DISEASE OF THE MONTH

Alport Syndrome and Thin Glomerular Basement Membrane Disease

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Alport syndrome (AS) is a generalized inherited disorder of basement membranes, manifested by hematuria, progressive nephritis with proteinuria and declining renal function, sensorineural deafness, and ocular abnormalities. The natural history of AS is gender-dependent: affected males typically have severe disease, while the course of AS in females tends to be mild. The first description of familial hematuria was provided by Guthrie (1) in 1902. Follow-up studies of this family by Hurst (2) and Alport (3) described the progressive nature of the nephropathy, its association with deafness, and the poorer prognosis in affected males. Electron microscopic investigations carried out by several groups in the early 1970s identified the glomerular basement membrane (GBM) as the site of the primary renal abnormality in AS (4–6). A series of reports between 1980 and 1990 established AS as an inherited disease of type IV collagen: immunohistologic studies revealing abnormal type IV collagen composition of AS basement membranes (7,8); mapping of an Alport locus to the X chromosome (9); cloning of a new type IV collagen gene (COL4A5) and its assignment to the same region of the X chromosome as the Alport locus (10); and, finally, identification of the first COL4A5 mutations in patients with X-linked AS (11).

Type IV Collagen
Genes and Proteins

Basement membranes are sheetlike structures that support endothelial and epithelial cells and are composed of various glycoproteins secreted by these cells, including type IV collagen, laminin, nidogen (entactin), and heparan sulfate proteoglycan. The type IV collagen family of proteins comprises six isomeric chains, designated α1 through α6(IV) (12). These chains show extensive sequence homology and share basic structural features, including (1) a major collagenous domain of approximately 1400 residues containing the repetitive triplet sequence glycine(Gly)-X-Y, in which X and Y represent a variety of other amino acids; (2) a carboxy-terminal noncollagenous (NC1) domain of approximately 230 residues; and (3) a noncollagenous amino-terminal sequence of 15 to 20 residues. The major collagenous domain of each chain contains about 20 interruptions of the collagenous triplet sequence, and each NC1 domain contains 12 completely conserved cysteine residues that participate in critical disulfide bonds.

Each type IV collagen molecule is a trimer composed of three α chains. The classical type IV collagen trimer isolated from the extracellular matrix of the murine Engelbreth-Holm-Swarm sarcoma has the composition (α1)3(α2). Type IV collagen α chains form trimers through associations between their carboxy-terminal NC1 domains, accompanied by folding of the collagenous domains into triple helices. Variable residues within the NC1 domains may determine which chains are able to associate. Type IV collagen triple helices form networks through various intermolecular interactions, including end-to-end linkages between the NC1 domains of two type IV collagen triple helices, covalent interactions between four triple helices at their amino-terminal ends, and lateral associations mediated by binding of the NC1 domains of one trimer to the collagenous region of another trimer. The result of these linkages is a nonfibrillar, open-network assembly that distinguishes type IV collagen from interstitial collagens like type I and type III, which lack carboxy-terminal NC1 domains in their mature forms and form fibrillar structures rather than networks. Type IV collagen networks associate with laminin assemblies through interactions mediated by nidogen to form basement membranes.

The six type IV collagen genes are distributed in pairs on three chromosomes. The human α1 and α2(IV) chains are encoded by the genes COL4A1 and COL4A2, respectively, on chromosome 13. COL4A3 and COL4A4 are located on chromosome 2 and encode the α3(IV) and α4(IV) chains of type IV collagen, respectively, whereas the α5(IV) and α6(IV) chains are encoded by the COL4A5 and COL4A6 genes on the X chromosome. The 5′ ends of each gene pair are adjacent to each other, separated by sequences of varying length containing motifs involved in the regulation of transcriptional activity. The five exons at the 3′ end of each gene encode the NC1 domain of the protein product, and most of the remaining exons encode the collagenous portion.

Tissue Distribution

Expression of the six type IV collagen α chains in human tissues has been studied using monospecific antibodies. The α1(IV) and α2(IV) chains are normally found in all basement membranes, and are completely concordant in distribution. The expression patterns of the α3(IV) and α4(IV) chains are also...
completely concordant with each other, but restricted in comparison to the α1(IV)/α2(IV) chains. The α3(IV) and α4(IV) chains are expressed in basement membranes that are demonstrably or potentially involved in AS, including GBM (Figure 1), several basement membranes of the cochlea, and ocular basement membranes such as anterior lens capsule, Descemet’s membrane, and Bruch’s membrane, but are absent from other basement membranes such as mesangium, vascular basement membranes, and epidermal basement membranes (EBM) (Table 1). All basement membranes that express the α3(IV) and α4(IV) chains also express α5(IV), although the converse is not true. For example, EBM express α5(IV) and α6(IV), but not α3(IV)/α4(IV).

Studies of the tissue expression of type IV collagen α chains thus suggest that several distinct networks of type IV collagen may exist in basement membranes: a ubiquitous network composed of the α1(IV) and α2(IV) chains, and other networks composed of α3(IV), α4(IV), and α5(IV) chains, or of α5(IV) and α6(IV) chains. For example, GBM appears to include separate α1/α2(IV) and α3/α4/α5(IV) networks, while EBM seem to contain separate networks of α1/α2(IV) and α5/α6(IV) chains. Biochemical studies of extracted basement membranes have recently lent support to this “separate networks” hypothesis (13,14). Whether these networks have different functional characteristics, or whether there are differences in their interactions with other matrix components or with cells, is not known, although the restricted distribution of the α3/α4/α5(IV) network suggests specialization of function.

Genetics of Alport Syndrome

The molecular genetics of two forms of AS have been established. The X-linked dominant form arises from mutations at the COL4A5 locus, primarily affecting the α5(IV) chain, and the autosomal recessive form results from mutations at the COL4A3 locus or the COL4A4 locus (Table 2). Pedigree and linkage analyses suggest that an autosomal dominant type of AS exists, although mutations causing autosomal dominant disease have yet to be identified (15).

X-Linked Alport Syndrome

X-linked Alport syndrome (XLAS) is the predominant form of the disease, accounting for approximately 80% of patients. Since the first report of COL4A5 mutations by Barker et al. (11) in 1990, more than 200 additional mutations have been described (16). However, COL4A5 mutations have been found in only approximately 50% of XLAS patients by exon screening methods such as analysis of single-strand conformational polymorphisms (17–19). It is possible that as yet unidentified intronic mutations in COL4A5 occur frequently in AS, or that the rate of mutation identification would improve with direct sequencing of PCR-amplified exon regions. Mutations in COL4A6 have not been found in AS patients, except for those with diffuse leiomyomatosis (see below), an observation that is consistent with the lack of expression of α6(IV) in normal GBM.

Major rearrangements at the COL4A5 locus, such as large deletions, account for approximately 10% of XLAS. A particular type of COL4A5 deletion is observed in families in which AS cosegregates with leiomyomas of the esophageal tract, tracheobronchial tree, and female genital tract. All patients with the AS-leiomyomatosis complex subjected to molecular genetic analysis have exhibited deletions of the 5’ end of COL4A5 that extend upstream to involve the first two exons of COL4A6, with the deletion breakpoint occurring in the second intron of COL4A6 (20, 21). The association between 5’ deletions of COL4A6 and leiomyomatosis is not presently understood.

Missense mutations, splice-site mutations, and small deletions (<10 bp) account for the majority of COL4A5 mutations. A common missense mutation involves replacement of a glycine residue in the collagenous domain of the α5(IV) chain by another amino acid. Such mutations are thought to interfere with the normal folding of the mutant α5(IV) chain into triple helices with other α(IV) chains. Glycine, which lacks a side chain, is the least bulky of amino acids; only glycine residues are small enough to allow three of them to fit into the interior of the tightly wound triple helix. The position of the substituted glycine, or the substituting amino acid itself, may determine

**Figure 1.** Expression of the α3, α4, and α5 chains of type IV collagen in normal glomerular basement membranes, as revealed by immunofluorescence using monospecific antibodies.
Table 1. Type IV collagen chain distribution in Alport target organs

<table>
<thead>
<tr>
<th>Chain</th>
<th>Kidney</th>
<th>Eye</th>
<th>Cochlea</th>
<th>Skin</th>
</tr>
</thead>
<tbody>
<tr>
<td>α1(IV)</td>
<td>All BM</td>
<td>All BM</td>
<td>All BM</td>
<td>All BM</td>
</tr>
<tr>
<td>Mesangium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α2(IV)</td>
<td>All BM</td>
<td>Lens capsule</td>
<td>Inner/outer sulci</td>
<td>None</td>
</tr>
<tr>
<td>Mesangium</td>
<td></td>
<td>Descemet's memb.</td>
<td>Descemet's memb.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bruch's memb.</td>
<td>Bruch's memb.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Internal limiting memb.</td>
<td>Internal limiting memb.</td>
<td></td>
</tr>
<tr>
<td>α3(IV)</td>
<td>GBM, BC, dTBM</td>
<td>Lens capsule</td>
<td>Inner/outer sulci</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Descemet's memb.</td>
<td>Descemet's memb.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bruch's memb.</td>
<td>Bruch's memb.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Internal limiting memb.</td>
<td>Internal limiting memb.</td>
<td></td>
</tr>
<tr>
<td>α4(IV)</td>
<td>GBM, BC, dTBM</td>
<td>Lens capsule</td>
<td>Inner/outer sulci</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Descemet's memb.</td>
<td>Descemet's memb.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bruch's memb.</td>
<td>Bruch's memb.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Internal limiting memb.</td>
<td>Internal limiting memb.</td>
<td></td>
</tr>
<tr>
<td>α5(IV)</td>
<td>GBM, BC, dTBM</td>
<td>Lens capsule</td>
<td>Inner/outer sulci</td>
<td>EBM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Descemet's memb.</td>
<td>Descemet's memb.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bruch's memb.</td>
<td>Bruch's memb.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Internal limiting memb.</td>
<td>Internal limiting memb.</td>
<td></td>
</tr>
<tr>
<td>α6(IV)</td>
<td>BC, dTBM</td>
<td>Unknown</td>
<td>Inner/outer sulci</td>
<td>EBM</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Descemet's memb.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Bruch's memb.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Internal limiting memb.</td>
<td></td>
</tr>
</tbody>
</table>

* BM, basement membranes; GBM, glomerular basement membrane; BC, Bowman’s capsule; dTBM, distal tubular basement membrane; memb., membrane; EBM, epidermal basement membrane.

Table 2. Inheritance of Alport syndrome

<table>
<thead>
<tr>
<th>Locus</th>
<th>Affected Gene Product</th>
<th>Chromosome</th>
<th>Inheritance</th>
</tr>
</thead>
<tbody>
<tr>
<td>COL4A5</td>
<td>α5(IV)</td>
<td>X</td>
<td>X-linked dominant Alport syndrome</td>
</tr>
<tr>
<td>COL4A5 + COL4A6*</td>
<td>α5(IV) + α6(IV)</td>
<td>X</td>
<td>X-linked Alport syndrome + leiomyomatosis</td>
</tr>
<tr>
<td>COL4A3</td>
<td>α3(IV)</td>
<td>2</td>
<td>Autosomal recessive Alport syndrome</td>
</tr>
<tr>
<td>COL4A4</td>
<td>α4(IV)</td>
<td>2</td>
<td>Autosomal recessive Alport syndrome</td>
</tr>
<tr>
<td>COL4A3?b</td>
<td>α3(IV)?</td>
<td>2</td>
<td>Autosomal dominant Alport syndrome</td>
</tr>
<tr>
<td>COL4A4?b</td>
<td>α4(IV)?</td>
<td>2</td>
<td>Autosomal dominant Alport syndrome</td>
</tr>
</tbody>
</table>

* The Alport syndrome-diffuse leiomyomatosis complex results from deletion mutations involving both COL4A5 and COL4A6. The breakpoint of the deletion within the COL4A6 gene must fall within its second intron in order to produce leiomyomatosis; if the deletion breakpoint occurs more distally within COL4A4, leiomyomatosis will not result.

b Autosomal dominant Alport syndrome has been mapped to region of chromosome 2 in which the COL4A3 and COL4A4 genes are located (15), but specific mutations have yet to be described. Benign familial hematuria due to a mutation in one allele of COL4A4 has been reported (70).

the ultimate effect of the mutation on folding. Other missense mutations of COL4A5 involve critical residues in the NC1 domain of α5(IV). For example, substitution of a conserved cysteine residue in the NC1 domain would prevent formation of a disulfide bond, which could destabilize heterotrimers or disrupt construction of networks involving α5(IV). COL4A5 mutations affecting the sites at which exons are spliced together in mRNA can result in skipping of a variable number of exons, with profound effects on the translated α5(IV) protein.

Small deletions or insertions may produce shifts of the transcriptional reading frame (nonsense mutations), resulting in nonfunctional protein.

Male patients with COL4A5 deletions consistently exhibit progression to end-stage renal disease (ESRD) and deafness during the second or third decade of life. Most of the missense, nonsense, and splicing mutations of COL4A5 described thus far are also associated with early progression to ESRD and deafness. Several missense mutations of COL4A5 have been
associated with late-onset (after the third decade) ESRD, or slowly progressive nephropathy with normal hearing. The severity of disease in a female heterozygous for a \textit{COL4A5} mutation probably depends on the extent of inactivation of the \textit{X} chromosome carrying the normal \textit{COL4A5} allele, as well as the nature of the mutation.

**Autosomal Recessive Alport Syndrome**

The existence of autosomal recessive AS (ARAS) was postulated by French investigators (22) and confirmed by Mochizuki \textit{et al.} (23), who identified mutations in the \textit{COL4A3} gene in two AS kindreds with autosomal recessive disease, and in \textit{COL4A4} in two other kindreds. Several other mutations in \textit{COL4A3} or \textit{COL4A4} mutations causing ARAS have since been reported (16).

ARAS should be suspected when an individual exhibits the typical clinical and pathologic features of the disease but lacks a positive family history, especially when a young female has findings indicative of severe disease such as deafness, renal insufficiency, or nephrotic syndrome. Sporadic cases of AS may, however, represent \textit{de novo} mutations at the \textit{COL4A5} locus or a germ-line \textit{COL4A5} mutation in the proband’s mother.

Phenotypic information on patients with ARAS is as yet somewhat sparse. On the basis of the available data, patients with \textit{COL4A3} mutations appear to progress to ESRD before age 30 and also have sensorineural deafness, regardless of gender.

### Type IV Collagen in Alport Basement Membranes

In males with XLAS, the GBM, distal tubular basement membrane (TBM), and Bowman’s capsules of males with XLAS usually fail to stain for the \(\alpha3(IV), \alpha4(IV), \text{ and } \alpha5(IV)\) chains, but do express the \(\alpha1(IV)\) and \(\alpha2(IV)\) chains (Table 3, Figure 2). The \(\alpha6(IV)\) chain is not expressed in Bowman’s capsule or distal TBM of XLAS males whose basement membranes lack \(\alpha5(IV)\) expression. Women who are heterozygous for XLAS mutations frequently exhibit mosaicism of GBM expression of the \(\alpha3(IV), \alpha4(IV), \text{ and } \alpha5(IV)\) chains, while expression of the \(\alpha1(IV)\) and \(\alpha2(IV)\) chains is preserved (Figure 2). EBM normally express the \(\alpha1(IV), \alpha2(IV), \alpha5(IV), \text{ and } \alpha6(IV)\) chains, but not the \(\alpha3(IV)\) or \(\alpha4(IV)\) chains (Table 1). Most males with XLAS show no EBM expression of \(\alpha5(IV)\) or \(\alpha6(IV)\), while female heterozygotes frequently display mosaicism (Table 3, Figure 3). Lens capsules of some males with XLAS do not express the \(\alpha3(IV), \alpha4(IV), \text{ or } \alpha5(IV)\) chains, while expression of these chains appears normal in other patients (24).

In patients with ARAS, GBM usually show no expression of the \(\alpha3(IV), \alpha4(IV), \text{ or } \alpha5(IV)\) chains, but \(\alpha5(IV)\) and \(\alpha6(IV)\) are expressed in Bowman’s capsule, distal TBM, and EBM (25) (Table 3, Figure 4). Therefore, XLAS and ARAS may be distinguishable by immunohistochemical analysis of renal biopsy specimens.

These observations indicate that a mutation affecting one of the chains involved in the putative \(\alpha3-\alpha4-\alpha5(IV)\) network can prevent GBM expression not only of that chain, but also of the

| Table 3. Immunohistochemical features of Alport basement membranes. |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| **Category**                | **GBM**                     | **BC**                      | **dTBM**                    | **EBM**                     |
| X-linked males              |                             |                             |                             |                             |
| \(\alpha3(IV)\)             | Absent                      | Absent                      | Absent                      | Normally absent             |
| \(\alpha4(IV)\)             | Absent                      | Absent                      | Absent                      | Normally absent             |
| \(\alpha5(IV)\)             | Absent                      | Absent                      | Absent                      | Absent                      |
| \(\alpha6(IV)\)             | Absent                      | Absent                      | Absent                      | Absent                      |
| females\(^a\)               |                             |                             |                             |                             |
| \(\alpha3(IV)\)             | Mosaic                      |                             |                             | Normally absent             |
| \(\alpha4(IV)\)             | Mosaic                      |                             |                             | Normally absent             |
| \(\alpha5(IV)\)             | Mosaic                      |                             |                             | Mosaic                      |
| \(\alpha6(IV)\)             | Mosaic                      |                             |                             | Mosaic                      |
| Autosomal recessive         |                             |                             |                             |                             |
| males and females           |                             |                             |                             |                             |
| \(\alpha3(IV)\)             | Absent                      | Absent                      | Absent                      | Normally absent             |
| \(\alpha4(IV)\)             | Absent                      | Absent                      | Absent                      | Normally absent             |
| \(\alpha5(IV)\)             | Absent                      | Present                     | Present                     | Present                     |
| \(\alpha6(IV)\)             | Absent                      | Present                     | Present                     | Present                     |

\(^a\) In some AS kindreds, staining of basement membranes for type IV collagen chains is entirely normal. Therefore, a normal result does not exclude a diagnosis of X-linked or autosomal recessive AS. AS, Alport syndrome. Other abbreviations as in Table 1.

\(^b\) Some obligate carriers have normal basement membrane immunoreactivity for type IV collagen chains. Thus, a normal result does not exclude the carrier state.
Expression of $\alpha$3, $\alpha$4, and $\alpha$5(IV) chains in glomeruli of patients with X-linked Alport syndrome. Note the complete absence of glomerular basement membrane staining in the male patient, and the segmental loss of staining in the female patient. In heterozygotes, distal tubular basement membranes may also show segmental loss of staining for these chains. It is important to recall that basement membrane staining for these chains is normal in some families with X-linked Alport syndrome (see "Type IV Collagen in Alport Basement Membranes"). In some instances, staining for these chains is diffusely reduced in intensity, rather than absent.

Other two chains. Similarly, a mutation involving the $\alpha$5(IV) chain can interfere with basement membrane expression of $\alpha$6(IV). The mechanisms that produce these effects remain under investigation. It is likely that at least some mutations interfere in various ways with the formation of trimers, leading to degradation of normal chains that have been prevented from forming trimers, or that have formed abnormal trimers. This kind of process accounts for abnormal type I collagen deposition in bone in osteogenesis imperfecta (26). In some instances, a mutation at one of the type IV collagen loci may result in reduced transcription of other type IV collagen genes, or may accelerate degradation of mRNA transcribed from these genes. Thorner et al. (27) found that kidneys of male dogs with Samoyed hereditary nephropathy, a canine form of AS that arises from a COL4A5 mutation, contained levels of mRNA for the $\alpha$3(IV) and $\alpha$4(IV) chains that were substantially less than the levels found in kidneys of unaffected males. However, Nakanishi et al. (28) found no differences in mRNA levels for $\alpha$3(IV) and $\alpha$4(IV) chains, as measured by competitive reverse transcription-PCR, in kidneys of men with COL4A5 mutations, when compared with normal male kidneys. Similarly, we have observed that dermal fibroblasts of males with XLAS express levels of $\alpha$6(IV) mRNA that are comparable to levels expressed in normal fibroblasts (29). In transgenic mice with $\text{COL4A3}$ mutations, renal mRNA levels for the $\alpha$4(IV) and $\alpha$5(IV) chains are not different from levels in normal mice (30, 31).

### Clinicopathologic Features and Correlations

#### Renal

The cardinal finding of AS is hematuria (Table 4). Affected males have persistent microscopic hematuria. Many also have episodic gross hematuria, precipitated by upper respiratory infections, during the first two decades of life. Hematuria has been discovered in the first year of life in affected boys, and is probably present from birth. Boys who are free of hematuria during the first 10 years of life are unlikely to be affected.

Females who are heterozygous for XLAS may have intermittent hematuria, and as many as 10% of obligate female heterozygotes never manifest hematuria. Hematuria appears to be persistent in both males and females with ARAS. The incidence and pattern of hematuria in individuals who are heterozygous for autosomal disease have yet to be characterized.

Proteinuria is usually absent during the first few years of life but eventually develops in males with XLAS and in both males and females with recessive disease. Proteinuria increases progressively with age, and may result in the nephrotic syndrome.
Significant proteinuria is relatively infrequent in females who are heterozygous for XLAS, but may occur.

Hypertension also increases in incidence and severity with age. Like proteinuria, hypertension is much more likely to occur in affected males than in affected females in the X-linked form of the disease, but there do not appear to be gender differences in the autosomal recessive form.

ESRD develops in virtually all affected males with XLAS, but the rate of progression shows significant inter-kindred variability. The rate of progression to renal failure is fairly constant among affected males within a particular family, although significant intra-kindred variability in the rate of progression to renal failure has occasionally been reported.

The prognosis in affected females with XLAS is generally benign, with most surviving into old age with clinically mild renal disease. Gross hematuria in childhood, nephrotic syndrome, and diffuse GBM thickening by electron microscopy are features suggestive of progressive nephritis in affected females (32). Sensorineural deafness and anterior lenticonus are also indicative of an unfavorable outcome in affected women. Many women with progressive nephritis maintain adequate renal function until late in life. As in women with other chronic nephritides, pregnancy does not appear to adversely affect renal function in those with mild disease, but may be associated with accelerated loss of function in those with more severe disease. Both males and females with ARAS appear likely to progress to ESRD during the second or third decade of life.

There are no pathognomonic lesions by light microscopy or direct immunofluorescence in AS. Indirect immunofluorescence of type IV collagen α chain expression in renal and/or skin basement membranes can be diagnostic, as discussed elsewhere. Electron microscopy frequently reveals diagnostic abnormalities, which are seen in patients with and without hearing loss or ocular involvement.

The pathognomonic fine structural feature of the kidney in AS is thickening of the GBM, with transformation of the lamina densa into a heterogeneous network of membranous strands, which enclose clear electron-lucent areas that may contain round granules of variable density measuring 20 to 90 nm in diameter (Figure 5). The origin of these granules is unknown, but they may represent degenerating islands of visceral epithelial cell cytoplasm. The altered capillary walls typically demonstrate epithelial foot process fusion, which may be extensive even in the absence of significant proteinuria. The epithelial aspect of the capillary wall is typically irregular.

This lesion occurs in most, but not all, patients with AS. Affected young males, heterozygous females at any age, and affected adult males on occasion, may have diffusely attenuated GBM measuring as little as 100 nm or less, rather than the pathognomonic lesion. Studies of males with human or canine AS have shown that the earliest manifestation of the GBM lesion is attenuation, and that the extent and severity of thickening and multilamellation increase with age (33,34). Heterozygous females may have normal-appearing GBM or, on the other end of the spectrum, diffuse GBM thickening and multilamellation, but most will display mild to moderate abnormalities (35).

Not all Alport kindreds demonstrate these characteristic ultrastructural features. Thick, thin, normal, and nonspecifically altered GBM have all been described. Although diffuse attenuation of GBM has been considered the hallmark of
alpha-3(IV)

alpha-4(IV)

alpha-5(IV)
(glomerulus)

alpha-5(IV)
(distal tubule)

Figure 4. Expression of α3, α4, and α5(IV) chains in glomeruli of a patient with autosomal recessive Alport syndrome. Note the complete absence of staining for the α3(IV) and α4(IV) chains. Staining of glomerular basement membranes for the α5(IV) chain is entirely negative, but staining for α5(IV) is preserved in Bowman’s capsule and distal tubular basement membranes. It is important to recall that basement membrane staining for these chains is normal in some families with autosomal recessive Alport syndrome (see “Type IV Collagen in Alport Basement Membranes”).

benign familial hematuria, some patients with this abnormality have progressive renal disease and a COL4A5 mutation (18). Rumpelt found a correlation between the percentage of GBM showing splitting of the lamina densa and the degree of proteinuria in Alport patients, suggesting that impaired permselectivity may be a functional consequence of the GBM alteration (34). Morphometric parameters such as mesangial volume fraction, cortical interstitial volume fraction, and percent global glomerular sclerosis are inversely correlated with creatinine clearance in AS (36,37). Similar relationships have been observed in other diseases, such as diabetic nephropathy and membranoproliferative glomerulonephritis. However, patients with AS show significantly greater impairment of filtration for any degree of structural change, in comparison with patients with diabetic nephropathy or membranoproliferative glomerulonephritis, suggesting that these morphologic abnormalities only partially account for reductions in creatinine clearance in AS. Decreased conductivity of water across the altered glomerular capillary wall could contribute to the decrement in filtration. Expansion of the cortical interstitium, a measure of interstitial fibrosis, is rarely observed before the age of 10 in males with AS (37).

Cochlear

Deafness is frequently but not universally associated with the Alport renal lesion, occurring in approximately 55% of males and 45% of females with the disease (38). In some families with the Alport nephropathy and apparently normal hearing, deafness may be a late and very slowly progressive phenomenon (39).

Hearing loss in AS is never congenital. Diminished hearing is usually detectable by late childhood or early adolescence in boys with XLAS. Hearing impairment in members of families with AS is always accompanied by evidence of renal involvement, there being no convincing evidence that deaf males lacking renal disease can transmit AS to their offspring. In its early stages, the hearing deficit is detectable only by audiometry, with bilateral reduction in sensitivity to tones in the 2000 to 8000 Hz range. In affected males, the deficit is progressive and eventually extends to other frequencies, including those of conversational speech. In females with XLAS, hearing loss is less frequent and tends to occur later in life. There do not appear to be gender differences in the incidence or course of deafness in ARAS.

Studies of brainstem auditory-evoked responses implicate the cochlea as the site of the aural lesion in AS. Impaired vestibular function also can be detected but is not of clinical significance. Only limited information regarding histologic alterations in the cochlea of Alport patients is available, because of the difficulty in obtaining representative pathologic specimens. Abnormalities of the stria vascularis and organ of Corti have been described (40,41).
Anterior lenticonus, in which the central portion of the lens protrudes into the anterior chamber, is virtually pathognomonic of AS. All reported patients with anterior lenticonus who have been adequately examined have exhibited evidence of chronic nephritis and sensorineural deafness (43). Anterior lenticonus is almost entirely restricted to AS families with progression to ESRD before age 30 and deafness. The lesion is bilateral in approximately 75% of patients. It is far more common in affected males but can occur in females. Anterior lenticonus is absent at birth, usually appearing during the second to third decade of life. Progressive distortion of the lens may occur, accompanied by increasing myopia. Lens opacities may be seen in conjunction with lenticonus, occasionally resulting from rupture of the anterior lens capsule. Marked attenuation and fracturing of the anterior lens capsule have been demonstrated by light and electron microscopy (44,45).

A variety of other ocular lesions have been reported in patients with AS. Perhaps the most common abnormalities are pigmented changes in the perimacular region, consisting of whitish or yellowish granulations surrounding the foveal area (46). These lesions are frequently accompanied by anterior lenticonus, but may occur in the absence of lenticonus. These lesions may represent abnormalities of Bruch’s membrane, the basement membrane that supports the retinal pigment epithelium.

Corneal endothelial vesicles (posterior polymorphous dystrophy) have been observed in AS patients by several investigators (47), and may indicate defects in Descemet’s membrane, the basement membrane underlying the corneal endothelium. Recurrent corneal erosion in AS patients was recently described and attributed to alterations of the corneal EBM (48).

**Leiomyomatosis**

The association of AS with leiomyomatosis of the esophagus and tracheobronchial tree has been reported in approximately 20 families (49). Affected females in these kindreds typically exhibit genital leiomyomas as well, causing clitoral hypertrophy with variable involvement of the labia majora and uterus. Bilateral posterior subcapsular cataracts also occur frequently in affected individuals in these kindreds.

Symptoms usually appear in late childhood and include dysphagia, post-prandial vomiting, retrosternal or epigastric pain, recurrent bronchitis, dyspnea, cough, and stridor. Leiomyomatosis may be suspected by chest x-ray or barium swallow, and confirmed by computed tomography or magnetic resonance imaging.

As noted above, all patients with the AS-diffuse leiomyomatosis complex have been found to have deletions that encompass the 5' ends of the COLA45 and COLA46 genes. In heterozygous females, expression of leiomyomatosis appears to occur more consistently than expression of the Alport nephropathy.

**Hematologic Defects**

In 1972, Epstein and coworkers described a family in which hereditary nephritis segregated with deafness and megathrombocytopenia, and reports of several similar families have subsequently appeared (50). In most of the families described, the disorder appears to have been transmitted as an autosomal dominant trait. Only two reports of this syndrome have in-
Figure 5. The characteristic ultrastructural abnormalities of Alport glomerular basement membranes (GBM) are evident in this biopsy specimen from a 5-year-old boy. The GBM shows both thinning and thickening. The lamina densa is split into multiple, interwoven strands that occasionally enclose round, electron-dense bodies. The epithelial aspect of the GBM is very irregular, and there is focal fusion of visceral epithelial cell foot processes. (Photomicrograph courtesy of Dr. Bernard Panner).

cluded results of electron microscopy of kidney biopsy specimens. One patient displayed focal thickening and splitting of the GBM, and GBM thickening and lamellation were present in another.

The association of these platelet defects, and in some families granulocyte abnormalities, with hereditary nephritis remains to be explained. There have been few reports of gene localization or mutation analysis in these families, except for negative reports regarding COLAAS involvement, so the genetic locus or loci involved remains unknown. Basement membranes of these patients show normal expression of type IV collagen α chains (51).

Diagnostic Issues

AS should be included in the initial differential diagnosis of patients with persistent microscopic hematuria, once structural abnormalities of the kidneys or urinary tract have been excluded. Electron microscopic examination of renal tissue remains the most readily available means for confirming a suspected diagnosis of AS. The presence of diffuse thickening and multilamellation of the GBM predicts a progressive nephropathy, regardless of family history. This approach has some limitations, however. Ultrastructural information alone does not establish the mode of transmission in a particular family. Thus, in a patient with a negative family history, electron microscopy cannot distinguish de novo X-linked disease from autosomal recessive disease. In some patients, the biopsy findings may be ambiguous (particularly females and young patients of either gender). Furthermore, families with progressive nephritis and COLAAS mutations in association with thin GBM have been described, indicating that the classic GBM lesion is not present in all AS kindreds.

It is not unusual for the pediatric nephrologist to see a child with hematuria and discover that multiple relatives also have hematuria, although none has ever undergone kidney biopsy. Because confirmation of AS in one member of a kindred with familial hematuria virtually establishes the diagnosis for other affected members, the person biopsied should ideally be the one most likely to exhibit unambiguous renal lesions. The natural history of the renal lesion suggests that older, male subjects are more likely to exhibit diagnostic ultrastructural abnormalities of GBM. In families in which a firm diagnosis of AS has been established, evaluation of individuals with newly recognized hematuria can be limited to ultrasound of the kidneys and urinary tract in most instances. In the absence of tumor or structural anomalies of the urinary tract, a diagnosis other than AS is unlikely.

Monoclonal antibodies directed against the α3, α4, and α5 chains of type IV collagen have recently become available, making it possible to reliably evaluate basement membranes for the presence or absence of these chains (Table 5) (52–54). Absence of these chains from GBM and distal TBM has not been described in any condition other than AS, making this a diagnostic finding on kidney biopsy. Because the α5(IV) chain
There are often clinical situations in which a firm diagnosis of Alport syndrome (AS) is expressed in the EBM, examination of skin biopsies by immunofluorescence for expression of \( \alpha_5(IV) \) is an additional tool for making a diagnosis of AS. In fact, given a male patient with a family history suggesting XLAS and clinical features characteristic of AS, examination of skin for \( \alpha_5(IV) \) expression may obviate the necessity for kidney biopsy. Unfortunately, a normal result does not exclude the diagnosis, because in some Alport kindreds with X-linked disease affected males express \( \alpha_5(IV) \) in their basement membranes. Heterozygous females frequently express \( \alpha_5(IV) \) mosaicly in epidermal and renal basement membranes (35,52,55). Although clearly mosaic expression of \( \alpha_5(IV) \) is diagnostic of the carrier state, a normal result does not exclude heterozygosity. A female member of an Alport kindred who does not have hematuria may still be a carrier, but is less likely to exhibit detectable mosaicism than a female with hematuria. Skin biopsy is probably not a good test for detecting asymptomatic carriers of \( \alpha_5(IV) \) mutations.

Renal expression of type IV collagen \( \alpha \) chains can serve to confirm a diagnosis of AS, and can in addition differentiate the X-linked and autosomal recessive forms of the disease (Table 3, Figures 2 and 4). As noted above, in most males with XLAS, renal basement membranes are devoid of the \( \alpha_3, \alpha_4, \) and \( \alpha_5(IV) \) chains, whereas females frequently show mosaic expression of these chains, most readily observed in GBM (35,52,54). In most males and females with ARAS, the GBM, Bowman’s capsule, and distal TBM show no expression of \( \alpha_3(IV) \) and \( \alpha_4(IV) \), whereas \( \alpha_5(IV) \) is expressed in Bowman’s capsule and distal TBM but not GBM (25). ARAS cannot be diagnosed by skin biopsy, since epidermal basement membrane expression of \( \alpha_5(IV) \) is preserved.

The ability to diagnose AS by skin biopsy may eventually reduce reliance on kidney biopsy for diagnosis of this disease. Skin biopsy is the initial diagnostic maneuver of choice when kidney biopsy is excessively risky, such as in patients with advanced renal insufficiency.

Genetic analysis provides the only means for reliably diagnosing the carrier state in asymptomatic female members of XLAS kindreds and for making a prenatal diagnosis of AS. There are often clinical situations in which a firm diagnosis of AS cannot be established, or in which it is not possible to determine the mode of transmission, despite careful evaluation of the pedigree and application of the full range of histologic methods. In these situations, genetic analysis has the potential to provide information essential for determining prognosis and guiding genetic counseling. Because reproductive decisions may be influenced by genetic counseling information, erroneous determination of the mode of inheritance of AS can have profoundly unfortunate consequences. The inheritance of AS in a family can be determined by linkage analysis, which does not require identification of a particular mutation.

At present, the clinical utility of molecular genetic analysis for diagnosis of AS is limited. The sheer size of the type IV collagen loci (>50 exons), combined with the great variety of mutations (most of which are missense alterations of a single base pair), presents a formidable obstacle, even when automated sequencing is available. Consequently, screening for \( COL4A3, COL4A4, \) and \( COL4A5 \) mutations is time consuming, expensive, and confined to a handful of research laboratories. As noted above, the current rate of identification of \( COL4A5 \) mutations in Alport kindreds is only about 50%. Improved sensitivity will enhance the utility of genetic analysis as a confirmatory test for suspected AS. Reimbursement for genetic analysis, including linkage studies, is an unresolved issue. As with other genetic disorders, presymptomatic identification of a mutation at one of the Alport loci may have implications regarding health and life insurance.

In the future, automation and miniaturization of DNA analytic techniques may result in a relatively simple, rapid, and inexpensive means of assaying a blood or tissue sample for mutations at genetic loci relevant to AS, replacing other diagnostic methods. For now, genetic analysis is appropriate, although not always practical, when there is ambiguity regarding the diagnosis of AS or the mode of transmission, or when prenatal testing is desired.

**Management**

*Why is the Alport Nephropathy Progressive?*

It is now clear that the tissue pathology of AS results from abnormalities of basement membrane expression of the \( \alpha_3, \alpha_4, \alpha_5, \) and possibly \( \alpha_6 \) chains of type IV collagen. These chains...
are usually absent from or underexpressed in the basement membranes of AS patients, so that the networks they form are absent, or, if present, defective in structure and function. The most straightforward demonstration of the consequences arising from the absence of these chains may be anterior lenticonus, in which the anterior lens capsule lacks the strength to maintain the normal conformation of the lens. Microhematuria, the first and invariable renal manifestation of AS, probably reflects GBM thinning and a tendency to develop focal ruptures, due to the absent or defective expression of the α3(IV) through α5(IV) chains. Episodic gross hematuria precipitated by infections, which is not uncommon during the first two decades of life, may reflect increased susceptibility of the Alport GBM to proteolysis (56).

The inexorable development of GBM thickening, proteinuria, and renal insufficiency in males with XLAS, and in both males and females with ARAS, is less readily explained, but some clues have been found. Unlike other glomerulopathies, AS is characterized by the accumulation of the α1(IV) and α2(IV) chains, along with types V and VI collagen, in the GBM (25, 56–58). These proteins appear to spread from their normal subendothelial location in the GBM, so that they come to occupy the full width of the GBM. As AS glomeruli undergo sclerosis, the α1(IV) and α2(IV) chains disappear from the GBM, but type V and type VI collagen persist and, in fact, accumulate. It is possible that the altered expression of the α1(IV) and α2(IV) chains, type V collagen and type VI collagen represents a compensatory response to the loss of the α3(IV), α4(IV), and α5(IV) chains from the GBM, or may simply reflect alterations in glomerular cellular behavior resulting from the absence of the signals normally provided to these cells by this network. In transgenic mice with ARAS due to partial deletion of COL4A3, renal mRNA bevels for the α1(IV) and α2(IV) chains progressively increase, suggesting activation of these genes (31). Whatever the underlying mechanism, the unrestrained deposition of certain collagens in the GBM may contribute to glomerulosclerosis in AS.

Is Effective Intervention Possible?

Clinical trials of therapy for the Alport nephropathy have not been conducted. The availability of canine and murine models of AS (30, 31, 59) should allow the testing of genetic or pharmacologic therapies, in order to select promising treatments for human trials (60). In a small study of dogs with canine hereditary nephropathy, beneficial effects of angiotensin-converting enzyme inhibition on renal function, renal structure, and survival were observed (61). AS resembles other chronic glomerulopathies in that deterioration of glomerular filtration rate is closely correlated with fibrosis of the renal interstitium (36, 37). Measurable increases in cortical interstitial volume are unusual in males with XLAS under age 10, but progressive expansion of the interstitium frequently occurs during the second decade of life (37). The processes driving interstitial fibrosis in AS have yet to be characterized. For example, studies of the expression of transforming growth factor-β or other growth factors in AS kidneys have not been reported. If the pathogenesis of interstitial fibrosis in AS is not specific to the disease, it is conceivable that therapies that interfere with interstitial fibrosis may be of benefit to AS patients, without correcting the primary abnormalities of type IV collagen expression.

Transplantation

At present, renal transplantation is the only available treatment for AS. The data of the North American Pediatric Renal Transplant Cooperative Study document equivalent allograft survival rates in patients with familial nephritis, compared to patients with other diagnoses (62). Anti-GBM nephritis involving the renal allograft is a rare but dramatic manifestation of AS, occurring in 3 to 4% of transplanted male Alport patients. Alport patients who develop posttransplant anti-GBM nephritis are usually male, always deaf, and likely to have reached ESRD before age 30. This profile describes the majority of Alport patients presenting for renal transplantation, limiting its predictive value. However, Alport patients with normal hearing or late progression to ESRD are at very low risk for the development of posttransplant anti-GBM nephritis. Females with XLAS also appear to at low risk for this complication.

The onset of posttransplant anti-GBM nephritis is usually within the first year after transplantation. Three-quarters of the allografts fail irreversibly, usually within a few weeks to months. Plasmapheresis and cyclophosphamide administration have been of limited benefit. Anti-GBM nephritis has recurred in most retransplanted patients, despite prolonged intervals between transplants and absence of detectable circulating anti-GBM antibodies before retransplantation.

The pathogenesis of posttransplant anti-GBM nephritis in AS patients is presumably based on exposure to antigens present in the donor GBM, for which the recipient has not established immune tolerance (7). The target(s) of anti-GBM antibodies in some of these patients has been determined, with variable results. Most of those with XLAS exhibit antibodies against the NC1 domain of the α5(IV) chain, but antibodies against α3(IV) NC1 have also been described (63, 64). In ARAS patients with anti-GBM nephritis, antibodies appear to target the α3(IV) NC1 domain (64).

Females who are heterozygous for COL4A5 mutations would not be expected to be at risk for the development of posttransplant anti-GBM nephritis, because the product of the normal COL4A5 allele would allow establishment of immunologic tolerance for α5(IV). Nevertheless, posttransplant anti-GBM nephritis has been reported in two females with AS, both of whom proved to have ARAS, due to COL4A3 mutations (65, 66).

There is evidence to suggest that mutations in the COL4A5 gene that prevent expression of an immunogenic gene product, thereby preventing the establishment of tolerance for α5(IV), are associated with an increased risk for the development of posttransplant anti-GBM nephritis (67). A recent review of the genetics of AS included seven patients with XLAS and posttransplant anti-GBM nephritis; six had large deletions of the gene, and one had a splicing mutation (16). Even if certain classes of COL4A5 mutation confer a higher risk of developing posttransplant anti-GBM nephritis, such data are currently of
limited value in planning transplantation. It is clear that Alport patients with COL4A5 deletions can undergo renal transplantation without developing anti-GBM nephritis, indicating that other factors, presently unknown, must influence the initiation and elaboration of the immune response to the allograft (16,68). At this time, the only way to determine whether a previously untransplanted Alport patient will develop posttransplant anti-GBM nephritis is to perform the transplant, although as noted above certain patients are at very low risk.

In view of our inability to predict the outcome of a first renal transplant in any particular Alport patient, regardless of whether that patient’s mutation has been characterized, and the typically excellent allograft survival in Alport patients, the use of living donor organs whenever available is recommended. The appropriateness of using a living donor kidney for a patient who has already lost an allograft to posttransplant anti-GBM nephritis should be carefully considered, given the high likelihood of recurrent disease and subsequent graft failure.

Should women who are heterozygous for COL4A5 mutations be allowed to serve as kidney donors? Clearly, those with proteinuria, hypertension, or renal insufficiency will not be allowed to donate, and the same should apply if any hearing loss is present. What about heterozygotes with hematuria but normal renal function and hearing? There is no long-term follow-up information on the impact of uninephrectomy in such women, although there does not seem to be a drastic decline in renal function over the first several years after transplant (69). The wishes of a heterozygous woman with asymptomatic microhematuria should be thoughtfully considered, but it must be assumed at this time that the risk to such an individual of ultimately developing significant renal insufficiency is substantially higher than it is for the usual kidney donor.

**Benign Familial Hematuria (Thin GBM Disease)**

Isolated glomerular hematuria may occur as a familial or sporadic condition, and is often associated with a renal biopsy finding of excessively thin GBM. Neither of the two terms for this condition, benign familial hematuria (BFH) or thin GBM disease, is entirely satisfactory. Familial hematuria is not always benign, and benign hematuria is not always familial. Thin GBM is a descriptive term, and because it is likely that several disorders that differ at the molecular level can be associated with this abnormality, and furthermore that in some instances it may be a normal variant, it is somewhat misleading to characterize thin GBM as a “disease.” These caveats having been stated, the term BFH is appropriate for familial, nonprogressive hematuria.

Like AS, BFH is an inherited disorder of GBM that is characterized clinically by persistent microscopic hematuria and episodic gross hematuria. BFH differs clinically from AS in several important respects: (1) it is only rarely associated with extrarenal abnormalities; (2) proteinuria, hypertension, and progression to ESRD are unusual; (3) gender differences in expression of BFH are not apparent; and (4) transmission is autosomal dominant in nature. BFH and early AS may be difficult to distinguish histologically, because diffuse GBM attenuation is characteristic of both. However, the GBM of BFH patients remains attenuated over time, rather than undergoing the progressive thickening and multilamellation that is pathognomonic of AS.

**Genetics**

BFH is usually transmitted as an autosomal dominant condition. A negative family history may not be reliable, because patients eventually diagnosed as having BFH are frequently unaware that they have relatives with hematuria. Lemmink et al. (70) recently described studies of a large Dutch BFH kindred, in which the disease locus was first mapped to chromosome 2 in the region of the COLA3 and COLA4 genes, and then affected individuals were found to be heterozygous for a missense mutation in COLA4. This landmark work demonstrates that mutations at type IV collagen loci are responsible for at least some cases of BFH. However, linkage to the COLA3 and COLA4 genes has been excluded in other BFH families, indicating that BFH is a genetically heterogeneous condition (71).

To date, immunohistologic studies of type IV collagen in GBM of patients with BFH or thin GBM disease have failed to uncover any abnormalities in the distribution of any of the six chains. Immunohistologic evaluation of GBM type IV collagen may be useful in the differentiation of BFH and AS (see below).

**Clinicopathologic Features and Correlations**

Individuals with BFH typically exhibit persistent microhematuria that is first detected in childhood. In some patients, microhematuria is intermittent, and may not be detected until adulthood. Episodic gross hematuria, often in association with upper respiratory infections, is not unusual. The hematuria of BFH appears to be lifelong.

Overt proteinuria and hypertension are unusual in BFH, but have been described (72,73). Some of these cases may represent variants of AS, in which the predominant abnormality of GBM is attenuation, rather than thickening and multilamellation. Other glomerular disorders such as IgA nephropathy may occur in patients with BFH or thin GBM disease, altering the expected natural history and histopathology of the condition (74).

Light and immunofluorescence microscopy are unremarkable in typical cases of BFH or thin GBM disease. Most patients with BFH exhibit diffuse thinning of the GBM as a whole, and of the lamina densa. Normal GBM thickness is age- and gender-dependent. Both the lamina densa and the GBM increase rapidly in thickness between birth and age 2, followed by gradual thickening throughout childhood, adolescence, and into adulthood (75). GBM thickness of adult men (373 ± 42 nm) exceeds that of adult women (326 ± 45 nm) (76). Thus, it is important to consider the age and gender of the patient when evaluating GBM width. Each electron microscopy laboratory should establish a consistent technique for measuring GBM thickness and determine its own means and standard deviations.
for GBM width, to make comparisons with published data meaningful.

The definition of “thin” GBM has varied in the literature, in part because of the use of different techniques to measure GBM width. When an electron microscopy laboratory’s normal values for GBM width are similar to those of Steffes et al. (76), a cutoff value of 250 nm will accurately separate adults with normal GBM from those with thin GBM. When the normal values are significantly higher, a cutoff value of 330 nm is appropriate (77). For children, the cutoff is in the range of 200 to 250 nm (250 nm is within 2 SD of the mean at age 11). It is useful to note that the intraglomerular variabilty in GBM width is small in thin GBM disease (72). Marked variability in GBM width within a glomerulus, in a patient with persistent microhematuria, should raise suspicion of Alport syndrome, although focal lamina densa splitting has been described in BFH.

Diagnostic Issues

Patients with persistent, isolated microhematuria are usually candidates for kidney biopsy, once structural urinary tract abnormalities, urinary tract stones, and tumors are excluded. If the patient’s family history indicates autosomal dominant transmission of hematuria, and there is no history of chronic renal failure, a presumptive diagnosis of BFH can often be made without kidney biopsy. When family history is negative or unknown, or there are atypical coexisting features such as proteinuria or deafness, renal biopsy may be extremely informative. A finding of thin GBM may be further characterized by examining the distribution of type IV collagen α chains in the kidney. Normal distribution of these chains provides supportive, although not conclusive, evidence for a diagnosis of BFH or thin GBM disease.

Management

Patients who are given a diagnosis of BFH or thin GBM disease should be reassured, but not lost to follow-up examination. The risk of chronic renal insufficiency appears to be small but real (72,73,78). Reasonable follow-up would include urinalysis and measurement of blood pressure and renal function every 1 to 2 years.

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