Mutational analysis of APOL1 in patients with Fechtner and Epstein syndromes: no evidence of a digenic etiology in MYH9-related disorders with renal disease

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Abstract: Mutations in MYH9 result in a group of clinically overlapping autosomal dominant macrothrombocytopenia syndromes, collectively termed the MYH9-related disorders (MYH9RD). Intriguingly, three of these disorders, Fechtner, Alport-like, and Epstein syndromes, are associated with the additional clinical phenotype of glomerulosclerosis. Recently, an abundance of studies have demonstrated that the APOL1 gene, contiguous to MYH9, is associated with a form of kidney disease in individuals of African ancestry. Given these findings related to kidney disease arising in mutations in two contiguous genes, this study aimed to determine whether APOL1 mutations could also be present in patients with Fechtner (FTNS) and Epstein (EPS) syndromes. This study used sequence analysis to investigate a discrete discovery set of FTNS patients, but did not identify second hit mutations in APOL1.

Keywords: APOL1, MYH9, Fechtner syndrome, Epstein syndrome

Introduction

Mutations in nonmuscle myosin heavy chain IIa, MYH9-cytoskeletal contractile protein, result in a spectrum of macrothrombocytopenia disorders collectively named “the giant-platelet” disorders, or the MYH9-related disorders (MYH9RD). These include May-Hegglin anomaly (MHA, MIM #155100), Sebastian platelet syndrome (SBS, MIM #605249), Alport-like syndrome (APSM), Epstein syndrome (EPS, MIM #153650), and Fechtner syndrome (FTNS, MIM #153640).¹² These disorders are generally considered to be rare in the population; however, as they can often be misdiagnosed as immune thrombocytopenia (ITP), the prevalence of MYH9RD may be underestimated.¹ While all patients share the clinical features of macrothrombocytopenia and characteristic leukocyte inclusions (Döhle-like bodies), some will also display high-tone sensorineural deafness and/or the development of pre-senile cataracts.¹–⁶ Of particular interest, a number of individuals with MYH9RD also develop kidney disease, which can range from glomerulosclerosis to end-stage renal disease.⁷–¹⁰ It should also be noted that MYH9 has been identified to play a role in nonsyndromic hereditary deafness.¹¹ In accordance with its role in hearing/deafness, MYH9 expression was identified in rat cochlea.¹² The N-terminus of the protein contains the motor domain, which binds to actin and...
ATP, while the C-terminus contains the regulatory region.\textsuperscript{14} Genotype-phenotype correlations with specific MYH9 mutations have been investigated by others.\textsuperscript{15,16} Affected individuals with a mutation within the motor domain, compared to the tail domain, of the MYH9 are at higher risk, but not certain risk, of having a more severe phenotype.\textsuperscript{15} For example, a small percentage of patients with the tail domain 1424 mutation have a more severe phenotype.\textsuperscript{15} Sekine et al demonstrated that nine patients with R702 mutations developed renal disease,\textsuperscript{16} whereas Pecci et al determined that 32% of patients with R702 mutations developed nephritis.\textsuperscript{15} Thus, while associations can be made, no universally acceptable genotype-phenotype correlation completely predicts the clinical spectrum of each MYH9 mutation.

Most recently, MYH9 was proposed as a potential risk factor in nondiabetic end-stage kidney disease (ESKD) or renal disease (ESRD).\textsuperscript{7} Mapping by admixture linkage disequilibrium (MALD) indicated that MYH9 maps to a chromosomal region that is associated with ESKD risk variants.\textsuperscript{7,8} African Americans have a 3.7 times increased risk of ESRD, significantly higher than individuals of European descent (US Renal Data System 2007). Various intrinsic single nucleotide polymorphisms (SNPs) have presented as kidney risk variants, but even after resequencing of the MYH9 gene, no functional mutations were discovered.\textsuperscript{17,18} A genome wide association study (GWAS) identified a region that contained both MYH9 and APOL1 and included a risk allele for focal segmental glomerulosclerosis (FSGS) in the African American population but not the European population.\textsuperscript{19} Given these ethnic differences, the authors suggested that APOL1 represented a possible disease-gene candidate because of its relationship in defining disease resistance to a lethal parasite, the trypanosome, which is common in Africa.\textsuperscript{19}

APOL1 is a member of a gene family consisting of six APOL genes, L1-L6,\textsuperscript{20,21} which are involved in cellular apoptosis,\textsuperscript{22} trypanosomal lysis,\textsuperscript{23} and lipid metabolism.\textsuperscript{24,25} APOL1 has the ability to remain sequestered as an intracellular protein or to be secreted.\textsuperscript{25} Freedman and colleagues studied novel SNP frequencies in two populations of African Americans: one containing 205 patients with FSGS, and another containing 180 control patients.\textsuperscript{26} They presented evidence that mutations in the last exon of APOL1 – rs73885319 (S342G) and rs60910145 (I384M) – were causative.\textsuperscript{26}

Based on the studies of APOL1 in the involvement in EKSD, we investigated its role in Fechtner and Epstein syndromes. The initial linkage block that was identified included the genes APOL1 and MYH9,\textsuperscript{8} which raised the possibility that FTNS and EPS are both digenic diseases. In fact, some forms of renal disease have been suggested result from digenic mutations.\textsuperscript{27,28} Hypothetically, a second hit, a mutation in APOL1, could be the causative difference among the macrothrombocytopenia disorders that have renal disease. Given the renal phenotype of MYH9RD and the renal disease now linked to the adjacent gene APOL1, we were curious to examine the possibility that mutations in both genes are necessary to give rise to the full spectrum of MYH9RD. We therefore performed mutational analysis of four DNA samples from individuals with Fechtner or Epstein syndromes.

**Methods**

**Patients and patient selection**

Genomic DNA from four MYH9RD individuals shown previously to harbor MYH9 mutations was analyzed for possible concurrent mutations in APOL1 (Table 1). Information regarding ethnicity and clinical history was obtained. Patient 1 was a pediatric patient of African descent who presented with a diagnosis of Fechtner syndrome and died as a result of end stage renal failure at the age of sixteen. Patient 2 was of French heritage and had been clinically diagnosed with Epstein syndrome. The patient’s mother had originally presented with symptoms of deafness and kidney failure requiring dialysis. Patient 3 was of Italian descent and diagnosed with Fechtner syndrome. Patient 4 was of French descent and presented with Fechtner syndrome. All samples collected were approved by the IRB.

**DNA sequence, mutation analysis**

Primers encompassing the coding regions and flanking intron/exon boundaries of APOL1 were designed to amplify the APOL1 transcript variant 2, NM_145343, as well as NM_003661.3, NM_001136540.1, and NM_001136541.1 (Table 2). The MYH9 primers have been previously described.\textsuperscript{2} For exons 1–6 of APOL1 and 7B-7E, reactions were performed in 20 μL of a final concentration of 1X PCR Buffer (GoTaq, Promega Corporation, Madison, Wisconsin, USA) containing 0.25 μM of each primer, and 40 ng of genomic DNA. For exon 7A, reactions were performed in 25 μL of a final concentration of 2.5 mM MgCl\textsubscript{2}, 1X PCR

<table>
<thead>
<tr>
<th>Table 1 MYH9 mutations</th>
<th>MYH9 protein alterations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>R702C</td>
</tr>
<tr>
<td>Patient 2</td>
<td>S96L</td>
</tr>
<tr>
<td>Patient 3</td>
<td>D1424H</td>
</tr>
<tr>
<td>Patient 4</td>
<td>R702G</td>
</tr>
</tbody>
</table>
Buffer, 0.2 mM dNTPs, 0.064 units of Amplitaq Gold (Applied Biosystems, Carlsbad, California, USA), and 40 ng of genomic DNA.

PCR reactions were carried out using the following conditions: 95°C for 10 minutes, followed by 35 cycles of 95°C for 30 seconds, 30 seconds for annealing at temperatures indicated in Table 2, and elongation at 72°C for 1 minute. This was then followed by a final elongation step at 72°C for 10 minutes. Amplified products were purified using Exosap (Affymetrix, Santa Clara, California, USA) and then Sanger sequenced in both orientations (Applied Biosystems 3730, MSSM Core Facility).

Sequencing data was analyzed using the program Sequencher 4.9 (Gene Codes Corp, Ann Arbor, Michigan, USA). Control DNA, obtained from an individual without evidence of disease, was also amplified and sequenced. Patient DNA was compared to a reference sequence NG_023228 and the cDNA sequences: NM_145343.2, NM_003661.3, NM_001136540.1, and NM_001136541.1. Sequencing variants were compared to dbSNP135 information tracks (UCSC Genome Browser Hg19 February 2009; http://www.genome.ucsc.edu/).

Results

For each MYH9RD patient, the APOL1 gene was sequenced to include all coding and non-coding exons and their intron and exon boundaries. No novel coding mutations were identified in any of the four patient-derived samples. As shown in Figure 1 and Table 3, a number of previously known SNPs were present. These included 10 intronic SNPs and five within coding exons. The five exonic SNPs predicted three non-synonymous changes (E166K, M244I, and R271K) and were all located in exon 7. Patients 1 and 4 possessed all five of these SNPs. Patient 4 was homozygous for all five, whereas

Table 2 PCR primer sequences for the amplification of APOL1 from genomic DNA

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Annealing temperature (°C)</th>
<th>Product size</th>
</tr>
</thead>
<tbody>
<tr>
<td>APOL1 EX1</td>
<td>GCTCCTCTAAGGGCAGCA</td>
<td>TGGCGCTACTTTCTGTC</td>
<td>55</td>
<td>350</td>
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<tr>
<td>APOL1 EX2</td>
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<td>GGGAGTGTAATTCGAGAG</td>
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<td>542</td>
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<tr>
<td>APOL1 EX3</td>
<td>AGACAGGTTGCTAGGGGTC</td>
<td>AATTCCGGGAAAGGCTTTTG</td>
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<td>497</td>
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<tr>
<td>APOL1 EX4/5</td>
<td>CTCTTGTCATTCAGGACAAC</td>
<td>GTTCTCTGGGGGCTCACTCA</td>
<td>55</td>
<td>531</td>
</tr>
<tr>
<td>APOL1 EX6</td>
<td>ACATCAAGCTGTCCAGGAA</td>
<td>GCCAGAAAGGCTACCTCACA</td>
<td>58</td>
<td>366</td>
</tr>
<tr>
<td>APOL1 EX7A</td>
<td>GTCTCAACTCTCTGACCCCTT</td>
<td>TCACCAAAACTCCCTCAC</td>
<td>62</td>
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<tr>
<td>APOL1 EX7B</td>
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<td>AAAAGTTTTCATTGCTCTTG</td>
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<tr>
<td>APOL1 EX7C</td>
<td>AGCCGACAGGAGGAATAGTC</td>
<td>TCTTCCCATTTCCCACTA</td>
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<tr>
<td>APOL1 EX7D</td>
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<td>TCTTCCGGAGACATTAACC</td>
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<td>535</td>
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<tr>
<td>APOL1 EX7E</td>
<td>AAGAAGACAGCGGCTCCACTA</td>
<td>CCATCACCACATTACGAGA</td>
<td>55</td>
<td>580</td>
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</tbody>
</table>

Figure 1 APOL1 single nucleotide polymorphisms SNPs.
Notes: This is the APOL1 transcript variant 2, NM_145343.2. Boxes represent exons, connecting lines represent introns. Translation start site is depicted by “ATG” and translational stop site is depicted by “Stop”. The SNP designations shown in red are exonic; those in black are intronic.
Patient 1 was homozygous for three of them (rs136174, rs136175, and rs136176). Patient 3 was heterozygous for the fourth SNP, rs136176. All patients, except for Patient 4 who was homozygous for the SNP, were heterozygous for the fifth exonic SNP, rs136177.

**Discussion**

MYH9RD represents a group of five autosomal dominant macrothrombocytopenias, which historically have been distinguished based on the constellation of associated clinical features beyond their commonly shared platelet defects. Gene discovery efforts, which first linked May-Hegglin Anomaly and Fechtner syndrome to the same region of chromosome 22q11-13, originally suggested a shared genetic etiology between these two disorders. Gene discovery then definitively demonstrated that all five clinical entities were, in fact, one allelic disorder. However, despite the seemingly clear understanding of the gene mutation(s) that causes MYH9RD, the correlation between genotype and phenotype remains unclear. Without this information, providing families and individuals with guidance regarding the natural history of their specific disease and/or designing future therapeutic strategies becomes difficult, if not impossible.

It should be noted that while we uncovered no evidence to support a second mutation influencing the origin of renal disease in this syndrome, a limitation of this study was the small number of patients analyzed and the fact that only one of these, Patient 1, was of African descent. Previous studies, which examined APOL1 mutations with regard to kidney disease, have been focused on individuals of African descent.
African descent. 26,32,33 Interestingly, Patient 1 in our study had the greatest frequency of SNPs.

Viewing this issue of a “simple” Mendelian trait as a possibly (more) complex disorder, one could also consider the possibility of digenic versus monogenic inheritance. This raises the question of whether the mutation of a second gene, especially an adjacent gene now also linked to a renal phenotype, results in or modifies the renal disease of MYH9RD. Examples of genetic disorders with a suggested digenic inheritance pattern are becoming more frequent and this increase may be a reflection of evolving sequencing technologies capable of sequencing entire genomes and exomes (reviewed by Vockley). 34

Conclusion

Based on our original hypothesis, we sequenced the APOL1 gene in four unrelated individuals with MYH9RD who all had evidence of kidney disease. The MYH9/APOL1 locus has generated a considerable interest given its association with both a rare monogenetic trait and a common kidney disease (reviewed by Rosset et al 35). While our analysis does not provide any evidence to support the requirement for digenic inheritance in MYH9RD renal disorders, the results are consistent with the concept that even “simple” Mendelian disorders can represent complex traits and – as was noted more than a decade ago – for many of these traits, “only a subset of all mutations reliably predicts phenotypes.”

Disclosure

The authors report no conflicts of interest in this work.

References