Original Article

Plausibility of the Application of SNPs Haplotype Analysis within the Renalase Gene in Children with a Family History of Chronic Kidney Disease

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ABSTRACT

Introduction: Renalase (RNLS) gene single nucleotide polymorphisms (SNPs) rs2296545 and rs10887800 are considered as one of the genetic risk factors for End stage renal disease (ESRD) in Egyptian children.

Aim of the study: Detect the association between the inferred haplotypes of the RNLS gene SNPs (rs2296545 and rs10887800) and ESRD in patients with a family history of chronic kidney disease (CKD).

Methods: The study population consisted of 65 Egyptian children (8 ESRD patients with a family history, 30 ESRD patients without family history and 27 controls). The distribution of RNLS haplotypes and linkage disequilibrium (LD) analysis between two SNPs were analyzed by LD2SNPing software.

Results: We found that ESRD patients with and without a family history had higher frequencies of C-G haplotype than controls. The odds ratio was 2.21(95% CI: 1.01-4.83) and 5.53(95% CI: 2.36 - 12.94) respectively. The C-A haplotype was significantly increased in the healthy controls and dialysis patients without a family history of CKD than patients with a family history, odds ratio of 0.25 (95% CI: 0.11-0.56) and 0.29 (95% CI: 0.11-0.71) respectively. Furthermore, Linkage disequilibrium analysis showed a higher degree of linkage between rs2296545 and rs10887800, D'=1.0 and r^2= 0.61].

Conclusion: The C-G haplotype represented by the SNPs rs2296545 and rs10887800 in the RNLS gene may have a role in the pathophysiology of CKD in patients with a family history.

Keywords: Haplotype analysis, Linkage Disequilibrium, ESRD, SNPs

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Introduction

Chronic kidney disease (CKD) is a major health issue that affects people all over the world. Despite its rarity in children, it can be a life-threatening condition with numerous long-term effects [1]. One of the risk factors for progression of CKD is having a family history of End stage renal disease (ESRD) [2].

Renalase (RNLS, FAD Dependent Amine Oxidase) is secreted into the blood from the kidney. It is encoded by RNLS gene which located on chromosome 10 (10q23.33) and has several polymorphic variants [3]. Recently, several studies suggested the association of SNPs within the RNLS gene with ESRD in adults [4-6]. One of these SNPs with identifier rs2296545 is reported for possible association with ESRD in Egyptian children with family history of CKD [7]. However, such assessment is based solely on a single nucleotide may not be sufficient to explain the risk of getting a disease and needs more enrichment. Therefore, there is a need to address the non-random association or linkage disequilibrium (LD) between two or more alleles and their haplotype frequencies, in particular those reported of involvement with CKD with family history.

Many developed software, such as LD2SNPing [8], PHASE [9], Haplo.stats [10] and Haploview [11], are used for inferring haplotypes and inspecting the nonrandom associations between two or more SNP variants with diseases.

To complement our recently published study [7], we assess the nonrandom association between rs2296545 & rs10887800 respective allele combinations in ESRD children with and without family history.

Methods

Study Participants

Thirty-eight children with ESRD and twenty-seven healthy controls were selected from the Pediatric Nephrology and Hemodialysis Unit of Al-Azhar University hospital. The study patients were divided into two subgroups: 8 ESRD patients with family history and 30 ESRD patients without a family history. As a cross sectional comparative study, only 8 patients with family history were available during period of study. Family history of chronic kidney disease included children with original kidney diseases and other forms of CKD (acquired, congenital, metabolic or unknown). Also, patients with original kidney diseases included first, second and third degree-relatives. The study was done during the period from March 2018 to October 2019 and was approved by the ethics committee of faculty of pharmacy, Ain Shams University (ACUC-FP-ASU), Egypt, under approval no. URHDIRB2020110301REC#42 and informed written consent was obtained in every case.

Haplotype analysis

Haplotypes were reconstructed by the LD2SNPing software from individual genotypes of rs2296545 and rs10887800 of the renalase gene [7]. Firstly, individual genotypes were input in two Excel file formats (.xls and .cvs). Then, LD2SNPing processed the input files of genotype data by estimating the frequencies of the haplotype SNP loci using an expectation-maximization algorithm (EM) [12]. The haplotype frequencies, Chi-square P. values, and LD statistics (D' and r2) of paired SNPs were produced. At this point,

comparisons among four haplotypes (G-A, G-G, C-G and C-A) in the studied groups and their respective frequencies as well as patterns of linkage disequilibrium were possible.

Statistical analysis

SPSS version 20 (SPSS Inc., Chicago, IL, USA) was used to evaluate the data. Groups with qualitative data were compared by using the Chi-square test. In addition, the association of the inferred haplotypes with a risk of family history of CKD was evaluated by odds ratio (OR) and 95% confidence interval (CI) which were calculated with non-conditioned logistic regression analysis. The p-value < 0.05 was considered significant.

RESULTS

The haplotype frequencies of the two renalase SNPs, rs2296545 and rs10887800, are showed for all studied groups. In general, haplotypes frequency distributions between all studied groups reveal significant differences. In details, the frequency of C-G haplotype was higher in ESRD patients with and without family history than healthy controls, and the C-A haplotype was significantly higher in the healthy controls. While In ESRD groups, the G-G haplotype had significantly higher frequency in patients without family history than those with family history of the disease **Table 1** and **Figure 1**.

To assess the strength of the association between haplotypes in all studied groups and ESRD, the test of the logistic regression analysis (OR and 95% CI) was applied **Table 2**. shows that the C-G haplotype was significantly high in ESRD patients with and without a family history when compared to healthy subjects with an Odds ratio of 2.21 (95% CI: 1.01 - 4.83) and 5.53 (95% CI: 2.36 - 12.94), respectively, while C-A haplotype showed increase in the healthy controls and patients without a family history of CKD than those with a family history, with an odds ratio of 0.25 (95% CI: 0.11 - 0.56) and 0.29 (95% CI: 0.11 - 0.71), respectively.

Analysis of linkage disequilibrium (LD) between renalase SNPs (rs2296545 and rs10887800) is shown in table 3. The results revealed that the D'/r² ratio has a significantly higher degree of LD at two sites in patients with a family history **Figures 2** and **3**.

Table 1: Haplotype frequency estimates in control, ESRD patients with and without a family history.

Haplotypes	Haplotype Frequency (%)			P. value
	Healthy Control	ESRD-FH	ESRD+FH	
G-A	35.2	18.3	50.0	**0.0006***
G-G	18.5	36.7	0.00	**< 0.0001***
C-G	13.0	28.3	37.5	**0.003***
C-A	33.3	16.7	12.5	**0.003***

The sequence of alleles depends on the following SNPs order respectively: rs2296545, rs10887800.
* P-value < 0.05 is significant. ESRD-FH=End-stage renal disease without a family history, ESRD+FH= End-stage renal disease with a family history.

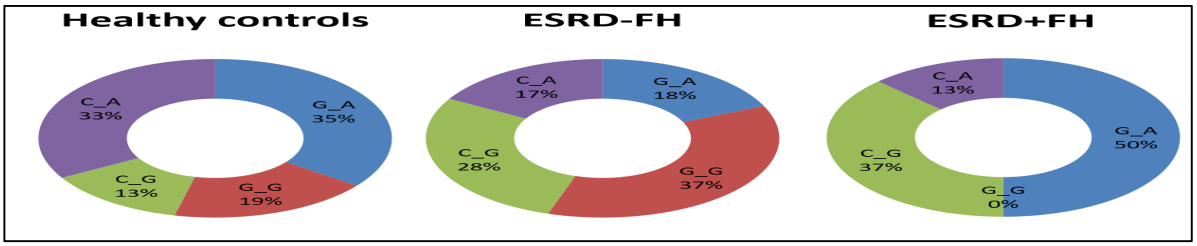

Figure 1: Haplotype distribution in the studied groups.

Table 2: Associations of haplotypes based on rs2296545 and rs10887800 with the risk of family history in Egyptian children with ESRD.

Haplo type	Frequency (%)			ESRD-FH vs. Controls		ESRD+FH vs. Controls		ESRD + FH vs. ESRD - FH	
	Healthy Control	ESRD -ve FH	ESRD +ve FH	OR (95% CI)	P. value	OR (95% CI)	P. value	OR (95% CI)	P. value
G-A	35.2	18.3	50.0	**1 (reference)**		**1 (reference)**		**1 (reference)**	
G-G	18.5	36.7	0.00	-	-	-	-	-	-
C-G	13.0	28.3	37.5	5.534 (2.365 - 12.948)	**0.0001***	2.2167 (1.016 - 4.833)	**0.04 ***	0.515 (0.251 - 1.058)	0.071
C-A	33.3	16.7	12.5	1.001 (0.443 - 2.264)	0.9968	0.2545 (0.115 - 0.560)	**0.0007***	0.290 (0.118 - 0.711)	**0.006***

The sequence of alleles depends on the following SNPs order respectively: rs2296545, rs10887800. OR: Odds Ratio; CI: Confidence Interval;
*P-value < 0.05 significant; ORs and 95% CIs were estimated by logistic regression analysis only for common haplotypes.
Haplotypes with frequency <1% were not addressed. ESRD-FH=End-stage renal disease without a family history,
ESRD+FH= End-stage renal disease with a family history.

Table 3: Linkage disequilibrium between (rs2296545- rs10887800) in ESRD patients. with /without family history.

Group		D'	r^2	X^2	P value
ESRD Patients	With family history	1.0	0.61	9.60	**0.002***
	without family history	0.048	0.001	0.090	0.765

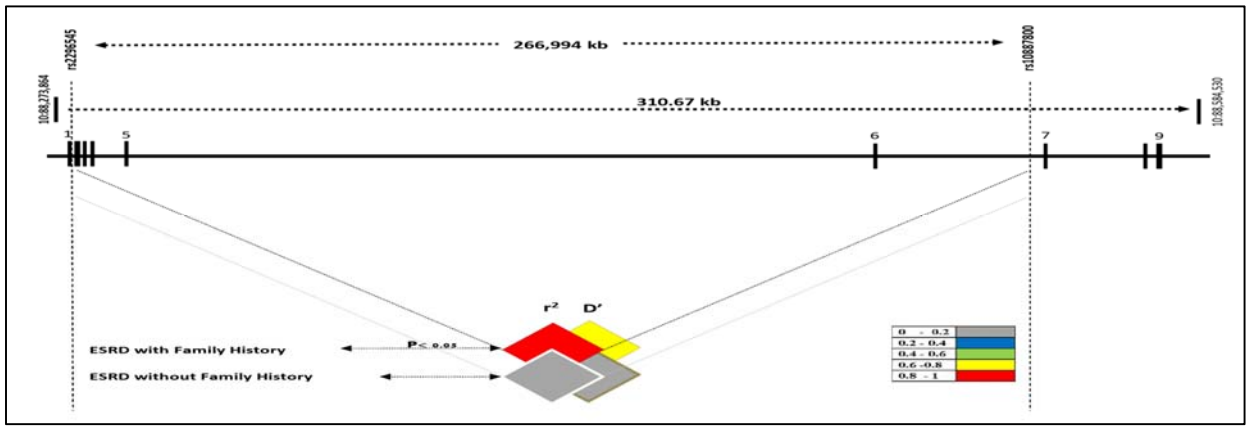

Figure 2: Linkage disequilibrium patterns (D' and r2) between analyzed SNPs in ESRD with/without family history in Egyptian children.

A genomic view of the renalase gene (310.67 kb) is shown in boldface horizontal line; exons are shown as vertical lines. Also, the locations of the SNPs used to construct haplotypes are indicated in exon 1 and intron 6. The left ligand represents a value by color.

Discussion

The association studies between renalase gene variants and susceptibility to familial CKD in Egyptian children are minimal. To date, the studies based on adults in the Egyptian population have demonstrated the importance of renalase gene variants on predisposition to ESRD development and hypertension **[4-8].** Also**,** Ghazy et al., **[7]** considered the association of renalase SNPs (rs2296545 and rs10887800) in children with a family history of CKD.

The current study investigated the association between renalase gene haplotypes and the susceptibility to familial CKD based on the previous results of Ghazy et al.**, [7].** Furthermore, linkage disequilibrium (LD) analysis was addressed between the two renalase gene SNPs (rs2296545 and rs10887800).

Our results highlighted that the C-G haplotype had a significant increase in dialysis children with and without a family history of CKD than the control group with an odds ratio of 2.21 (95% CI: 1.01 - 4.83) and 5.53 (95% CI: 2.36 - 12.94), respectively and this might consider a risk factor for the incidence of CKD. On the other hand, the C-A haplotype increased in the healthy controls and dialysis patients without a family history of CKD than patients with a family history. The odds ratio was 0.25 (95% CI: 0.11 - 0.56) and 0.29 (95% CI: 0.11 - 0.71), respectively, indicating a significant increase in the protective effect against familial aggregation of CKD.

In the same way, but in different disease, Li et al., **[13]** reported that the C-G haplotype was linked with predisposition to preeclampsia compared to women with or without pregnancy in the Chinese population. Also, reports by Li et al., **[14]** highlights that the C allele of rs2296545, in the haplotypes G-C and A-C, is markedly associated with hypertension and hypertensive patients with concomitant coronary heart disease (CHD) respectively.

Generally, we are aware that if a patient has a strong family history of CKD, this will lead to early initiation of treatment. Therefore, through our preliminary results, we advise parents with a positive family history to undergo early screening of their offspring for inspection of pathogenic/ benign combinations of haplotype variants. And it is worth mentioning here, that despite of the limited proportion of patients with family-history due to scarcity, it does not reduce the value and importance of the study.

Lastly, we recommend checking the association between the renalase gene polymorphisms and their relationship to other factors than family history (e.g., etiology and age of ESRD). Also, it is necessary to extend this type of investigation and do more SNVs enrichment analysis to define driver from passenger variants behind the disease and assess how they do mediate their pathogenic and/or benign effect.

Conclusion

The C-G haplotype of the renalase gene may carry a susceptibility role in patients suffering from end-stage renal disease (ESRD), while the C-A haplotype may be a protective factor.

ABBREVIATIONS

CI	Confidence interval		
CKD	Chronic Kidney Disease	**LD**	Linkage disequilibrium
CHD	Coronary Heart Disease	**OR**	Odds Ratio
ESRD+FH	End Stage Renal Disease with a Family History	**RNLS**	Renalase
		SNPs	Single Nucleotide Polymorphisms
ESRD –FH	End Stage Renal Disease without a Family History		

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AUTHORS' CONTRIBUTIONS

The submitted manuscript is the work of the author & co-author. All authors have contributed to authorship, have read and approved the manuscript.

Conception and design of study:
2nd and 4th authors.

Acquisition of data:
2nd author.

Analysis and/or interpretation of data:
4th and 1st authors.

Drafting the manuscript:
4th and 1st authors.

Revising the manuscript critically for important intellectual content:
3rd and 2nd.

Approval of the version of the manuscript to be published:
All authors revised and approved the manuscript content.

STATEMENTS

Ethics approval and consent to participate
This study protocol and the consents were approved and deemed sufficient by the Ethical Committee of Faculty of Pharmacy; Ain Shams University (ACUC-FP-ASU), Egypt, under approval number URHDIRB2020110301 and informed written consent was obtained in every case from their legal guardians.

Consent for publication
"Not applicable"

Availability of data and material
"Not applicable"

Conflict of interest
The authors declare no conflict of interest.

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