Journal of Genetics (2020)99:33
https://doi.org/10.1007/s12041-020-1188-9

RESEARCH ARTICLE

CG/CA genotypes represent novel markers in the *NPHS2* gene region associated with nephrotic syndrome

LEILA ESMAELI CHAMGORDANI, NASIM EBRAHIMI, FARZANE AMIRMAHANI and SADEQ VALLIAN*

Genetics Division, Faculty of Biological Sciences and Technologies, Department of Cellular and Molecular Biology and Microbiology, University of Isfahan, Isfahan, Iran
*For correspondence. E-mail: svallian@sci.ui.ac.ir.

Received 15 November 2019; revised 10 January 2020; accepted 14 January 2020

Abstract. Nephrotic syndrome (NS) is considered as a primary disease of the kidney that represents a heterogeneous group of glomerular disorders occurring mainly in children. It is generally divided into steroid-sensitive and steroid-resistant forms, depending upon the patient's response to steroid therapy. Among the genes involved, the *NPHS2* gene has been reported as the causative gene in steroid resistant form of nephrotic syndrome. In the present study, heterozygosity rate, allelic frequency and linkage of rs2274625 and rs3829795 markers were investigated in the *NPHS2* gene region. To determine the SNP alleles, tetra-primer ARMS PCR was used. After genotyping rs2274625 and rs3829795 polymorphic markers in 120 unrelated individuals and nine trios families, the data were analysed using various computer programs such as UCSC Genome Browser, dbSNP and SNPper. Based on the statistical analysis of the results, for rs2274625 marker, allele frequency for C and T alleles was 97% and 3%, respectively. For rs3829795 marker allele frequency for G and A alleles was 55% and 45%, respectively. The values of heterozygosity index for the examined markers were 5% for rs2274625 and 45/8% for rs3829795. Consequently, two informative haplotypes, CG/CA, were identified in the *NPHS2* gene region through combination of these two markers. These haplotypes can serve as appropriate tools for the identification of heterozygous carriers and linkage analysis of nephrotic syndrome disease in the Iranian families with an affected child.

Keywords. *NPHS2* gene; nephrotic syndrome; single-nucleotide polymorphism; linkage; Iranian population.

Introduction

Nephrotic syndrome (NS) is a nonspecific kidney disorder that belongs to glomerular heterogeneous disease. It can affect individuals of all ages, but mostly occurs in children (Choi *et al.* 2017). The annual occurrence of this disease is 4.7 cases in every 100,000 people worldwide (Paranjape 2018). The symptoms of the disease include severe proteinuria, hypoalbuminaemia, hyperlipidaemia, and edema (Shin *et al.* 2018). Depending on the patients' response to steroid treatment, nephrotic syndrome is divided into two groups, steroid sensitive and steroid resistant (Caridi *et al.* 2005). The most affected people with this disease are steroid-sensitive NS (SSNS), and about 20% of children and 40% of adults are steroid-resistant NS (SRNS) (Benoit *et al.* 2010; Tory *et al.* 2014; Bullich *et al.* 2015). To date, at least 30 different genes have been identified for this type of disease (SRNS) (Wang *et al.* 2017). Major portion of SRNS is caused by inherited structural defects in proteins of the glomerular filtration barrier. SRNS is inherited as both autosomal recessive (AR) or autosomal dominant (AD) manner (Yang *et al.* 2012). The main reason of congenital and childhood onset SRNS is mutations in nephrin (*NPHS1*) and podocin (*NPHS2*) genes, respectively with an AR inheritance (Bullich *et al.* 2015; Thomas *et al.* 2018).

The present study focusses on the SRNS that has been created by *NPHS2* gene mutation which is located on 1q25-31 chromosome (Ranganathan 2016). The gene size is 25 kb and the coding area of the gene has a length of 1149 bp with eight exons (Franceschini *et al.* 2006; McKenzie *et al.* 2007). NPHS2 encodes a 42 kDa membrane integral protein called Podosine. This protein is a member of Stomatin proteins family including 383 amino acids (Ruf *et al.* 2004; Gbadegesin *et al.* 2007). Podosine protein is expressed in slit podocytes of kidney diaphragm and it has a fundamental role in filtration of glomerular wall (Dong *et al.* 2015). Podocytes contain cellular groups which consist of a cell body, major processes and foot processes (FPs), which have a major role

Published online: 20 April 2020

in selective permeability, structure stability and filtration of glomerulus wall (Reiser and Altintas 2016). Most NS types are caused by impairments in podocytes and mutations in *NPHS2* caused early proteinuria, disappearing of FPs, separation of podocytes from the glomerular basement membrane (GBM), and finally, irreversible loss of podocytes (Kerjaschki 2001; Greka and Mundel 2012).

More than 100 pathogenic mutations and 25 polymorphic variants have been identified in *NPHS2* gene (Baylarov *et al.* 2019). The reported mutations exist throughout the gene and include any type of alternations such as deletion, missense and nonsense mutations (Caridi *et al.* 2005). Because of the large number of mutations, timing of their analysis, and high cost of direct analysis as well as wide range of unknown mutations in the Iranian population, indirect analysis of the polymorphic markers with linkage analysis could be used as an alternative method for molecular diagnosis of genetic diseases (Nadeali *et al.* 2014).

Single-nucleotide polymorphism (SNP) markers are the most common polymorphisms in the human genome and they exist in the average distance of 290 bp in the genome (Jazaeri *et al.* 2016). Each SNP indicates a single-nucleotide change in the genome. A single-nucleotide position is usually considered as a SNP when the frequency of its recessive allele in the population is 1% or more (Ebrahimi and Vallian Borujeni 2016). SNPs have many known advantages in linkage analysis including high frequency, distribution, stability, intense linkage, and even high speed and power of SNP determination systems. Because the frequencies of the alleles and haplotypes of SNPs is different from one population to another, markers should be analysed separately for each population and informative haplotypes and markers should be introduced specifically for the understudied population (Ebrahimi and Vallian borujeni 2017; Fazeli and Vallian 2013).

To date, no study has been performed on the polymorphic markers in *NPHS2* gene region in the Iranian population. Among the SNPs related to *NPHS2* gene, rs2274625 and rs3829795 have shown high heterozygosity and high allele frequency in some populations; thus, allele and haplotype frequencies as well as linkage disequilibrium (LD) of two aforementioned markers in the *NPHS2* gene region were investigated for the first time in a group of Iranian population (Isfahan).

Materials and methods

In silico studies and SNP selection

An *in silico* analysis with various significant databases such as UCSC Genome Browser (https://genome.ucsc.edu/), dbSNP (http://www.ncbi.nlm.nih.gov/SNP) and SNPper (http://snpper.chip.org/bio/snpper-enter) was carried out on the polymorphic markers in the *NPHS2* gene region. As two important characteristics of genetic markers are MAF and heterozygosity rate for their informativeness in linkage analysis (Jones and Ardren 2003), the markers with high minor allele frequency (MAF), high heterozygosity and physical distance close to the gene including rs2274625 and rs3829795 were selected.

Sample collection and genotyping

Peripheral blood samples were collected from 120 unrelated healthy individuals and nine-family trios in the selected Iranian population. The families were consisted of both parents and one or two healthy children. The reason for choosing family trias in this study was to increase the accuracy and precision of the subsequent statistical analysis. Genomic DNA was extracted from peripheral blood leukocytes using standard salting out method (Miller *et al.* 1988).

The selected rs2274625 marker was genotyped using tetra-primer amplification refractory mutation system PCR (ARMS-PCR) (Ye *et al.* 2001). This technique is based on the use of two allele-specific inner primers and two outer primers to amplify three different length fragments from template DNA in a single PCR reaction followed by gel electrophoresis for the designation of genotypes (Amirmahani *et al.* 2017; Jamalvandi *et al.* 2018). Large fragment contains the SNP and illustrates the control band and the two smaller fragments indicate each of the two allele-specific products (figure 1). Two inner primers produce allele-specific fragments and the specificity was created through a mismatch at the 3′ end corresponding to the site of the SNP. Intentional mismatches to enhance the specificity were at position, from the 3′ end as well. The proper selection of primers were obtained using various software packages such as Oligo7 (https://www.oligo.net/) and Primer1 (http://primer1.soton.ac.uk/), which assess appropriate primers for a given sequence. The sequences of primers are shown in table 1. ARMS-PCR was used for genotyping rs3829795. Four primers were used for each DNA sample of which three were added in each PCR tube and two PCR reaction were carried out for each person (figure 2). In fact, the differences between these two methods are the number of primers in each tube and the number of PCR amplification for each sample.

The PCR reaction were carried out for rs2274625 and rs3829795 in optimal conditions using an Eppendorf thermal cycler machine (Eppendorf, Germany). The PCR conditions for rs2274625 marker was 25 μL total volume containing 50 ng of template DNA, 0.25 μL of 10 pmol primers for forward and reverse outer primers, 1 μL for forward and reverse inner primers. Two μL of 50 mM $MgCl_2$ and 1 μL of 10 mM dNTP, 2.5 μL 10× buffer and 0.2 μL of 5 U *Taq* DNA polymerase. PCR condition of rs3829795 marker was 25 μL total volume containing 50 ng of template DNA, 1 μL of 10 pmol primers for forward outer and reverse inner primers, 0.25 μL for reverse outer primer. 1 μL of 50 mM $MgCl_2$ and, 1 μL of 10 mM dNTP, 2.5 μL 10× buffer and 0.2 μL of 5 U *Taq* DNA polymerase.

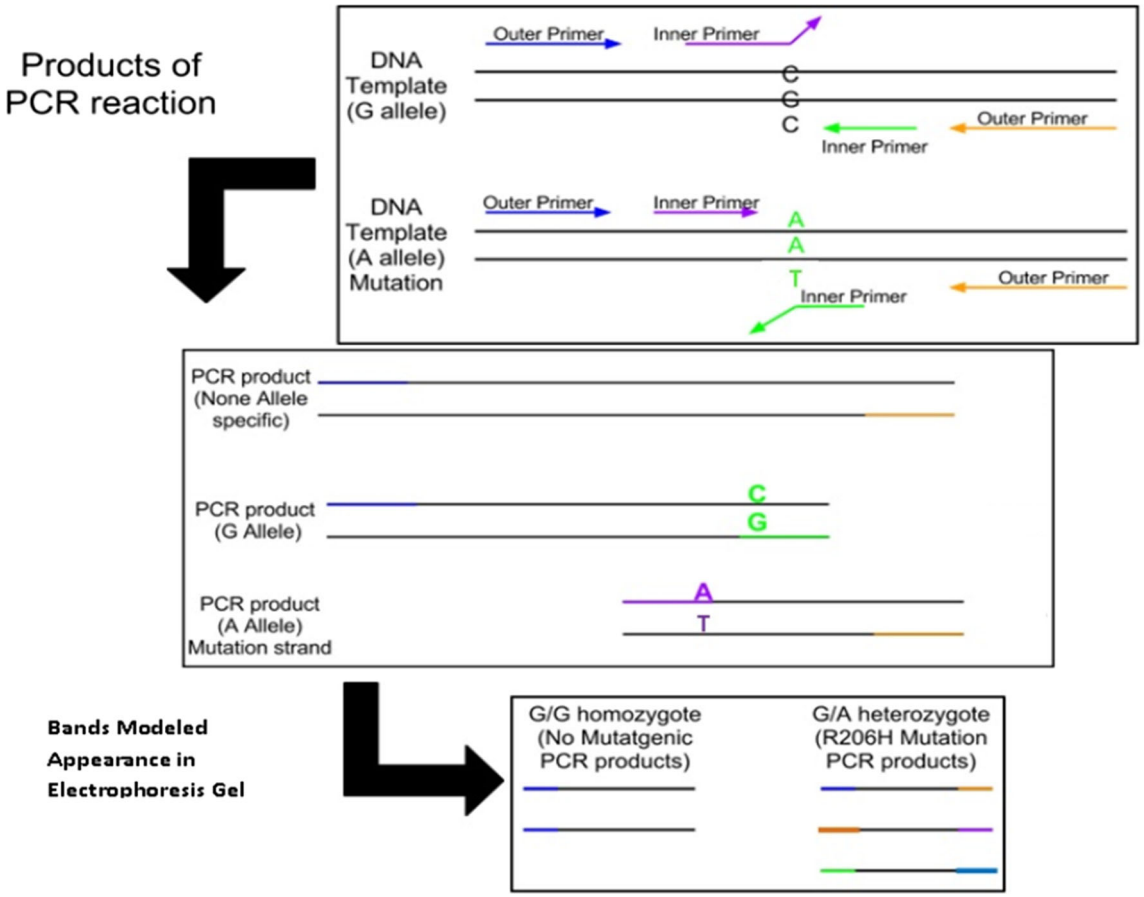

Figure 1. Schematic illustration of SNP genotyping using tetra-primer ARMS PCR. The two outer primers amplify a large fragment of the gene as an integral control. Two inner primers were designed to specific amplification of the alleles (Ye *et al*. 2001).

Table 1. The sequences of the designed primers used for genotyping rs2274625 and rs3829795 SNP markers.

Polymorphism	Primer name	Primer sequence	Amplicon size	Amplicon type
rs2274625 (G>A)	Forward outer primer (FO)	5′-CTGTGGATCACTGAGGGGAG-3′	With RO: 416	Control
	Reverse outer primer (RO)	5′-CAAGCACGGTTAAGCATAGAAC-3′	With FO: 416	Control
	Forward inner primer (FI)	5′-ATCCTAATCTTTCAAGGCCAAC-3′	With RO: 174	G Allele
	Reverse inner primer (RI)	5′-GGGGAGTTATTAGCATCGGA-3′	With FO: 283	A Allele
rs3829795 (G>A)	Forward outer primer (FO)	5′-CATCAACATCAGGCATAAGCAT-3′	With RO:292	Control
	Reverse outer primer (RO)	5′-ACAAAAGGTCATCGAATTAGGGT-3′	With FO: 292	Control
	Reverse G primer (RG)	5′-CCTTTCTCTCCTCCCTCCG-3′	With FO: 201	G Allele
	Reverse A primer (RA)	5-′ CCTTTCTCTCCTCCCTCCA-3′	With FO: 201	A Allele

Primary denaturation was carried out at 94°C for 5 min, followed by 30 cycles including 40 s denaturation at 94°C; annealing temperature at 59°C for 40 s, extension at 72°C for 50 s and finally a 10 min final extension at 72°C for two markers. The PCR products were separated on 2% agarose gel electrophoresis and genotyped (figure 2).

Statistical analysis

The approximation of the allele frequency as well as the observed and expected heterozygosity was determined using the GENEPOP website (Raymond 1995). Analyses were computed under the Hardy–Weinberg equilibrium (HWE) since its existence is essential for the accuracy and precision of human genetic statistical analyses. *P* value is a standard for detecting the deviation from HWE. In this study, the HWE *P* values were examined for the two studied markers using an accurate test of the PowerMarker software. Also, the haplotype frequency for 120 unrelated healthy individuals was evaluated using the PowerMarker software (https://brcwebportal.cos.ncsu.edu/powermarker/) and for nine families, FBAT software (https://gaow.github.io/genetic-analysis-software/f/fbat/) was used. To provide a better

description of the earned outcomes from the haplotype estimation, linkage disequilibrium (LD) for unrelated individuals was also carried out using the PowerMarker. LD was measured by using the standardized D′. The D′ is the LD relative to its maximum value for a given set of allelic frequencies for the pair of sites and was calculated using PowerMarker as well. In this regard, D′ is a normalized value of LD. Span of D′ is between +1 and –1. D′=1 represents that LD is complete and LD is present (Zhao *et al.* 2007). When D′ is 0, it is evident that the presence of linkage equilibrium is between two loci. As D′ could not supply substantial prediction in the probable performance for multiallelic markers, another multiallelic LD statistics, chi-square (χ^2) was used (Zhao *et al.* 2005). Chi-square determines the direct assessment of the strength of the correlations between alleles located at the two loci. To approximate D′ and χ^2, PowerMarker software was used. The null hypothesis for random relation between pairs of alleles at the two loci (D′= 0) was determined by χ^2 value. The χ^2 value for two pairwise markers was compared with χ^2 obtained from the chi-square chart ($P \leq 0.05$). Phasing of haplotypes for the markers was computed using the PowerMarker, which calculates the most plausible haplotypes according to the prepared genotypic data. The study was approved by biosafety committee of University of Isfahan for the use of human blood samples.

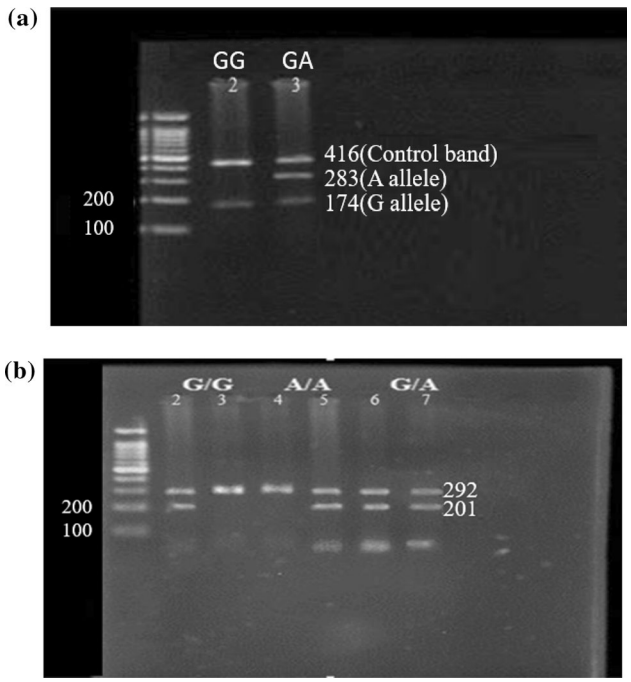

Figure 2. Electrophoretic pattern of (a) rs2274625 by the tetra-Primer ARMS PCR and (b) rs3829795 by conditional ARMS PCR. (a) In both lanes, 416 bp band represents as an internal control. Also, 283 and 174 bp specific bands show A and G alleles, respectively. Lane 2 represents the GG homozygous, while the other one shows the GA heterozygous sample. (B) 292 bp band shows the internal control. Specific 201 bp bands illustrate G or A alleles based on the primer. Lanes 2 and 3 show the GG homozygous samples, lanes 4 and 5 represent AA homozygous and lanes 6 and 7 are GA heterozygous samples.

Results

In the present study, two markers including rs2274625 and rs3829795 in the *NPHS2* gene region were genotyped in 120 unrelated healthy individuals of the Iranian population. Based on the results, the observed heterozygosity degrees of alleles were 7 and 55 for rs2274625 and rs3829795, respectively (table 2). HWE was evaluated for the indicated markers; therefore, HWE departure could influence the statistical analyses accuracy. *P* value of 1 and 0.4620 were obtained for rs2274625 and rs3829795, respectively, and HWE was verified for these markers.

Moreover, by using PowerMarker and FBAT software, the haplotype frequency was computed. As shown in table 3, among the four identified possible haplotypes, two with frequencies ≥5% were considered as informative haplotypes in the Iranian population. Haplotypes were estimated at the family level using FBAT software and the results showed that G–C and A–C haplotypes have frequencies more than 5%. These findings were consistent with the results of studies on nonrelative healthy individuals at the population level. These results introduced rs2274625 and rs3829795 combination as an informative haplotype. As the presence of relatively high proportion of informative haplotypes in the studied population, the LD pattern for the pairing of these two markers was evaluated using PowerMarker program. This program computes average D′ and χ^2 (Liu and Muse 2005). As shown in table 4, the values of D′ for two possible pairing of markers are higher than zero. Also, the χ^2 values were higher than χ^2 values obtained from the chi-square

Table 2. Allele frequency, heterozygosity and homozygosity of rs2274625 and rs3829795 SNP markers in 120 unrelated healthy individuals in the Iranian population.

Polymorphism	Allele	Frequency	Heterozygosity		Homozygosity	
rs2274625	G	0.97	Observed	Expected	Observed	Expected
	A	0.03	7	6.82	113	113.17
rs3829795	Allele					
	G	0.55	55	59.54	65	60.45
	A	0.45				

Table 3. Haplotype frequencies for rs2274625 and rs3829795 SNP markers in 120 unrelated healthy individuals in the Iranian population.

Haplotype	Frequency
rs2274625–rs3829795	
C–G	0.55147
C–A	0.42219
A–T	0.02733
G–T	0.00002

Table 4. Analysis of D′ and χ^2 values for pairing of the rs2274625 and rs3829795 markers in the NPHS2 locus in the Iranian population.

Pairing of markers	D′	*P* value	χ^2
rs2274625–rs3829795	1	0	8.96

table ($P<0.05$) (Fazeli and Vallian 2009). These results confirmed the LD for the two studied markers.

Discussion

NS is a primary disease that belongs to a group of glomerular heterozygous diseases (Kodner 2009). It is the most frequent cause of proteinuria in children and is appearing as a major cause of uremia. To date, more than 50 different genes have been identified and that the mutations in the *NPHS2* gene is associated with SRNS (Sharif and Barua 2018). The disease is usually inherited in an autosomal recessive pattern. NPHS2 encoded an integral membrane of 42 kDa protein with 383 amino acids, named Podocin (Caridi *et al*. 2005). Also, several polymorphisms of *NPHS2* gene have been reported in association with NS (Mishra *et al.* 2014; Joshi *et al.* 2017). Therefore, the markers of the *NPHS2* gene may be applied as bio-markers to evaluate individualized risk of the diseas. Because of more accuracy of haplotype analysis for genotype–phenotype correlations than individual SNPs, it is beneficial to do this procedure (Judson *et al.* 2000; Yang *et al*. 2012). Also, it is more informative to analyse the markers in groups although each of them could be evaluated separately. Additionally, haplotype analysing could be more informative than analyses of individual markers independently, while multiple markers in a unique chromosome were applied to find their association with a disease (Crawford and Nickerson 2005). Principally, the molecular diagnosis of nephrotic syndrome is based on direct analysis of gene mutations and indirect examination by linkage analysis (Crawford and Nickerson 2005). Sequencing and direct identification of mutations due to the several number of known mutations in the *NPHS2* gene and the probability of *de novo* mutations in this gene is a costly and time-consuming process. Hence, the alternative method for genetic diagnosis is based on association study using polymorphic markers that are used to determine the carriers (heterozygote) as well as prenatal diagnosis in families with affected children (Männikkö *et al.* 1997).

Table 5. Minor allele frequencies of rs2274625 and rs3829795 in different populations based on the obtained data from this study and the information available on Ensemble database (Zhang *et al.* 2019).

Population	SNP	
	rs2274625 MAF (A)	rs3829795 MAF (A)
Japanese	0.367	0.375
Chinese	0.353	0.443
Chinese	0.299	0.367
Indian	0.257	0.348
Mexican	0.233	0.273
European	0.217	0.392
Italian	0.181	0.393
African	0.053	0.298
Kenya	0.003	0.287
Nigerian	0	0.206
Iranian	0.03	0.4458

In the present study, among the present markers in the *NPHS2* gene region, one intronic marker, rs2274625, and another marker in the promoter region, rs3829795, were investigated. The allele and heterozygosity degree of these markers were computed in the Iranian population and these results were compared with those in other population. The allele frequency variation at polymorphic markers has determined to be useful for the study of genetic relationship between human populations (Fazeli and Vallian 2013). The results on the rs2274625 showed that C allele with 97% frequency and T allele with 3% frequency had the highest and lowest frequencies, respectively, in the selected population of the Iran. However, for rs3829795, the highest allele frequency was observed for the G allele with 55.42% frequency and A allele had the lowest frequency of 44.58%. Moreover, the observed heterozygosity percentage for rs2274625 and rs3829795 markers in the Iranian population were 7% and 55%, which was respectively higher and lower than the expected heterozygosity. As shown in table 5, according to the International HapMap project database (http://hapmap.ncbi.nlm.nih.gov), the highest frequency for the recessive allele of the rs2274625 was for the Japanese population, while the Iranian population showed the highest frequency of the recessive allele for rs3829795. However, for both markers, Nigerian population have less allele frequencies than other populations. Comparison of the results of this study with the available data of the International HapMapProject database shows that the allelic frequency of rs2274625 marker in the Iranian population is less than that of the Japanese, Chinese, Indian, Mexican, European,

Italian, African populations. While the allele frequency of this marker in the Iranian population is more than Kenya and Nigeria. On the other hand, the allelic frequency of the rs3829795 marker in the Iranian population is higher than European, Japanese, Chinese and Nigerian populations (Zhang *et al.* 2019).

The samples of our study consisted of unrelated healthy individuals and family trios. Data combination when collected through various study designs, such as family trios and unrelated samples, creates more precision and is affordable compared to separate data analysing (Crawford and Nickerson 2005). Haplotype phasing was performed for the two markers combination, which could increase the heterozygosity and informativeness of the markers in the form of haplotypes for applications of the linkage analysis (Haghighatnia *et al.* 2012). Haplotype frequencies estimation for the studied markers in families and unrelated individuals demonstrated two of the four haplotypes to be common in the Iranian population with frequencies ≥5%. Therefore, they can be used as informative haplotypes to identify pathogenic alleles of the *NPHS2* gene in families with NS. In addition, the estimation of D′ and χ^2 for the pair of markers showed a LD in *NPHS2* genomic region. Finally, due to the fact that these markers are is in the intronic and promoter regions of the gene, the presence of the LD between the two markers indicated their linkage with the gene, which could play a role as an important property in the indirect detection of pathogenic alleles of *NPHS2* gene.

In conclusion, this study was conducted to determine the characteristics of rs2274625 and rs3829795 markers in the Iranian population. The results showed that rs2274625, due to its linkage to the *NPHS2* gene, and rs3829795 with a high allelic frequency and heterozygosity degree could be used in indirect diagnosis of nephrotic syndrome. The combination of these two markers resulted in the identification of two C–G and C–A informative haplotypes in the *NPHS2* gene. In fact, these haplotypes could be used as a means of identifying carriers as well as prenatal diagnosis of nephrotic syndrome in Iranian families. These markers could be also used as single-nucleotide markers in linkage studies to detect mutations in the *NPHS2* gene in the molecular diagnosis of the disease.

Acknowledgements

This study was supported by Department of Research of University of Isfahan through an Internal Postgraduate Fund (pajoohaneh).

References

Amirmahani F., Motovali-Bashi M. and Samani Z. G. 2017 Association scrutiny between the miRNA 148a/152 polymorphisms and risk of breast cancer in Isfahan population. *Health Biotechnol. Biopharma* **1**, 50–60.

Baylarov R., Baylarova R., Berdeli A., Bayramov R. and Haziyev E. 2019 *NPHS2* gene sequencing results in children of the Azerbaijani population with different types of nephrotic syndrome caused by chronic glomerulonephritis. *Bratisl. Lek. Listy* **120**, 102–105.

Benoit G., Machuca E. and Antignac C. 2010 Hereditary nephrotic syndrome: a systematic approach for genetic testing and a review of associated podocyte gene mutations. *Pediatr. Nephrol.* **25**, 1621–1632.

Bullich G., Trujillano D., Santín S., Ossowski S., Mendizábal S., Fraga G. *et al.* 2015 Targeted next-generation sequencing in steroid-resistant nephrotic syndrome: mutations in multiple glomerular genes may influence disease severity. *Eur. J. Hum. Genet.* **23**, 1192.

Caridi G., Perfumo F. and Ghiggeri G. M. 2005 NPHS2 (Podocin) mutations in nephrotic syndrome. Clinical spectrum and fine mechanisms. *Pediatr. Res*. **57**, 54R.

Choi Y. W., Kim Y. G., Song M.-Y., Moon J.-Y., Jeong K.-H., Lee T.-W. *et al.* 2017 Potential urine proteomics biomarkers for primary nephrotic syndrome. *Clin. Proteom*. **14**, 18.

Crawford D. C. and Nickerson D. A. 2005 Definition and clinical importance of haplotypes. *Annu. Rev. Med.* **56**, 303–320.

Dong L., Pietsch S., Tan Z., Perner B., Sierig R., Kruspe D. *et al.* 2015 Integration of cistromic and transcriptomic analyses identifies Nphs2, Mafb, and Magi2 as Wilms' tumor 1 target genes in podocyte differentiation and maintenance. *J. Am. Soc. Nephrol.* **26**, 2118–2128.

Ebrahimi N. and Vallian Borujeni S. 2016 Analysis of genetic variation of rs1542705 marker in *SMPD1* gene region as an informative marker for molecular diagnosis of Niemann-Pick disease in Isfahan population. *J. Arak. Uni. Med. Sci.* **19**, 1–10.

Ebrahimi N. and Vallian Borujeni S. 2017 Analysis of allele frequency and genotyping of rs67992843 marker in *SMPD1* gene region associated with Niemann Pick disease in Isfahan population. *SJIMU* **25**,151–160.

Fazeli Z. and Vallian S. 2009 Estimation haplotype frequency of Bgl II/Eco RI/VNTR markers at the PAH gene region in Iranian population. *Int. J. Hum. Genet*. **9**, 115–121.

Fazeli Z. and Vallian S. 2013 Molecular phylogenetic study of the Iranians based on polymorphic markers. *Gene* **512**, 123–126.

Franceschini N., North K. E., Kopp J. B., Mckenzie L. and Winkler C. 2006 *NPHS2* gene, nephrotic syndrome and focal segmental glomerulosclerosis: a HuGE review. *Genet. Med.* **8**, 63.

Gbadegesin R., Hinkes B., Vlangos C., Mucha B., Liu J., Hopcian J. *et al.* 2007 Mutational analysis of NPHS2 and WT1 in frequently relapsing and steroid-dependent nephrotic syndrome. *Pediatr. Nephrol.* **22**, 509–513.

Greka A. and Mundel P. 2012 Cell biology and pathology of podocytes. *Annu. Rev. Physiol*. **74**, 299–323.

Haghighatnia A., Vallian S., Mowla J. and Fazeli Z. 2012 Genetic diversity and balancing selection within the human phenylalanine hydroxylase (PAH) gene region in Iranian population. *Iran J. Public Health* **41**, 97.

Jamalvandi M., Motovali-bashi M., Amirmahani F., Darvishi P. and Goharrizi K. J. 2018 Association of T/A polymorphism in miR-1302 binding site in CGA gene with male infertility in Isfahan population. *Mol. Biol. Rep*. **45**, 413–417.

Jazaeri A., Karimi Moghadam A. and Vallian Borujeni S. 2016 Evaluating the association between Rs1800624 in RAGE gene and multiple sclerosis in Isfahan population. *SSJU* **23**, 923–931.

Jones A. G. and Ardren W. R. 2003 Methods of parentage analysis in natural populations. *Mol. Ecol*. **12**, 2511–2523.

Joshi B. B., Mistry K. N., Gang S., Koringa P. G. and Joshi C. G. 2017 Characterization of *NPHS2* gene polymorphisms associated to steroid resistance nephrotic syndrome in Indian children. *Gene* **628**,134–140.

Judson R., Stephens J. C. and Windemuth A. 2000 The predictive power of haplotypes in clinical response. *Pharmagenomics* **1**, 15–26.

Kerjaschki D. 2001 Caught flat-footed: podocyte damage and the molecular bases of focal glomerulosclerosis. *J. Clin. Invest.* **108**,1583–1587.

Kodner C. 2009 Nephrotic syndrome in adults: diagnosis and management. *Am. Fam. Physician* **80**, 1129–1134.

Liu K. and Muse S. V. 2005 PowerMarker: an integrated analysis environment for genetic marker analysis. *Bioinformatics* **21**, 2128–2129.

Männikkö M., Kestilä M., Lenkkeri U., Alakurtti H., Holmberg C., Leisti J. *et al.* 1997 Improved prenatal diagnosis of the congenital nephrotic syndrome of the Finnish type based on DNA analysis. *Kidney Int.* **51**, 868–872.

McKenzie L. M., Hendrickson S. L., Briggs W. A., Dart R. A., Korbet S. M., Mokrzycki M. H. *et al.* 2007 NPHS2 variation in sporadic focal segmental glomerulosclerosis. *J. Am. Soc. Nephrol.* **18**, 2987–2995.

Miller S., Dykes D. and Polesky H. 1988 A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* **16**,1215.

Mishra O. P., Kakani N., Singh A. K., Narayan G., Abhinay A., Prasad R. *et al.* 2014 NPHS2 R229Q polymorphism in steroid resistant nephrotic syndrome: is it responsive to immunosuppressive therapy? *J. Trop. Pediatr.* **60**, 231–237.

Nadeali Z., Karimi A. and Vallian-Borujeni S. 2014 Analysis of genetic variation of rs4148326 marker located in *UGT1A1* gene region: an informative marker for molecular diagnosis of Crigler-Najjar syndrome. *Iranian J. Basic Med. Sci.* **20**, 880–885.

Paranjape J. 2018 Diet in nephrotic syndrome. *J. Renal. Nutr. Metab.* **4**, 80–81.

Ranganathan S. 2016 Pathology of podocytopathies causing nephrotic syndrome in children. *Front Pediatr.* **4**, 32.

Raymond M. 1995 GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J. Hered.* **86**,248–249.

Reiser J. and Altintas M. M. 2016 Podocytes. F1000Res 5.

Ruf R. G., Schultheiss M., Lichtenberger A., Karle S. M., Zalewski I., Mucha B. *et al.* 2004 Prevalence of WT1 mutations in a large cohort of patients with steroid-resistant and steroid-sensitive nephrotic syndrome. *Kidney Int.* **66**, 564–570.

Sharif B. and Barua M. 2018 Advances in molecular diagnosis and therapeutics in nephrotic syndrome and focal and segmental glomerulosclerosis. *Curr. Opin. Nephrol. Hypertens* **27**,194–200.

Shin J. I., Kronbichler A., Oh J. and Meijers B. 2018 Nephrotic syndrome: genetics, mechanism, and therapies. *Biomed Res.* 2018, article ID 6215946.

Thomas M. M., Abdel-Hamid M. S., Mahfouz N. N. and Ghobrial E. E. 2018 Genetic mutation in Egyptian children with steroid-resistant nephrotic syndrome. *J. Formos Med. Assoc*. **117**, 48–53.

Tory K., Menyhárd D. K., Woerner S., Nevo F., Gribouval O., Kerti A. *et al.* 2014 Mutation-dependent recessive inheritance of NPHS2-associated steroid-resistant nephrotic syndrome. *Nat. Genet.* **46**, 299.

Wang F., Zhang Y., Mao J., Yu Z., Yi Z., Yu L. *et al.* 2017 Spectrum of mutations in Chinese children with steroid-resistant nephrotic syndrome. *Pediatr. Nephrol.* **32**, 1181–1192.

Yang J., Cai L., Huang H., Liu B. and Wu Q. 2012 Genetic variations and haplotype diversity of the UGT1 gene cluster in the Chinese population. *PLoS One* **7**, e33988.

Ye S., Dhillon S., Ke X., Collins A. R. and Day I. N. 2001 An efficient procedure for genotyping single nucleotide polymorphisms. *Nucleic Acids Res.* **29**, e88–e88.

Zhang J., Chen H., Kornreich R. and Yu C. 2019 Prenatal diagnosis of Tay-Sachs disease. *In* Prenatal diagnosis, pp. 233–250. Springer.

Zhao H., Nettleton D., Soller M. and Dekkers J. 2005 Evaluation of linkage disequilibrium measures between multi-allelic markers as predictors of linkage disequilibrium between markers and QTL. *Genet. Res.* **86**, 77–87.

Zhao H., Nettleton D. and Dekkers J. C. 2007 Evaluation of linkage disequilibrium measures between multi-allelic markers as predictors of linkage disequilibrium between single nucleotide polymorphisms. *Genet. Res.* **89**, 1–6.

Corresponding editor: Indrajit Nanda