ORIGINAL ARTICLE

IL-13 rs20541 (R130Q) Single Nucleotide Polymorphism in a Sample of Egyptian Children Suffering from Idiopathic Nephrotic Syndrome

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ABSTRACT

Background: *Idiopathic nephrotic syndrome (INS) is the most frequent glomerular disease affecting children. Its pathogenesis is not completely evident, it is probably due to immunological disturbance that increases cytokines production and alters the glomerular permeability. Interleukin 13 (IL-13) has been implicated in INS pathogenesis by changing the permeability of the glomerular basement membrane and inducing proteinuria.* ***Objective:*** *To investigate the association between rs20541 (R130Q) single nucleotide polymorphism (SNP) in IL-13 gene, idiopathic nephrotic syndrome susceptibility and steroid treatment response in Egyptian children.* ***Methodology****: This cross sectional case-control study was performed on 50 INS children aged 2-15 years following up in the Nephrology Unit of the Pediatric department at Benha University Hospitals and 50 healthy age and sex matched children as controls. All candidates were subjected to clinical evaluation. Genotyping of the selected SNP was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).* ***Results:*** *A highly significant frequency of GA genotype, p=0.017, OR= 1.933 (1.126-3.319) and AA genotype, p=0.001, OR=6.660 (2.283-19.433) as well as A allele, p<0.001, OR=2.281(1.551-3.354) were observed in the INS patients with a higher significant frequency of AA type p=0.013, OR=3.660 (1.308-10.238) and A allele, p=0.009, OR=1.947 (1.178-3.218) in steroid resistant patients.* ***Conclusion:*** *The results showed that IL-13 polymorphism (rs20541) seems to play a role in the pathogenesis of INS. The A allele is a risk factor for the disease and moreover, it has a relation to steroid responsiveness.*

INTRODUCTION

Nephrotic syndrome (NS) is the most common cause of kidney disease in children with its incidence increases annually. It is a glomerular disorder characterized by heavy proteinuria, hypoalbuminemia, generalized edema and hyperlipidemia[1]. NS may be congenital or acquired either primary (idiopathic); the most common type in children or secondary to systemic diseases and infections[2].

Steroids are the mainstay for pediatric NS treatment with a high success rate despite of relapses. Steroids responsiveness is a valuable prognostic indicator for idiopathic nephrotic syndrome (INS) as steroid-sensitive nephrotic syndrome (SSNS) children have favorable long-term outcomes than those with steroid-resistant nephrotic syndrome (SRNS) who relatively have lower renal survival rates[3].

The precise pathophysiology of INS is not fully clarified, it has been considered as a T-cell dysfunction disease mediated by a number of T-cell cytokines including IL-2, IL-8, IL-13, TNFα, IFNγ and vascular endothelial growth factor (VEGF) that alter the podocyte function increasing the glomerular permeability to serum proteins and resulting in proteinuria[4].

Implication of B-cell in INS pathogenesis was evidenced although its exact role is still unclear[5]. It was also suggested that podocytes play a direct role in the disease development[6].

IL-13 is one of cytokines with elevated level in INS patients' sera. It consists of 132 amino acids, secreted by various T-cell subsets including activated T-helper cells, memory and naïve T-cells[7], basophils, mast cells and dendritic cells[8] with its gene located on chromosome 5q[9].

IL-13 increases CD80 expression on podocytes' surface. CD80 is a T-cell co-stimulatory molecule can interact with CD28 on CD4+ T-cells resulting in their activation, bind with cytotoxic T-lymphocyte associated (CTLA)-4 terminating the T-cell response or CTLA-4 on T-regulatory blocking their maturation into T-effector phenotype; therefore it is involved in both activation and inhibition of T-cell response[10,11].

Podocytes constitute a final barrier to urinary protein loss through their foot processes (FPs) and the slit diaphragms (SDs) which are modified adherence

junctions act as the main selectively permeable barrier in the kidney[11]. SDs composed of a vast number of proteins including nephrin and podocin, two important protein molecules in maintaining the SD integrity and the podocyte foot processes attachment to the glomerular basement membrane[12].

IL-13 down-regulats expression of nephrin and podocin resulting in SD functional destabilization, consequent podocyte foot process effacement and increased urinary albumin excretion[12].

In podocytes, IL-13 influences intracellular protein passage, stimulates secretion of pro-cathepsin L and creates acidic environment by inducing H^+ influx. Acidity enhances cleavage of pro-cathepsin L into cathepsin L and activates its proteolytic activity resulting in degradation of basement membrane proteins and alteration of glomerular permeability[13].

Gene variants in IL-13 frequently associated with allergic disorders, most commonly bronchial asthma[14]. As being involved in INS pathogenesis its polymorphisms could influence the disease susceptibility, clinical course and the response to treatment[15]. One of IL-13 SNPs is a coding SNP in exon 4 (rs20541 G/A) at the +2044 position or the Arg130Gln as it resulted in replacement of arginine (R) 130 with glutamine (Q)[16].

The study aimed to assess the association between IL-13 SNP rs20541 with INS susceptibility and response to steroid treatment in Egyptian children.

METHODOLOGY

Study design and subjects:

The present cross sectional case control study was conducted on 50 children aged from 2-15 years diagnosed clinically as INS and following up at the Nephrology Unit in the Pediatric Department, Benha University Hospitals. Patients were diagnosed by the presence of generalized edema, proteinuria, hypo-albuminemia with or without hyperlipidemia according to the International Study of Kidney Disease in Children (ISKDC)[17].

Patients were divided into steroid sensitive nephrotic syndrome (SSNS) (n=25) and steroid resistant nephrotic syndrome (SRNS) (n=25). Steroid sensitive was defined as the disappearance of proteinuria (normal protein excretion trace or negative for at least three consecutive days or <5 mg/m2/h and serum albumin ≥35 g/l) within the first 4week course of full dose steroid therapy. Steroid resistance was defined as the persistence of proteinuria after a 4week course of full dose steroid therapy.

Fifty healthy children with matched age and sex were selected as controls.

All subjects underwent full clinical evaluation (complete history taking and physical examination) by an experienced pediatrician and tested for blood urea, serum creatinine, total protein, albumin level, complete lipid profile and urine protein: creatinine ratio.

Inclusion criteria:

Patients included in the study have normal or impaired kidney function tests and not indicated for dialysis. They have normal serum complement levels, without electrolyte disturbance e.g. hypokalemia or hypocalcemia, without renal anomalies and had no gross haematuria during their clinical course.

Exclusion criteria:

Patients with hypertension, low serum complement, electrolyte disturbance, renal anomalies, gross hematuria, poor compliance and those indicated for dialysis or not on regular follow up were excluded.

Blood samples were collected after ethical approval by Benha Faculty of Medicine Ethical Committee, article No. (RC722022) and taking a written informed consent from all subjects' parents.

IL-13 Gene detection and genotyping:

Gene detection and typing were carried out at the Medical Microbiology and Immunology Department, Benha Faculty of Medicine, Benha University.

A sample of 2ml venous blood was collected in blood collection tube containing EDTA. Genomic DNA was extracted using Thermo Scientific™ GeneJET Whole Blood Genomic DNA Purification Mini Kit (Thermo Scientific, USA), according to the manufacture instructions. The purified DNA was stored at -20°C for further analysis.

Gene amplification was performed in a 50µL reaction mixture containing 5µL purified DNA, 1µL of each primer [F-primer: 5′-CTTCCGTGAGGACTGAATGAGACGGTC-3′ and R-primer: 5′-GCAAATAATGATGCTTTCGAAGTTTCAGTGGA-3′][18], 25µL Hot Start Taq 2X Master Mix (Biolabs, Inc., UK) and 18µL nuclease free water. The amplification parameters were initial denaturation 5min at 95°C followed by 35 cycles of denaturation 30s at 95°C, annealing 30s at 62°C and extension 1 min. at 72°C and final extension 10 min. at 72°C. The amplified PCR products were electrophoresed on 2% agarose gel stained with ethidium bromide and visualized on an UV transilluminator at 235pb using a 100pb ladder (Thermo Scientific, USA) as a marker .

The resulting PCR products were subjected to digestion using NlaIV restriction enzyme (Biolabs, Inc., UK), yielded three bands (178bp, 32bp and 25bp) for the wild homozygous genotype (GG), two bands (210bp and 25bp) for the mutant homozygous genotype (AA) and four bands (210bp, 178bp, 32bp, 25bp) for the heterozygous mutant genotype (GA), the digested fragments were separated using 2% agarose gel and visualized under ultraviolet light (Fig. 1).

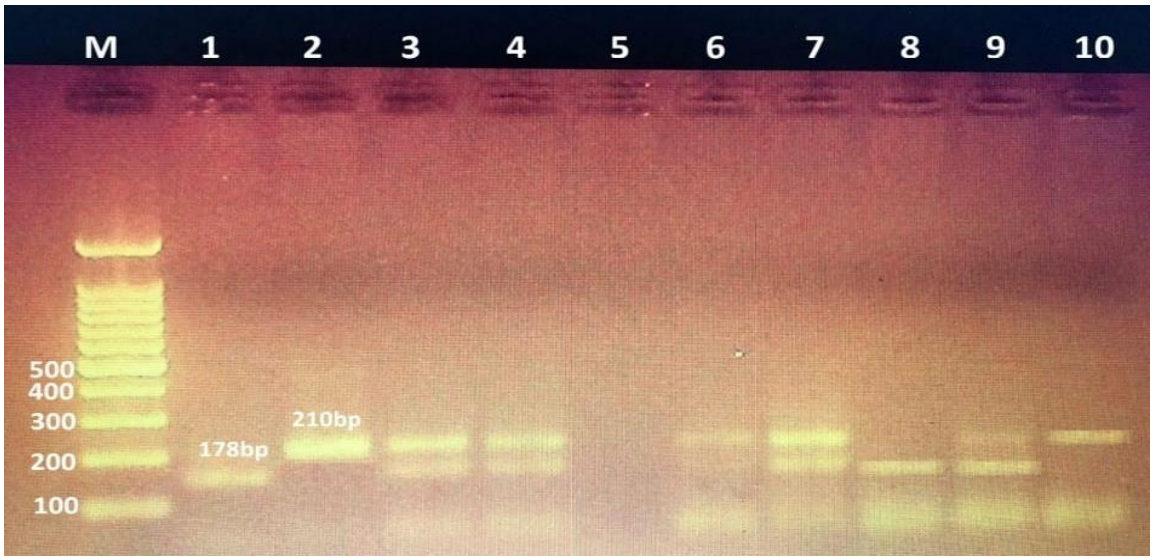

Fig.1: PCR-RFLP analysis of IL-13 SNP (rs20541) genotypes after digestion with NlaIV. Lanes 1and 8 show the homozygous wild (GG) genotype, lanes 2, 6 and 10 show the homozygous mutant (AA) genotype and lanes 3, 4, 7 and 9 show the heterozygous mutant (GA) genotype while lane 5 show no bands being not loaded. M is the molecular weight marker (100 bp, Thermo Scientific, USA).

Statistical analysis

The collected data was analyzed using Statistical package for Social Science (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0 Armonk, NY: IBM Corp.). Deviations from Hardy–Weinberg equilibrium expectations were determined using the goodness of fit between the observed and expected genotype frequencies. Polymorphisms and genotype frequencies were evaluated by gene counts. Student T Test was used to assess the statistical significance of the difference between the study groups. Mann Whitney Test (U test) was used to assess the statistical significance of the difference of the non-parametric variables. Chi-Square test was used to examine the relationship between the qualitative variables. Regression analysis: Logistic regression analysis was used for prediction of risk factors, using generalized linear models. P value was considered significant if <0.05 at confidence interval 95%.

RESULTS

Clinical and laboratory data:

The present study included 50 INS cases diagnosed according to the International Study of Kidney Disease in Children (ISKDC)[17], aged 2-15 years with a mean age of 8±3.6 years and 50 healthy controls of matched age and gender. The clinical and laboratory data showed that except for age there was a significant difference ($p<0.05$) between INS patients and controls at all parameters. INS was associated with a significantly lower weight, height, body mass index (BMI), serum protein and albumin and a significantly higher serum urea, creatinine, cholesterol and urinary protein/creatinine ratio when compared to controls (Table 1).

Table 1: Clinical and laboratory parameters of INS patients and controls

		Control (N=50)	INS (N=50)	*P*
Age (year)	Mean ±SD	8.7±3.5	8±3.6	0.362
	Median (range)	9 (2-14.5)	8 (2-14.5)	
Weight (Kg)	Mean ±SD	43.2±14.6	24.7±9	<0.001
	Median (range)	41 (17-69)	22.8 (10.5-45)	
Height (cm)	Mean ±SD	136.2±18.9	122.6±18.4	<0.001
	Median (range)	139 (80-163)	124 (77-155)	
BMI	Mean ±SD	22.6±3.6	16.2±3.9	<0.001
	Median (range)	22.32 (15.71-31.57)	14.95 (7.8-24.5)	
Blood urea (mg/dl)	Mean ±SD	11.7±3	33.7±33.9	<0.001
	Median (range)	11.4 (7.1-18)	21 (9-200)	
S. creatinine (mg/dl)	Mean ±SD	0.7±0.1	1.5±1.4	0.012
	Median (range)	0.7 (0.5-0.9)	0.9 (0.14-5.5)	
Total S. protein (g/dl)	Mean ±SD	7±0.5	5.5±1.3	<0.001
	Median (range)	7 (6.3-7.9)	5.5 (2.9-7.5)	
S. albumin (g/dl)	Mean ±SD	4.6±0.6	3±1.2	<0.001
	Median (range)	4.8 (3.5-5.4)	3.1 (0.5-5)	
S. cholesterol (mg/dl)	Mean ±SD	116.8±34	224±68.3	<0.001
	Median (range)	105 (56-175)	206 (121-400)	
Urinary protein/creatinine	Mean ±SD	0.049±0.028	3.39±1.194	<0.001
	Median (range)	0.05 (0.01-0.1)	3.1 (1.3-6.3)	

IL-13 rs20541G/A SNP association with INS susceptibility

The genotype distribution in INS subjects revealed that the heterozygous mutant type (GA) was the most frequent 23(46%) followed by the homozygous wild type (GG) 15(30%) and the homozygous mutant type (AA) 12(24%) while in the control subjects the GG genotype was the most frequent 32(64%) followed by the GA genotype 17(34%) and the AA genotype 1(2%). The G allele was the most frequent among cases 53(53%) and controls 81(81%) compared to the A allele frequency 47(47%) in patients and 19(19%) in controls.

The frequency of genotypes distribution was statistically significant (p<0.05%); (GG vs. GA): p=0.017, OR= 1.933 (1.126-3.319) and (GG vs. AA): p=0.001, OR=6.660 (2.283-19.433) as well as alleles distribution (G vs. A): p<0.001, OR=2.281(1.551-3.354) when patient group was compared to control group. The genotypes and alleles distribution frequency of IL-13 rs20541SNP, among patients and controls were in H.W. equilibrium (Table 2).

Table 2: IL-13 rs20541 genotypes and alleles distribution frequency in INS patients and control groups

		Control N=50 (%)		INS N=50 (%)		*P*	OR (95% CI)
IL13rs20541	**GG**	32	64.0	15	30.0		1 **(Reference)**
	GA	17	34.0	23	46.0	0.017	1.933 (1.126-3.319)
	AA	1	2.0	12	24.0	0.001	6.660 (2.283-19.433)
	G	81	81.0	53	53.0		1 **(Reference)**
	A	19	19.0	47	47.0	<0.001	2.281 (1.551-3.354)
	HW p	0.459		0.588			

OR: odds ratio, CI: confidence interval, HW p: Hardy-Weinberg proportion**,** *p*<0.05 is significant, R: reference; regression analysis test was used.

Association of IL-13 rs20541G/A SNP with steroid treatment:

The genotypes and alleles distribution among INS patients revealed that in steroid resistant INS group the GA genotype showed the highest frequency 12(48%) followed by AA genotype 9(36%) and GG genotype 4(16%) while in steroid sensitive INS group the GG and the GA genotypes frequencies were equal 11(44%) each whereas the AA genotype frequency was 3(12%). Allele distribution frequency revealed that G allele was more frequent in steroid sensitive cases 33(66%) than in the steroid resistant 20(40%), in contrast to A allele which

was more frequent 30(60%) in the steroid resistant patients than in the steroid sensitive 17(34%).

Distribution frequency of GA genotype was statistically insignificant (p>0.05%); (GG vs. GA): p= 0.119, OR=1.969 (0.840-4.617) while distribution frequency of AA genotype was statistically significant (p<0.05%); (GG vs. AA): p=0.013, OR=3.660 (1.308-10.238) as well as alleles distribution frequency (G vs. A): p=0.009, OR=1.947 (1.178-3.218) when the steroid resistant and the steroid sensitive INS patients groups were compared (Table 3).

Table (3): IL13-rs20541 genotypes and alleles distribution frequency among INS patients according to steroid response

		Steroid sensitive N=25 (%)		Steroid resistant N=25 (%)		*P*	OR (95% CI)
IL13rs20541	**GG**	11	44.0	4	16.0		1 **(Reference)**
	GA	11	44.0	12	48.0	0.119	1.969 (0.840-4.617)
	AA	3	12.0	9	36.0	0.013	3.660 (1.308-10.238)
	G	33	66.0	20	40.0		1 **(Reference)**
	A	17	34.0	30	60.0	0.009	1.947 (1.178-3.218)

OR: odds ratio, CI: confidence interval, $p<0.05$ is significant, R: reference; regression analysis test was used.

DISCUSSION

Several studies have evaluated IL-13 gene SNPs in the coding sequence and the promoter region and reported their association with allergic phenotypes; asthma, allergic rhinitis and atopic dermatitis in different ethnic populations [19-23], however, little data exists concerning their association with INS.

To our knowledge there is only one study assessed the association between IL-13 SNP (rs20541), INS susceptibility and steroid response in Arab children performed in Kuwait[24] while there in no previous studies carried out in Egypt.

The current study revealed a significant genotypic and allelic distribution under condition of Hardy Weinberg equilibrium when patient and control groups were compared. It exhibited a statistically significant higher frequency of the heterozygous mutant (AG) genotype p=0.017, OR= 1.933 (1.126-3.319) and the homozygous mutant (AA) genotype p=0.001, OR=6.660 (2.283-19.433) in INS patients compared to controls. The A allele frequency in INS patients was higher than in controls with a statistical significance p<0.001, OR=2.281(1.551-3.354).

The present study, reported that the A allele was a risk factor for INS. Functional studies exhibited that the IL-13 variant has different biochemical and conformational properties that altered its functions in comparison with its wild type[25]. As substitution of the major (G) allele by the minor (A) allele in IL-13 SNP rs20514 leads to replacement of the positively charged arginine by the neutral glutamine that induces conformational changes at the site of interaction with the IL-13 receptor augmenting the IL13/IL13R interaction resulting in increasing IL-13 activity and its downstream signalling pathway [26, 27].

This study exhibited a statistical significant higher frequency of the AA genotype, p=0.013, OR=3.660 (1.308-10.238) and the A allele p=0.009, OR=1.947 (1.178-3.218) in comparing the steroid resistant and the steroid sensitive INS patients.

In contrast to our results Al-Rushood et al.,[24] reported no association between IL-13rs20541 SNP and INS susceptibility in Kuwaiti children as there was no statistical significant association (p<0.05) between IL-13 rs20541SNP genotypes and alleles distribution in comparing the INS subjects and controls. Comparing genotypes and alleles frequencies between steroid sensitive and steroid resistant INS patients only the heterozygous mutant genotype showed statistical significance (p= 0.04).

Wei et al.,[15] assessed six IL-13SNPs including rs20541SNP in INS Singapore children and showed no significant difference in both genotypes and alleles frequencies of IL-13 polymorphisms between INS cases and normal controls with no significant association to steroid responsiveness.

Tenbrock et al.,[28] evaluated IL-13 polymorphism at position110 in German INS patients, reported no polymorphisms frequency valuable variations between the different NS clinical courses and concluded that IL-13(110SNP) do not seem to affect the clinical course of NS.

Gillespie et al.,[29] did not find any association between IL-13 SNPs and INS predisposition in British children.

These conflicting results can be explained by the compounding effects of multiple factors involved in the predisposition and pathogenicity of INS such as the environmental, epigenetic and genetic factors. Also, it may return to the complex interactions of the genetic and epigenetic variations in different ethnicities that

may influence the overall impact of the tested SNPs, and may result in different biological outcomes.

An animal study was carried out by Lai et al.,[12] who directly addressed the role of IL-13 in the rats by overexpressing it and reported that nephropathy was induced, with changes in the structure and gene expression of podocyte similar to those seen in human diseases.

Van den Berg et al.,[30] showed that stimulation of IL-13 receptors present on glomerular epithelial cells, resulted in decrease in the transepithelial electrical resistance indicating a possible direct effect of IL-13 on the podocytes and its function to maintain circulatory albumin and prohibit albuminuria; the hallmark of INS.

CONCLUSION

IL-13 gene (rs20541, G/A) polymorphism may influence susceptibility to idiopathic nephrotic syndrome and might affect steroid response in INS patients. Further studies with a larger patient sample are required to confirm these findings. Given the effect of environment and ethnicity on genetics, further investigation is required to elucidate the role of rs20541 SNP in IL-13 gene among INS patients of different ethnicities.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

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