Brief report

Seeking confidence in the diagnosis of systemic AL (Ig light-chain) amyloidosis: patients can have both monoclonal gammopathies and hereditary amyloid proteins

Raymond L. Comenzo, Ping Zhou, Martin Fleisher, Bradley Clark, and Julie Teruya-Feldstein

Investigators in the United Kingdom have shown that hereditary amyloidosis can be misdiagnosed as Ig light-chain (AL) amyloidosis because family history is an ineffective screen, and tissue staining used to type amyloid is unreliable. Misdiagnosis of AL can lead to inappropriate use of chemotherapy and failure to diagnose a hereditary disease. Over a 3-year period we sought to determine how often both possible sources of amyloidosis occurred in the same patient. We employed an algorithm based on established data and patterns of amyloidosis in order to focus the screening effort. Of 178 consecutive patients referred for amyloidosis, 54 were screened by polymerase chain reaction techniques with primers designed to detect transthyretin, apolipoprotein AI, apolipoprotein AII, fibrinogen Aα, and lysozyme variants. Three patients (6% of those screened and 2% of symptomatic patients) had both a monoclonal gammopathy and a hereditary variant. These results justify further study of screening for hereditary variants in patients with apparent AL, and highlight the need for practical techniques for identifying fibrils extracted from tissue. (Blood. 2006;107: 3489-3491)

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Introduction

In the report of the first autologous stem cell transplantation (SCT) trial for systemic Ig light-chain (AL) amyloidosis, the authors wrote that patients with hereditary amyloidosis “are never candidates for dose-intensive melphalan.” In hereditary amyloid, the mutant protein is often hepatic in origin, and the standard treatment is liver transplantation, not high-dose chemotherapy. In AL, the precursor protein is an immunoglobulin light chain produced by clonal plasma cells, and standard treatment is cytoreductive chemotherapy.

The issue of misdiagnosis of AL has been raised by several investigators. The national amyloidosis center in the United Kingdom reported that, of 350 patients thought to have AL, 10% had hereditary variants instead, including patients who had understandably failed SCT. The investigators noted that hereditary variants have variable penetrance, making family history an ineffective screen, and that the immunohistochemical (IHC) staining techniques used to type tissue amyloid as derived from Ig light chains were unreliable, although IHC staining for non-Ig amyloid-forming proteins such as transthyretin (TTR) or fibrinogen Aα may be useful. The lack of reliable staining for all types of amyloid limits the utility of IHC approaches.

Most hereditary variants are due to point mutations causing amino acid replacements. In the series of 350 patients from the United Kingdom, the identification of hereditary variants was based on polymerase chain reaction (PCR) amplification and sequencing of potentially mutated genes. Obviously the issue of diagnostic confidence would be moot if the amyloid protein in each case could be extracted easily from tissue and evaluated for identity. Currently, such techniques are not practical because, given the variability of deposits, obtaining enough tissue for protein extraction or for immunogold electronmicroscopy is problematic. In this study, we asked how often patients with symptomatic amyloidosis might have both a monoclonal gammopathy and a hereditary variant, employing a screening algorithm based on patterns of presentation of hereditary and AL amyloidosis. Our results support the need for routine DNA-based screening and for new methods for typing amyloid.

Study design

Patients and screening

Between June 1, 2002, and August 1, 2005, patients referred for assessment of systemic amyloidosis were evaluated for monoclonal gammopathy and organ involvement with amyloid as previously described. Approval was obtained from the institutional review board of the Memorial Sloan-Kettering Cancer Center for these studies and patients gave written informed consent. We screened all patients in the following categories for hereditary variants whether or not they had a monoclonal gammopathy: (1) African Americans were screened for the presence of a mutant transthyretin (the Val122Ile variant of TTR occurs in 4% of African Americans); (2) patients with dominant peripheral nervous system involvement were screened for the variants shown in Table 1 (peripheral neuropathy is a common presentation of AL amyloidosis and several hereditary variants)
Table 1. Genes, primers, PCR conditions, and amplicon sizes

<table>
<thead>
<tr>
<th>Gene (exon)</th>
<th>Forward</th>
<th>Reverse</th>
<th>Anneling temperature, °C (no. cycles)</th>
<th>Amplicon size, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transthyretin* (2)</td>
<td>gtaacctttgctgctccgga</td>
<td>gataaaaaaccgcctttgqgg</td>
<td>58(35)</td>
<td>271</td>
</tr>
<tr>
<td>Transthyretin (3)</td>
<td>ggtgattattcttgctcagc</td>
<td>ctctggagctgtgtaactcac</td>
<td>58(35)</td>
<td>226</td>
</tr>
<tr>
<td>Transthyretin (4)</td>
<td>tcatcttgtctacatgtcggc</td>
<td>gtttaaagttgtaaaagttgc</td>
<td>58(35)</td>
<td>340</td>
</tr>
<tr>
<td>Apolipoprotein AI (2)</td>
<td>cccccctaggggcagccgggg</td>
<td>ttagttcagactggccgtctggg</td>
<td>65(35)</td>
<td>255</td>
</tr>
<tr>
<td>Apolipoprotein AI (3)</td>
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<td>cagatgtgctggcagccggtcacc</td>
<td>65(35)</td>
<td>392</td>
</tr>
<tr>
<td>Apolipoprotein AI (4)</td>
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<td>aacctgctagagagctagccaatc</td>
<td>65(35)</td>
<td>370</td>
</tr>
<tr>
<td>Fibrinogen A‡ (H9251)</td>
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<td>gaccccccttcaccccgtgtgcat</td>
<td>60(35)</td>
<td>217</td>
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<tr>
<td>Apolipoprotein AI (4)</td>
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<td>45(35)</td>
<td>161</td>
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<tr>
<td>Lysosome† (2)</td>
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<td>gataaaaaaccgcctttgqgg</td>
<td>54(35)</td>
<td>242</td>
</tr>
</tbody>
</table>

*The Online Mendelian Inheritance in Man identifier (OMIM ID) for transthyretin (TTR) is + 176300. There are more than 100 amyloidogenic TTR variants. Common ones include Val30Met, Thr60Ala, Ile84Thr, and Val122Ile (see Connors et al).† The OMIM ID for apolipoprotein AI is *107680. Most variants are nonneuropathic and cause cardiac and cutaneous amyloidosis. Common ones include Gly26Arg, Leu60Arg, Trp50Arg, Leu60Pro, Arg173Pro, Leu174Ser, Ala175pro, and a deletion variant with a 2-bp insertion at position 60.‡ The OMIM ID for fibrinogen A is + 134820. There are at least 3 amyloidogenic variants: Arg554Leu, Glu526Val, and a frameshift mutation that causes termination at codon 548. § The OMIM ID for apolipoprotein AI is + 176760. The amyloidogenic variants involve stop codon mutations at position 78 to glycine, serine, or arginine, with abnormal extension of the protein; these are usually nonneuropathic.

PCR assays

Genomic DNA was extracted from mononuclear cells as previously described. Primer pairs were designed for transthyretin, apolipoprotein AI, apolipoprotein AI, fibrinogen A, and lysozyme (Table 1).1,3,18-21 PCR amplicons were sequenced at our core facility and results scanned with Chromas Version 1.45 (Griffith University, Queensland, Australia) and evaluated by BLAST (Genbank).

Immunohistochemical staining for TTR

Tissue sections stained for the identification of amyloidosis by Congo red dichroism were also stained for TTR in patients with both a monoclonal gammopathy and hereditary TTR variants and in patients suspected of gammopathy and hereditary TTR variants and in patients suspected of.

Results and discussion

One hundred and seventy-eight consecutive patients were evaluated for amyloidosis and the diagnosis was confirmed in 96%, most of whom were symptomatic (Table 2). In an attempt to determine how often both a monoclonal gammapathy and a hereditary variant...
occurred in the same patient, patients in 4 categories were screened and 1 patient with both proteins was identified in 3 of the 4 categories. They represented 6% of those screened and 2% of symptomatic patients. One was African-American, one had peripheral neuropathy, and the third was referred for evaluation of hereditary amyloidosis and was diagnosed with Durie-Salmon stage I multiple myeloma. All had bona fide monoclonal gammopathies, not faint bands on urine immunofixation, and all had a variant TTR (Table 2). TTR staining indicated that in 2 cases amyloid was likely due to variant TTR and in one it was not (Table 2).

The amyloidoses are diseases caused by protein misfolding. The most common type encountered by hematologists is due to monoclonal immunoglobulin light chains (AL) and can be difficult to diagnose and treat in a timely fashion. The median survival even with the most aggressive therapy is less than 5 years. Diagnostic confidence is critical in order to plan therapy, and hereditary and senile cardiac variants are not treated with cytotoxic therapies or SCT.

Our results speak to the need for reliable tools to aid in the diagnosis and management of patients with amyloidosis. Combined with the recent report from the United Kingdom, the data indicate that the issue of diagnostic confidence is important because 1 patient can have both a monoclonal gammapathy and a hereditary variant, representing 2 possible sources of amyloid-forming proteins. It should also be recalled that the incidence of monoclonal gammapathy of undetermined significance (MGUS) increases with age and that hereditary variants in the United States usually present in older patients. Both MGUS and Val122Ile mutant TTR are also more common in African Americans.

Given the implications for patients and their families if AL is misdiagnosed and a hereditary mutation not identified, we agree with our colleagues in the United Kingdom that all new cases of amyloidosis should be screened for both AL and hereditary variants. The PCR technique we used is reliable and easily implemented in human genetics laboratories. In addition, innovative and improved techniques are needed for typing amyloid from tissue biopsies. Finally, the issue of diagnostic confidence becomes even more critical as new therapies are evaluated for both AL and hereditary disease.

**Acknowledgments**

We thank the many amyloid patients who contributed to this work. We also thank Dr Stephen D. Nimer for continued help and encouragement, the MSKCC Hematology-Oncology fellows for caring for our patients, Joanne Santorsa, RN, for assistance in bone marrow studies, and Dr Kenneth Offit and colleagues in the Department of Human Genetics at MSKCC for their encouragement.

**References**

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