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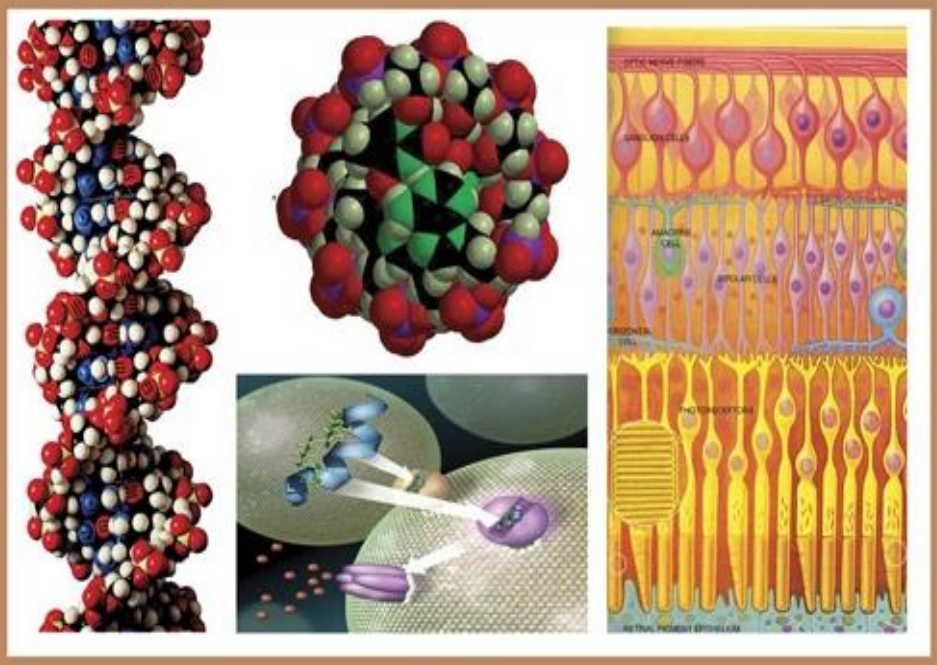

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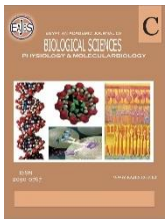

A Genetic Risk Factor in Egyptian Children with A Family History of End-Stage Renal Disease

Ahmed Ghazy[1], Manal Abdel- Salam[2], Mohamed, I. Rady[3]and Mahmoud A. Khalifa[1*]

1-Bioinformatics & Molecular Biology Unit, Zoology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt.

2- Nephrology Unit, Pediatric Department, Faculty of Medicine, Al-Azhar University, Cairo, Egypt.

3-Cytology and Histology Lab., Zoology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt.

*E. Mail : mahmoud.khalifa@azhar.edu.eg

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ABSTRACT

Background: Family history of end-stage renal disease (ESRD) is a significant risk factor for the subsequent development of nephropathy. Recently, several studies have shed light on single-nucleotide polymorphisms (SNPs) within the renalase gene that is believed to be associated with this disease.

Objective: Investigate the association of the susceptibility of rs2296545 and rs10887800 genotypes/alleles within the renalase (RNLS) gene in children with family history of ESRD.

Methods: The study included eight children on regular hemodialysis with a family history of chronic kidney disease (CKD), thirty children their ages range from 4-18 years without a family history, and 27 controls. We evaluated demographic and biochemical information, and renalase genotypes/alleles of rs2296545 and rs10887800 by the Sanger sequencing method. All data, including genotypes and alleles frequencies, were analyzed.

Results: The statistical evaluation of the rs2296545 showed significant increase relatively in CC genotypes and C alleles in dialyzed patients with a family history of CKD to those without family history with odds ratio of 7.70 (95%CI=3.35-17.72) and 3.16(95% CI=1.77-5.63); respectively. In rs10887800, there was a significant increase in GC and GG genotype distribution in dialyzed patients without a family history of CKD compared to the control group, odds ratio of 2.095 (95% CI=1.0057 - 5.265) and 2.301 (1.0057 - 5.265) (1.0322 - 4.254); respectively.

Conclusion: rs2296545 C allele may be considered as one of the genetic risk factors for ESRD pathogenesis in children with family history, and its corresponding G allele may have a protective role.

INTRODUCTION

As one of the public health problems in Egypt, pediatric chronic kidney disease (CKD) is the result of a variety of causes (Safouh *et al.* 2015).). However, the degree of CKD varies geographically due to environmental and genetic factors.

Late by end of the last century, a study on family history of CKD highlighted end-stage renal disease (ESRD) as a risk factor (Ferguson *et al*., 1988). At the onset of this century, few reports indicated that family members of patients with end-stage renal disease (ESRD) have increased prevalence of CKD (Freedman *et al*., 2001, Gumprecht *et al.*, 2003 and Freedman *et al.*, 2005). In 2012, a study by McClellan *et al*. showed up the associations between a family history of ESRD and various demographic and clinical features in the REGARDS (Reasons for Geographic and Racial Differences in Stroke) study. While some studies have shown that genetic predisposition to end-stage renal disease is strongly linked to race (Kao *et al.*, 2008; Freedman *et al.*, 1999).

As of 2012, few studies have shed light on single nucleotide polymorphisms (SNPs)/ variants (SNVs) within the RNLS gene and their possible association with CKD and ESRD. For instance, rs2296545, a SNP position in exon 1 that as reported by Ahlawat *et al*., is a susceptibility variant in hypertensive nephrosclerosis subjects from the North Indian population (Ahlawat *et al.*, 2012), a risk factor for developing the end-stage renal disease (ESRD) in the Egyptian population (Rezk *et al.*, 2015 and Abou Zaghla *et al.*, 2020) and a risk factor for hypertension in ESRD in Egyptian population (Abou Zaghla *et al.*, 2020 and Ghazy *et al.*, unpublished data).

Also, the SNP position, rs10887800 in intron 6 within the same gene is reported as a susceptibility variant in various diseases, such as developing ESRD in Egypt (Abdallah and Sabry, 2013; Kandil *et al.*, 2018 and Abou Zaghla *et al.*, 2020) and for hypertension in ESRD Polish Caucasian (Stec *et al.*, 2012).

Here, we investigate the distribution of renalase gene SNVs, rs2296545 and rs10887800, by Sanger sequencing among dialysis cases with/ without family history of CKD in Egyptian children.

MATERIALS AND METHODS

Subjects*:*

This is a case-control study. Samples were selected from the Pediatric Nephrology & Hemodialysis Unit from Al-Zahraa Hospital, Al-Azhar University, Cairo, Egypt between March 2018 to August 2019.

The Subjects Included Two Groups:

Hemodialysis Group: Included 38 children (eGFR < 15 mL/min/ 1.73 m2) on regular hemodialysis more than three months at the time of the study (Becker *et al.*, 2012), for 4 hours, three times weekly, with polysulfone low flux membrane dialyzer by 4008 Fresenius and 4008 S-classic machines. Verbal consent of the parents was taken, and they had the right to withdraw from the study at any tone without giving any reasons. We divided the study cases into two subgroups**:** 8 dialyzed patients with a family history of CKD and 30 without those.

Control group: Included 27 healthy children matched age and sex with ESRD.

The Inclusion Criteria: children with ESRD on regular hemodialysis their ages ranged from (2-18) years.

The Exclusion Criteria: We exclude children with chronic diseases other than CKD.

Ethical Consideration: This study was approved by Faculty of Pharmacy, Ain Shams University (ACUC-FP-ASU)**,** Egypt, under approval Number URHDIRB2020110301**.**

Demographic and Biochemical Analysis:

Demographics data of all subjects were collected as age, gender, BMI, presence of hypertension, blood pressure, and mortality. Venous peripheral blood was collected after an overnight fast of at least 12 h before the mid-week HD session and divided into two portions; one portion was directly collected on potassium

ethylene diamine tetraacetic acid (K3EDTA) for hematological and genotyping studies, while the other portion for biochemical studies, centrifuged to obtain serum:(creatinine, urea, potassium, sodium, calcium, phosphate).

Genetic Analysis:

DNA was isolated from EDTA whole blood sample using Qiaamp DNA Mini Kit. Cat. No. 51304, Lot No. 148026863, Qiagen, Germany, and then stored at −20 ℃ till the time of use. Thermocycler Gene Amp 9700 (USA) was used to amplify a specific fragment of renalase gene (RNLS) using two pairs of oligonucleotide primers as described by (Zhao *et al.* 2007). The following primers for amplification: for rs2296545 forward 5'GGAAGTCCCCGATCACGT GAC-3' and reverse 5'TGCTGTGTGGG ACAAGGCTGA-3' and for rs10887800 forward 5'CAGGAAAGAAAGAGTTG ACAT-3'and reverse 5'AAGTTGTTCC AGCTACTGT-3'. The polymerase chain reaction (PCR) thermal program was set as follows: Initial denaturation at 94 ℃ for 1 min, then 35 cycles of denaturation at 94 ℃ for 1 min, annealing at 60 ℃ for 1 min, extension at 72 ℃ for 1 min and final extension step at 72 ℃ for 10 min. PCR amplifications were carried out in 25 µl reaction volume including 0.5 µl of each primer (10 p mole/µl), 12.5 µl 2× PCR Master Mix solution (i-Taq™), 10.5 H2O and one µl of template DNA.

The standard PCR products were kept at four ℃ until being electrophoresed and purified using the QIA quick PCR purification kit protocol. After purification of the PCR product, a second PCR was performed using Bigdye Terminator V5.1 Cycle Sequencing Kit. DNA sequencing was carried out using 3500 genetic analyzers (Applied Biosystems). The proofread generated sequences from the sequencer in this study by MEGA 7.0.14 software (Kumar *et al.*, 2016), and the polymorphisms were identified.

Statistical Analysis:

We used statistical software Package for Social Science (IBM SPSS) version 20. Qualitative data were presented as numbers and percentages while quantitative data were presented as mean and standard deviations (X±SD) when parametric and median with interquartile range (IQR) when non-parametric. The comparison between two groups with qualitative data was done by using the Chi-square test. The comparison between two independent groups with quantitative data was done by using an independent t-test when the data were parametric, Mann-Whitney and Kruskal-Wallis tests when the data were nonparametric.

We used Haploview 4.2 for Hardy–Weinberg equilibrium **(**HWE) test, genotype analysis, and allele frequencies. The association of renalase gene SNPs with a family history of CKD was evaluated by odd risk and 95% confidence interval (CI) which were calculated with non-conditioned logistic regression analysis. General clinical data and various biochemical investigators and the relationship between renalase SNP genotypes were compared by linear regression. The p-value was considered significant as $P < 0.05$.

RESULTS

Demographic Analysis:

The demographic and clinical characteristics of the studied groups are mentioned in Table 1. There were no significant differences in gender, BMI, SBP, DBP, in addition, there were no significant differences in the hematological and biochemical investigations between ESRD subjects with a family history of CKD and those without. However, there was a significant decrease in age and a high percentage of mortality rate in patients with a family history of CKD.

Table 1: Baseline characteristics among studied groups

Variables		Patients without a family history of ESRD N= (30)	Patients with a family history of ESRD N= (8)	P. value
Demographics parameters				
Age (year)		11.9±2.6	9.7±2.6	**0.02***
Gender	Female	12(40.0%)	3(37.5%)	0.6
	Male	18(60.0%)	5(62.5%)	
BMI (kg/m2)		16.63±3.9	15.48±2.3	0.2
Hypertension (%)		14(46.6%)	3(37.5%)	0.3
SBP (mm Hg)		126.52±24.97	121±30.8	0.3
DBP (mm Hg)		85±19.038	80±24.49	0.2
Mortality (%)		2(6.0%)	2(25.0%)	**0.0006****
Hematological parameters				
Hemoglobin (g/dL)		9.31±2.01	8.71±1.44	0.2
RBCs (10^6/mm^3)		3.425±0.68	3.41±0.84	0.4
Hematocrit (%)		28.74±6.18	26.48±5.35	0.1
MCV (fL)		84.09±8.218	78.58±6.88	0.1
MCH (pg)		27.27±2.959	26.07±3.180	0.3
MCHC(g/dL)		32.45±1.807	33.15±2.56	0.4
Platelet count(10^3/mm^3)		204±54.09	219±76.21	0.2
TLC (10^3/mm^3)		6.148±1.62	6.56±2.25	0.2
ESR (mm)		38(22-50)	19(10- 65)	0.2
Biochemical parameters				
Urea (mg/dl)		157.3±72.06	189.14±80.36	0.1
Creatinine (mg/dL)		8.698±2.836	7.10±2.035	0.09
Calcium (mg/dL		8.739±1.102	8.54±1.19	0.3
Phosphate (mg/dL)		6.504±1.493	7.12±1.48	0.1
Potassium (mEq/l)		5.357±1.107	5.64±0.81	0.2
Sodium (mEq/l)		139±4.88	143.28±6.019	0.06

Abbreviations: **SBP**: Systolic Blood Pressure, **DBP**: Diastolic Blood Pressure, **BMI**: Body Mass Index, **MCV**: Mean cell volume **MCHC**, mean corpuscular hemoglobin concentration **MCH:** mean corpuscular hemoglobin, **TLC:** total leucocyte count, ESR, erythrocyte sedimentation rate, RBCs red blood corpuscles. * p. value <0.05 is significant.

Genotype Identification of each SNP:

Flanking region of renalase gene SNPs (rs1088780 and rs2296545) was specifically targeted using pairs of primers and amplified using PCR protocol (see Materials and Methods). The size length of the amplified PCR products was 554 and 209 bp (Figs. 1 and 2). A total of 65 PCR products of the renalase gene SNPs were sequenced by classical Sanger sequencing on the sequencer described in Materials and Methods. The raw sequence files were subjected to quality assessment and assembly against a reference sequence to determine the genotype (Figs. 3 and 4).

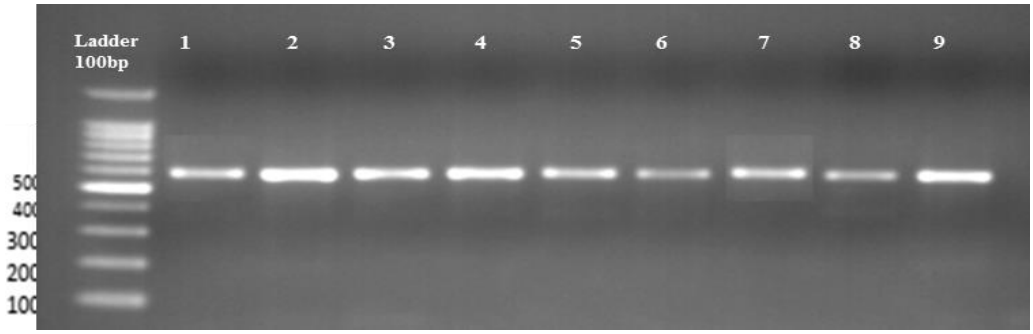

Fig. 1. Agarose gel electrophoresis (2% - 1X TBE) introducing PCR products of flanking region of rs1088780 (554bp). Lane 1 contains 100 base pair DNA ladder markers**.**

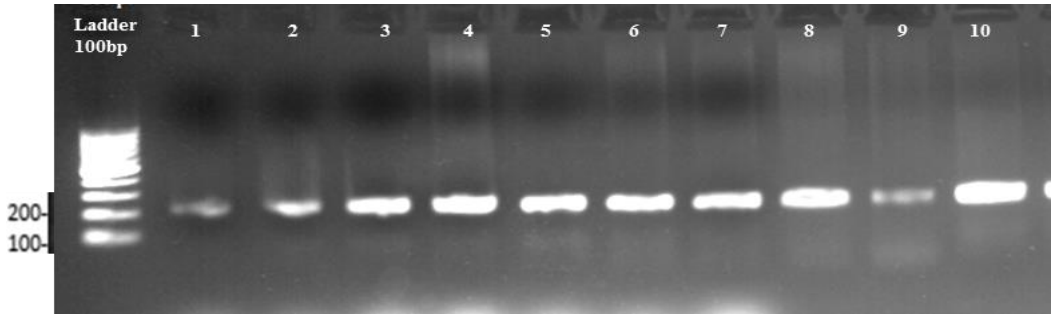

Fig. 2. Agarose gel electrophoresis (2% - 1X TBE) introducing PCR products of flanking region of rs2296545 (209bp). Lane 1 contains 100 base pair DNA ladder markers.

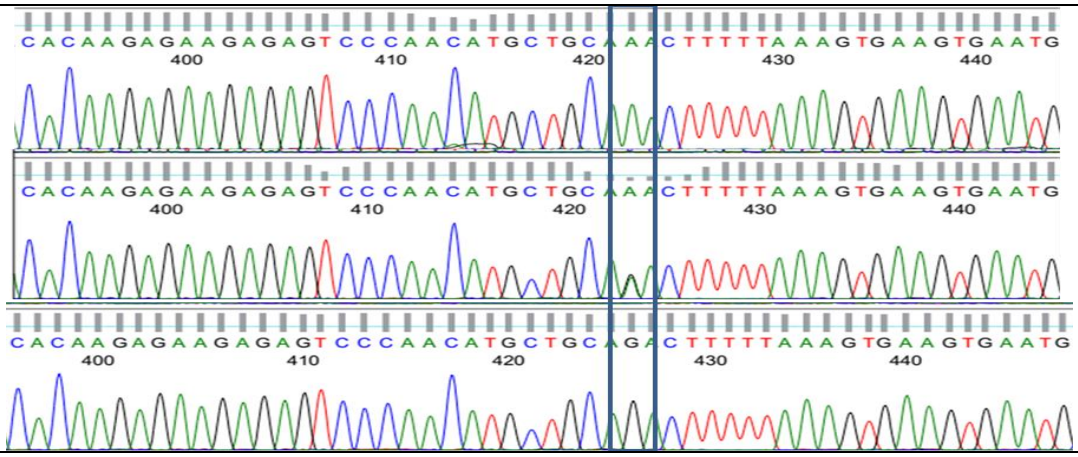

Fig.3. A Sanger sequencing chromatogram generated by ABI Genetic Analyzer showing 3 different genotype sequences of PCR products of renalase gene SNP (rs10887800).

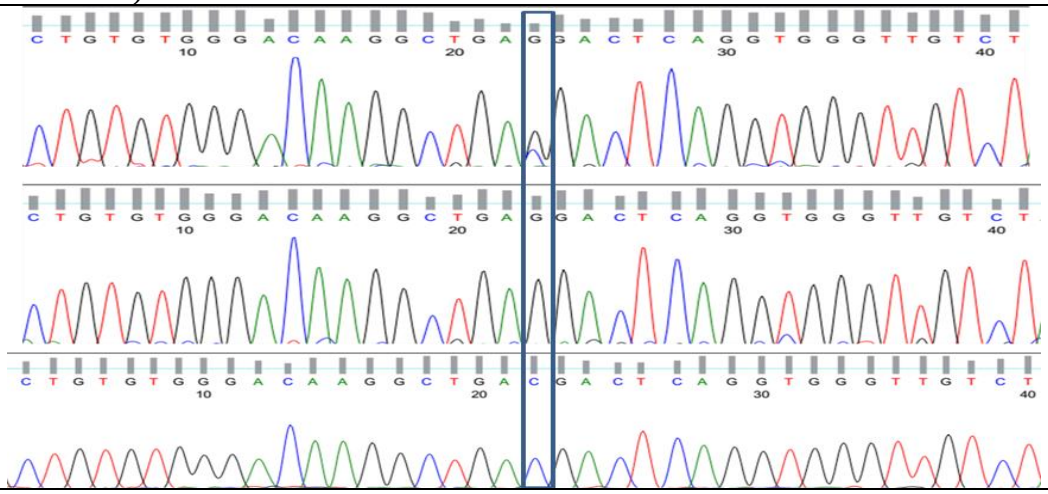

Fig. 4. A Sanger sequencing chromatogram generated by ABI Genetic Analyzer showing 3 different genotype sequences of PCR products of renalase gene SNP (rs2296545).

Hardy–Weinberg Equilibrium (HWE) test of SNPs:

In this study, tests of Hardy–Weinberg equilibrium (HWE) were carried out for two loci of renalase gene polymorphism (rs2296545 and rs10887800) among controls as shown (Table 2). The genotype distribution of renalase polymorphisms rs2296545and rs10887800 was checked in the control

group, and the results showed their consistency with HWE (P=0.61 and 0.73, respectively). Therefore, our control group was a typical Mendelian population.

Table 2: Comparison between observed genotypes frequencies of the control in our study and expected frequency obtained using Hardy-Weinberg.

SNPs	Genotype	n=27	Observed results (Current Study)	Expected results (HWE)	X^2	p.
rs2296545	**CC**	13	0.481	0.469	0.25	0.61
	CG	11	0.407	0.431		
	GG	3	0.111	0.100		
rs10887800	**AA**	8	0.296	0.291	0.11	0.73
	GA	13	0.48.1	0.497		
	GG	6	0.222	0.212		
Total		27	1.000	1.000		

HWE = Hardy-Weinberg equilibrium; n=Number,
P. Value is depending on the X^2 test, *p. value <0.05 is significant,

Analysis of Renalase Genotyping:

Table (3) shows significant differences in genotype distribution and allele frequency of renalase gene (rs2296545) between groups (control and patients with and without a family history of ESRD) (Figures 5 and 6), while there are no significant differences in genotypes and alleles of renalase rs10887800.

For the rs2296545 SNP, table (4) shows a significant increase in the distribution of genotypes (GC and CC) and frequency of C allele in the patients with a family history of CKD with odds ratio 6.94 (95%CI=3.19-15.08), 7.70 (95%CI=3.35-17.72), and 3.16(95% CI=1.77-5.63) compared to the group without a family history of ESRD. However, there was no significant difference in the distribution of genotypes and alleles of rs10887800 SNP among the patient groups.

Furthermore, in comparison, the patients without a family history of CKD and control groups were compared in terms of genotypes and allele frequencies; there was no significant difference for rs2296545 polymorphism. However, there was a significant increase in the frequencies of renalase (rs10887800) GG and GA genotype in patients without a family history of CKD compared to the control group with an odds ratio of 2.095 (95% CI=1.0322 - 4.254) and 2.301 (95% CI=1.0057 - 5.265) (Table 5).

Table 6 shows that there is a significant increase in the CC genotype and C allele in the patients with a family history of CKD when compared with healthy subjects with an Odds ratio of 13.45(95% CI: 3.35-17.72) and 3.618 (95% CI: 2.017-6.489). However, there was no significant difference in the distribution of genotypes and alleles of rs10887800 SNP among patients with a family history of ESRD and control.

Table 3: Genotype distributions and allele frequencies of renalase rs2296545 and rs10887800 polymorphisms between patients with and without a family history of ESRD.

Renalase SNPs	Healthy	Patients (N=38)		P. value
		Without a family history of ESRD (N=30)	With a family history of ESRD (N=8)	
rs2296545				
GG	13(48.2%)	15(50%)	1(12.5%)	< 0.0001**
GC	11(40.7%)	9(30%)	4 (50%)	0.08
CC	3(11.1%)	6(20%)	3 (37.5%)	0.0003**
G Allele	37(68.5%)	39(65%)	6(37.5%)	< 0.0001**
C Allele	17(31.5%)	21(35%)	10(62.5%)	
rs10887800				
AA	8(29.6%)	5(16.7%)	2(25%)	0.166
GA	13(48.1%)	17(56.7%)	4(50%)	0.6490
GG	6(22.2%)	8(26.6%)	2(25%)	0.8369
A Allele	29(53.7%)	27(45.0 %)	8(50.0%)	0.4434
G Allele	25(46.3%)	33(55.0%)	8(50.0%)	

OR: Odds Ratio; CI: Confidence Interval; * p. value <0.05 is significant

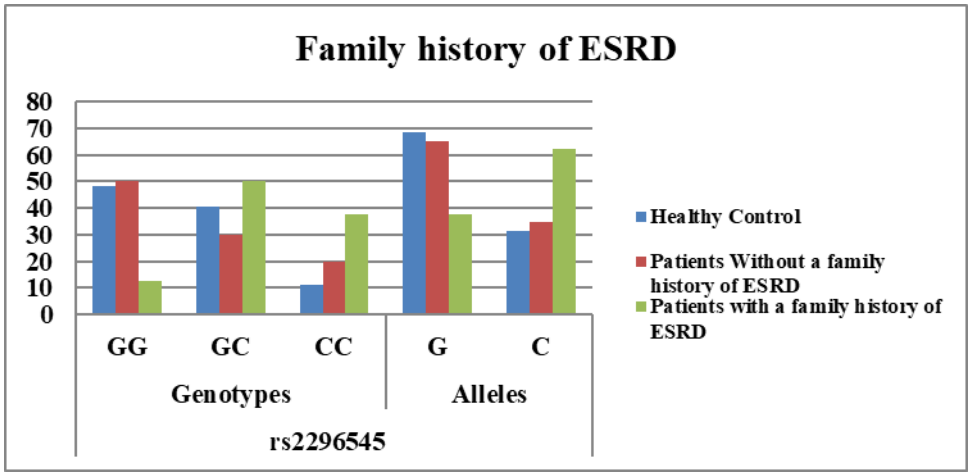

Fig. 5: Genotype distributions and allele frequencies of renalase rs2296545 polymorphism in control and patients with and without a family history of ESRD.

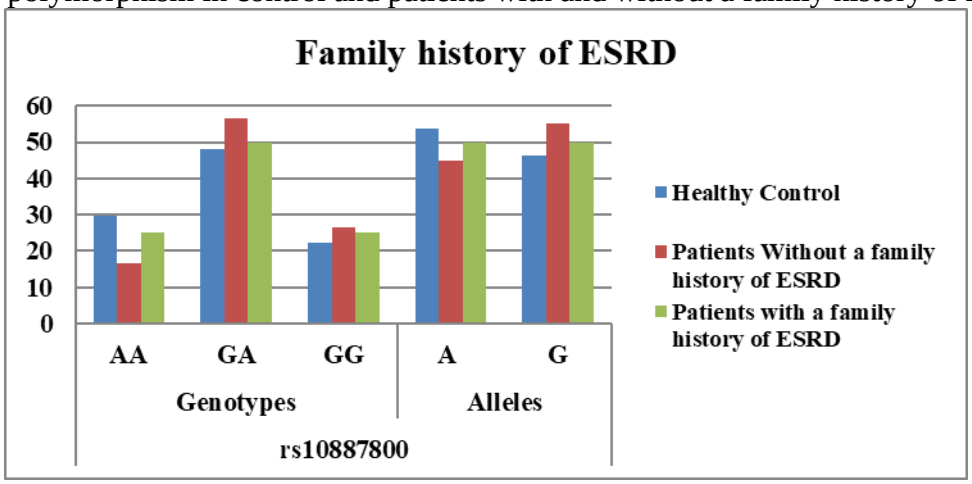

Fig. 6: Genotype distributions and allele frequencies of renalase rs10887800 polymorphism in control and patients with and without a family history of ESRD.

Table 4: Comparison of genotype distributions and allele frequencies of renalase rs2296545 and rs10887800 polymorphisms between patients with and without a family history of ESRD.

Renalase SNPs	Patients (N=38)		OR (95% C.I)	P. value
	Without a family history of ESRD (N=30)	With a family history of ESRD (N=8)		
rs2296545				
GG	15(50%)	1(12.5%)	1(reference)	
GC	9(30%)	4 (50%)	6.94(3.19-15.08)	**<0.0001****
CC	6(20%)	3 (37.5%)	7.70(3.35-17.72)	****<0.0001**
G Allele	39(65%)	6(37.5%)	1(reference)	
C Allele	21(35%)	10(62.5%)	3.16(1.77-5.63)	**0.0001****
rs10887800				
AA	5(16.7%)	2(25%)	1(reference)	
GA	17(56.7%)	4(50%)	0.596(0.289 - 1.230)	0.161
GG	8(26.6%)	2(25%)	0.629(0.276 - 1.432)	0.27
A Allele	27(45.0 %)	8(50%)	1(reference)	
G Allele	33(55.0%)	8(50%)	0.818(0.469 - 1.426)	0.479

* P. value <0.05 is significant, OR: Odds Ratio; CI: Confidence Interval.

Table 5: Comparison of genotype distributions and allele frequencies of renalase rs2296545 and rs10887800 polymorphisms between healthy Control and patients without a family history of ESRD.

Renalase SNPs	Healthy Control (N=27)	Patients without a family history of ESRD (N=30)	OR (95% C.I)	P. value
rs2296545				
GG	13(48.2%)	15(50%)	1(reference)	
GC	11(40.7%)	9(30%)	0.70 (0.379 -1.299)	0.2
CC	3(11.1%)	6(20%)	1.74 (0.756 - 4.025)	0.1
G Allele	37(68.5%)	39(65%)	1(reference)	
C Allele	17(31.5%)	21(35%)	1.14 (0.635 - 2.059)	0.6
rs10887800				
AA	8(29.6%)	5(16.7%)	1(reference)	
GA	13(48.1%)	17(56.7%)	2.095 (1.0322 - 4.254)	**0.04***
GG	6(22.2%)	8(26.6%)	2.301 (1.0057 - 5.265)	**0.04***
A Allele	29(53.7%)	27(45.0 %)	1(reference)	
G Allele	25(0.46.3%)	33(55.0%)	1.4348 (0.8223 - 2.5035)	0.203

* P. value <0.05 is significant, OR: Odds Ratio; CI: Confidence Interval.

Table 6: Comparison of genotype distributions and allele frequencies of renalase rs2296545 and rs10887800 polymorphisms between healthy control and patients with a family history of ESRD.

Renalase SNPs	Healthy Control (N=27)	Patients with a family history of ESRD (N=8)	OR (95% C.I)	P. value
rs2296545				
GG	13(48.2%)	1(12.5%)	1(reference)	
GC	11(40.7%)	4 (50%)	4.878(2.291 - 10.38)	**< 0.0001****
CC	3(11.1%)	3 (37.5%)	13.45(5.342 - 33.88)	**< 0.0001****
G Allele	37(68.5%)	6(37.5%)	1(reference)	
C Allele	17(31.5%)	10(62.5%)	3.618 (2.017 - 6.489)	**< 0.0001****
rs10887800				
AA	8(29.6%)	2(25%)	1(reference)	
GA	13(48.1%)	4(50%)	1.250(0.6446 - 2.424)	0.5090
GG	6(22.2%)	2(25%)	1.363(0.624 - 2.977)	0.4364
A Allele	29(53.7%)	8(50%)	1(reference)	
G Allele	25(46.3%)	8(50%)	1.173(0.673 - 2.045)	0.5714

* P. value <0.05 is significant, OR: Odds Ratio; CI: Confidence Interval.

Comparative Analysis of Renalase Genotypes with General Clinical and Biochemical Data:

We evaluated the clinical and biochemical characteristics in participants with ESRD among different genotypes in the renalase gene SNPs (rs2296545 and rs10887800) in an exploratory analysis (Tables 7 and 8). Body mass index (BMI) varies considerably among genotypes of rs2296545, while there are no significant variations in biochemical parameters among different genotypes. However, there are no statistically significant differences in clinical and biochemical characteristics among different genotypes at the rs10887800 locus.

Table 7: Clinical and biochemical characteristics of participants with ESRD in different genotypes of rs2296545 SNP in the renalase gene.

NO	Variable	CC	CG	GG	P value
1	BMI (kg/m2)	17.09±4.10	14.81±2.36	17.37±2.94	**0.02***
2	SBP (mm Hg)	123.33±26.64	131.00±25.58	122.86±24.30	0.58
3	DBP (mm Hg)	82.67±20.52	87.00±20.58	81.43±17.73	0.79
4	Hemoglobin (g/dL)	9.37±1.94	8.81±1.02	9.35±2.56	0.57
5	Urea (mg/dl)	158.93±78.61	164.50±43.57	174.57±95.34	0.45
6	Creatinine (mg/dL)	8.98±2.32	6.66±1.85	9.57±3.47	0.06
7	Calcium (mg/Dl	8.30±1.06	9.10±0.96	9.00±1.20	0.33
8	Phosphate (mg/dL)	6.53±1.62	6.88±1.46	6.43±1.16	0.36
9	Potassium (mEq/l)	5.28±1.23	5.91±0.79	5.31±0.79	0.26
10	Sodium (mEq/l)	139.27±5.08	139.70±3.53	141.71±7.61	0.11

Abbreviations: SBP: Systolic Blood Pressure**, DBP:** Diastolic Blood Pressure**, BMI:** Body Mass Index

Data are represented as Mean ± SD; the data were analyzed by a Kruskal-Wallis test. * p. value <0.05 is significant.

Table 8: Clinical and biochemical characteristics of participants with ESRD in different genotypes of rs10887800 SNP in the renalase gene.

NO	Variable	AA	AG	GG	P. value
1	BMI (kg/m^2)	16.11±2.5	16.69±4.319	17±1.9	0.8
2	SBP (mm Hg)	121.66±31	126.25±23.3	120±28	0.8
3	DBP (mm Hg)	81.66±28	83.75±17.46	81±21	0.7
4	Hemoglobin (g/dL)	9.03±1	9.18±2.004	9.4±2.3	0.9
5	Urea (mg/dl)	212.5±118	150.18±62.4	156±40	0.5
6	Creatinine (mg/dL)	8.69±4.3	8.65±2.396	7.2±2	0.4
7	Calcium (mg/dL	8.35±0.8	8.58±1.121	8.8±1	0.5
8	Phosphate (mg/dL)	5.71±1.8	7.02±1.481	6.9±1.4	0.3
9	Potassium (mEq/l)	5.15±0.8	5.78±1.042	5±1.1	0.2
10	Sodium (mEq/l)	139.5±5.3	139±5.657	143±4.5	0.3

Abbreviations: SBP: Systolic Blood Pressure**, DBP:** Diastolic Blood Pressure**, BMI:** Body Mass Index

Data are represented as Mean ± SD and median (IQR); the data were analyzed by a Kruskal-Wallis test. * P. value <0.05 is significant.

DISCUSSION

The possible effects of the aforementioned genotypic variants are being evaluated to reveal their nature and whether they have a pathogenic, protective, or neutral tone. Here, we attempt to highlight the effect of both rs2296545 and rs10887800 on the risk of family history with ESRD in Egyptian children. Also, as possible additional step to routine biochemical examinations, if not a prognostic signal, inspecting these genetic loci for candidate alleles that are possibly characterizing those suspicious

cases aiming to improve strategies for prognosis.

Our data assessment clearly indicates that rs2296545 genotypes/alleles CC/C are higher significantly in dialysis cases with a family history of CKD than those without family history and healthy control. The latter perhaps explains the association of such a genetic variant with the development of kidney failure in Egyptian children with a family history of CKD. In the same context, previous studies have dealt with this genetic factor and association with different causal cases as; essential hypertension in the Han Chinese population (Zhao *et al.*, 2007), hypertensive nephrosclerosis in the North Indian population (Ahlawat *et al.*, 2012), risk factor for hypertension in the Egyptian population with ESRD (Abou Zaghla *et al*., 2020, and Ghazy *et al.*, unpublished data), or just risk factor with CKD (Rezk *et al.*, 2015).

Moreover, regarding rs10887800, results show that GG and GA genotype are higher significantly in dialysis patients without a family history of CKD compared to healthy control. This indicates that this variant plays no role in the incidence of CKD through family history of the Egyptian children. In the same context, several studies announced that GG genotype and G allele might increase its susceptibility with development of ESRD in Egyptian patients **(Abdallah and Sabry, 2013)** and a risk factor for developing hypertension in ESRD **(Stec *et al.* 2012 and Ghazy *et al.*, unpublished data).** On the contrary, reports on an Egyptian case study by **Abou Zaghla *et al.* (2020)** and **Kandil *et al.* (2018)** indicate that carriers of the AA genotype might be at risk of developing ESRD.

These findings ascertain that the C allele has a risk factor that in part play an essential role in the development and pathogenesis of CKD in patients with family history. So, we recommend that the patients diagnosed with CKD should undergo testing for these SNPs that may predict the early susceptibility against the progression to ESRD. This may be best for children with a family history of this disease to protect against the risk of CKD.

In conclusion, CC genotype and C allele of rs2296545 of the renalase gene polymorphism may be a susceptibility locus for incidence of CKD in the patients with family history.

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ARABIC SUMMARY

عامل خطورة وراثي في الأطفال المصريين الذين لديهم تاريخ عائلي للإصابة بمرض الداء الكلوي بمراحله الأخيرة

أحمد غازي[1]، منال عبد السلام[2] ، محمد ابراهيم راضى[3] ،محمود أمين خليفة[1]

1 .وحدة المعلوماتية الحيوية والبيولوجيا الجزيئية - قسم علم الحيوان - كلية العلوم (بنين) - جامعة الأزهر – القاهرة - مصر

2.وحدة الكلى – قسم طب الاطفال - كلية الطب (بنات) - جامعة الأزهر – القاهرة – مصر

3. معمل الخلية والأنسجة - قسم علم الحيوان - كلية العلوم (بنين) - جامعة الأزهر – القاهرة - مصر

E-mail: mahmoud.khalifa@azhar.edu.eg

الخلفية العلمية: يعد التاريخ العائلي للإصابة بالمرحلة الأخيرة من مرض الكلى عامل خطورة كبير للتطور اللاحق لاعتلال الكلى. تم مؤخرًا نشر العديد من تعدد أشكال النيوكليوتيد المفردة (SNP) داخل جين الرينياليز الذي يستهدف هذا المرض.

الهدف: التحقق من ارتباط الأنماط الجينية rs2296545 و rs10887800 لجين الرينياليز مع خطر التاريخ العائلي للإصابة بمرض الكلى في المرحلة الأخيرة في الأطفال المصريين.

الطريقة: اشتملت الدراسة على ثمانية أطفال خضعوا لغسيل الكلى المنتظم مع تاريخ عائلي للإصابة بمرض الكلى المزمن، وثلاثين طفلاً تتراوح أعمارهم بين 4-18 عامًا دون تاريخ عائلي، و27 ضوابط صحية. قمنا بتقييم المعلومات الديموغرافية والكيميائية الحيوية وتم تحديد الأنماط الجينية rs2296545 و rs10887800 لـجين الرينياليز بواسطة قراءة التتابع النيوكليوتيدية بطريقة سنجر. قمنا بتحليل جميع البيانات بما في ذلك الأنماط الجينية وترددات الأليلات.

النتائج: بالنسبة للمعرف الجيني rs2296545 ، وجدنا زيادة ملحوظة للنمط الجيني CC وأليل C في المرضى الذين لديهم تاريخ عائلي من الاعتلال الكلوي بمراحله الأخيرة من أولئك الذين ليس لديهم بمعاملات خطورة هي 7.70 و 3.16، على التوالي. بالإضافة إلى ذلك، فيما يتعلق بـ rs10887800 ، كانت هناك زيادة ملحوظة في توزيع النمطين GC و GG في المرضى الذين ليس لهم تاريخ عائلي من الاعتلال الكلوي بمراحله الأخيرة مقارنة بالمجموعة الصحية الضابطة بمعاملات خطورة 2.095 و 2.301 على التوالي.

الخلاصة: يمكن اعتبار أليل C بالنسبة للمعرف الجيني rs2296545 بجين الرينياليز أحد عوامل الخطورة الوراثية الجديدة لأمراض الداء الكلوي بمراحله الأخيرة في الأطفال المصريين الذين لديهم تاريخ عائلي، وقد يكون أليله المقابل G عاملاً وقائيا.