the normal inactive X does not allow the expression of the normal allele of the gene, producing the severe disease phenotype in a female in X-linked diseases [17].

Although the detailed breakpoint of the *COL4A5* gene was not examined in the present study, the disruption of one copy of the gene due to the balanced translocation between the chromosome X and 1 may cause the large structural abnormalities on the encoded protein, α5(IV). In addition, a skewed X-inactivation pattern has been shown to be responsible for the severe phenotypic expression of disease in woman carriers of X-linked disease genes [18, 19]. In the present study, we could not show a skewed expression status of the genes (*AR*, *PGK* and *FMR1*) on X chromosome, because the

polymorphic regions of three loci were homozygous in our patient. However, replicating R-banding chromosomal analysis revealed that the normal X chromosome was late replicating in all the cells examined, indicating that the normal X chromosome was preferentially inactivated, whereas the der(X)t(X;1), bearing the disrupted *COL4A5* gene, was active. Collectively, the large structural abnormalities of α5(IV) protein and the preferential inactivation of the normal X chromosome might have a pivotal role for the severe phenotype seen in our patient.

Although the replicating R banding chromosomal analysis showed the normal X chromosome was inactivated in the lymphocyte examined, 10-20% of glomerular capillaries expressed α5(IV) protein. It is known that not all genes on the X chromosome undergo inactivation. Therefore, it is possible that the results of the replicating R banding chromosomal analysis did not necessarily reflect the degree of inactivation of the normal *COL4A5* gene. Alternatively, the degree of X inactivation and/or the mRNA splicing of *COL4A5* in lymphocytes and glomeruli may have been different in our patient.

Our patient showed primary amenorrhea, which was probably due to X-autosome translocations. The close association between gonadal dysfunction and breakpoints on Xq13-26 (the critical region) has been reported [20]. Recently, it was reported that the critical region was interrupted in Xq21, and was divided into the proximal part (critical region I) and the distal part (critical region II) [21] Gonadal dysgenesis in female sex chromosome aberrations would primarily be attributable to chromosome pairing failure during meiotic prophase. It is hypothesized that severe pairing failure causes degeneration of nearly all oocytes before puberty, leading to primary amenorrhea [22]. The *COL4A5* gene (Xq22.3) is within the critical region II. Therefore, the disruption of the *COL4A5* gene due to X-autosome translocations in female patients may cause gonadal dysgenesis (primary amenorrhea) by the above mentioned mechanism, as well as severe Alport syndrome. In another words, we should consider the possibility of X-autosome translocations when female Alport syndrome patients showed gonadal dysfunction.

In conclusion, chromosomal abnormalities should be considered in female patients with severe form of Alport syndrome, especially for cases with gonadal dysfunction including primary amenorrhea.

Besides Alport syndrome, there are several X-linked diseases affecting the kidney, including Dent disease (*CLCN5, OCRL*), Fabry disease (*GLA*), hypophsphatemic rickets dominant (*PHEX*), Lesh-Nyhan syndrome (*HPRT*), nephrogenic diabetes insipidus (*AVPR2*), and oral facial digital syndrome 1 (*OFD1*) [23]. Therefore, we should examine chromosomal abnormalities including X-autosome translocations when female patients with the above mentioned X-linked diseases show severe phenotype and other abnormalities including gonadal dysgenesis.

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(1,824 words without abstract)

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Figure legends

Fig. 1. Renal biopsy findings.

A. A patient with frequent-relapsing nephrotic syndrome in remission (Control). **B.-D.** The patient in the present study. **A.** Electron microscopy revealed normal appearance of glomerular basement membrane (GBM). **B.** Electron microscopy revealed a widespread basket-weave pattern of GBM. **C.** Normal pattern of the type IV collagen α2 chain in GBM. **D.** mosaic pattern of type IV collagen α5 chain in GBM (the GBM α5(IV) expression ratio: 13.7%).

Fig. 2. G-banding chromosomal analysis.

A. G-banding chromosomal analysis showed 46, X, t(X;1)(q22.3;p36.32) balanced translocation. **B.** Ideograms of chromosomes X and 1 and their derivatives der(X) and der(1) with inidications of breakpoints (arrows).

Fig. 3. Fluorescence in situ hybridization (FISH) analysis and R-banded chromosomal analysis.

A. FISH analysis of the patient's chromosomes with the probe for exon 1 of *COL4A5* demonstrated signals (Rhodamine) on the normal X chromosome and the der(X) (arrows). Cohybridization with the probe for X chromosome-specific alpha satellite DNA (DXZ1: Spectrum green) was used to indicate the normal and derivative X chromosome (arrowheads). **B.** FISH analysis with the probe for exon 51 of *COL4A5* showed signals (Rhodamine) on the normal X chromosome and the derivative chromosome 1, indicating the disruption of one copy of the gene. **C.** R-banding chromosomal analysis demonstrated that the normal chromosome X (black arrow) was stained less intense than the derivative chromosome X (white arrow), indicating that the normal X chromosome was preferentially inactivated.

Fig.1

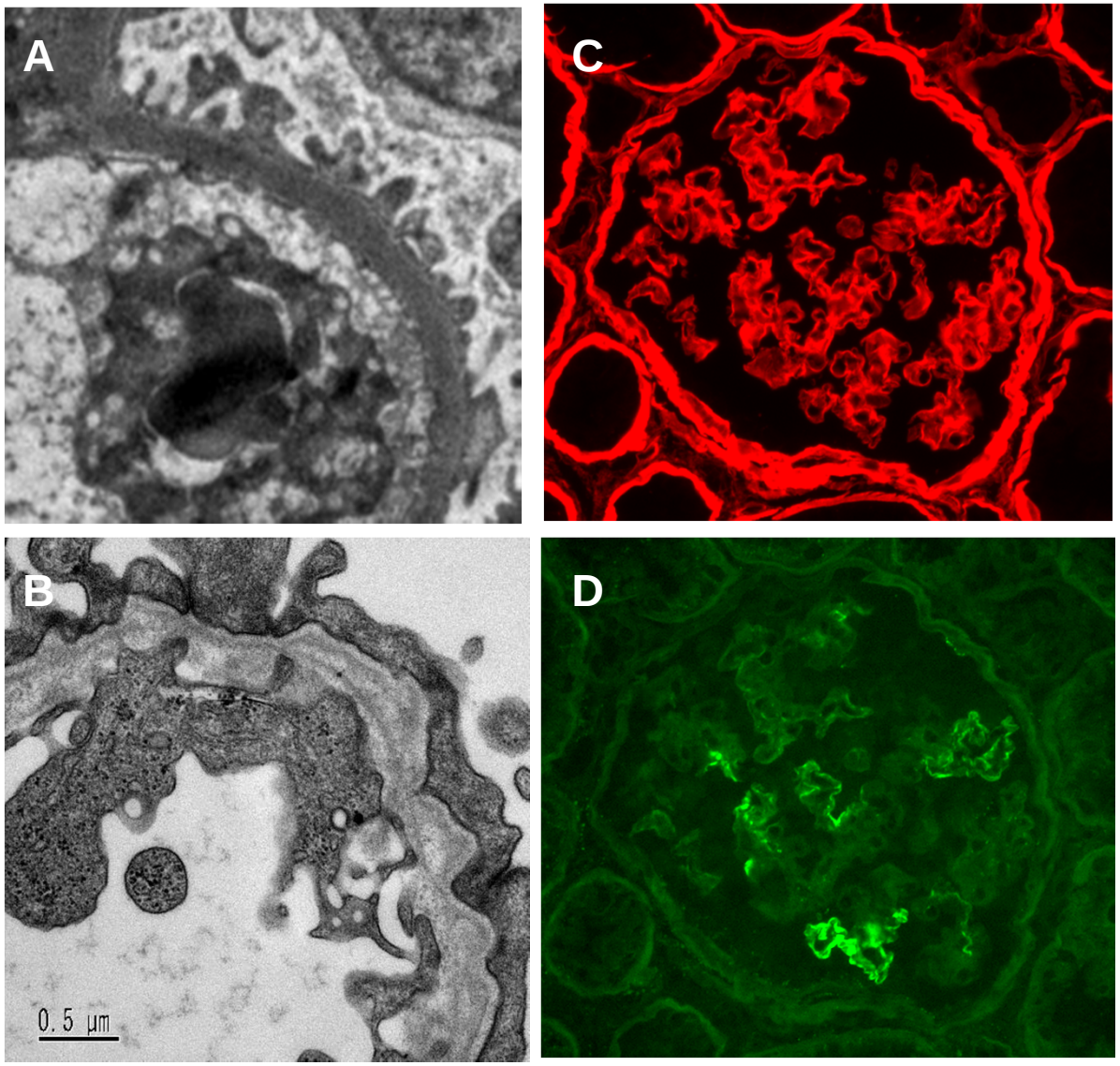

Fig.2

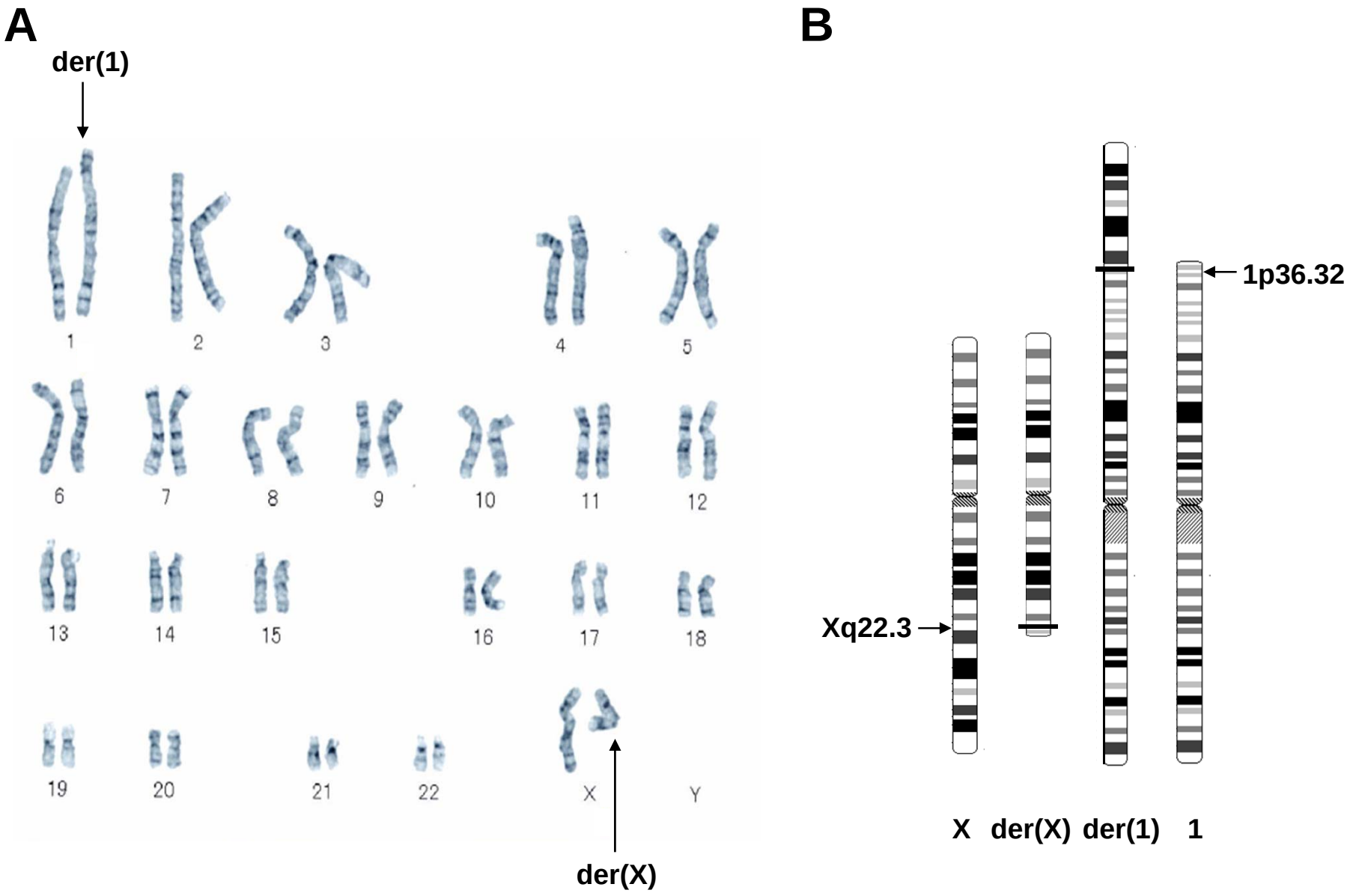
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Fig.3