Somatic mosaicism for a mutation of the COL4A5 gene is a cause of mild phenotype male Alport syndrome

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【 学位論文題目 】

Somatic mosaicism for a mutation of the COL4A5 gene is a cause of mild phenotype male Alport syndrome(COL4A5 遺伝子に体細胞モザイク変異を認めた軽症男性 Alport 症候群患者の分子生物学的研究)

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学位論文の内容要旨

Somatic mosaicism for a mutation of the *COL4A5* gene is a cause of mild phenotype male Alport syndrome

COL4A5 遺伝子に体細胞モザイク変異を認めた軽症男性
Alport 症候群患者の分子生物学的研究

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INTRODUCTION

Alport syndrome (AS) was first described in 1927 as a hereditary nephritis characterized by hematuria and sensorineural deafness. Now we know that it is an inherited basement membrane disorder with a progressive nephropathy leading to end-stage renal disease (ESRD) in virtually all affected males and many affected females, hearing loss in 60–80% and ocular lesions in 25–40% of affected males. The prevalence of AS is estimated to be in the range from 1:5,000 to 1:50,000 live births. Patients with AS constitute about 2% of the population receiving renal transplant worldwide.

About 80% of Alport syndrome patients have the X-linked form of the disease (XLAS), which is caused by mutations in *COL4A5*, the gene encoding the α5 chain of type IV collagen [α5(IV)], located on X chromosome. Autosomal recessive Alport syndrome (ARAS) accounts for about 15% of affected individuals and arises from mutations in both alleles of *COL4A3* or *COL4A4*, which respectively encode the α3(IV) and α4(IV) chains, and are located on chromosome 2. About 5% of patients have autosomal dominant Alport syndrome (ADAS) due to heterozygous mutations in *COL4A3* or *COL4A4*.

There are two groups of patients with different progression of the disease. Juvenile AS is defined as mean age at onset of ESRD ≤ 30 years, and adult AS > 30 years.

In the X-linked form of AS females have a more variable phenotype than males, ranging from intermittent microscopic hematuria to ESRD. Proteinuria is present in 75% of the carriers.

There is no pharmacological or biological agent which has been definitely shown to prevent or delay the development of terminal renal failure in patients with AS. Alport nephropathy is essentially cured by renal transplantation, and before that – dialysis.

Diagnosis of Alport syndrome

Diagnosis of AS is made basically on the examination of renal biopsy specimen. The pathognomonic ultrastructural lesion of Alport syndrome consists of thickening of the glomerular basement membrane (GBM), splitting of the lamina densa,

scalloping of the epithelial aspect of the GBM ("basket weave" changes), and disappearance of podocyte foot processes. In males with XLAS, these changes typically first appear during childhood, and the extent of GBM displaying these alterations increases progressively with age. In females, the extent of GBM thickening ranges from focal to diffuse.
In around 90% of male patients with XLAS negative immunohistochemical staining for α3(IV), α4(IV) and α5(IV)-chains is found. Females usually show an interrupted, discontinuous pattern of α3(IV), α4(IV) and α5(IV) staining in the GBM.
The definitive diagnosis of AS can be made by molecular analysis of type IV collagen genes, and discovery of underlying mutation.

Pathogenesis of Alport syndrome

Type IV collagen occurs only in the basement membranes (BMs) and comprises up to six genetically distinct alpha-chains designated α1(IV) to α6(IV). Three α(IV)-chains assemble to form triple helical molecules (protomers) that further associate to form supramolecular networks. Only three isoforms (protomers) have so far been identified in BMs: the$[\alpha1(IV)]_2\alpha2(IV)$, $\alpha3(IV)\alpha4(IV)\alpha5(IV)$, and $[\alpha5(IV)]_2\alpha6(IV)$ protomers. The α1(IV) and α2(IV)-chains are present ubiquitously in BMs, whereas the α3(IV), α4(IV), α5(IV), and α6(IV)-chains have a restricted tissue distribution.
The pathogenesis of AS is best described for its renal manifestations.
Two distinct collagen networks have been identified in the GBM, an embryonic network consisting of the $[\alpha1(IV)]_2\alpha2(IV)$ protomers and a postnatal predominant $\alpha3(IV)\alpha4(IV)\alpha5(IV)$ network. The basis of the progressive renal abnormalities is related to the normal developmental switch from the embryonic to the mature network. In general, when one of the α3 - α5(IV)-chains is changed or absent due to a mutation in the underlying gene, all three fail to accumulate in the GBM. Disregard of which gene is affected, mutations that alter triplehelical collagen molecule cause an arrest in the developmental switch and persistence of the $[\alpha1(IV)]_2\alpha2(IV)$ network in the GBM. The embryonic network exhibits a reduced stability and is more susceptible to proteolysis. The consequence is progressive renal failure.

Analogical mechanism of basement membranes pathology underlie the ocular and cochlear manifestations of AS.

Somatic mosaicism

A mosaic or mosaicism denotes the presence of two genetically distinct cell populations in one individual who has developed from a single fertilized egg. Mosaicism can exist in both somatic cells and germ line cells. If the event leading to mosaicism occurs during development, it is possible that both somatic and germ line cells will become mosaic. In this case, both somatic and germ line tissue populations would be affected, and an individual could transmit the mosaic genotype to his or her offspring. Conversely, if the triggering event occurs later in life, it could affect either a germ line or a somatic cell population. If the mosaicism occurs only in a somatic cell population, there would be no risk of passing on the mosaic genotype to offspring. If the mosaicism occurs only in a germ line cell population, the individual would be unaffected, but his or her offspring could be affected.
In female somatic cells one of two X chromosomes is randomly inactivated. As a consequence of clonal inheritance of the inactive X, human females are mosaics, a composite of two populations of cells that differ as to which X chromosome is expressed. The size of the mosaic patch differs from tissue to tissue. Because most of the kidney is formed after the onset of X inactivation and because branches may consist of clonal populations of cells, the patch size might be relatively large in kidneys.
Immunostaining of Alport heterozygote glomeruli with an antibody to α5(IV)-chain shows the mosaic pattern, with a block of labeled cells, most likely normal clones, and blocks that are not labeled, most likely mutant clones. It is widely assumed that the variability in renal outcomes in women with XLAS is a consequence of the variable balance of wild-type and mutant alleles resulting from random X-chromosome inactivation.

AIM OF THE STUDY

Among clinical nephrologists it is well known that some male X-linked Alport syndrome patients show a relatively mild phenotype, but few molecular investigations have been conducted to clarify the mechanism of this phenomenon. We report a male XLAS case with mild clinical symptoms and somatic mosaicism as a possible explanation.

RESULTS

Our patient is an 8-year-old male with no family history of kidney disease. At the age of 3 years hematuria was detected and kidney biopsy performed.
Electron microscopy of the GBM showed typical findings for AS, but surprisingly the immunohistochemical analysis of the glomerulus showed a mosaic α5(IV) staining.
Patient presented with slight proteinuria. Auditory test showed no hearing loss, and he had no ocular abnormalities. Karyotype analysis revealed a normal, 46 XY, result. Direct sequencing of the *COL4A5* gene in the DNA from leukocytes, urine sediments, hair roots and skin unexpectedly disclosed heterozygous mutation at the intron 43 splicing acceptor site (c. 3998-2 a/t). Splicing acceptor site inactivation and heterozygosity was confirmed by analysis of mRNA extracted from urine sediments. By RT-PCR we amplified two products – one normal, and other with exon 44 skipping.
Restriction enzyme assay demonstrated heterozygosity for all tissues analyzed and therefore proved somatic mosaicism in our patient. By semiquantitative capillary electrophoresis we have been able to compare expression of wild type and mutant alleles in different tissues.

CONCLUSIONS

Although there are reports of somatic mosaicism in XLAS males with mild phenotype, our study for the first time presented somatic mosaicism case with a comprehensive analysis of genomic DNA, mRNA and α5(IV) expression in different tissues.

神戸大学大学院医学系研究科（博士課程）

論文審査の結果の要旨

受付番号	甲 第2097号	氏名	KROL RAFAL PRZYBYSLAW
論文題目 Title of Dissertation	Somatic mosaicism for a mutation of the *COL4A5* gene is a cause of mild phenotype male Alport syndrome *COL4A5* 遺伝子に体細胞モザイク変異を認めた軽症男性 Alport 症候群患者の分子生物学的研究		
審査委員 Examiner	主査 Chief Examiner 西尾久英 副査 Vice-examiner 前田士人 副査 Vice-examiner 清野進		

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INTRODUCTION

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CASE REPORT

The patient was an 8-year-old male with no family history of kidney disease. At the age of 3 years hematuria was detected and kidney biopsy performed. Electron microscopy of the GBM showed typical findings for AS, but surprisingly the immunohistochemical analysis of the glomerulus showed a mosaic α5(IV) staining.

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Although there are reports of somatic mosaicism in XLAS males with mild phenotype, the study for the first time presented somatic mosaicism case with a comprehensive analysis of genomic DNA, mRNA and α5(IV) expression in different tissues.

The candidate, having completed studies on X-linked Alport syndrome, with a specialty in its molecular genetics, and having advanced the field of knowledge in the area of inheritance trait including somatic mosaicism, is hereby recognized as having qualified for the degree of Ph.D.(Medicine).