APOL1 renal risk alleles in patients on chronic hemodialysis in Northwest of Iran

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ABSTRACT

Introduction: Apolipoprotein L1 (APOL1) gene’s risk variants located on chromosome 22 are newly discovered factors for the development of chronic renal failure among African-American. These risk alleles were developed on the African continent as an evolutionary defense against sleep sickness due to Trypanosoma brucei rhodesiense and then spread with human migrations.

Objectives: In the present study, we sought to examine these risk variants in a group of hemodialysis patients of Northwest of Iran.

Patients and Methods: Two hundred patients receiving hemodialysis in different centers of the city (Tabriz in Northwest of Iran) were allocated randomly from a total number of 825 patients. The assessment of APOL1 polymorphisms (rs73885319, rs60910145, and rs71785313) was conducted using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method. Patients’ demographic data, history, and their biochemical parameters were recorded based on their last measurement.

Results: No proposed renal risk variants of APOL1 gene in our hemodialysis population were found. All the participants had a wild genotype.

Conclusion: The results of our study match with reports from Europe and Asia. In the paleoanthropological point of view, our results do not support African human migration hypothesis.

Implication for health policy/practice/research/medical education:
The appearance of renal risk variants of APOL1 gene (G1, and G2) protect the host against African sleep sleekness; however, predispose carriers to kidney disease. In the present study, we did not find any of these variants in a group of hemodialysis population.


Introduction
APOL1, a key apolipoprotein component of high-density lipoprotein (HDL) molecule, is unique to humans and some primates. It has tryppanolytic activity and protects them against African trypanosomes. This is one of the solid defense mechanisms against Trypanosoma which causes sleeping sickness (1-4). However, one of the Trypanosoma species (T. brucei rhodesiense) overcomes the lytic effects of APOL1 by expressing a serum resistance-associated (SRA) protein (1). It binds to and inactivates APOL1 in the endosomal and lysosomal compartments, allowing the trypanosomes to proliferate (5,6). This evolutionary burden leads to the emergence of two common human-derived APOL1 variants (G1 and G2) on chromosome 22. These polymorphisms did change the APOL1-SRA binding domain (7) and did restore its trypanosomes lytic activity. APOL1 genetic variants that have lytic activity against T. brucei rhodesiense predispose the host to various type of kidney diseases including focal segmental glomerulosclerosis (FSGS), HIV-associated nephropathy (HIVAN), lupus nephritis, hypertensive nephropathy (HTN), and accelerated progression of diabetic kidney disease (6-8), particularly when allelic frequencies did raise in the population and permitted homozygosity (5).
End-stage renal diseases (ESRD) is an important public health burden, affecting over half a million people in the United States. Approximately 14% of Americans with African ancestry carry 2 APOL1 risk alleles that predispose them to develop ESRD at rates 4 to 5 times higher than European-Americans, accounting for the high chronic kidney disease (CKD) burden in this population (6). The appearance of APOL1 renal risk alleles (APOL1-RRV) has had evolutionary origins, selection for protection against T. brucei endemic to sub-Saharan Africa that causes sleeping sickness. However, they are associated with 5–29 times higher odds of severe kidney disease, such as nondiabetic with an impressive odd ration of 16.9 for idiopathic FSGS (6,9,10).

**Objectives**

It is needed to explore the differences in APOL1 variability among different races to provide more evidence on future genetic studies on APOL1-related kidney disease. APOL1-RRV has impact on innate immunity, vulnerability to CKD and its severity, and the risk of progress to ESRD. The Northwest of Iran was speculated as a gateway for prehistoric human migration toward Russia and Europe. The aim of this study is to assess the prevalence of APOL1-RRV in Azarian hemodialysis patients in Northwest of Iran.

**Patients and Methods**

**Sample collection**

A total of 200 patients with End Stage Renal Disease (ESRD) on maintenance hemodialysis were recruited from dialysis Units of Imam Reza, Sina, Army and 29 Bahman Hospital at Tabriz from May 2017 to April 2018. The sample size was calculated based on the 12% prevalence of chronic kidney failure in population (error rate= 0.05, Power= 80%). The total population of East Azerbaijan in Northwest of Iran is approximately estimated around 3.9 million. Close to half of this population lives in Tabriz, which is a metropolitan large city. The total number of patients on chronic hemodialysis treatment in East Azerbaijan is 1,380, of which 820 of them are receiving chronic hemodialysis in city of Tabriz. Patients with ESRD on chronic hemodialysis who were 18 years of age or older were included in the present study. We excluded subjects with diabetic nephropathy, obstructive nephropathy, and autosomal dominant polycystic kidney disease (ADPKD). Other clinical data including age, gender, weight, height, dialysis vintage, kidney biopsy, if any, the cause of kidney failure, family history of kidney disease and hypertension were collected by chart review or face to face interview.

**APOL1 genotyping**

Genomic DNA was extracted from whole blood samples (5 mL) according to the Samadi Shams et al protocol (11). The APOL1 genotype at the G1 and G2 loci was determined using PCR–restriction fragment length polymorphism (RFLP) method. G1 comprises rs73885319 (Ser342Gly) and rs60910145 (Ile384Met) missense mutations. The G2 variant is characterized by 2 amino acids (6 base pair) deletion (N388/Y389) (12, 13). APOL1 high-risk genotypes defined as having 2 risk alleles (G1/G1, G1/G2, or G2/G2) and the low-risk genotypes as having 1 or no risk alleles (G1/G0, G2/G0, G0/G0). PCR reaction was performed to amplify a fragment (458bp) containing 3 variant sites using APOL1 forward (5’-AGACGAGCCGAGCCAATCTTC-3’) and reverse (5’- CACCATTGCACCTCAACCTGGC −3’) primers (12). PCR reactions (25 µL) was done in an initial denaturation step (at 94 °C for 2 minutes) that was followed by 30 steps of denaturation temperatures at 94 °C for 1min, annealing at 66°C for 1 minute, and extension step at 72°C for 1min, and a final extension step at 72°C for 2 minutes. PCR products were run on 1/5% Agarose gel to determine product size. After approval of product size, an independent RFLP test was accomplished for each of the SNPs (Table 1). For each RFLP reaction, PCR product (2 µL) was digested with the enzyme (10 units) and the fragments were separated on a 2% agarose gel.

**Ethics issues**

The study was conducted in accordance with Tenets of the Declaration of Helsinki. The present investigation was approved by the committee of clinical research ethics of Tabriz University of Medical Sciences (Ethical code: IRTBZMED.REC.1396.121). The protocol of the study was clarified to all participants and written informed consent was achieved from the patients. This study was extracted from a thesis of residency in internal medicine of Tabriz University of Medical Sciences, Tabriz, Iran (# 57845).

**Statistical analysis**

Data were given as mean ± SD for normally distributed variables. Median (with maximum and minimum values) was used for non-parametric analysis. Statistical analysis was performed using SPSS statistical software, version 16.0 (SPSS, Chicago, IL). P < 0.05 was considered significant.

**Results**

A total of 200 non-diabetic patients (142 men and 58 women) on hemodialysis were included in this study. The patients were on hemodialysis for a median of 36 months (minimum: 1, maximum: 264) months. Their mean age

<table>
<thead>
<tr>
<th>APOL1 genotype</th>
<th>SNPs</th>
<th>Restriction enzymes</th>
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<tbody>
<tr>
<td>G1</td>
<td>rs73885319 (G1&lt;sup&gt;342G&lt;/sup&gt;, A&gt;G Substitution)</td>
<td>HindIII</td>
</tr>
<tr>
<td></td>
<td>rs60910145 (G1&lt;sup&gt;384M&lt;/sup&gt;, T&gt;G Substitution)</td>
<td>Nsil</td>
</tr>
<tr>
<td>G2</td>
<td>rs71785313, 6 bp deletion</td>
<td>MluCl</td>
</tr>
</tbody>
</table>

**Table 1. Restriction enzymes used in the present study**

was 56 ± 16 years. The causes of ESRD were hypertension in 164 (82%), glomerulonephritis in 16 (8%), and unknown in 20 (10%) patients. The mean ± SD of cholesterol, triglycerides, and HDL-C levels were 149±40, 157±35, and 37.5±12 (mg/dL), respectively. The genetic study identified that all of the studied hemodialysis participants were G0 homozygous, neither of them was identified as containing G1 or G2 risk alleles (Figure 1).

Discussion
Advanced chronic renal failure is a prominent phenotypic presentation/association of APOL1-RRV. We assess the prevalence of this gene in our region. In our study, no APOL1 risk alleles in our sampling group of ESRD patients in Northwestern part of Iran were found. To have higher yield, those patients with diagnosis of diabetic nephropathy, ADPKD, and obstructive uropathy had excluded. Our findings were compatible with the data from 1000 Genomes projects and other studies indicated that G1 and G2 were not present in any chromosomes of Japanese, Chinese, Indians (14,15), and Europeans (6, 16) (Figure 2).

APOL1 gene is unique to humans and some primates and protects them against African trypanosomiasis. ApoL1 protein lyzes trypanosomes mediated by osmotic swelling of parasite lysosomes through a pore-forming mechanism. However, T. brucei rhodesiense produced virulent factor (SRA) that neutralizes APOL1 by binding to its C-terminus. Both APOL1 risk variants alter the SRA binding amino acid within the C-terminal of APOL1 thus preserving lytic activity (6,17).

The exact mechanism by which APOL1 variants cause kidney disease remains unknown. Vascular endothelial dysfunction and impaired renal microcirculation have been proposed as potential mechanisms (18). APOL1 is localized in podocytes, proximal tubular epithelial cells, and small-artery endothelium (19-21). APOL1 – RRV that produced by these cells may induce cytotoxic damage (1,22). In humans, APOL1 circulates in the blood (5-μg/mL). Its level is not increased with RRV (19,21), but became less bound to HDL and consequently, easily filtrates. A direct toxic effect of filtrated APOL1-RRV on proximal tubular cells and apoptosis of podocytes (22) are another pathogenic possibility (5).

Increased chloride permeability with resultant lysosomal swelling and lysosomal rupture could be another underlying mechanism of the podocyte injury (1, 23). In Drosophila model, expression of APOL1-G1 in nephrocytes increased endocytosis of albumin. The same mechanism may create podocytes damage in human (24). APOL1 also transports cations (K+), (2+) across lipid bilayers and APOL1-RRV by decreasing intracellular K+ through the aberrant activity of the stress-activated protein kinases, p38 MAPK, and JNK signaling triggers renal cells damage (25). Moreover, APOL1-RRV reduces mitochondrial catalase and superoxide dismutase 2 (SOD2) and predisposes the cell to oxidative damage by reactive oxygen species (ROS) and causes mitochondrial dysfunction (26).

Nicotinate phosphoribosyl transferase gene, responsible
for NAD biosynthesis, is also down-regulated by APOL1-RRV (26). The robust associations of APOL1 with HIVAN suggests the inflammation as a trigger that potentiates glomerular injury (27,28). Common inflammatory milieu in HIV acts as a second hit that induces the expression of the APOL1 in macrophages, endothelial, and epithelial cells (28), but interestingly IFN-α does not have any role in this interaction (4).

Approximately 40% of individuals with two APOL1-RRV never develop overt kidney disease (29,30). Individual differences in downstream apoptosis and autophagy signaling (29) and genetically differences in ubiquitin-like protein modifier that destined proteasomal protein degradation are proposed possibilities (31).

Trace of this APOL1-RRV could be helpful in paleoanthropology study and for some theories such as “Out-of-Africa” hypothesis. In paleo-anthropological point of view, our finding probably is not in favor of “Out-of-Africa” or replacement hypotheses that believes “modern humans” evolved only in sub-Saharan Africa, then they spread to Asia and Europe (32,33). This hypothesis has recently come under skepticism by another discipline such as archeological and paleoanthropological evidence. Study of the Early Upper Paleolithic (EUP) tool traditions of Eurasia do not support the arrival of human from sub-Saharan Africa to that region and the findings are more in favor of a continuum of a local succession of tool making traditions (33). It is also suggested that paleoarts objects of EUP era, in Eurasia, attribute to “Neanderthals” (34,35).

Conclusion
APOL1-RRV emerged in sub-Saharan Africa as a protection against T. brucei rhodesiense but increased the risk of chronic kidney disease. A negative result of our study was similar to other reports from Europe and Asia. In the anthropological point of view, our results do not support African human migration hypothesis.

Study limitations
Relatively small sample size was a limitation of the present study.

Authors’ contribution
MRA designed the study and selected the cases. ER did sampling and recorded the patients’ information. MH ad VE performed experimental analysis. SZV, RT and MRA performed the interpretation of the data and prepared the manuscript. All authors read and signed the final paper.

Conflicts of interest
The authors declare no conflict of interest.

Ethical considerations
Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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