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Polymorphic variants of MIF gene and prognosis in steroid therapy in children with idiopathic nephrotic syndrome

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Nephrotic syndrome (NS) is the most common reason of proteinuria in children and can be caused by the pathology of renal glomeruli. Steroid therapy is typically used in this disorder. It has been shown that MIF is a cytokine which counteracts the immunosuppressive properties of glucocorticoids. The aim of this study was looking for a correlation between *MIF* polymorphisms and genetic susceptibility to steroid resistance in children with INS (Idiopathic NS). Methods: The study was performed in 71 patients with INS including SRNS (steroid resistance nephrotic syndrome) (41) and SSNS (steroid sensitive nephrotic syndrome) (30) and in 30 control subjects. We employed Sanger sequencing and capillary electrophoresis. Linkage disequilibrium was made using Haploview and PHASE. Results: We didn't observe a statistical significance between SNPs detected in patients with INS and controls. Our studies revealed statistical significance for two polymorphisms: rs2070767C>T and rs2000466T>G between patients with SRNS and SSNS. The results for rs34383331T>A are close to being statistically significant. Statistical significance was revealed for CATT5/CATT6 genotype in SRNS group *vs* SSNS group (OR=4.604, 95%CI=1.356–15.632, p=0.0168). We found that the frequency of 5/X-CATT genotype compared with X/X-CATT genotype was significantly higher in SRNS patients *vs* SSNS (OR=3.167, 95%CI=1.046–9.585, p=0.0426). In linkage disequilibrium analysis we didn't show involvement in susceptibility to INS and steroid sensitive phenotype. Conclusions*:* Our results suggest that the role of *MIF* polymorphisms in the susceptibility to positive response to steroid therapy is still unresolved. It indicates that MIF may be involved in indirect and complex molecular mechanisms of steroid activity in hormone-dependent metabolic pathways in children with INS. Because of ambiguous findings, pleiotropic features of this cytokine require that more research should be undertaken.

Key words: nephrotic syndrome, steroid resistance, *MIF* gene, single nucleotide polymorphism, short tandem repeat

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INTRODUCTION

Nephrotic syndrome (NS) is the most common reason of proteinuria (more than 50 mg/kg/day or protein/creatinine ratio >200mg/mmol) in children and can be caused by the pathology of renal glomeruli (Van Hausen & Kemper, 2011; Abid *et al*., 2012). The most common proteinuria in children can be associated with the idiopathic nephrotic syndrome (INS), which includes several histological variants: minimal change nephrotic syndrome (MCNS), (80%), diffuse mesangial proliferation (DMP) and focal segmental glomerulosclerosis (FSGS) (Banaszak *et al*., 2011; Harambat *et al*., 2011). Since the 1950s steroid treatment is the most frequently used therapy of INS. (Hodson *et al*., 2000). However, not all patients show positive response to the therapy and approximately 10-20% of patients don't respond to it. The variety of reactions to this therapy divides the patients to steroid sensitive (SS) and steroid resistant (SR) (Mehls & Hoyer, 2011; Abid *et al*., 2012).

Actions of glucocorticoids (GCs) include the genomic and nongenomic mechanisms. Genomic reactions of GCs are associated with glucocorticoid receptor α (GR) (Gross *et al*., 2009; Mehls & Hoyer, 2011). Nongenomic mechanisms include interactions of GCs with cell membrane, cytosolic GR and with cell membrane-bound GR (Smoak & Cidlowski, 2004; Stahn & Buttgereit, 2008; Alangari, 2010).

MIF (macrophage migration inhibitory factor) is a pleiotropic proinflammatory cytokine produced by many cell types such as: T lymphocytes, monocytes/macrophages, vascular endothelia. It is also released from the pituitary which suggests that MIF is also an endocrine factor (Berdeli *et al*., 2005; Gómez *et al*., 2007), (Fig. 1). Because of its widespread properties it is a crucial mediator of many immune and autoimmune diseases such as: juvenile idiopathic arthritis (JIA), Crohn disease, diabetes type 1, glomerulonephritis, septic shock, inflammatory lung disease and cancer (Berdeli *et al*., 2005; Stosic-Grujicic *et al*., 2009). Although most proinflammatory cytokines are inhibited by GCs, it was shown that MIF is released from macrophages by very low concentrations of GCs (dexamethasone or hydrocortisone at 10^{-14}M) in a rodent cell line (Donn & Ray, 2004; Barnes & Adcock, 2009). Therefore, it may suggest that MIF is a factor which suppresses the efficiency of steroid treatment (Stosic-Grujicic *et al*., 2009). It is assumed that many polymorphisms in the *MIF* genes associated with immune

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Abbreviations: INS idiopathic nephrotic syndrome; GCs glucocorticoids; GR glucocorticoid receptor; MIF macrophage migration inhibitory factor; SSNS steroid sensitive nephrotic syndrome; SRNS steroid resistance nephrotic syndrome; SNP single nucleotide polymorphisms; STR short tandem repeat; LD linkage disequilibrium; RA rheumatoid arthritis

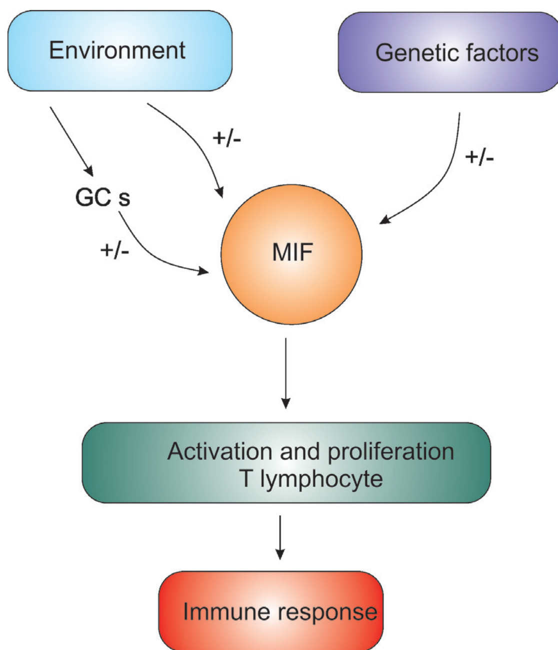

Figure 1. MIF — a mediator of the immune response.

response may influence many immune and autoimmune diseases and have a prognostic value.

The aim of this study was to analyze an association between the discovered *MIF* polymorphisms and glucocorticoid resistance of children with idiopathic nephrotic syndrome in the Polish population, by sequencing all of the exons in the *MIF* gene, with about 100bp of the neighboring intronic sequences and the 5', 3' flanking DNA regions, as well as the promoter DNA.

MATERIALS AND METHODS

Patients and controls. Seventy one (29 girls and 42 boys) patients at the ages from 3 to 19 years old (average age — 10.1±4.4) that were hospitalized because of idiopathic nephrotic syndrome in the years 2008–2010 at the Department of Cardiology and Children Nephrology at the Medical University of Poznan were the subject of this study. Based on steroid treatment during the induction treatment, thirty patients (13 girls and 17 boys) that had the steroid sensitive nephrotic syndrome (SSNS) and forty one children (16 girls and 25 boys) with steroid resistance nephrotic syndrome (SRNS) were selected. Histopathological classification of kidney biopsies of the SRNS children was performed by an experienced pathologist. They were also correlated with age and gender (Table 1). Thirty patients were included as controls. The mean age of the control subjects was 10.1±4.4. Protocol of the study was approved by the Local Ethical Committee of Poznan University of Medical Sciences. Written agreement was obtained from patients and controls. All participating subjects were of Polish origin.

Genotyping. DNA isolation and PCR assay. Genomic DNA was extracted from 200 µl of peripheral leukocytes using a QIA Amp DNA Blood Mini Kit (Qiagen, Hilden, Germany). The DNA was precipitated and resuspended in 200 µl of the AE buffer and stored at –20°C until needed.

The following sequences obtained from GenBank were used as reference sequences of MIF (Gene ID:4282): NG_012099.1: 5001-5845 (genomic). Each of

Table 1. Histopathological classification and correlation with age and gender.

SRNS				
Histopathology	Age of illness onset		Gender	
	<6 years old	>6 years old	Female	Male
MCD (n=3) 7.3%	1	2	3	–
FSGS (n=7) 17.1%	7	–	4	3
MP (n=12) 29.3%	7	5	2	10
MCD, N (n=2) 4.9%	2	–	0	2
MP, N (n=1) 2.4%	1	–	1	–
MP, GS (n=1) 2.4%	1	–	–	1
MPGM (n=1) 2.4%	–	1	1	–
DMP (n=1) 2.4%	1	–	1	–

MCD, minimal change disease; FSGS, focal segmental glomerulosclerosis; MP, mesangial proliferation; N, nephritis; GS, glomerulosclerosis; MPGM, membrane proliferative; DMP, diffuse *mesangial* proliferation

the MIF exons, about 100bp of the neighboring intronic sequences and the 5', 3' flanking DNA regions, as well as the promoter region were amplified by PCR. PCR primers were designed using Primer 3 Input (version 0.4.0.). The PCR reaction consisted of nuclease-free water, PCR Buffer + $MgCl_2$ 10X, d NTP mix 5mM, primer mix (F+R) 12.5 µM, GC Rich Solution 5X, FastStart Taq DNA Polymerase, genomic DNA. The PCR conditions were as follows: 95°C/4min, followed by 40 cycles of: 95°C/30sec, annealing temperature depending on the primer, 72°C/1min, and then 72°/7min. The PCR products were resolved using a 1.5% agarose gel stained with the ethidium bromide.

DNA fragment length analysis. Because of Short Tandem Polymorphism (STR) in the promoter region, the forward primer created for this region was labeled fluorescently with FAM-6 dye at the 5' end. To estimate the length of the resulting reaction products, capillary electrophoresis was carried out on Genetic Analyzer 3110 (Applied Biosystems) in the presence of GeneScan 600 LIZ marker. The results were analyzed using Peak Scanner™ software (Applied Biosystems).

DNA sequencing. PCR products were sequenced using the Big Dye™ Terminator Cycle Sequencing Kit 3.1 and AmpliTaq® polymerase (Applied Biosystems). Detection and sequencing were carried out with a 3130 ABI Genetic Analyzer (PE Applayed Biosystems). Results were analyzed using Sequencing Analysis 5.2. The data were screened for genetic variations in the *MIF* gene using FASTA Sequence Comparison at the U. of Virginia (www.fasta.bioch.virginia.edu) and the GenBank entry NM_002415 as the reference sequence from the National Center for Biotechnology Information SNP database (dbSNP): www.ncbi,nlm.nih.gov/SNP (accesses on September 15, 2012). The SNP data for each sample were used to calculate study-specific SNP genomic and allelic frequencies.

Statistical analysis. The Hardy-Weinberg equilibrium (HWE) assumption was assessed for both the patient and control groups by comparing the observed numbers of each genotype with those expected under the HWE for the estimated allele frequency. Moreover, the Odds Ratio (OR) and 95% Confidence Intervals (95% CI) were calculated for the association between the alleles and genotypes and the risk of INS or initial steroid

Table 2. Genotype distribution and allele frequencies of MIF polymorphisms for children with INS including SRNS and SSNS and controls.

MIF	Position	n	Genotypes			p	OR(95%CI)	Alleles		p	OR(95%CI)
rs755622	-173		G/G	G/C	C/C			G	C		
G>C	Controls	30	16 (53.3%)	13 (43.3%)	1 (3.3%)	0.495	0.687 (0.283-1.666) GC *vs* GG	45 (75.0%)	15 (25.0%)	0.718	0.873 (0.431-1.765)
	INS	71	43 (60.6%)	24 (33.8%)	4 (5.6%)			110 (77.5%)	32 (22.5%)		
	SSNS	30	15 (50.0%)	13 (43.3%)	2 (6.7%)	0.196	0.453 (0.164-1.256) GC *vs* GG	43 (71.7%)	17 (28.3%)	0.222	0.566 (0.256-1.251)
	SRNS	41	28 (68.3%)	11 (26.8%)	2 (4.9%)			67 (81.7%)	15 (18.3%)		
rs112568463	-74		A/A	A/T	T/T			A	T		
A>T	Controls	30	27 (90.0%)	3 (10.0%)	0 (0.0%)	0.358	0.397 (0.075-2.091) AT *vs* AA	57 (95.0%)	3 (5.0%)	0.365	0.410 (0.080-2.092)
	INS	71	68 (95.8%)	3 (4.2%)	0 (0.0%)			139 (97.9%)	3 (2.1%)		
	SSNS	30	27 (90.0%)	3 (10.0%)	0 (0.0%)	–	–	57 (95.0%)	3 (5.0%)	–	–
	SRNS	41	41 (100%)	0 (0.0%)	0 (0.0%)			82 (100%)	0 (0.0%)		
rs2096525	Intron 1 (255)		T/T	T/C	C/C			T	C		
T>C	Controls	30	16 (53.3%)	13 (43.3%)	1 (3.3%)	0.364	0.398 (0.142-1.118) TC *vs* TT	45 (75.0%)	15 (25.0%)	0.714	0.838 (0.413-1.699)
	INS	71	44 (62.0%)	23 (32.4%)	4 (5.6%)			111 (78.2%)	31 (21.8%)		
	SSNS	30	15 (50.0%)	13 (43.3%)	2 (6.7%)	0.117	0.398 (0.142-1.118) TC *vs* TT	43 (71.7%)	17 (28.3%)	0.150	0.521 (0.233-1.164)
	SRNS	41	29 (70.7%)	10 (24.4%)	2 (4.9%)			68 (82.9%)	14 (17.1%)		
rs2070766	Intron 2 (657)		C/C	C/G	G/G			C	G		
C>G	Controls	30	16 (53.3%)	12 (40.0%)	2 (6.7%)	0.516	0.744 (0.315-1.760) CG+GG *vs* CC	44 (73.0%)	16 (27.0%)	0.589	0.800 (0.399-1.602)
	INS	71	43 (60.6%)	24 (33.8%)	4 (5.6%)			110 (77.5%)	32 (22.5%)		
	SSNS	30	15 (50.0%)	13 (43.3%)	2 (6.7%)	0.145	0.464 (0.176-1.227) CG+GG *vs* CC	43 (71.7%)	17 (28.3%)	0.222	0.566 (0.256-1.251)
	SRNS	41	28 (68.3%)	11 (26.8%)	2 (4.9%)			67 (81.7%)	15 (18.3%)		
rs2070767	3'UTR (899)		C/C	C/T	T/T			C	T		
C>T	Controls	30	18 (60.0%)	10 (33.0%)	2 (7.0%)	0.644	0.659 (0.101-4.286) TT *vs* CC	46 (77.0%)	14 (23.0%)	1.000	0.995 (0.487-2.031)
	INS	71	41 (57.7%)	27 (38.1%)	3 (4.2%)			109 (76.8%)	33 (23.2%)		
	SSNS	30	21 (70.0%)	7 (23.0%)	2 (7.0%)	0.047	3.00 (1.043-8.627) CT *vs* CC	49 (82.0%)	11 (18.0%)	0.315	1.633 (0.722-3.695)
	SRNS	41	20 (73.0%)	20 (49.0%)	1 (2.0%)			60 (73.0%)	22 (27.0%)		
rs2000466	3'UTR (1298)		T/T	T/G	G/G			T	G		
T>G	Controls	30	17 (56.7%)	12 (40.0%)	1 (3.3%)	0.660	0.802 (0.337-1.909) TG+GG *vs* TT	46 (76.7%)	14 (23.3%)	0.854	0.918 (0.447-1.882)
	INS	71	44 (62.0%)	23 (32.4%)	4 (5.6%)			111 (78.2%)	31 (21.8%)		
	SSNS	30	14 (46.7%)	14 (46.7%)	2 (6.7%)	0.028	0.321 (0.119-0.869) TG+GG *vs* TT	42 (70.0%)	18 (30.0%)	0.0633	0.440 (0.196-0.989)
	SRNS	41	30 (73.2%)	9 (22.0%)	2 (4.9%)			69 (84.1%)	13 (15.9%)		

rs34383331	3'UTR (1515)			T/T	T/A	A/A			T	A		
T>A	Controls	30		17 (57.0%)	12 (40.0%)	1 (3.0%)	0.650	0.791 (0.324-1.929) TA *vs* TT	46 (77.0%)	14 (23.0%)	1.000	0.956 (0.467-1.956)
	INS	71		43 (60.6%)	24 (33.8%)	4 (5.6%)			110 (77.5%)	32 (22.5%)		
	SSNS	30		14 (47.0%)	14 (47.0%)	2 (7.0%)	0.0514	0.362 (0.135-0.968) TA+AA *vs* TT	42 (70.0%)	18 (30.0%)	0.103	0.480 (0.216-1.066)
	SRNS	41		29 (71.0%)	10 (24.0%)	2 (5.0%)			68 (83.0%)	14 (17.0%)		

responsiveness using Fisher's exact test. The p value of ≤0.05 was considered to indicate statistical significance. Linkage disequilibrium (LD) between SNPs polymorphisms of *MIF* gene was examined by pair-wise comparisons of D' using Haploview version 4.1. Where necessary, correction for multiple testing was done. Haplotype frequencies between SNP and STR polymorphisms of the *MIF* gene in the promoter region were calculated using the PHASE version 2.1.

RESULTS

Single Nucleotide Polymorphisms.

Seventy one patients with INS, including SRNS and SSNS, and thirty controls were genotyped. The genotypic and allelic frequencies for SNP and STR are shown in Table 2 and in Table 3. The frequency of all genotypes studied did not exhibit deviation from the HWE between all investigated groups (p>0.05).

As shown in Table 2, we didn't observe a statistical significance between the detected SNPs in patients with INS and controls. Our studies revealed statistical significance for two polymorphisms: rs2070767C>T and rs2000466T>G between patients with SRNS and SSNS. The results for rs34383331T>A are close to being statistically significant. We found that CT genotype of rs2070767C>T is associated with the risk of SRNS vs SSNS. The OR for SRNS children with the CT genotype compared with CC genotype was 3.00 (95%CI=1.043–8.627, p=0.047). In addition, we evaluated a correlation of rs2000466T>G with SRNS vs SSNS (OR=0.321, 95%CI=0.119–0.869, p=0.028). The distribution of TG genotype is about 2-fold higher in SSNS children than in SRNS (46.7% and 22.0%, respectively), and the occurrence of the GG genotype is about 1.5-fold higher in SSNS children than in SRNS (6.7% and 4.9%, respective-

Table 3. Data from MIF -794 (CATT)5-8 microsatellite (rs5844572) in patients with INS including SRNS and SSNS and controls.

Polymorphisms	Genotypes	INS n=71 (%)	Controls n=30 (%)	OR (95%CI)	p value
rs5844572	5/5	6(8.45%)	2(6.67%)	1.292(0.246-6.800)	1.00
CATT 5-7	5/6	21(29.58%)	8(26.67%)	1.155(0.444-3.006)	0.815
	5/7	4(5.63%)	2(6.67%)	0.836(0.145-4.828)	1.00
	6/6	25(35.21%)	8(26.67%)	1.495(0.581-3.843)	0.490
	6/7	13(18.31%)	10(33.33%)	0.448(0.170-1.181)	0.122
	7/7	2(2.82%)	0(0%)	–	1.00
	Alleles				
	5	37(26.06%)	14(23.33%)	1.158(0.572-2.345)	0.727
	6	84(59.15%)	34(56.67%)	1.108(0.602-2.039)	0.757
	7	21(14.79%)	12(20%)	0.694(0.317-1.521)	0.406
	Genotypes	SRNS n=41 (%)	SSNS n=30 (%)	OR (95%CI)	p value
	5/5	2(4.88%)	4(13.33%)	0.333(0.057-1.954)	0.233
	5/6	17(41.46%)	4(13.33%)	4.604(1.356-15.632)	0.017
	5/7	2(4.88%)	2(6.67%)	0.718(0.095-5.408)	1.00
	6/6	13(31.71%)	12(40%)	0.696(0.621-1.861)	0.616
	6/7	6(14.63%)	7(23.33%)	0.563(0.168-1.890)	0.371
	7/7	1(2.4%)	1(3.33%)	0.725(0.044-12.076)	1.00
	Alleles				
	5	23(28%)	14(23.33%)	1.281(0.594-2.761)	0.567
	6	49(59.8%)	35(58.33%)	1.061(0.539-2.087)	0.865
	7	10(12.2%)	11(18.33%)	0.619(0.244-1.568)	0.345

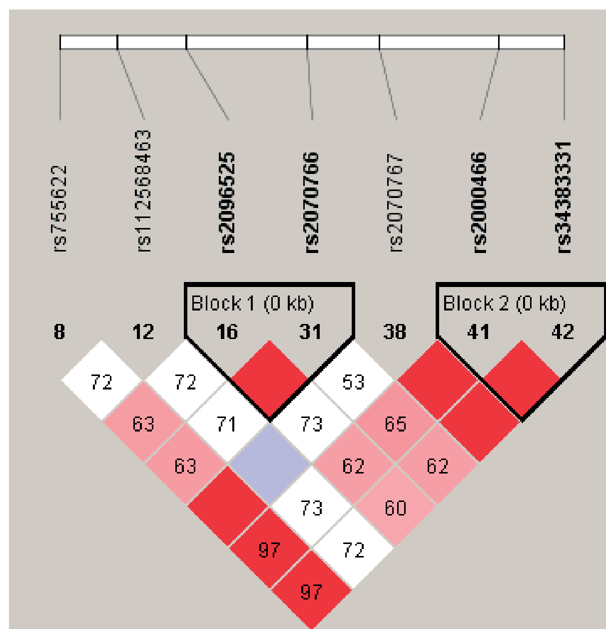

Figure. 2. Relative positions and LD estimates between 7 MIF polymorphisms in the analyzed population (controls and INS). Colored squares correspond to D′ values with numerical estimates given within the squares.

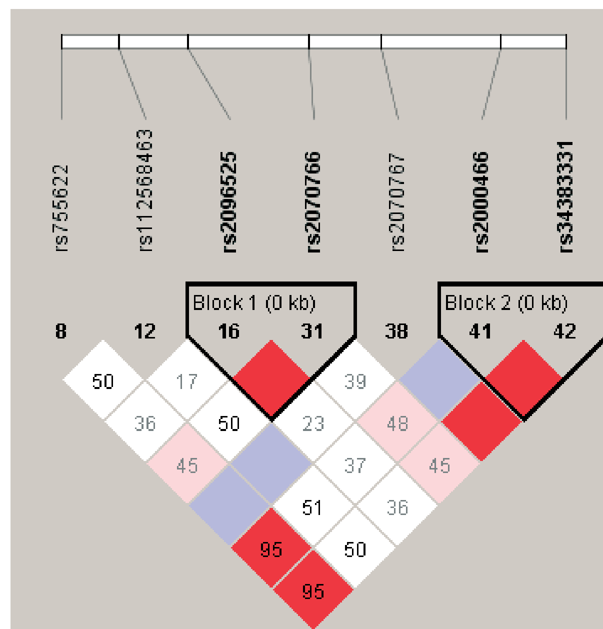

Figure. 3. Relative positions and LD estimates between 7 MIF polymorphisms in the analyzed population (SRNS and SSNS). Colored squares correspond to D′ values with numerical estimates given within the squares.

ly). A similar situation was observed for rs34383331T>A for SRNS vs SSNS (OR=0.362, 95%CI=0.135-0.968, p=0.0514) (Table 2).

Short Tandem Repeat in the promoter region

The microsatellite DNA (rs5844572, CATT5-8) in the *MIF* gene has four CATT repeat units (5, 6, 7 and 8 CATT repeats). In our research we didn't detect any 8 CATT repeats (Table 3). We observed the highest distribution of the CATT5/CATT6 genotype in SRNS patients (41.46%) and CATT6/CATT7 genotype in controls (33.33%). Our studies revealed a statistical significance for the genotype CATT5/CATT6 in SRNS group *vs* SSNS group (OR=4.604, 95% CI=1.356–15.632, p=0.0168). We didn't evaluate the genotype CATT7/CATT7 in controls, whereas it was present in others (INS — 2.82%, SRNS — 2.4%, SSNS — 3.33%). In addition, in Table 3 we make the association between tetranucleotide repeat polymorphism and INS and SRNS and SSNS. We found that the frequency of 5/X-CATT genotype compared with X/X-CATT genotype was significantly higher in SRNS patients *vs* SSNS (OR=3.167, 95% CI=1.046–9.585, p=0.0426). In addition, there were no significant differences in other groups. We also made

Table 4. The association between tetranucleotide repeat polymorphism between groups INS vs controls and SRNS vs SSNS.

Overall	Genotype (n)			OR (95%CI)	Genotype (n)			OR (95%CI)
	5/5	5/X	X/X		Y/Y	7/Y	7/7	
				0.333 (0.057-1.954)				0.725 (0.044-12.076)
SRNS	2(4.88%)	19(46.34%)	20(48.78%)	p=0.233 (5/5 *vs* others)	32(78.05%)	8(19.51%)	1(2.44%)	p=1.00 (7/7 *vs* others)
n=41								
SSNS	4(13.33%)	6(20%)	20(66.67%)	3.167 (1.046-9.585)	20(66.67%)	9(30%)	1(3.33%)	0.556 (0.184-1.676)
n=30				p=0.0426 (5/X *vs* X/X)				p=0.397 (7/Y *vs* Y/Y)
INS	6(8.45%)	25(35.21%)	40(56.34%)	1.292 (0.248-7.347)	52(73.24%)	17(23.94%)	2(2.82%)	0.490 (0.1968-1.2218)
n=71				p=1.00 (5/5 *vs* others)				p=0.151 (7/Y *vs* Y/Y)
Controls	2(6.67%)	10(33.33%)	18(60%)		18(60%)	12(40%)	0(0%)	
n=30								
X - 6 or 7 repeats			5/7;6/7;7/7	„high expression" genotypes				
Y - 5 or 6 repeats			5/5;5/6;6/6	„low expression' genotypes				

Table 5. The correlation between rs755622 G>C and rs5844572(CATT5-8) in children with INS including SRNS and SSNS and controls.

Genotypes	SRNS	SSNS	OR (95%CI)	p value
(CATT)5/5_GG	1(2.4%)	4(13.3%)	0.163(0.0172-1.536)	0.155
(CATT)5/5_GC	1(2.4%)	0(0%)	-	1.00
(CATT)5/6_GG	16(39%)	2(6.7%)	8.96(1.8719-42.888)	0.002
(CATT)5/6_GC	1(2.4%)	2(6.7%)	0.35(0.030-4.050)	0.570
(CATT)5/7_GC	2(4.9%)	2(6.7%)	0.718(0.095-5.408)	1.00
(CATT)6/6_GG	11(26.8%)	9(30%)	0.856(0.302-2.427)	0.795
(CATT)6/6_GC	2(4.9%)	3(10%)	0.462(0.072-2.951)	0.645
(CATT)6/7_GC	5(12.2%)	6(20%)	0.556(0.152-2.027)	0.509
(CATT)6/7_CC	1(2.4%)	1(3.3%)	0.725(0.044-12.076)	1.00
(CATT)7/7_CC	1(2.4%)	1(3.3%)	0.725(0.044-12.076)	1.00
Genotypes	Controls	INS	OR (95%CI)	p value
(CATT)5/5_GG	2(6.7%)	5(7%)	1.061(0.194-5.796)	1.00
(CATT)5/5_GC	0(0%)	1(1.4%)	-	0.307
(CATT)5/6_GG	8(26.7%)	18(25.4%)	0.934(0.354-2.464)	1.00
(CATT)5/6_GC	0(0%)	3(4.2%)	-	0.553
(CATT)5/7_GC	2(6.7%)	4(5.6%)	0.836(0.145-4.828)	1.00
(CATT)6/6_GG	6(20%)	20(28.2%)	1.569(0.558-4.408)	0.462
(CATT)6/6_GC	2(6.7%)	5(7%)	1.061(0.195-5.796)	1.00
(CATT)6/7_GC	9(30%)	11(15.5%)	0.428(0.156-1.176)	0.108
(CATT)6/7_CC	1(3.3%)	2(2.8%)	0.841(0.073-9.638)	1.00
(CATT)7/7_CC	0(0%)	2(2.8%)	-	1.00

(CATT)5/5_GG — low risk; (CATT)7/7_CC — high risk

a correlation between two promoter polymorphisms: rs755622G>C and rs5844572 (CATT5-8) (Table 5). We observed a significant association only in groups SRNS *vs* SSNS for CATT5/CATT6_GG genotypes (OR=8.960, 95%CI=1.872–42.888, p=0.002). The frequency of CATT5/CATT6_GG genotypes was 5.8-fold higher in the SRNS groups when compared with SSNS (39.0% and 6.7% respectively).

Linkage disequilibrium and haplotype analysis

In haplotype blocks for patients with INS and controls, SRNS and SSNS, we observed a strong linkage disequilibrium between four of the seven *MIF* polymorphisms analyzed. The program revealed two blocks (Fig. 2, Table 7). Our results for patients with INS and controls showed that none of the analyzed SNPs is associated with INS (p>0.05). We observed four haplotypes, two in the first block and two in the second block (estimated population frequency >0.05). The haplotype analyses for steroid resistance phenotype also revealed two blocks (Fig. 3, Table 8). We observed three haplotypes in one block and two haplotypes in the second block. We observed that two markers (rs112568463A>T and rs2000466T>G) revealed correlation with steroid resistance phenotype (p=0.0407 and p= 0.0438 respectively, Table 9). In addition, we found that the GA haplotype (rs2000466T>G, rs34383331T>A) may be protective in regard to the steroid resistance phenotype in INS children (p=0.0438), although the association was not sufficiently strong to survive adjusting the significance level for multiple comparisons using 10000 permutations. Also, Table 6 presents *MIF* haplotypes for polymorphisms located in the promoter region: –173G/C (rs755622) and –794 CATT (rs5844572). As we can see, the C allele is in a high linkage disequilibrium with CATT7 allele. The C allele is more frequently associated with CATT7 than with CATT5 or CATT6 in all groups. Accordingly, the G allele is more frequently associated with CATT6 than with CATT5 or CATT7 in

Table 6. MIF Haplotype Frequencies in patients with INS including SRNS and SSNS and controls (according to PHASE).

SRNS				SSNS			
index	haplotype	E(freq)	S.E	index	haplotype	E(freq)	S.E
1	5 G	0.262717 (26.2%)	0.008079	1	5 G	0.222951 (22.2%)	0.012248
2	5 C	0.005576 (0.6%)	0.008079	2	5 C	0.010383 (1%)	0.012248
3	6 G	0.553182 (55.3%)	0.008856	3	6 G	0.490779 (49.1%)	0.013661
4	6 C	0.056574 (5.7%)	0.008856	4	6 C	0.092555 (9.3%)	0.013661
5	7 G	0.001174 (0.1%)	0.003632	5	7 G	0.002937 (0.3%)	0.006939
6	7 C	0.120777 (12.1%)	0.003632	6	7 C	0.180396 (18%)	0.006939
INS				Controls			
index	haplotype	E(freq)	S.E	index	haplotype	E(freq)	S.E
1	5 G	0.249132 (24.9%)	0.006095	1	5 G	0.233005 (23.3%)	0.002319
2	5 C	0.004389 (0.4%)	0.006095	2	5 C	0.000328(0.03%)	0.002319
3	6 G	0.525231(52.5%)	0.006046	3	6 G	0.512798(51.2%)	0.008737
4	6 C	0.073361(7.3%)	0.006046	4	6 C	0.053869(5.4%)	0.008737
5	7 G	0.000286(0.03%)	0.001392	5	7 G	0.004197(0.4%)	0.009279
6	7 C	0.147602(14.8%)	0.001392	6	7 C	0.195803(19.6%)	0.009279

Table 7. The value of D′, LOD and R^2.

INS and controls	L1	L2	D′	LOD	R^2
	rs2096525	rs2070766	1.0	32.92	0.946
	rs2000466	rs34383331	1.0	33.9	0.972
SRNS and SSNS					
	rs2096525	rs2070766	1.0	19.36	0.844
	rs2000466	rs34383331	1.0	23.51	0.96

all groups. Unfortunately we didn't observe any differences between the groups.

DISCUSSION

The present study was designed to investigate association between MIF cytokine polymorphisms in the Polish children from Wielkopolska with INS and their response to steroids.

MIF is a proinflammatory cytokine which plays an important role in many immune and inflammatory diseases so that it can be a good prognostic factor in many therapies (Berdeli *et al.*, 2005). It was shown that MIF also plays an important role in kidney diseases (Lan, 2008). Moreover it counter-regulates glucocorticoids effect by suppressing their therapeutic effects (Lolis, 2001; Berdeli *et al.*, 2005). It was found that glucocorticoids induce the synthesis of IϰB, whereas MIF reduces IϰB. Consequently NF-ϰB translocates to the nucleus as a free factor where it initiates transcription of specific genes. This is one of the ways to enhance an inflammatory response. Similarly, glucocorticoids also inhibit activity of the phospholipase A_2 (PLA_2) but MIF counteracts by stimulating the ERK1/2 kinases pathways, activating PLA_2 releasing arachidonic acid and as a consequence leukotriens and prostaglandins are expressed (Renner *et al.*, 2005; Bucala, 2013). Several studies have reported a functional significance of the *MIF* gene polymorphisms. Our results show that frequency of the CT genotype rs2070767C>T, which is located in the 3'UTR region, is significantly higher in the SRNS group than in SSNS children. We also found that other two polymorphisms located in the 3'UTR region (rs2000466T>G and rs34383331T>A) are correlated with SSNS when compared with SRNS. In contrast to previous findings, however, no evidence of rs2070767C>T, rs2000466T>G and rs34383331T>A were determined in patients with INS, and SSNS or SRNS. Li Gao *et al.* (2007) examined six polymorphisms of the *MIF* gene in Acute Lung Injury. They explained the influence of polymorphisms in regulatory elements in the 3'UTR on mRNA stability as well as subcellular localization of transcripts.

Zheng X *et al.* (2012) examined whether polymorphisms of the *MIF* gene are associated with Behçet's disease (BD) in the Han Chinese population. They showed significant association of two SNPs: rs755622G>C and rs2096525T>C with BD. Additionally, they suggested that the involvement of *MIF* in BD may be through regulation of its mRNA expression (Zheng *et al.*, 2012). Berdeli et al. (Berdeli *et al.*, 2005) showed correlation of the MIF –173C allele with the INS and SRNS in children in the Turkish population. Similar results were obtained by Vivarelli *et al.* (2008) in respect to children from Italian population. Polymorphism rs755622G>C in the promoter region has been correlated with a high level of MIF protein as the transition of G to C creates an activator protein 4 response element (Berdeli *et al.*, 2005, Gómez *et al.*, 2007). However, these findings are not in the agreement with our results. This observation is in agreement with Choi's findings which showed no association between the rs755622G>C MIF polymorphism and clinical parameters, renal histology and steroid responsiveness (Choi *et al.*, 2011). It seems possible that these results are due to variations in ethnic and geographic distribution of INS. Similar results were shown for other inflammatory diseases. Berdeli *et al.* (2006) did not reveal the role of –173C allele in susceptibility to JRA (juvenile rheumatoid arthritis) in children from the Turkish population. On the other hand, Donn *et al.* (2002) presented association between –173C allele and JIA (juvenile idiopathic arthritis) in the UK population. Positive results were also shown in the Chinese population with RA (rheumatoid arthritis) (Liu *et al.*, 2012; Xie *et al.*, 2012).

Several studies have been carried out on the influence of short tandem repeat (STR) CATT5-8 (rs5844572) in the promoter region of *MIF* and its influence on many inflammatory diseases (Renner *et al.*, 2005; Li *et al.*, 2012). It was shown in these studies that the number of this tetranucleotide repeat is correlated with higher *MIF* expression taking into account population diversity (Bucala, 2013). In our study we found that the frequency of 5/X-CATT genotype compared with X/X-CATT genotype was significantly higher in the SRNS patients *vs* SSNS. In addition, we observed correlation between two promoter polymorphisms in *MIF*, which showed that the C allele is in a high linkage disequilibrium with CATT7 allele and the G allele is more frequently associated with CATT6. Similar results were obtained by Sreih *et al.* (2011) who showed dual effect of the *MIF* gene according to the severity of SLE. They revealed that the presence of high expression of *MIF* haplotype 7C correlates with a lower form of SLE in Caucasians and lower range in African-Americans. They showed that increased MIF production due to genetic factors may correlate with protection against autoimmune response associated with SLE. According to our research it is suggested that it may be associated with a positive response in steroid therapy. In contrast to previous findings, Gázquez *et al.* (2012) showed no association of functional variants in the promoter region of *MIF* gene with disease susceptibility or hearing loss progression in patients with Méniére's disease. However, the findings

Table 8. Haplotype associations between groups SRNS i SSNS

SSNS i SRNS				
Haplotype	Frequency	Case, Control Frequencies	Chi Square	P Value
Block 1				
TC	0.775	0.817, 0.717	2.001	0.1572
CG	0.197	0.171, 0.233	0.858	0.3544
TG	0.028	0.012, 0.050	1.809	0.1786
Block 2				
TT	0.775	0.829, 0.700	3.317	0.0686
GA	0.218	0.159, 0.300	4.063	0.0438*

Table 9. Single marker association between groups SRNS and SSNS

Name	Assoc. Allele	SRNS, SSNS Ratio Counts	SRNS, SSNS Frequencies	Chi square	P value
rs755622	G	67:15, 43:17	0.817, 0.717	2.001	0.1572
rs112568463	A	82:0, 57:3	1.000, 0.950	4.188	0.0407
rs2096525	T	68:14, 46:14	0.829, 0.767	0.858	0.3544
rs2070766	C	67:15, 43:17	0.817, 0.717	2.001	0.1572
rs2070767	T	22:60, 11:49	0.268, 0.183	1.402	0.2364
rs2000466	T	69:13, 42:18	0.841, 0.700	4.063	0.0438
rs34383331	T	68:14, 42:18	0.829, 0.700	3.317	0.0686

of tour study do not support the previous research in autoimmune diseases. Baugh *et al.* (2002) identified that the low expression of 5-CATT repeat allele was correlated with low inflammation in RA. They also analyzed rs755622G>C but it wasn't associated with RA (Baugh *et al.*, 2002). A similar situation was observed in the Japanese population with ulcerative colitis (UC). Shiroeda *et al.* (2010) showed that genotype 5/5-CATT was a decreased risk for UC, but additional genotype 7/7-CATT was correlated with chronic continuous phenotype and distal colitis phenotype. They didn't find association with polymorphism rs755622G>C.

Histological correlation with age and gender showed that all cases of FSGS (Focal Segmental Glomerulosclerosis) occurred in children under 6 years old and 83.3% cases of MP (Mesangial Proliferation) were observed in boys (Table 1). We did not find any association between histological type of glomerulonephritis and SNPs. However, we observed that the most frequent types: FSGS and MP, were weakly correlated with the decreased number of repeats in the STR polymorphism (CATT5/6, CATT6/6 and CATT5/6_GG, CATT5/6_ GG, CATT6/6_GG) (not shown).

In addition, Matsumoto *et al.* (2005) showed that MIF levels in the serum increased in patients with FSGS and steroid-resistance NS. However, further work is required to establish the association with INS. Moreover, according to Bucala (2013), MIF interacts with Jab1 (Jun activation domain-binding protein 1), which is a co-activator of the transcription factor AP-1. AP-1 participates in transcription of many proinflammatory cytokines. The ability of MIF to switch off this pathways suggests that MIF can also play a role in anti-inflammatory mechanisms (Donn & Ray, 2004; Bucala, 2013), which may indicate pleiotropic features of MIF.

In summary, the study presented here was designed to determine the genetic factors of INS as well as SRNS. We discovered seven SNPs polymorphisms and one STR polymorphism in the *MIF* gene. Our results suggest that the influence of *MIF* polymorphisms on positive response to the steroid therapy is rather weak. Because of ambiguous findings, pleiotropic features of this cytokine require that more research should be undertaken. In addition, most polymorphisms in our studies are in nonfunctional variants. This suggests that they may be in linkage disequilibrium with polymorphisms of other genes or because of localization in regulatory elements in the 3'UTR region, their mRNA stability might be influenced. In summary, MIF may be involved in complex and indirect molecular mechanisms of steroid resistance in children with INS.

Conflict of interest

No conflict declared.

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