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The effect of APOL1 risk variants on emergent outcomes in kidney disorders: A meta-analysis of individual participant data

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Abstract. *Kidney diseases associated with APOL1 polymorphisms are human immunodeficiency virus-associated nephropathy, idiopathic focal segmental glomerulosclerosis, hypertension-attributed chronic kidney disease, lupus nephritis and sickle cell nephropathy. This research aimed to investigate the risk of genetic variants on disease contribution.*

Methods. In this individual participant data meta-analysis, eighteen patients with kidney dysfunction and at risk of APOL1 genotype were investigated. Clinical features, laboratory data at initial presentation, management and outcomes were collected. The paper has written based on searching PubMed Central and Google Scholar to identify potentially relevant articles. Median, percentage, mean ± standard deviation (SD), two-tailed t and chi-square tests were used for statistical analyses. Moreover, relative risk, odds ratio for statistical analyses were used.

Results: The average age of patients at the time of diagnosis in APOL1-associated kidney disorders was 41.09 ± 20.63 years (ranging from 8 years to 70 years). Relative risk for kidney failure and persistent hemodialysis therapy in APOL1-associated nephropathy patients with renal risk variants (RRVs) were assessed 1.13 and odds ratio of 1.5 with 95% CI of 0.08-26.86 and the value of 0.0764 by chi-square test but there was no significant statistical result in this research (p-value of 0.782). The relative risk for patients of allograft failure with RRVs was assessed 1,0 odds ratio of 1,0 95% CI of 0.06-15.99 and p-value of 0.81.

Conclusion: The present study revealed the risk and odds of APOL1 gene effect on the onset of kidney failure with replacement therapy in patients at risk of APOL1 genotype but results were not significant statistically. Future clinical research is required for investigating APOL1 gene effect on non-African ancestry.

Keywords: *Apolipoprotein L1, graft failure, hemodialysis, kidney disease, renal risk variants.*

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Фатіме Шамехі Амірі

Вплив варіантів ризику APOL1 на виникаючі наслідки при порушеннях роботи нирок: мета-аналіз даних окремих учасників

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Резюме. *Захворювання нирок, асоційовані з поліморфізмом APOL1, включають в себе ВІЛ-асоційовану нефропатію, фокальний сегментарний гломерулосклероз, гіпертонічну хворобу, хронічну хворобу нирок, вовчаковий нефрит та серповидноклітинну нефропатію. Це дослідження мало на меті дослідити роль генетичних варіантів APOL1 у ризику захворювань.*

Методи. У цьому мета-аналізі даних окремих учасників було досліджено вісімнадцять пацієнтів з дисфункцією нирок та ризиком генотипу APOL1. Були зібрані клінічні особливості, лабораторні дані при первинному обстеженні та результати лікування і спостереження. Стаття написана на основі пошуку PubMed Central та Google Scholar для виявлення потенційно релевантних робіт. Для статистичного аналізу використовували t-тест для порівняння середніх значень зі стандартним відхиленням (SD) та 2-тест для порівняння якісних показників. Крім того, для статистичного аналізу використовували відносний ризик, коефіцієнт шансів.

Результати. Середній вік пацієнтів на момент встановлення діагнозу порушень функції нирок, пов'язаних з APOL1, становив 41,09 ± 20,63 років (8-70 років). Коефіцієнт відносного ризику та шансів розвитку термінальної стадії хронічної хвороби нирок (ХХН) з необхідністю лікування гемодіалізом у хворих з APOL1-асоційованою нефропатію, становили 1,5 (95% ДІ 0,08-26,86), p = 0,782. Відносний ризик відторгнення алотрансплантату склав 1,0 (95% ДІ 0,06-15,99) p = 0,81.

Висновки. У цьому дослідженні не було виявлено ризику впливу гена APOL1 на розвиток ХХН у пацієнтів із ризиком генотипу APOL1. Необхідні майбутні клінічні дослідження для визначення впливу гена APOL1 у пацієнтів неафриканського походження.

Ключові слова: *аполіпопротеїн L1, хронічна хвороба нирок, гемодіаліз, ризик.*

Introduction. A common genetic predisposition to kidney disease in African Americans and sub-Saharan African ancestry is the possession of apolipoprotein L1 (APOL1). APOL1 risk variants regulate the production of APOL1. APOL1 circulates in blood and it is found in the kidney [1]. APOL1 protein that involves in the autophagic pathway encodes the APOL1 gene. APOL1 is present in podocytes, renal tubule cells, and glomerular endothelial cells [2]. Normally, high-density lipoprotein (HDL) particles have potent anti-inflammatory, antioxidative, and antithrombotic properties caused by several components carrying these properties. These are enzymes, apolipoproteins, complements, and other components. APOL1 is a minor component of HDL-C particles in the more dense fractions HDL3b/c. APOL1 concentrations have been suspected to be a surrogate of other antioxidative and anti-inflammatory proteins, such as paraoxonases, solely. In 2010, the description of an association of certain APOL1 genetic variants (called G1 and G2) with the susceptibility to nondiabetic chronic kidney disease (CKD) significantly contributed to the understanding of the 3—4-fold higher incidence rates for end-stage renal disease (ESRD) in African ancestry compared with whites. About 13% of African ancestry carries high-risk genotypes. This frequency is drastically increased to around 70% in patients of this ethnicity with focal segmental glomerulosclerosis (FSGS), human immunodeficiency virus (HIV) - associated nephropathy, and to 45% in patients with hypertension-associated kidney disease. These risk alleles are practically nonexistent in nonblack populations. Sequencing studies in that gene region did not reveal other independent risk variants than G1 and G2 [3]. The discovery of variants of the APOL1 gene, which encode the lipoprotein L1 and confer the risk for kidney diseases, was one of the most significant advances in decades in the knowledge of the pathogenesis of common kidney diseases [4].

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Objectives. *How this study might work.* The frequencies of APOL1 variants and their associations with chronic kidney disease vary substantially with populations from Africa. Therefore understanding the underlying relationships could have an impact on therapeutic options and prevention of the creation of APOL1-associated nephropathy.

What does this study? Circulating APOL1 is a pre-β-high density lipoprotein particles and its gene on chromosome 22 comprises putative sterol response elements in the promoter region, through which the transcription of genes involved in lipid metabolism is presumed to be activated. Given to genetic risk is only disease-causing in the setting of an appropriate environmental stressor, this suggests the APOL1 variants are conditional mutations, where the function or dysfunction of the variants is the only disease-causing when exposed to the appropriate environmental condition. Genetic testing is necessary to investigate the relationship between genotype-phenotype and understand to contribute to disease.

Methods. *Eligibility Criteria.* Among screened 6212 full-text articles obtained in this research paper, 6066 articles were excluded due to unrelated subjects, review articles and other studies. Then 146 full-text articles were eligible and 134 articles were excluded due to not case reports (n = 133). Furthermore, a case report was excluded due to the absence of genotype testing. All case reports were obtained via electronic search in PubMed Central (PMC) and Google Scholar database. These 12 articles included 20 case reports that were examined 20 patients at risk APOL1 genotype and renal dysfunction for systematic review and meta-analysis synthesis.

Type of participants. Patients with kidney disorders in APOL1 risk genotype e.g., FSGS, HIV-associated nephropathy (HIVAN), sickle cell nephropathy, and other diseases, which span standard pathologic classifications, and additional clinical (e.g., eGFR decline) and histopathologic phenotypes in all ages and both male and female were considered in this study. Patients with atypical diabetes mellitus (DM) and CKD, those with nephropathy with short diabetes duration or lacking diabetic retinopathy may benefit from APOL1 genotyping to detect patients more likely to have the disease in the FSGS spectrum.

Primary outcomes. Risk CKD, ESKD progression (need to persistent hemodialysis) and graft loss according to Banff 2013 classification [5] were primary outcomes in this study. Pathologic features and Banff score include interstitial inflammation (i), total inflammation (ti), tubulitis (t), arterial inflammation (V), glomerulitis (g), capillaritis (ptc), c4d deposition in ptc, interstitial fibrosis (ti), tubular atrophy (ct), arterial intimal thickening (cv), transplant glomerulopathy (cg), arteriolar hyalinosis (ah) and mesangial matrix increase (mm).

Secondary outcomes. Decreased eGFR and elevated UACR for detecting proteinuria were secondary outcomes in this study.

Information sources. The paper has written based on advanced searching via PMC and Google Scholar databases to identify articles published from inception to April 2020. The mentioned search used the following terms of APOL1-associated nephropathy, APOL1-associated kidney disease and advanced search with APOL1 and kidney. Moreover, the author reviewed references of all included articles and performed hand-searching of related journals to identify the additional relevant studies.

Study selection. The search strategy was used to obtain titles and abstracts of studies that might be relevant to the review. The 1335 and 4894 titles and abstracts were screened via electronic search in PMC and Google Scholar by author, respectively. Total records of 6229 articles were screened and after deduplication 6212 articles identified. Of them, 6066 articles were excluded due to non-related subjects, review articles, others and 146 full-text articles were considered for eligibility. However, studies and reviews that might include relevant data or information on studies were retained initially. The 133 articles were excluded due to not case reports and one case report was discarded because of the absence of genotype testing. Then 20 case reports that were examined 20 patients at risk APOL1 genotype and renal dysfunction were included for qualitative and quantitative synthesis.

Data collection and analysis. Data extraction was carried out by the author and studies that reported in non-English language journals were to be translated before assessment. Where more than one publication of one study existed, reports were grouped and the publication with the most complete data was included. All patients at APOL1 risk genotype with decreased eGFR that gene analysis of APOL1 performed in those were considered in this research. Clinical features such as age, sex, different symptoms and physical signs were extracted from this study. Furthermore, biochemical variables of serum creatinine (SCr), eGFR, urine protein, genetic testing at initial presentation, imaging, management and outcomes were collected.

Atypical diabetes. Diabetes mellitus was classified as type 1 DM, type 2 diabetes, hepatocyte nuclear factor 1α (HNF1A) diabetes, ketosis-prone diabetes and latent autoimmune diabetes of adulthood. Atypical DM diagnosis is suspected clinically through recognition of a characteristic phenotype that differs from typical type 1or type 2 diabetes. Ketosis-prone diabetes has been the ongoing difficulty of classifying this heterogeneous group. A useful classification system designated the AB classification scheme, has been proposed. In this system individuals are categorized into one of four groups, depending on the presence or absence of islet cell autoantibodies (A+ or A-, respectively) and the presence or absence of β cell functional reserve, once the period of metabolic decompensation has resolved (B+ or B-, respectively). A+ B- and A-B- individuals are distinct subgroups with differing genetic and immunologic underpinnings, but they share the clinical characteristics of type 1 diabetes, including reduced β cell secretory function. A+B+ and A-B- individuals share the features of type 2 diabetes, with preservation of β cell function over time. Patients in the largest of these four groups A-B+ resemble with the type 2 diabetes, obese phenotype and account for 50% of all those with ketosis-prone diabetes. In this group, persons are often Afro-Caribbean or Hispanic Ancestry and male predominance. Insulin therapy can always use as initial therapy in these patients [6].

Genetic definition. APOL1 high-risk status was defined as the presence of 2 risk alleles (G1/G1, G2/G2 or G1/G2) and APOL1 low-risk status was defined as having 1 or 0 risk allele (G1/G0, G2/G0, G0/G0), representing a recessive model. In other words, kidney-risk variants are additive (0 vs. 1 vs. 2) or recessive model (0/1 vs. 2). In African Americans, APOL1 alleles G1 and G2 have been hypothesized to contribute to the elevated cardiovascular disease (CVD) risk. This suggestion might be biologically plausible, as APOL1 is a major apo component of HDL3 particles, which plays a key role in cholesterol transport and the limitation of low-density lipoprotein (LDL) oxidation. These two APOL1 alleles are also associated with a higher incidence of CKD (including FSGS, collapsing glomerulopathies and arterionephrosclerosis) and a more progressive course of the disease in the African American population [7]. The APOL1 gene is induced by proinflammatory stimuli and has roles in innate immunity. The best-characterized function of APOL1 is protection against African sleeping sickness which is caused by Trypanosoma brucei. Plasma APOL1 is a constituent of HDL3, and when these HDL particles are ingested by susceptible trypanosomes, APOL1 kills the parasites by forming pores in lysosomal membranes, leading to membrane rupture. viral infections have largely been described as triggers for rather than protection against the development of collapsing glomerulopathy in individuals with high-risk APOL1 genotypes including HIV, parvovirus B19, cytomegalovirus or the BK polyomavirus [8]. The APOL1 protein which is present in the circulation at a concentration of approximately 0.3 mg/dl. Higher rates of chronic kidney disease and ESKD in those carrying two risk variants have been reported. In addition, the risk of graft loss following a kidney transplant is higher those carrying two APOL1 risk variants but not in recipients carrying two risk variants, suggesting that the donated kidney may carry a predisposing factor for the development of kidney disease.

Definition of kidney dysfunction. Acute kidney injury (AKI), acute kidney disease (AKD) and CKD can form a continuum whereby initial kidney injury can lead to persistent injury eventually leading to CKD. AKI is defined as an abrupt decrease in kidney function occurring over 7 days or less whereas CKD is defined by the persistent of kidney disease for a period of > 90 days. AKD is defined as acute or subacute damage and/or loss of kidney function for a duration of between seven and 90 days after exposure to an AKI initiating event. Recovery from AKI within 48 h of the initiating event typically heralds rapid reversal of AKI (16th ADQI consensus report of 2017). CKD is classified zero to seven stages (stages of 0, 1, 2, 3a, 3b, 4 and 5) according to estimated glomerular filtration rate (eGFR) and kidney damage such as proteinuria (>200 mg/day or protein to creatinine ratio > 200 mg/g creatinine) or albuminuria (urinary albumin excretion ≥ 30 mg/day or albumin to creatinine ratio ≥ 30 mg/g creatinine). eGFR is defined according to creatinine clearance (CrCl), Cockcroft-Gault equation, modification of diet in renal disease (MDRD) and chronic kidney disease-epidemiology collaboration (CKD-EPI). CrCl in 24-hr urine collection is expressed using urine creatinine (mg per deciliter or micromole per liter) multiplication by urine volume (milliliter or liter) divided on plasma creatinine (milligram per deciliter or micromole per liter) multiplied 1440 and it's unit is expressed with milliliter per minutes (ml/min). The Cockcroft-Gault equation is expressed as CrCl=(140-age)×wt divided on SCr×72, multiplication by 0.85 if female. MDRD equation given by: estimated GFR=175×Standardized SCr $^{-1.154}$×age $^{-0.203}$×1.212 [if black] ×0.742 [if female] where eGFR is expressed as ml/min/1.73m^2 of body surface area and SCr is expressed as mg per dl. The CKD-EPI equation, expressed as a single equation, is eGFR=141×min (Scr/κ,1) α × max (Scr/κ,1) - 1.209 × 0.993age × 1.018 [if female] - 1.159 [if black], where κ is 0.7 for females and 0.9 for males, α is -0.329 for females and 0.411 for males, min indicates the minimum Scr/kor 1 and max indicates the maximum of Scr/κ or 1. Proteinuria, albumin-to-creatinine ratio (ACR) is greater than 2.5 mg/mmol in men or 3.5 mg/mmol in women, or a protein-to-creatinine ratio (PCR) is greater than 15 mg/mmol is sufficient for diagnosis of CKD (random PCR < 15 mg/mmol: normal; 15-49 mg/mmol: trace proteinuria; 50-99 mg/mmol: significant proteinuria; 100-300 mg/mmol: high proteinuria; > 300 mg/mmol: nephrotic range proteinuria). Proteinuria may be classified as moderately increased albuminuria (3-30 mg/mmol creatinine or severely increased albuminuria (greater than 30 mg/mmol creatinine). The normal PCR in children and adolescents is less than 0.3. In infants and younger children, the PCR is higher with the upper normal limit of 0.5. PCR above 3 is found in patients with nephrotic syndrome. The daily protein excretion rate (PER) can be determined from spot urine PCR, based on sex, age and weight using the following equations: PER (g/m^2/day)=0.63* (PCR) [9-12].

Assessment of risk of bias and quality in included articles. Case reports were analyzed using criteria developed by the Joanna Briggs Institute Critical Appraisal tool for case reports that has different assessment tools for each study design in question. The evaluation tool has 8 items for case reports.

Statistical analysis. Data were entered in Microsoft Excel 2010 software. Categorical variables are recorded as frequency (N) and percentage (%). The continuous variables were determined as to whether they were normally distributed using the Kolmogorov-Smirnov or Shapiro-Wilk test. Continuous variables with normal distribution reported as mean ± standard deviation (SD). Nonparametric variables are expressed as the median and interquartile range (Q1, Q3 and IQR). Comparisons between continuous variables with normally distributed (ND) data assessed by two-tailed one-sample t-test analysis. Relative risk and Odds ratio for assessing effect measures of the risk factor on outcomes of disease were used. Furthermore, for the association between the APOL1 risk factor and outcomes, a chi-square test was used. Significance was assessed with p-value of < 0.05.

Results. *Results of the search and study selection.* After searching electronic databases author identified 6229 records. After duplicated articles were removed and titles and abstracts screened, the author retrieved 6212 full-texts articles for further assessment after deduplication. Then 146 articles were eligible and 134 articles were discarded due to not case reports and absence of genotype testing. Of these, 12 published articles (20 case reports) were included and enrolled to participate in this study (Fig. 1).

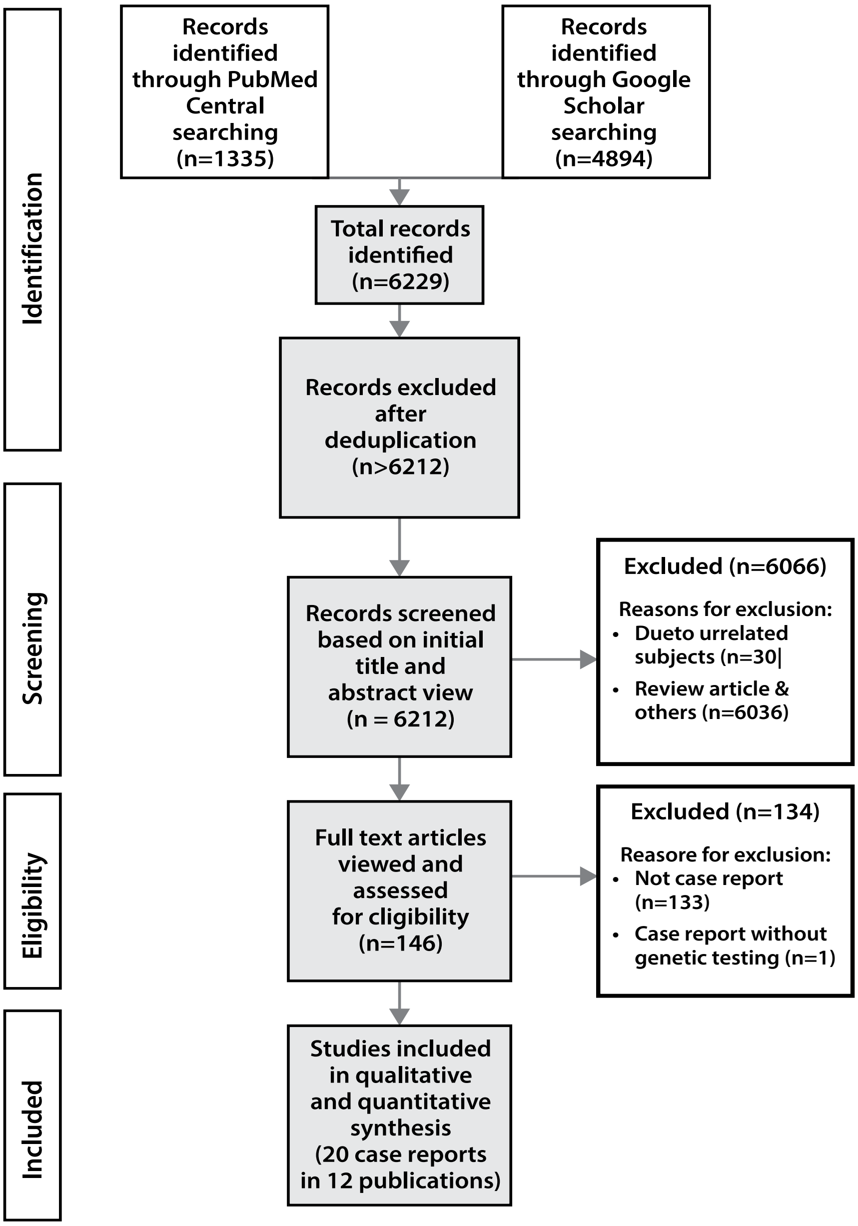

Fig. 1. Workflow diagram of the included studies in this research.

Study characteristics. Twelve published articles (20 case reports or participants) were considered for inclusion in this research. Randomized data were planned with a systematic review and meta-analysis design in this retrospective study and those articles were collected via a non-randomized method. Sample sizes ranged from 20 to 21 patients in this study that one patient excluded from this study.

Participants were referred to a single center in eight case reports and these situations were not mentioned in twelve case reports.

Participants. All patients included in this study had kidney diseases associated with the APOL1 risk genotype. These patients had elevated serum creatinine levels or decreased eGFR that gene analysis for APOL1 performed in these patients. Patients were excluded from the study if they had no genetic testing and urologic disease.

Risk of bias and quality in the included studies. Assessment of risk of bias and quality of included articles performed using Joanna Briggs Institute critical appraisal tools for case reports (Table 1).

Table 1

JBI critical appraisal tool for case reports included articles of APOL1-associated nephropathy case reports in the present study

Items	Koffman-R-2014	Koffman-D-2014	Koffman-2012	Khalighi-case report 1	Khalighi-case report 2	Shah	Zwang	Attias	Chang-R-A 1	Chang-R-A 2	Chang-R-B 1	Chang-R- C	Chanh-D-A	Chang-D-B	Chang-D-C	Watanabe	Besse	Tillman	Abid	Larsen
1. Demographic characteristics described	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
2. Subject history described	UC	Y	Y	Y	Y	UC	Y	UC	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
3. The pre-intervention clinical condition described	Y	Y	Y	Y	Y	UC	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	UC	Y	Y
4. Diagnostic tests or assessment methods and result	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
5. Intervention/ treatment described	Y	Y	Y	Y	Y	UC	Y	Y	Y	Y	Y	Y	UC	UC	UC	Y	Y	Y	Y	Y
6. The post-intervention clinical condition described	Y	Y	Y	UC	UC	Y	Y	UC	Y	UC	UC	UC	UC	UC	UC	UC	Y	UC	N	Y
7. Adverse events	Y	UC	UC	UC	UC	UC	Y	UC	UC	Y	UC	UC	UC	UC	UC	UC	Y	Y	UC	UC
8. Takeaway lesson	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Score total	7/8	7/8	7/8	6/8	6/8	4/8	8/8	5/8	7/8	7/8	6/8	6/8	5/8	5/8	5/8	6/8	8/8	6/8	6/8	7/8

Abbreviation: N, no; UC, unclear; Y, yes.

Based on these criteria, two patients presented with 8 scores (2/20, 10%), six patients with 7 score (6/20, 30%), seven patients with 6 score (7/20, 35%), four patients with 5 score (4/20, 20%) and one patient with 4 scores in this research (1/20, 5%).

Patients' Characteristics. Among screened 6212 full-text articles obtained in this research paper, 6066 articles were excluded due to unrelated subjects, review articles and other studies. Then 146 full-text articles were eligible and 133 articles were excluded due to not case report (n=133). Furthermore, a case report was discarded due to the absence of genotype testing. Finally12 published articles were included in this study. These 12 articles included 20 case reports that were examined 20 patients at risk APOL1 genotype and renal dysfunction for qualitative and quantitative synthesis [13-24]. Eleven of twenty patients (11/20, 55%) were from Afro-American ethnicity, four of twenty patients (4/20, 20%) from the Caribbean, three of twenty patients from white ancestry (3/20, 15%) and one patient (1/20, 5%) from Asian and black race. Causes of APOL1-associated nephropathy in this research were different that have been described in Table 2.

Table 2

Causes and genotypes of APOL1-associated nephropathy in the present study

Author-cause	Cause	Relation	Loss of Protective factor	Second hit	APOL1 genotype
Koffman-Recepient-2014	ESKD with unknown origin	Monozygotic twin			G1G2
Koffman-Donor-2014	Post-donation kidney failure	Monozygotic twin			G1G2

Table 2 continuation

Author-cause	Cause	Relation	Loss of Protective factor	Second hit	APOL1 genotype
Koffman-2012	Lupus flare				G2G2
Khalighi- case report 1	Amyloid nephropathy			Parvovirus B19 positive IgG	G1G2
Khalighi-case report 2	Amyloid nephropathy				G1G2
Shah-Donor	deceased			CMV IgG positive	G1G2
Zwang-Donor	Post-donation Nephrotic Sx & ESKD due to FSGS	Donation to her brother		CMV IgG positive EBV IgG positive positive ANA (1:80)-speckled	G1G1
Attias	MM & FSGS			Monotypic plasma cell interstitial nephritis	G1G2
Chang –case report-R-A1	DM & HTN			CMV& EBV positive	G1G1
Chang-Case report-R-A2	APCKD & DM			EBV positive	G0G0
Chang-Case report-R-B1	HTN & secondary FSGS		Interferon administration	EBV positive	G0G0
Chang-Case report-R-C1	HTN		Interferon administration	EBV positive Cancer& hepatic metastasis	G0G0
Watanabe	Nephrotic Sx & FSGS	Family hx of kidney disease of unknown cause in her paternal uncle			High-risk allele
Besse	HTN during pregnancy	Positive history parvovirus B19 infection in her daughter one month before presentation Donation from her sister		Acute parvovirus B19 infection	G1G1
Tillman	CKD progression due to NSAID	Positive history of ESKD in her mother	Environmental factors e.g. high dose of NSAIDs, hydrocodone, cocaine	Positive pharmacogenomics test of CYP2D6-5/17 CYP2C9-1/8	G1G1
Chang-D-A	HTN				G1G1
Chang-D-B	HTN			EBV, CMV	G1G1
Chang-D-C	HTN			EBV, CMV	G1G2
Abid	SAVI, NS			APOL1-associated nephropathy	G1G2
Larsen	AKI/CKD, DM, HTN	Fraternal twin	Positive ANAs, Sjogren-syndrome related antigen A	COVID-19, Cancer	G1G1

Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; AKI, acute kidney injury; ANA, antinuclear antibody; CKD, chronic kidney disease; CMV, cytomegalovirus; COVID-19, coronavirus disease-2019; DM, diabetes mellitus; EBV, Epstein-Barr virus; ESKD, end-stage kidney disease; HTN, hypertension; FSGS, focal segmental glomerulosclerosis; MM, multiple myeloma; NS, nephrotic syndrome; NSAIDs, non-steroidal anti-inflammatory drugs; SAVI, Stimulator of interferon genes (STING)-associated vasculopathy with onset in infancy; Sx, syndrome.

The average age of the patients was 41.90 ± 20.63 years (ranging from 8 years to 70 years) at the time of diagnosis in APOL1-associated kidney disorders. Among the patients, there were 10/20 (50%) males and 10/20 (50%) females. The average age of male and female patients at the time of the APOL1-associated kidney disease diagnosis were 40 ± 22.79 old years (ranging from 4 weeks to 66 years old) and 43. 8 ± 18.02 (ranging from 8 years to 70 years), respectively. There was no statistical significance for age between two sex levels in ApoL1-associated nephropathy (p-value: 0.7) [**Supplementary Table S1**].

Patients Complaints. The symptoms in this study were not mentioned in six patients (6/20, 30%) and three patients (3/20, 15%) presented with lower extremity edema. Two of twenty patients (2/20, 10%) presented with shortness of breath, cough and there was a history of hypertension in eleven of twenty patients (11/20, 55%) with APOL1-associated nephropathy [**Supplementary Table S2**]. In signs of APOL1-associated nephropathy, there was high blood pressure in eight of twenty patients (8/20, 40%) (Table 3).

Table 3

Distribution of symptoms and signs in Apol1-associated nephropathy

Symptoms	Number	Percentage	Signs	Number	Percentage
Not determined	6/20	30%	Fever	2/20	10%
Lower extremity edema	3/20	15%	BMI≥25 kg/m^2	4/20	20%
Shortness of breath	2/20	10%	High BP	8/20	40%
Weight gain	1/20	5%	Wt loss	1/20	5%
Low back pain	1/20	5%	PER	1/20	5%
Dyspnea	1/20	5%	Abnormal cardiac examination	1/20	5%
Cough	2/20	10%	Edema	1/20	5%
Polyarthralgia	1/20	5%			
Periorbital edema	1/20	5%			
Hand-Foot swelling	1/20	5%			

Abbreviations: BMI, body mass index; BP, blood pressure; PER, papuloerythematous rash.

Seventeen of twenty patients (17/20, 85%) were in the high-risk allele group and three of twenty patients belonged to the low-risk allele group (3/20, 15%). Five of seventeen (5/17, 29.4%) patients with high-risk alleles were hypertensive and three of three (3/3, 100%) participants with low-risk allele were presented with high BP. There were elevated body mass index (BMI) in six of twenty patients (6/20, 30%) in this research that four of six (4/6, 66.6%) patients belonged to high-risk allele group and two of those (2/6, 33.3%) belonged to the low-risk allele group. Two of twenty patients (2/20, 10%) had a fever in the presented study. One of twenty patients (1/20, 5%) presented with weight loss, pitting edema, papuloerythematous rash and chronic low back pain (Table 4) [**Supplementary Table S3**].

Table 4

Distribution of symptoms and signs in two high and low-risk allele groups in Apol1-associated nephropathy

Symptoms	The high-risk allele (Number, Percent)	The low-risk allele (Number, Percentage)	Signs	High risk allele (Number, Percent)	Low risk allele (Number, percentage)
Not determined	4/17 (23.5%)	2/3 (66.6%)	Fever	2/17 (11.7%)	0
Lower extremity edema	2/17 (11.7%)	1/3 (33.3%)	BMI≥25 kg/m2	4/17 (23. 5%)	2/3 (66.6%)
Short of breath	2/17 (11.7%)	0	High BP	5/17 (29.4%)	3/3 (100%)
Weight gain	1/17 (11.7%)	0	Wt loss	1/17 (11.7%)	0
Low back pain	1/17 (11.1%)	0	PER	1/17 (11.7%)	0
Dyspnea	1/17 (11.7%)	0	Abnormal cardiac examination	1/17 (11.7%)	0
Cough	1/17 (11.7%)	0	Edema	1/17 (11.7%)	0
Polyarthralgia	1/17 (11.7%)	0			
Periorbital edema	1/17 (11.7%)	0			
Hand-Foot swelling	1/17 (11.7%)	0			

Abbreviations: BMI, body mass index; BP, blood pressure; PER, papuloerythematous rash; Wt, weight.

Laboratory data. Metabolic syndrome definition according to National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) is diagnosed co-occurrence of greater or equal than of three of five metabolic abnormalities: abdominal obesity (BMI ≥ 25 kg/m^2 or waist circumference > 40 inches in male and > 35 inches in female), hyperglycemia, hypertension (HTN), and dyslipidemia in combination. Three of twenty patients (3/20, 15%) with APOL1-associated nephropathy had three components of metabolic syndrome in this study. These patients had DM, HTN and high BMI of more or equal 25 kg/m2 with each of those in high and low-risk allele groups. There were elevated SCr in thirteen of twenty patients (13/20, 65%) with an average of 3.47±2.42 mg/dl. There were elevated SCr in ten of thirteen patients (10/13, 76.9%) with an average of 4.11±2.42 mg/dl in high-risk alleles group and elevated SCr in three of thirteen patients (3/13, 23%) with an average of 1.36±0.04 mg/dl in low-risk allele group. Furthermore, there were raised blood urea nitrogen in three of thirteen patients (3/20, 15%) and hypercholesterolemia in two of twenty patients (2/20, 10%). Five of twenty patients (5/20, 25%) presented with hypoalbuminemia and four of twenty patients (4/20, 20%) presented with decreased eGFR. There was 24-hr urine protein excretion more than 200 mg in three of twenty patients (3/20, 15%) with an average of 2133.33±1108 mg/day and high spot urine protein creatinine ratio in eight of twenty patients (8/20, 40%) with HR allele group that semiquantitative proteinuria (4+) was observed in one patient of apol1-associated nephropathy. The average of high PCR in the high-risk allele group was assessed at 11.47±10.19. There were high PCR in three of twenty patients (3/20, 15%) with an average of 9.9±3.61 g/g in the low-risk allele group (Fig. 2) [**Supplementary Table S4 a-c**].

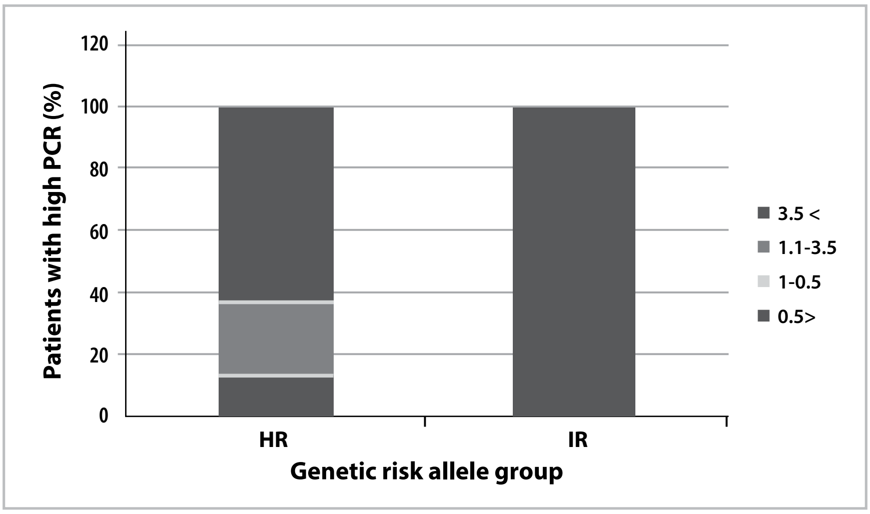

Fig. 2. Distribution of different PCR in two genetic risk allele groups. APOL1 genetic variants with two alleles risk (homozygous or heterozygous carriers) are considered high risk and renal risk variant with 0 or 1 risk allele is considered as low risk. HR, high risk; LR, low risk; PCR, protein to creatinine ratio.

Genetic testing. The 2 single nucleotide polymorphism (SNPs) in the APOL1 G1 and the insertion/deletion polymorphism for the G2 risk allele were genotyped. There were seventeen of twenty patients (17/20, 85%) in the high-risk alleles group and three of twenty patients (3/20, 15%) in the low-risk alleles group. Patients' genotypes were categorized as noncarriers (0), heterozygous carriers (1 or G0/G1, G0/G2), homozygous carriers (2 or G1G1, G2G2) or compound heterozygous carriers (2 or G1G2). There was compound heterozygosity in eight of twenty patients (8/20, 40%) as G1G2, seven of twenty patients (7/20, 35%) with homozygous carriers as G1G1, three of twenty (3/20, 15%) with homozygous carriers as G0G0, one of twenty patients (1/20, 5%) as G2G2 and high-risk allele (not determined) in this research (Fig. 3).

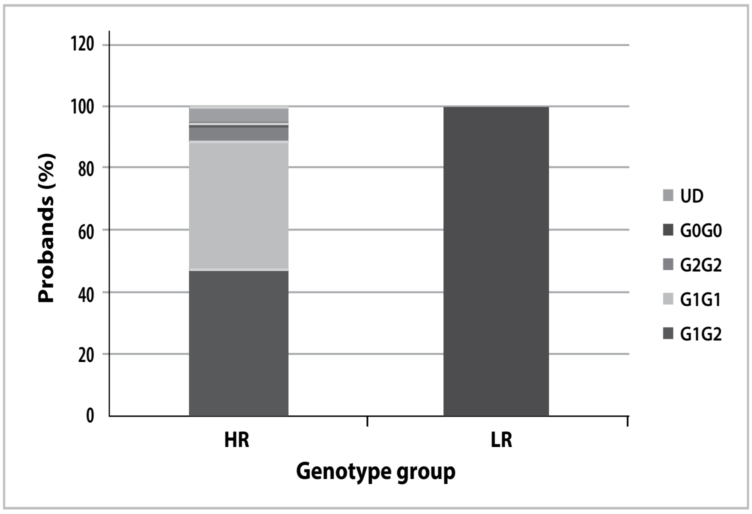

Fig. 3. Schematic distribution of high and low allele risk groups in participants of included cases in this research. HR, high risk; LR, low risk; UD, undetermined.

Eight of seventeen patients (8/17, 47%) were in high-risk alleles group in the male gender and nine of seventeen patients (9/17, 52.9%) belonged to high-risk alleles group in the female gender. The average age of patients was assessed 34.63±22.47 and 41.5±17.62 years old in male and female levels in high-risk alleles group at the time of diagnosis, respectively. Three of twenty patients (3/20, 15%) were in low-risk alleles group that average age of these patients was assessed 61.5±0.5 years old in male gender that encompassed two males and one female gender. There was no statistical significance for age between two sex levels in high-risk alleles group in APOL1-associated nephropathy (p-value: 0.52) (Fig. 4, 5) [**Supplementary Table S5**].

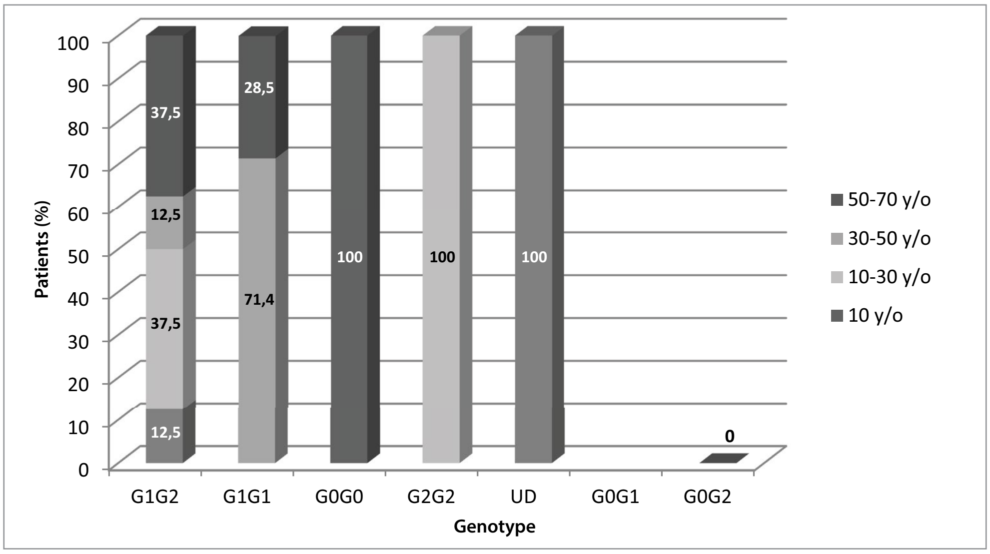

Fig. 4. Distribution of age of patients in high and low-risk allele groups in included case reports in this research. UD, undetermined.

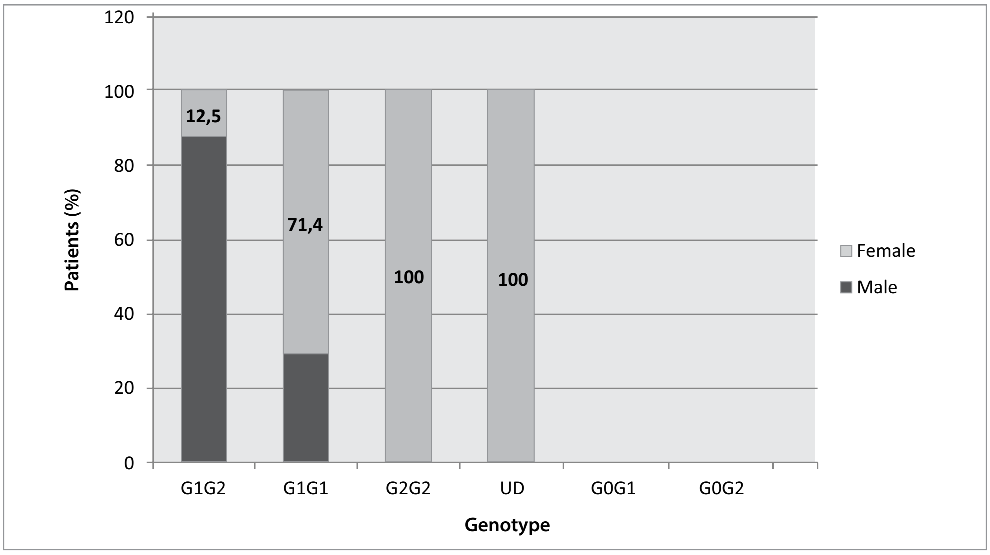

Fig. 5. Distribution of gender in high and low-risk allele groups in included case reports; UD, undetermined. HR, high risk; LR, low risk.

Imaging. In this study, there was normal renal ultrasonography in four patients (4/20, 20%) and one of twenty patients (1/20, 5 %) revealed bilaterally increased echogenicity in kidneys. Moreover, there were abnormal positron emission tomography (PET) scan and cerebral magnetic resonance imaging (MRI) in two of twenty patients (2/20, 10%). A chest x-ray in one of twenty patients (1/20, 5%) showed right subsegmental atelectasis and small right-sided pleural effusion at the initial presentation. A Chest CT scan revealed diffuse parenchymal and interstitial abnormalities in one of twenty patients (1/20, 5%). Bone x-ray, abdominal computed tomography (CT) scan, abdominopelvic computed tomography angiography (CTA) and whole-body MRI were normal in one of twenty patients (1/20, 5%) [**Supplementary Table S6**].

Pathology. A renal biopsy performed in thirteen of twenty patients (13/20, 65%) with APOL1-associated nephropathy. Glomerulitis in ten; tubular atrophy in five; interstitial fibrosis in four; tubulitis, inflammation in the area of interstitial fibrosis and tubular atrophy (i-IFTA), intimal arteritis, peritubular capillaritis in three;

foot process effacement in two and mesangial matrix expansion (mm) in one of thirteen kidney biopsies were seen. Pathologic diagnosis of FSGS was seen in seven (7/13, 53.8%) and collapsing glomerulopathy in six of thirteen kidney biopsies (6/13, 46.1%). Acute kidney injury was seen in four (4/13, 30.7%) and amyloid nephropathy in two of thirteen (2/13, 15.3%) biopsy-proven pathologic features. Interstitial fibrosis, tubular atrophy, FGGS, suspicious to FSGS and pathologic findings in favor of systemic lupus erythematosus were seen in one of thirteen biopsy-proven pathologic features in Apol1-associated nephropathy (1/13, 7.6%). It is worth noting that the APOL1 genotype performed on biopsy material from one patient and analyzed as APOL1 genotype. Bone marrow (BM) aspiration was normal in one of twenty patients (1/20, 5%) and BM biopsy revealed findings in favor of indolent multiple myeloma in one of twenty patients (1/20, 5%) with Apol1-associated nephropathy. Skin biopsy in one of twenty patients (1/20, 5%) revealed dermal perivascular and interstitial inflammation with vasculopathy.

Treatment. Treatment modalities in this study included intravenous (IV) methylprednisolone in five of twenty patients (5/20, 25%), cyclophosphamide, bortezomib, oral corticosteroids and IV dexamethasone in three of twenty patients (3/20, 15%), intravenous immunoglobulins (IVIg), interferon in two of twenty patients (2/20, 10%) and other drugs in one of twenty patients (1/20, 5%). Hemodialysis performed in eleven of twenty patients (11/20, 55%) and two of twenty patients treated with plasma exchange (2/20, 10%). Seven of twenty patients (7/20, 35%) underwent kidney transplantation. Of these, three patients (3/7, 42.8%) had preserved graft and four patients developed graft loss in the post-renal transplant period (4/7, 57.1%) [**Supplementary Tables S7a-b**].

Outcomes and Follow UP. Primary endpoints of APOL1-associated nephropathy are as follows. Four of twenty patients (4/20, 20%) with APOL1-associated nephropathy developed CKD as a primary end-point in this research. All participants harboring both high and low-risk alleles groups encompassed two copies of renal risk variants (4/4, 100%). Two of four CKD patients were from Caribbean ancestry, one of four patients (1/4, 25%) had black and Afro-American descent. The average age of CKD in these patients was assessed 35.75±22.08 years old with an equal ratio of gender involvement. The average of initial and follow up SCr was accounted for 1.44±0.32 and 2.29±0.21 mg/dl, respectively. The average time of CKD onset in these patients was assessed 5±3.52 years after the initial presentation. Elven of twenty patients (11/20, 55%) underwent hemodialysis therapy and eight of eleven patients (8/11, 72.7%) stayed on hemodialysis therapy during follow-up. The average of hemodialysis duration (vintage) in patients with kidney renal transplant (KRT) was assessed 1.23±1.03 years. The average vintage in high and low-risk allele groups was assessed at 0.67±0.47 and 2.72±0.52 years, respectively. The average age of ESKD and hemodialysis onset in these patients was assessed 48.93±16.49 years old that four of eleven patients (4/11, 36.3%) were male and seven of eleven patients were female (6/11, 54.5%). The average age of ESKD and hemodialysis onset were 52.25±18.71 and 47.04±17.74 years old in male and female were 52.25±18.71, respectively. Eight of eleven patients (8/11, 72.7%) had two renal risk variants (RRVs) and three of eleven participants consisted of 0 or 1 RRVs in this primary end-point (3/11, 27.2%). The genotype of high-risk allele groups encompassed four of eight (4/8, 50%) patients G1G1, three of eight (3/8, 37.5%) with G1G2 and one of eight (1/8, 12.25%) patients with G2G2 genotypes. The average age of ESKD and hemodialysis onset were 43±23 and 44.21±14.05 years old in high-risk allele groups in male and female levels, respectively. While the average age of ESKD and hemodialysis onset were assessed 61.5±0.5 years old in low-risk allele group at male levels. Median and IQR time to ESKD and hemodialysis onset in high-risk allele groups were assessed 0.75 and 0.95 years (Q3-Q1:1.33-0.375), respectively. The median and IQR time of ESKD and hemodialysis onset were assessed 1 and 2.41 years (Q3-Q1=2.91-0.5). Median and IQR time to ESKD and hemodialysis onset in low-risk allele groups were assessed 1 and 1.16 years (Q3-Q1:1.66-0.5), respectively. The average time to ESKD and initial hemodialysis was assessed 2.72±0.52 years in low-risk allele group. Eight of eleven patients (8/11, 72.7%) in the present study stayed on persistent hemodialysis and had an average age of 49.5±17.97 years. Five of eight patients (5/8, 62.5%) were female and three of eight patients (3/8, 37.5%) were males. The average age of patients on persistent hemodialysis was assessed 49.3±20.13 and 49.6±16.54 years old in male and female during follow up, respectively. Six of eight patients (6/8, 75%) had two RRVs and two of eight participants consisted of 0 or 1 RRVs in this primary end-point (2/8, 25%). The genotype of high-risk allele groups included three of eight (3/8, 37.5%) patients with G1G2, one of eight patients (1/8, 12.5%) with G1G1 and one of eight (1/8, 12.5%) patients with G2G2 genotypes. The average age of persistent hemodialysis was 43.5±22.5 years old and 46 ±16.65 years old in high-risk allele groups in male and female levels, respectively. Median and IQR time to persistent hemodialysis assessed 1.08 and 1.16 years (Q3-Q1:1.66-0.5), respectively. Relative risk and odds ratio for ESKD and persistent hemodialysis therapy in APOL1-associated nephropathy patients with RRVs were assessed 1.13, the odds ratio of 1.5 with 95% CI of 0.08-26.86; the value of 0.0764 by chi-square test and p-value of 0.782 (not significant). In this research, seven of twenty patients (7/20, 35%) with apol1-associated nephropathy were received kidney transplantation as recipients and five of twenty patients (5/20, 25%) were accounted as kidney transplant donors. Four of five kidney donors (4/5, 80%) were deceased donors and one of five patients (1/5, 20%) was living donor. Ancestry of recipients (7/12, 58.3%) in the present study comprised two of seven recipients from

Afro-American, Caribbean, White and one of seven recipients (1/7, 14.2%) from the Asian race. Ancestry of kidney donors (5/12, 41.6%) included four of five deceased kidney donors (4/5, 80%) from Afro-American and one of those (1/5, 20%) with living kidney donors had Caribbean ethnicity. The time of kidney transplantation in these patients was assessed with an average of 1.44±1.02 years. Five of recipients (5/7, 71.4%) were received their graft from deceased kidney donors and two recipients (2/7, 28.5%) from living donors that two recipients (2/7, 28.5%) were received a transplanted kidney from related donors and others (5/7, 71.4%) were non-related donors (one sibling from his fraternal twin and another sister sibling from her sister). Here, one interesting point is the organ donation of a donor to her brother and then underwent kidney transplantation from a deceased donor due to post-donation kidney failure at seven years after kidney donation. Three of seven recipients (3/7, 42.8%) were homozygous G0 and G1 carriers and one of seven patients (1/7, 14.2%) possessed heterozygous G1G2. Four patients were female (4/7, 57.1%) and three patients were male (3/7, 42.8%). The average age of patients with allograft kidney was assessed 48±19.09 and 48.18±9.96 years old in male and female, respectively. The average age of kidney replacement therapy onset in individuals with two copies of APOL1 RRVs was assessed 42.9±4.58 years old in female level vs. the average age of 61.5±0.5 years old in the male in 0 to 1 RRVs. Two of seven patients (2/7, 28.5%) were from African-American, Caribbean, White and one patient (1/7, 14.2%) from the Asian race. The average age of five kidney donors was assessed 37±17.45 years with male to female ratio of four to one in kidney donors (M/F ratio:4/1). Three of five kidney donors (3/5, 60%) were genotyped as G1G2 and two of five kidney donors (2/5, 40%) as homozygous G1carriers. The average age of kidney donors with two copies of RRVs was assessed 34.75±18.85 years old. Four of five kidney donors (4/5, 80%) were deceased kidney donors and one of five kidney donors (1/5, 20%) was living kidney donors. The average age of deceased kidney donors was assessed 41±17.34 years old. Half of four deceased kidney donors were genotyped homozygous G1 carriers and another half as heterozygous G1G2. Four of seven patients (4/7, 57.1%) developed allograft failure that average time-onset of allograft failure was assessed 3.03±1.58 years. The average age of allograft failure was assessed 51.29±15.54 years with equal distribution for both sexes. Two of four patients (2/4, 50%) with allograft failure were from white and one patient (1/4, 25%) from Asian and Caribbean ethnicity. Two of four patients (2/4, 50%) with allograft failure had homozygous G0, one of four patients (1/4, 25%) with homozygous G1 genotypes and one patient (1/4, 25%) was inherited heterozygous G1G2 genotype (Fig. 6).

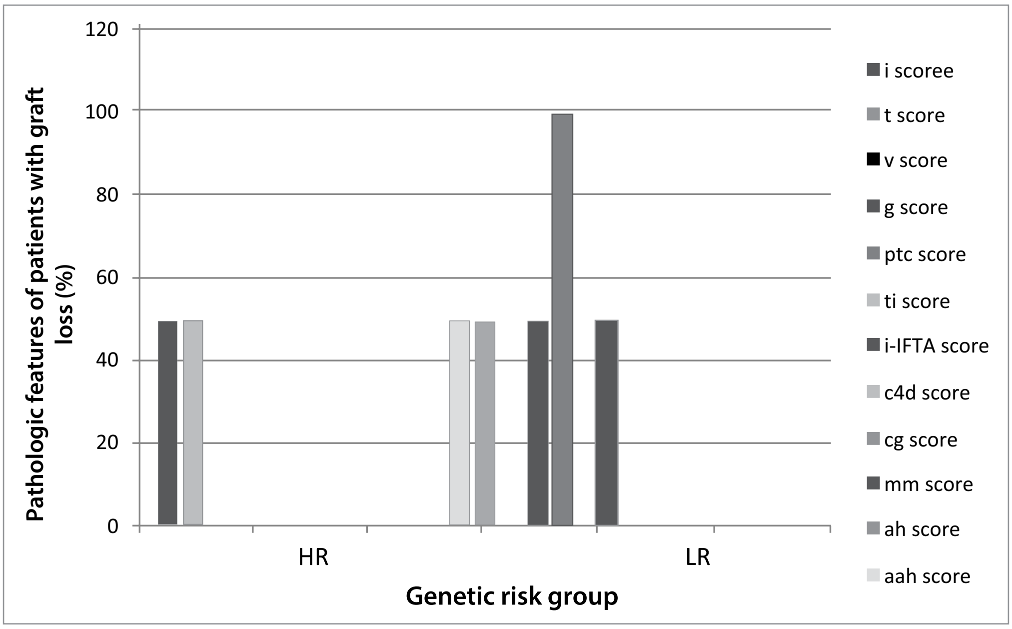

Fig. 6. Distribution of pathologic features in high and low-risk allele group in included case reports.

Patients with allograft failure had different patterns of histologic graft biopsy. One patient with graft loss revealed Banff score of ci3ct3 in third kidney biopsy at five years post kidney transplantation. Another patient showed acute cellular rejection (Banff grade 1B) 19 months after transplantation. Allograft failure with Banff score 2 in involved glomeruli was observed in one patient at 14 months after kidney transplantation. Allograft failure with Banff grade 1A due to acute cellular rejection was seen in the other patient at 21 months after kidney transplantation. The relative risk for patients of allograft failure with RRVs was assessed 1, the odds ratio of 1, 95% CI of 0.06-15.99 and p-value of 0.81 (not significant) (Fig. 7).

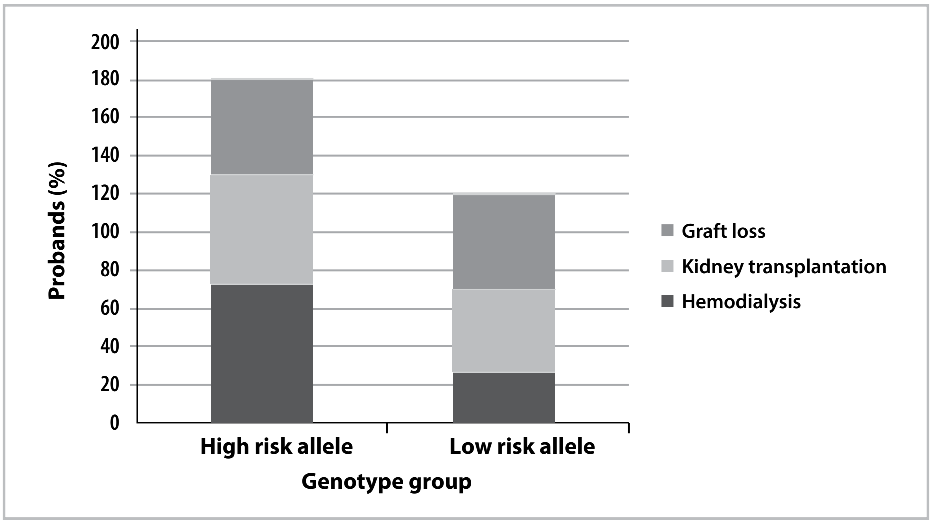

Fig. 7. Distribution of primary end-points of participants in the high and low-risk allele groups in this research.

There were kidney donor profile index (KDPI) in two of five kidney donors and one donor of a recipient in the present study. The average of KDPI in two deceased donors was assessed 60±12 percent and in deceased donor of one recipient was assessed 4% [**Supplementary Table S8a-d**].

Secondary end-points. In this research, initial eGFR in six of twenty patients (6/20, 30%) were measured. In two patients (2/6, 33.3%), eGFR was measured using MDRD and one of six patients (1/6, 16.6%) with CKD-EPI and CrCl methods were measured, respectively. In two of six patients (2/6, 33.3%) eGFR method was not reported. The average of decreased eGFR measurement was assessed 42.25±30.43 ml/min/1.73m^2 at initial presentation and the average of spot urinary PCR were assessed 7.2±6.24 g/g in patients with APOL1-associated nephropathy. Initial eGFR measurement was checked in three of four patients (3/4, 75%) with CKD and only one patient had eGFR measurement in follow up. Baseline spot protein to creatinine ratio and timed 24-hr urine protein collection were measured in two (2/4, 50%) and one of four (1/4, 25%) of CKD patients, respectively. Furthermore, data of follow up proteinuria is scant. There were decreased eGFR in one of four patients (1/4, 25%) and increasing proteinuria in two of four patients (2/4, 50%) during follow up with ApoL1-associated nephropathy in this research.

Discussion. APOL1 protein that encoded by the human APOL1 gene is one of the risk factors for non-diabetic renal disease (NDRD) in the African ancestors population. Secretory APOL1 circulates in high-density lipoprotein 3 (HDL3) complexes and is expressed in various tissues. Genetic studies show that the two G1and G2 alleles of the APOL1 gene are significantly associated with the risk of various types of kidney diseases [**25**]. The presence of APOL1 high-risk genotypes, comprising any combination of two APOL1 kidney risk alleles, increases the risk for several kidney diseases compared with APOL1 low-risk individuals (defined as those carrying zero or one APOL1 kidney risk allele). These diseases include NDRD, hypertension-attributed (HA-APOL1) associated nephropathy, FSGS, HIV-associated nephropathy [**26**], focal global glomerulosclerosis with interstitial and vascular changes (overlapping with the pathologic pattern formerly termed arterionephrosclerosis), sickle cell nephropathy, lupus nephritis associated with collapsing glomerulopathy and unexplained ESKD [**27**]. We know that analysis of the APOL1 high-risk genotypes in the African-American study of kidney disease in hypertension demonstrated that APOL1 high-risk individuals with hypertension and reduced eGFR tended to have heavier proteinuria and faster GFR loss compared with APOL1 low-risk genotypes in African ancestors. The present study revealed high BP (66.6%) in HR allele group vs. LR allele group (33.3%). Moreover, proteinuria has been seen in 40% of participants with HR allele group vs. LR allele group in this research as such 83.3% of those presented with nephrotic-range and 16.6% of those with subnephrotic proteinuria in HR allele group that is in agreement with the paper by Kopp et al [**28**]. Risk factors for kidney disease in APOL1-associated nephropathy include a history of premature birth, hypertension and obesity. There were a high BMI of more or equal 25 kg/m^2 in six of twenty (6/20, 30%) patients with APOL1-associated nephropathy that 20% of them belonged to the HR allele group and 10% of others to low risk allele group. Furthermore, metabolic syndrome was seen in 15% (3/20) of patients in this research. Our study revealed an increased risk of 1.13 and odds of 1.5 for ESKD in high-risk allele group in this research but the study by Franceschini et al. said individuals carrying two APOL1 risk genotype (high risk; 13% Africans Americans and 2% of U.S. Hispanics/Latinos of Caribbean background) have an increased risk for ESKD (odds ratio of ~7) and for FSGS odds ratio of 10 to 29 including HIV nephropathy [**29**]. The present study showed a lower mean age of ESKD and hemodialysis onset in high vs low-risk allele group. Furthermore, it revealed that heterozygous (HTZ) probands in the high-risk allele group have lower age rather than low-risk allele group. These results were in agreement with the study by Kanji et al the genetic variation in APOL1 has been associated with earlier onset of ESRD in African Americans without diabetes mellitus as the etiology of ESRD [30]. Clinical APOL1 testing in kidney transplantation possesses specific importance. First transplanted kidneys from living and deceased APOL1 high-risk donors fair slightly worse than non-APOL1 high-risk kidneys. Nevertheless, the allograft survival differences are modest, and the benefits of a transplant outweigh the hazards of remaining on dialysis. Second and importantly APOL1 high-risk genotype vs. low-risk genotype have lower eGFR at follow up and a faster decline in eGFR during the period after kidney donation [31]. Donor APOL1 genotype effects on allograft survival in deceased donor kidney transplant (DDKT) from black donors seem comparable with (or stronger than) established risk factors for allograft failure, such as HLA match, cold ischemia time, and sensitization on the basis of panel reactive antibodies. However, only 30% of the population possess APOL1 high-risk genotypes and may be at risk for early graft failure. Transplantation of a kidney from a healthy living donor with two APOL1 nephropathy risk variants has been associated with FSGS with early allograft failure in recipients as well as subsequent ESKD in the donor [32]. At present, some physicians are testing potential living donors for APOL1 risk alleles before kidney donation [27, 33]. Our study showed a lower mean age of KRT onset in high-risk allele group vs low-risk allele group, i.e. patient at the time of kidney transplantation had younger age in high-risk genotype. The majority of probands of the high-risk allele group possessed heterozygous G1G2 vs. low renal risk variants with homozygous G0G0. Moreover, four of seven patients (57.1%) with allograft kidney developed graft failure during post-transplant period follow-up and the really APOL1 genotype wasn't revealed risk on allograft failure. This result is in disagreement with the study by kanji et al [30]. Moreover, this result was in agreement with the study by Wei et al. that has demonstrated younger African American study of kidney disease and Hypertension (AASK) participants with high-risk genotype had the greatest KRT risk [34]. Genetic factors such as sickle cell trait have been investigated mostly among African Americans like of APOL1 genetic factors. Many studies have been shown that sickle cell trait (SCT) has an impact on the normal functioning of the kidneys among African Americans with some studies indicating a significant association between SCT and CKD. These studies revealed that genetic variants of APOL1 and non-muscle myosin heavy chain IIA (MHY9) in sickle cell disease (SCD) cause the progression of CKD to ESRD. The mean survival of patients with ESRD and SCD (sickle cell nephropathy) is estimated to be 4 years, even with dialytic treatment [34]. In the study by Masekoameng et al, they have not found a significant association between APOL1 and CKD in SCT patients, indicating that there is no evidence that SCT influences the relationship between APOL1 and CKD [35]. In our study sickle cell nephropathy did not was the cause of APOL1-associated nephropathy in the patients. Moreover, this point should be considered that the prevalence of SCD and SCT in the south area of our country (Iran) is high, but no study has been reported from Iran about APOL1 genetic factors at those populations. The only study of APOL1-associated nephropathy in Iran has been performed by Rikhtegar et al in the northwest of Iran. This descriptive, cross-sectional study examined the existence and frequency of G1 and G2 alleles of the APOL1 gene in Tabriz ESRD patients and concluded that polymorphisms of APOL1 in Tabriz ESRD patients are absent and this genetic change in this region is not a risk factor for CKD and progression to ESRD [36]. Recently, the subject of ESKD risk in living kidney donors possesses specific importance in APOL1-associated nephropathy in the African-Americans race. Reeves-Daniel et al in an original article investigated APOL1 risk variants in 106 Africans-Americans (AA) deceased organ donors and graft survival assessed in 136 resultant kidney transplants. The mean follow-up was 26.4±21.8 months. Twenty-two of 136 transplanted kidneys (16%) were from donors with two APOL1 nephropathy risk variants. Twenty-five grafts failed (18.3%) and eight (32%) had two APOL1 risk variants. Kidneys from AA deceased donors harboring two APOL1 risk variants failed more rapidly after renal transplantation than those with zero or one risk variants. If replicated, APOL1 genotyping could improve the donor selection process and maximize long-term renal allograft survival [37]. Moreover, Cohen et al predicted that the role of APOL1 genotype in living kidney donor outcomes will be equally important, if not more so. APOL1 shares structural and functional similarities with the Bcl2 family of proteins involved in apoptosis. Apoptosis may lead to glomerulosclerosis. Development of subclinical APOL1-associated kidney disease in native kidneys, with subsequent loss of graft function after donation in the presence of cold ischemia and nephrotoxic medications including calcineurin inhibitors. Additional risk factors for post-kidney transplant allograft failure include deceased donor category, preservation and recipient issues and exposure to BK polyomavirus infection. These factors could accelerate graft loss in the donor's kidneys harboring two APOL1 risk variants [38]. Kidney donor risk index (KDRI) compares a variety of donor factors to summarize the risk of graft failure after kidney transplant into a single number. The KDRI expresses the relative risk of kidney graft failure for a given donor compared to the median kidney donor from last year; values exceeding 1 have higher expected risk than the median donor and vice versa. The KDPI is a remapping of the KDRI onto a cumulative percentage scale, such that a donor with a KDPI of 80% has the higher expected risk of graft failure than 80% of all kidney donors recovered last year and can be used to compute KDPI and KDRI for a hypothetical or actual donor

[39]. KDPI > 85% is thought to be equivalent to an extended criteria donor (ECD) kidney (while the KDPI is based on 10 parameters, the historical ECD definition is based on only 4 parameters), but almost twice fewer donors were classified as ECD according to KDPI> 85% in comparison with the historical ECD [39]. The KDRI downgrades the quality of all kidneys donated by African ancestors. However, only of the population possess APOL1 high-risk genotypes and may be at risk for early graft failure [32]. Our study revealed allograft failure in four of seven transplanted kidneys (57.1%) during 3 years follow up that fifty-seven percent of these patients consisted of high-risk alleles in their APOL1 genetic test. This finding shows a high prevalence in comparison with the study by Reeves-Daniel et al that has related graft failure was 16% (22/136). Furthermore, relative risk and odds ratio for allograft failure in the present study was assessed 1 that showed no association between risk effect and odds of allograft failure on two copies of RRVs in APOL1-associated kidney disease. In the present study, donors of two transplanted kidneys (2/7, 28.5%) developed post-donation kidney failure in seven years following transplant nephrectomy and both donors belonged to the high-risk allele group. One of two donors (1/2, 50%) underwent renal transplantation from a deceased donor during follow up. Eleven of twenty patients (55%) with APOL1-associated nephropathy had viral infections (CMV, EBV and parvovirus B19) that two of them (2/11, 18.1%) were in hemodialysis patients and others in kidney transplant patients. Viral infections found in five deceased and one living kidney donors that the frequency of high and low-risk alleles in these patients was equal (three HR vs. three LR alleles). It appears that viral infections to operate as a second hit in more of half of these patients for developing APOL1-associated nephropathy. Endogenous and locally acting (not circulating) APOL1 protein appears to cause nephropathy based on data from kidney transplantation, cell biology and animal models. Most individuals with 2 APOL1 KRVs do not develop nephropathy, a modifier is required. Proven modifiers include HIV-induced alterations in the immune response and administration of interferons. In these settings, APOL1 expression levels are increased via the toll-like receptor 3 (TLR)-dependent pathway. B-cell lymphoma 2 (BCL-2)-antagonist/killer1 (BAK1) is a nuclear-encoded mitochondrial outer membrane protein. APOL1 is also present in mitochondria and its G1 and G2 KRVs can induce mitochondrial dysfunction. APOL1 KRVs induce cell death by mitochondrial translocation and opening of the mitochondrial permeability pore on the inner membrane, other pathways may exist. APOL1 is co-expressed with the mitochondrial outer membrane protein BAK1, involved in mitochondrial dynamics via stabilization of phosphate dikinase regulatory protein 1(pDRP1) on the mitochondrial outer membrane. Bax/Bak-dependent release of DDP/TIMM8a protein in the intramitochondrial space promotes DRP1-mediated mitochondrial fission and mitoptosis by redistributing pDRP to the mitochondrial outer membrane and inducing mitochondrial fission [40]. Another lesson in APOL-associated nephropathy to learn is a significant interaction effect between APOL1 high-risk genotype and particulate matter (PM) 2.5 for kidney disease. For demonstrating this hypothesis, Paranjpe et al performed a cohort study and enrolled 4800 black participants and found a significant interaction between PM2.5 and APOL1 ($p<0.001$). This suggests that although both PM 2.5 and APOL1 independently increase kidney disease in blacks, the effect of PM2.5 is worse in APOL1 high-risk individuals. Here, air pollution as an environmental factor may serve as a second hit for accentuating kidney disease in APOL1 predisposed individuals that inherited high risk [41].

Conclusions. APOL1-associated nephropathy is inherited in an autosomal recessive fashion, only a minority with high-risk genotypes develop CKD in their lifetime. Given to different distribution of ancestry and high prevalence of hypertension-attributed ESKD in the present study, this entity should be considered in other populations who are at risk of APOL1 genotype inheritance. Furthermore, our study revealed an increased risk of ESKD in patients with two copies of renal risk variants of APOL1 genotype. Prevalence of allograft failure in the present study was higher than previous studies but there was no risk associated with renal risk variants of the APOL1 gene.

Abbreviations. AA, Africans-Americans; African American study of kidney disease and Hypertension (AASK); AKD, acute kidney disease; AKI, Acute kidney injury; BAK1, B-cell lymphoma 2 (BCL-2)-antagonist/killer1; BM, bone marrow; BMI, body mass index; CKD, chronic kidney disease; CKD-EPI, chronic kidney disease-epidemiology collaboration; CMV, cytomegalovirus; CT, computed tomography; CTA, computed tomography angiography; CVD, cardiovascular disease; DDKT, deceased donor kidney transplant; DM, diabetes mellitus; pDRP1, phosphate dikinase regulatory protein 1; EBV, epstein-barr virus; ECD, extended criteria donor; eGFR, estimated glomerular filtration rate; ESKD, end-stage kidney disease; ESRD, stage renal disease; FGGS, focal global glomerulosclerosis; FSGS, focal segmental glomerulosclerosis; HA-APOL1, hypertension-attributed apolipoprotein 1; HDL, high density lipoprotein; HIV, human immunodeficiency virus; HIVAN, human immunodeficiency virus-associated nephropathy; HNF1A, hepatocyte nuclear factor 1α; HR, high risk; HTN, hypertension; HTZ, heterozygous; IF, interstitial fibrosis; IV, intravenous; KDPI, kidney donor profile index; KDRI, kidney donor risk index; KRT, kidney renal transplant; KRVs, kidney risk variants; LDL, low density lipoprotein; LR, low risk; MDRD, modification of diet in renal disease; MHY9, myosin heavy chain IIA; MRI, magnetic resonance imaging; NCEP-ATP III, National Cholesterol Education Program Adult Treatment Panel III; ND, normally distributed; NDRD, non-diabetic renal disease; PCR, protein-to-creatinine ratio; PER, protein excretion rate; PET, positron emission tomography; PM, particulate matter; PMC, PubMed central; RRVs, renal risk variants; SCD, sickle cell disease; SCr, serum creatinine; SCT, sickle cell trait; SD, standard deviation; SNP, single nucleotide polymorphism; TA; tubular atrophy; TLR, toll-like receptor.

Ethics approval and consent to participate. Authors of published articles stated that research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. They described that subjects (or their parents or guardians) were given their informed consent and the study protocol was approved by the institute's committee on human research.

Data availability. The author requested that the datasets become active in Figshare repository in DOI: 10.6084/m9.figshare.12587351 after article publication.

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