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A female with X-linked Alport syndrome and compound heterozygous *COL4A5* mutations

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Abstract

Background: Females with X-linked Alport syndrome have a single *COL4A5* mutation, germ cell mosaicism in affected tissues and typically develop renal failure later or less often than males. Women with two mutations are exceedingly rare, and usually have consanguineous parents or uniparental disomy. We describe here a 20 year old female who inherited two different *COL4A5* variants, one from her father (c.2677G>C) and one from her mother (c.384 +1 G>A).

Case Diagnosis: The index case had normal renal function, proteinuria and no clinically-detectable hearing loss, or ocular abnormalities.

Her father and paternal uncle developed end-stage renal disease at 37 and 28 years respectively, together with hearing loss, but not lenticonus or central retinopathy.

Her mother had mildly impaired renal function, proteinuria, hearing loss but no ocular abnormalities. Her maternal grandfather and 22 year-old brother, both with this mutation, developed renal failure by 28 years with hearing loss, or had proteinuria and hearing loss respectively.

Conclusions: The index case has clinical features consistent with germ cell mosaicism of two *COL4A5* mutations associated with adult-onset renal failure but no ocular abnormalities. Her risk of renal failure is high, but the rate of progression to end-stage disease depends on the underlying mutations, and disease modification with renin-angiotensin blockade.

Keywords: X-linked Alport syndrome, female, *COL4A5* mutation

INTRODUCTION

Alport syndrome affects one in 10,000 individuals, and 85% of patients have X-linked disease due to mutations in the *COL4A5* gene. Clinical features include hematuria, proteinuria, renal failure, hearing loss, lenticonus and a central fleck retinopathy, peripheral coalescing retinopathy, and retinal thinning [1-2]. All affected males eventually develop renal failure, most have a hearing loss, and many have lenticonus and central retinopathy.

In contrast, most women with X-linked Alport syndrome have hematuria, and some develop proteinuria, hearing loss and peripheral retinopathy [3,4]. Eventually, 15% of females will develop renal failure, but lenticonus and central retinopathy are rare [5]. Clinical expression is different in males and females because female tissues demonstrate ‘lyonisation’ where only one X chromosome is expressed in any cell. This means that affected tissues have two cell populations, one with the mutant allele, and the other with the wildtype.

In X-linked Alport syndrome, genetic mutations are typically different in each family, and clinical features in males depend on the mutation characteristics and location [6-8]. Thus, large deletions, rearrangements, nonsense mutations, missense mutations near the carboxy terminus and some splicing mutations, which account for more than half of all disease-causing variants, result in early onset end-stage renal disease (before about the age of 30), often with hearing loss, lenticonus and retinopathy. Missense mutations near the collagen chain amino terminus produce late onset renal failure and hearing loss, but not the ocular abnormalities. Overall, clinical features correlate well with mutations and are consistent within families. Inconsistencies occur where the physical examination has been incomplete or there is coincidental disease. Clinical features correlate less well with mutations in females because of

lyonisation, but in general women with the most severe disease have the features seen in affected adult males from the same family or with the same mutation. Thus, an affected female from a family with X-linked Alport syndrome due to a mutation that is not associated with lenticonus will not develop lenticonus.

Most females with X-linked Alport syndrome have only one mutation in the *COL4A5* gene, but we describe here a 20 year-old female who inherited two *COL4A5* mutations, one from each parent.

Case report

The index case (III-3) was a 20 year-old female whose father and mother both had X-linked Alport syndrome caused by different *COL4A5* mutations (Figure 1a, Electronic Supplementary Table 1).

This study was approved by the Human Research Ethics Committee of Northern Health according to the standards of the Declaration of Helsinki, and all participants provided signed informed consent.

All members of the patient's family were assessed for hematuria, proteinuria and renal function, and examined by an ophthalmologist for the characteristic ocular features of Alport syndrome. These included examination for the 'oil drop' sign of anterior lenticonus using a hand-held retinoscope, and optic fundi examination for the central and peripheral retinopathies by direct ophthalmoscopy, slit lamp biomicroscopy with a 78D lens, and indirect ophthalmoscopy with a 20D lens. All immediate family members also underwent retinal photography with at least one view centred on the foveola, and with red-free manipulation to facilitate demonstration of the fleck retinopathy. They also had optical coherence tomography (OCT, Zeiss) to measure retinal thickness.

Family members provided samples of peripheral blood from which DNA was extracted. All 51 exons and splice sites of the *COL4A5* gene were screened for pathogenic mutations by high throughput unidirectional tagged-primer Sanger sequencing, in a 384 well format, using robotics at the DNA Laboratory, Guy's and St Thomas' Hospital Trust. PCR products were generated using Qiagen Multiplex Buffer at an annealing temperature of 56^{o}C, and cleaned using AMPure magnetic beads (Agencourt Bioscience Corporation). Sequence analysis used BigDye cycle

sequencing kit v3.1 (Applied Biosystems, Foster City, California) and products were cleaned using CleanSEQ magnetic beads (Agencourt Bioscience Corporation). Sequences were detected using an ABI Prism 3730 DNA analyser (Applied Biosystems) and sequence traces examined using Mutation Surveyor software (SoftGenetics). The presence of any variant was determined from the reference sequence (NM_000495.1) and confirmed by bidirectional tagged-primer Sanger DNA sequencing.

The index case had inherited two *COL4A5* mutations: c.2677G>C from her father and c.384+1G>A from her mother. She had first presented as a 4 year-old with episodes of macroscopic haematuria, and now had persistent microscopic haematuria, mild proteinuria (0.3 g/24 hours) but normal renal function (eGFR > 90 ml/min/1.73m^2), and normal blood pressure. She had no clinically detectable hearing loss (but had not undergone formal audiometry), corneal abnormalities, lenticonus, central or peripheral retinopathy, or retinal thinning on OCT (Figure 1b). She was currently undergoing treatment with ACE inhibitors.

The patient's renal biopsy at the age of 20 years demonstrated that 2 out of 8 glomeruli were globally sclerosed, and there was mild patchy interstitial fibrosis and tubular atrophy. Electron microscopy demonstrated a glomerular basement membrane (GBM) with a basket weave appearance, lamellation, subepithelial frilling, small intramembranous deposits and thinning (Figure 1c).

Her father, II-2, had the c.2677G>C mutation which corresponded to p.Gly893Arg in exon 31. He was now 44 years old, and had developed end-stage renal disease at 37 years, when he underwent renal transplantation. He had first presented with macroscopic haematuria at the age of 5, and had a renal biopsy as a child. He noted a hearing loss from the age of 20, and wore hearing aids, but had no lenticonus or

central retinopathy. However, he had peripheral retinopathy and retinal temporal thinning on OCT. His mother was 68 years old and had haematuria but her renal function was not known.

The patient's paternal uncle, II-1, also had the c.2677G>C mutation. He was 43 years old, and had been diagnosed at 1 year of age with haematuria and proteinuria. He first noted hearing loss at 20 years, and had started on dialysis at 28 years. He had a renal transplant two years later. He now had no lenticonus or central retinopathy. However, he had what were probably steroid-related cataracts, peripheral retinopathy and retinal thinning.

The patient's mother (II – 3) had the c.384 +1 G>A mutation. She was 47 years old, and had hematuria, proteinuria and an eGFR of 73 ml/min/1.73 m^2. She had mild hearing loss, no lenticonus, no central or peripheral retinopathy and no retinal thinning. She was currently undergoing treatment with ACE inhibitors. Her own father had developed renal failure at 28 years of age and died at 31. He had hearing loss, but it is not known if he had ocular abnormalities.

Our patient had two brothers. One (III-1) had no mutation and no features of Alport syndrome. The other (III-2) was 22 years old, and had the maternal mutation (c.384 +1 G>A), with haematuria, 0.3 g proteinuria /day, but a normal eGFR and normal blood pressure. He had a mild hearing loss, but no retinopathy and normal retinal thickness. He was currently treated with ACE inhibitors.

A younger sister (II-4) was 16 years old and also had the paternal mutation. She had haematuria but no proteinuria, normal renal function and no clinically detectable hearing loss or ocular abnormalities.

DISCUSSION

The index case was a 20 year-old female with X-linked Alport syndrome who had inherited two different *COL4A5* mutations, one from each parent. There are no previous reports of a female with different *COL4A5* mutations. Most young women with Alport syndrome and renal impairment have recessive disease [9].

In males with X-linked Alport syndrome, clinical features can be predicted from the location and nature of *COL4A5* mutations, and are consistent in different males within a family and in males from different families with the same mutation [6-7]. However in females, the clinical features depend not only on the underlying mutations but also on germ cell mosaicism or the extent to which each variant is expressed in the basement membranes of the glomerulus, cochlea, lens capsule and retina.

In this case, both the maternal and paternal mutations resulted in adult-onset renal failure in males. The maternal mutation (c.384 +1 G>A) was associated with late onset renal failure and hearing loss but no ocular features in the mother, and renal failure at 28 years of age in the maternal grandfather. Splicing mutations, such as this, do not produce the severe disruption seen with, say, nonsense mutations. The paternal mutation, c.2677G>C, resulted in a glycine being substituted by an arginine in the carboxyl half of the collagen IV α5 chain. Arginine substitutions usually produce more severe disease than substitutions with other amino acids [10]. However, this variant was also associated with adult- onset renal failure, with hearing loss, but not lenticonus or central retinopathy in the father and uncle. Both men, however, had retinal thinning and mild peripheral retinopathy [2]. The thinned retina

appears to be sensitive for Alport syndrome in males, and may be useful diagnostically. While the thinning occurs in the same location as a macular hole, it has no other visual significance [11].

Thus, both mutations in the index case were associated with adult-onset renal failure without lenticonus or retinopathy. Her clinical features were expected to be of intermediate severity, and indeed, at the age of 20, she had mild proteinuria, but normal renal function, no clinically-detectable hearing loss, a normal lens, and normal retinal appearance and thickness. Hearing loss is difficult to assess without formal audiometry, and proteinuria and renal function are likely to deteriorate later, but were currently controlled with ACE inhibitor treatment [12]. Her renal biopsy already demonstrated glomerular sclerosis with interstitial fibrosis, and her GBM ultrastructural appearance was identical to that seen in males with X-linked disease.

Interestingly, the index case's phenotype appears less severe than for many females with autosomal recessive Alport syndrome who often develop renal failure in their twenties, together with hearing loss, lenticonus and retinopathy. However this may reflect an ascertainment bias if females with recessive disease are usually identified because of their extrarenal characteristics. It is more likely that the clinical phenotype in recessive mutations, as for X-linked Alport disease, depends on the characteristics of the underlying mutations [13].

Generally, females with X-linked disease and two *COL4A5* mutations are from consanguineous families with homozygous mutations. Other explanations include uniparental disomy with duplication of the X chromosome, or a single mutation in a female with Turner syndrome. There are occasional reports of females with other X-linked disease due to two mutations [14], where the clinical features usually resemble the male phenotype.

Genetic testing now represents the ‘gold standard’ for the diagnosis of Alport syndrome and to confirm the mode of inheritance. With more widespread testing, individuals with different combinations of mutations, for example, *COL4A5* and *COL4A3* or *COL4A4* variants, resulting in a combination of X-linked Alport syndrome and Thin membrane nephropathy, will be identified.

We were unable, despite attempts, to test for collagen IV α3α4α5 expression in the glomerular membrane in our patient’s renal biopsy because of the way the tissue had been treated.

Affected members of this family who have proteinuria are currently treated with renin-angiotensin blockade. There is now evidence that commencing ACE inhibitors even before the onset of microalbuminuria delays the onset of renal failure by many years, and should be considered in patients with high risk mutations or microalbuminuria [12,15].

Genetic counselling is different for females with X-linked disease and two mutations. A female with X-linked disease is usually advised that, on average, half her children will inherit the mutation, and half her sons will develop renal failure, but her daughters have only a 15% risk of renal failure at the age of 60 [8]. In contrast, all the offspring of a woman with two *COL4A5* mutations will inherit a mutation, and all her sons will eventually develop end-stage kidney disease.

Conclusions: Clinical features in X-linked Alport syndrome depend on the location and nature of the causative mutations, even in females with two mutations. The female described here is highly likely to develop end-stage kidney disease, but not lenticonus or retinopathy, and renin-angiotensin system blockade should delay her progression to end-stage disease.

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LIST OF TABLES AND FIGURES

A: Family tree indicating inheritance of paternal (black, c. 2677G>C) and maternal (striped, c.384 +1 G>A) mutations

B: Optical coherence tomography of the left retina (cross-section on left and thickness on right) in a. father; b. mother; and c. index case indicating temporal thinning in father (271 µm and 234 µm in inner and outer temporal retina) but not in mother or index case (310 µm and 263µm, 302 µm and 267 µm respectively).

C: Electron micrographs of renal biopsy from the index case indicating lamellation (i, black arrow), vacuolation intramembranous deposits (i, white arrow), subepithelial frilling (ii, white arrow) and thinning.

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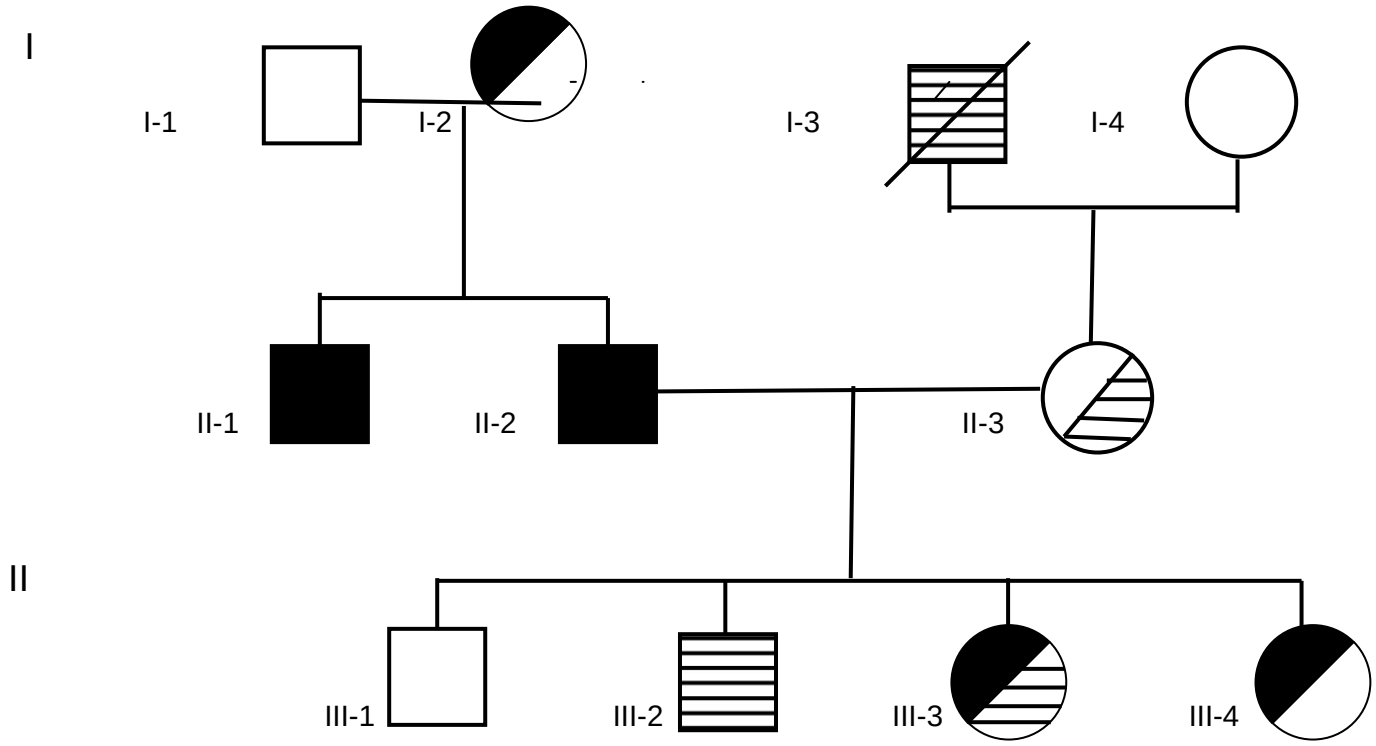
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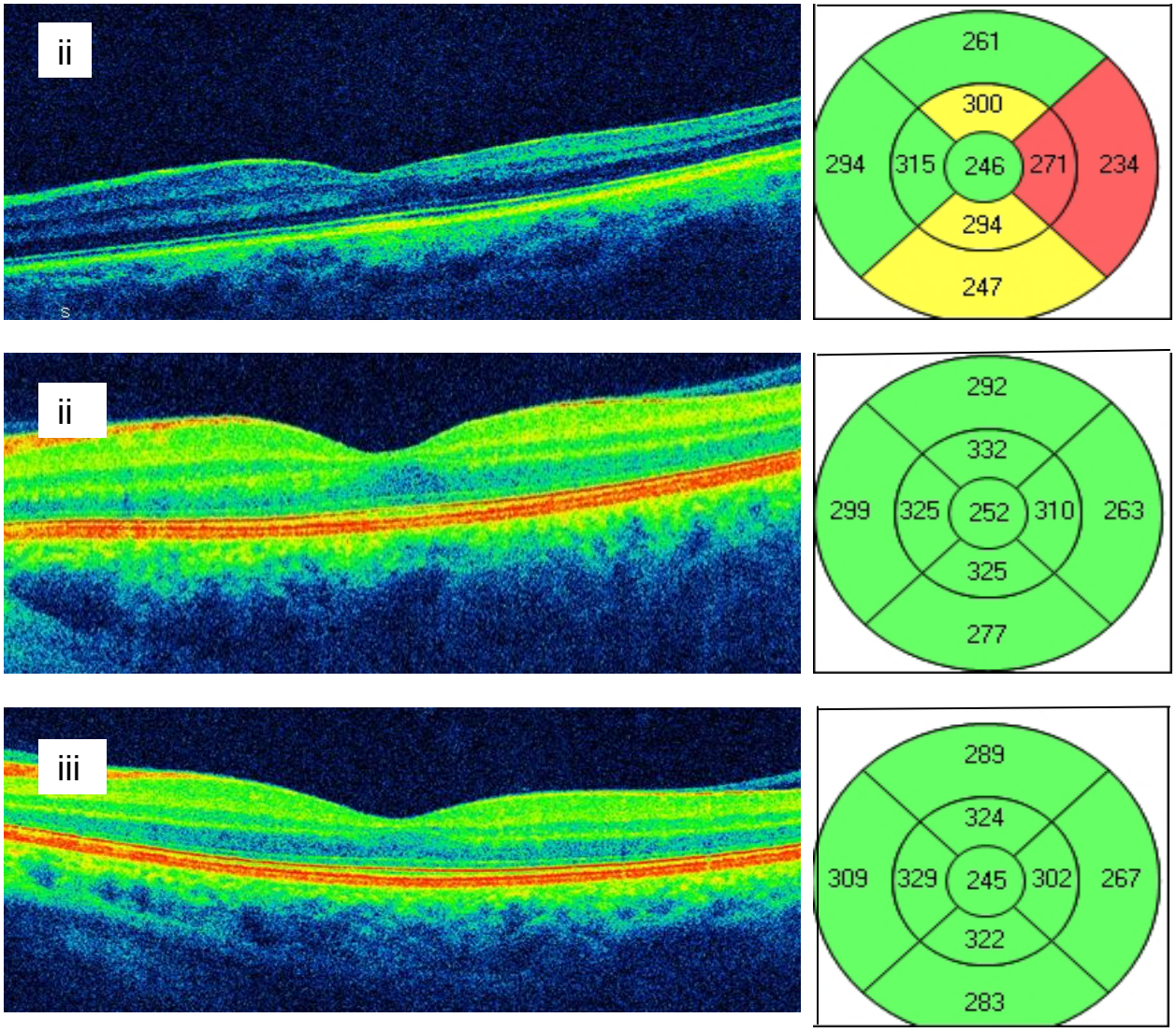
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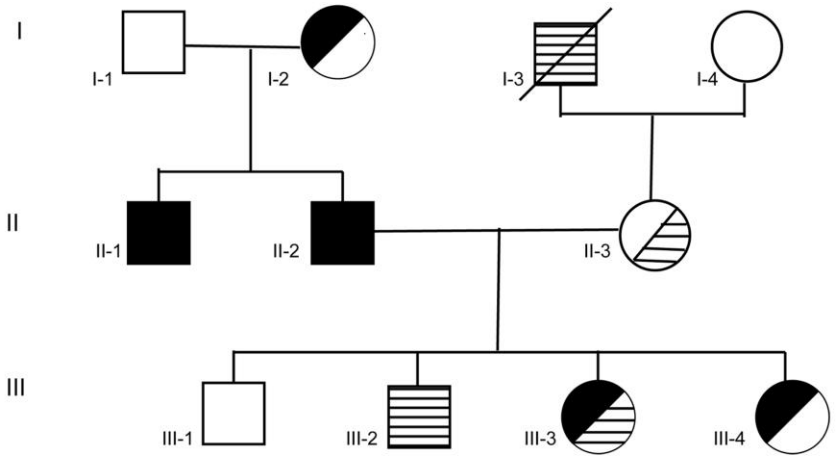
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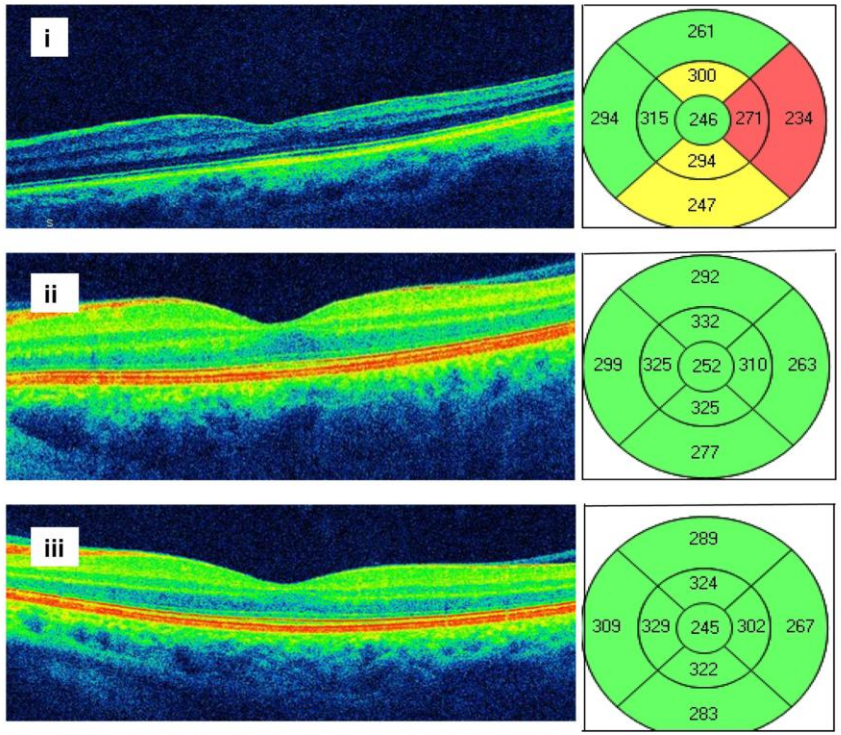
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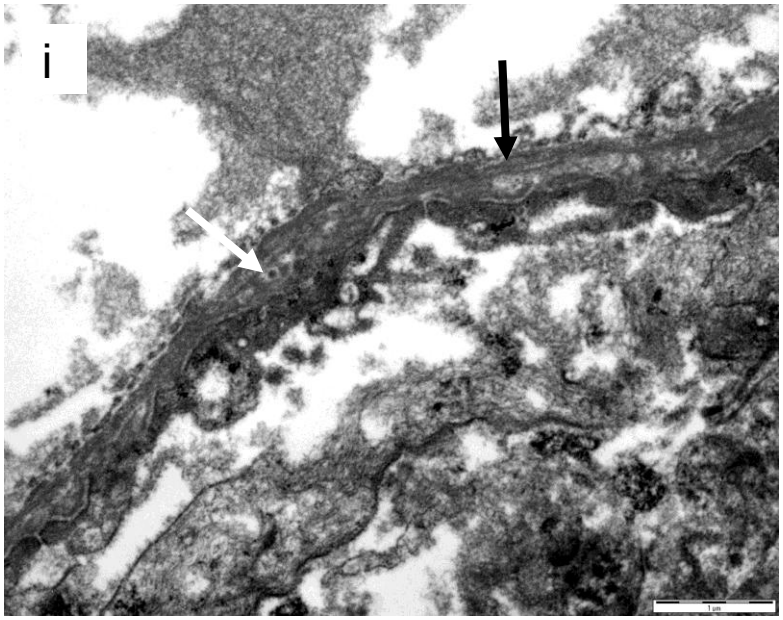
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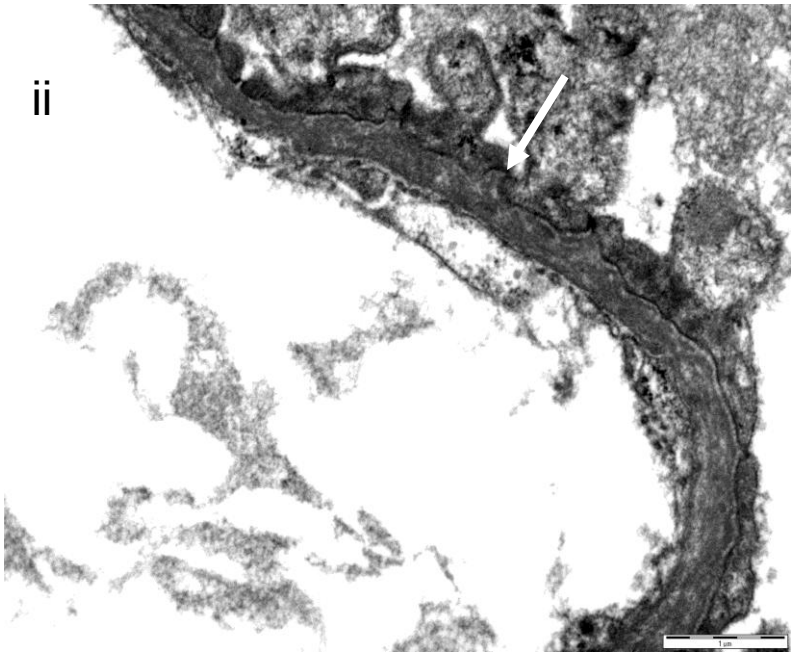
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