Journal of
Gastroenterology and Hepatology Research

Online Submissions: http: //www.ghrnet.org/index./joghr/
doi: 10.17554/j.issn.2224-3992.2021.10.1015

Journal of GHR 2021 June 21; **10(3)**: 3504-3509
ISSN 2224-3992 (print) ISSN 2224-6509 (online)

ORIGINAL ARTICLE

STAT4 Gene Polymorphism in Children with Type-1 Autoimmune Hepatitis

Ola G. Behairy[1], Nazih M. Alnady[1], Shuzan Ali Mohammed[2], Walaa A. Ibrahim[3]

1 Pediatrics department, Faculty of Medicine, Benha University, Egypt;
2 Medical Biochemistry department, Faculty of Medicine, Benha University, Egypt;
3 M.B.B.Ch., Faculty of Medicine, Benha University, Egypt.

Conflict-of-interest statement: The authors declare that there is no conflict of interest regarding the publication of this paper.

Correspondence to: Ola Galal Ali Behairy, Department of Pediatrics, Faculty of Medicine, Benha University, Benha, Qualiopia, Egypt.
Email: olaped99@yahoo.com
Telephone: +22890125159

Received: May 23, 2021
Revised: May 30, 2021
Accepted: June 2, 2021
Published online: June 21, 2021

ABSTRACT

BACKGROUND: The signal transducer and activator of the transcription 4 (*STAT4*) gene plays an important role in macrophages, dendritic cells, activated peripheral blood monocytes, and Th1-dependent liver injury.

AIM: To assess the association of signal transducer and activator of transcription (STAT4) single gene polymorphisms (SNPs) (rs7574865, rs7582694) with type-1 autoimmune hepatitis (AIH) in Egyptian children.

MATERIALS AND METHODS: 125 children diagnosed as AIH type-1and 125 control healthy children were included for genotyping of STAT4 SNPs (rs7574865 and rs7582694).

RESULTS: STAT4 (rs7574865) GT and TT genotypes increased the risk of AIH type-1 development by 2 and 4 folds regarding the GG genotype ($p < 0.019$, 0.001 respectively). T allele individuals had about 2.5 folds increased risk of the disease compared to those with G allele ($p < 0.001$). STAT4 (rs7582694) GC and CC genotypes increased the risk of AIH type-1 by 1.76 and 3.8 folds regarding the GG genotype ($p < 0.037$, 0.015 respectively). The C allele had about 1.88 folds increased risk of AIH type-1 development than those with the G allele ($p = 0.002$). Dominant model of either SNPs and the recessive model of rs7574865 only are significant predictors of AIH type-1.

CONCLUSION: Minor alleles of both STAT4 SNPs (rs7574865 T allele, rs7582694 C allele) are associated with an increased risk of type-1 AIH. The disease could be predicted by the dominant model of either SNPs and by the recessive model of rs7574865.

Key words: Autoimmune hepatitis; Signal transducer and activator of transcription 4; Children

Behairy OG, Alnady NM, Mohammed SA, Ibrahim WA. STAT4 Gene Polymorphism in Children with Type-1 Autoimmune Hepatitis. *Journal of Gastroenterology and Hepatology Research* 2021; **10(3)**: 3504-3509 Available from: URL: http://www.ghrnet.org/index.php/joghr/article/view/3173

INTRODUCTION

Autoimmune hepatitis (AIH) is an immune-mediated inflammatory liver disease, affecting both kids and teens, most often females. In children, the clinical presentation of AIH may be acute, chronic, or silent, and must be supposed and excluded in any child presented with signs of acute, prolonged, or severe liver disease. The existence of unique autoantibodies distinguishes the two forms of AIH are (type-1 and type-2), when AIH is left untreated, and sometimes even with treatment, AIH gradually progresses to cirrhosis. A combination of clinical symptoms, laboratory evaluation, histopathology, and the absence of other more common liver diseases is used to render the

diagnosis of AIH. The best outcome for AIH relies on early diagnosis and the early start of immunosuppressant therapy[1].

A dynamic association of genetic, immunological, and environmental factors contributes to the loss of immunotolerance to hepatic antigens, causing a progressive inflammatory necrotizing and fibrotic disease, characterizes the current hypothesis of AIH pathogenesis[2].

AIH Susceptibility is partly determined by the existence of human leukocyte antigen (HLA) related genes, mostly allelic variants of DRB1[3]. Non-HLA susceptibility genes can also lead to, and remain to be, elucidated as hereditary susceptibility to AIH. With the advent of genome-wide association studies (GWAS), several complex hereditary autoimmune diseases, such as type 1 diabetes and rheumatoid arthritis (RA), have seen a significant rise in genetic findings[4].

One of the STAT family members is the signal transducer and activator of transcription 4 (STAT4), which spans 120 kb on human chromosomes 2q32.2-q32.3. The gene is made up of 24 exons and encodes the STAT4 transcription factor that locates the cytoplasm[5], after its phosphorylation and translocation to the nucleus, STAT4 control the expression of different genes as transcription factor. STAT4 activation is practically observed in many use models of liver damage in the liver as well as in chronic liver diseases in the human[6], also STAT4 is expressed in dendritic cells, macrophages and activated peripheral blood monocytes[7]. STAT4IS activated by interleukin 12 (IL2) resulting in differentiation of T helper (Th) 1and Th 17, monocyte activation and interferon-alpha (IFN-alpha) which induce autoimmunity, so STAT4 may play a key role in the production of autoimmune diseases[8].

Given the limited number of studies on the relationship between single gene polymorphisms (SNPs) of STAT4 (rs7574865 and rs7582694) and the risk of AIH, especially in children[9], this study aimed to assess such association of STAT4 (rs7574865 and rs7582694) SNPs with AIH type-1 in Egyptian children.

PATIENTS AND METHODS

This comparative cross-sectional study included 250 children divided into 2 groups. An AIH group included 125 children diagnosed using ESPGHAN score[10] who were attending Pediatric Hepatology Clinics in Benha University Hospitals and National Liver Institute−Menoufia University, in the period between October 2015 and October 2020. Any child with other chronic liver diseases and/or comorbidity as cardiovascular, renal, or central nervous systems affection was excluded. The second group is the control group; included 125 healthy children selected from the general population of matched age and sex with the patient group. Control subjects were children attending outpatient clinic for routine checkup for the purpose of sports training or school routine examination.

Ethical permission and consent were obtained from the parents or health care providers of each child. Approval of the study was obtained from the Ethical Committee of Scientific Research, Faculty of Medicine, Benha University. This study was carried out according to the guidelines of the Declaration of Helsinki[11].

Methods

Each patient underwent full history taking, comprehensive clinical examination, abdominal ultrasonography, and routine laboratory investigations including:

Complete blood picture by Sysmex-XP300, prothrombin time (PT), activated partial thromboplastin time (aPTT) were done by TEClot PT-S on Coatron A4 instrument (Germany), and liver function tests; aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), total and direct bilirubin and serum albumin were done by Biosystem A1A-auto analyser-Spain.

Patients were tested for antinuclear antibody (ANA), anti-smooth muscle antibody (ASMA), liver-kidney microsomal antibody (LKM-1), anti-soluble liver antigen, anti-liver cytosol type 1 (LC1), and anti-mitochondrial antibody (AMA) titer were performed by indirect immunofluorescence technique using NOVA Lite Rat Liver, Kidney, Stomach (INOVA Diagnostic Inc, Germany). Serum immunoglobulin G (IgG): By radial immunodiffusion using (IgG--NLRID, RN004.3, Binding Site, Birmingham, United Kingdom). To exclude other causes of liver diseases before assigning patients for liver biopsy; all patients were subjected to serological tests for viral hepatitis B, E and C: Hepatitis B surface antigen (HBs Ag), Anti-HEV IgM and anti-HCV antibody (HCV-Ab) and cytomegalovirus IgM as well as anti-Epstein-Barr virus (EBNA-IgM and VCA-IgM) antibodies by enzyme linked immunosorbent assay (ELISA) were done and confirmation was done by using PCR (Biokit, Spain). Wilson's disease was excluded by estimating serum ceruloplasmin, 24 hr. urinary copper before and after penicillamine and the presence of Keyser-Fleischer rings. An ultrasound-guided liver biopsy was performed for all patients using the Menghini aspiration needle to obtain an adequate core containing at least 11 portal tracts **(Hepafix Luer Lock Braun Melsungen AG, Melsungen, Germany)**. Formalin-fixed, paraffin-embedded biopsy specimens were cut and stained with; hematoxylin and eosin to assess the histological activity of liver disease using the METAVIR scoring system[12], which comprises five stages: F0 (no fibrosis), F1 (minimal fibrosis, portal fibrosis without septa), F2 (moderate fibrosis, portal fibrosis with few septa), F3 (severe fibrosis, septal fibrosis with many septa but no cirrhosis) and F4 (cirrhosis). Sections were stained with Mason-Trichrome to assess fibrosis stage, Perls' Prussian blue stain to show iron deposition, and periodic acid chief stain to exclude alpha 1 anti-trypsin deficiency. Two pathologists blindly evaluated the slides.

Genotyping of *STAT4* (rs7574865 and rs7582694) SNPs:

Sampling: A venous blood sample (2 ml) was taken from each subject on ethylene diamine tetra-acetic acid (EDTA) and kept at -20°C till genotyping.

Steps

1. DNA extraction: DNA was extracted from 200 µl EDTA blood sample; using Purelink® Genomic DNA mini kit (Invitrogen, Life Technologies) according to the manufacturer's instructions. The extracted DNA concentration was measured by Nanodrop Spectrophotometer 2000 (Thermo-Fisher Scientific, Wilmington, USA). Optical density (OD) was taken at wave-lengths 260 and 280 nm. An OD ratio (260 / 280) provides an estimate of DNA purity. Pure DNA preparations have a ratio of 1.7 - 2.0[13]. The extracted DNA was used for amplification of both SNPs; rs7574865 by conventional PCR (followed by RFLP) and rs7582694 by real-time PCR.

2. Genotyping of rs7574865: NA amplification for rs7574865 was done in 25µl reaction / a sample with forward primer (FP): 5'-AAAGAAGTGGGATAAAAAGAAGTTTG-3' and reverse primer (RP): 5'-CCACTGAAATAAGATAACCACTGT-3'[14]. Amplification mix contained 12.5µl Dream Taq Green PCR Master Mix (2X), 3µl DNA template, 1.5µl FP, 1.5µl RP, and up to 25µl nuclease-free water. The master mix and primers were supplied by Thermofisher Scientific. Amplification was done in Veriti™ Thermal Cycler (Applied Biosystems). The PCR conditions were:

initial denaturation (5 min at 95°C), 35 cycles (denaturation; 30 sec at 95°C, annealing; 30 sec at 56°C, extension; 1 min at 72°C) and then final extension (5 min at 72°C). PCR products (10µl) and 100 base-pair ladder (5µl) were resolved in 2.5% agarose gel stained with 0.3 ug/ml ethidium bromide to check the PCR product at 147 bp fragment. Bands were visualized using UV transilluminator (254 nm) and imaged with a digital camera 8 megapixel and analyzed by computer software (Alpha InoTech Gel Documentation System) (Figure 1A).

RFLP was done for *STAT4* (rs7574865) by Fast-digest HpaI restriction enzyme (ThermoFisher Scientific) in 30µl total volume by mixing: 10µl of PCR products + 1µl HpaI restriction enzyme + 2µl 10X buffer + 17µl nuclease-free water. The digestion mixtures were incubated at 37°C for 2 hours then inactivated by incubation at 65°C for 10 min. Post-digestion bands were visualized with the same conditions described for bands of PCR products before digestion. Post-digestion; the T allele gave 2 fragments (122 bp and 25 bp), while the G allele was uncut (147 bp). The small band (25 bp) was lost in the gel (Figure 1B).

3. Genotyping of rs7582694: Allele discrimination of rs7582694 was performed using the TaqMan SNP Genotyping assay (Applied Biosystem, Foster City, California, USA) using Step one Real-Time PCR System (Applied Biosystem, Foster City, USA). The amplification mix contained 10µl Taqman Universal PCR master mix No AmpErase (2X), 1µl working SNP assay (20X), 20 ng DNA template, and up to 20µl nuclease-free water. The thermal cycling conditions were: pre-PCR read (15 sec at 60°C), Amplitaq Gold enzyme activation (10 min at 95°C), 40 cycles (denaturation; 15 sec at 92°C, annealing/extension; 1 min at 60°C) and finally Post-PCR read at 60°C for 15 sec.

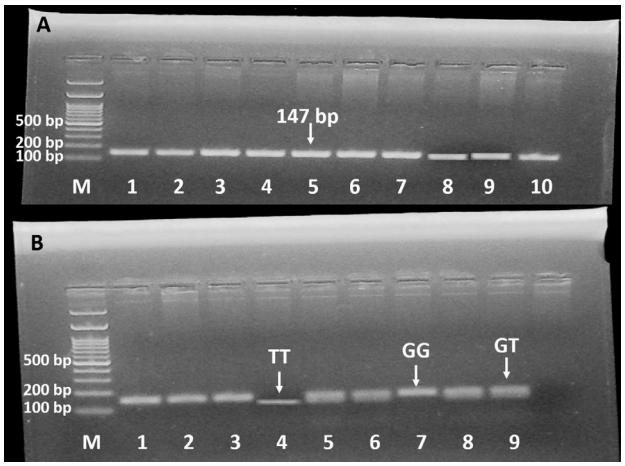

Figure 1 PCR products of amplified STAT4 (rs7574865) before (1-A) and after (1-B) HpaI restriction enzyme.

Statistical analysis

The collected data were tabulated and analyzed using SPSS version 16 software (SpssInc, Chicago, ILL Company). Categorical data were presented as number and percentages while quantitative data were expressed as mean ± standard deviation (SD), median, and range. Chi-square (X^2), Fisher's exact test (FET), and Kappa test were used to analyze categorical variables. Continuous data were tested for normality using the Shapiro-Wilks test assuming normality at $p > 0.05$, using student "t" test to assess difference among 2 independent groups regarding normally distributed variables or Man Whitney U test for non-parametric ones. Binary logistic regression analysis was run to detect the predictor allele of AIH type-1. P ≤0.05 was considered significant (S). Hardy-Weinberg equilibrium was calculated by an online calculator comparing the observed and expected genotype frequencies. For rs7582694, both control and patient groups were in Hardy-Weinberg equilibrium (HWE) (X^2 = 0.039, p = 1, and X^2 = 0.24, p = 0.062, respectively), but they were not in HWE for rs7574865 (X^2 = 14.47 and =10.7, respectively) with $p < 0.01$ for both groups.

RESULTS

Study population characteristics

The mean age of the studied AIH patients was 9.9 ± 3.7 years ranged from 4-16 years old. They were 68 (54.4%) females and 57(45.6%) males with a female to male ratio 1.2:1. While the control children were 65 females (52%) and 60 males (48%) with mean age 9.3 ± 4.3 years ranged from 4 to 16 years old. The AIH patients were presented clinically with jaundice (72.8%), fatigue (33.6%), abdominal pain (19.2%), abdominal distention (35.2%), fever (9.6%), faltering of growth (8.8%), and gastrointestinal bleeding (9.6%). Abdominal ultrasonography of patients revealed hepatomegaly (64.8%), splenomegaly (44%), and ascites (12%). All studied patients were AIH type 1 (anti-LKM-1, anti-liver cytosol type1 antibody (anti-LC-1) or AMA negative). Ninety-five patients (76%) were ANA positive and 100 patients (80%) were ASMA positive with titer ranged from 1:20 to 1:160.

Table 1 Baseline biochemical characteristics among the studied groups.

Variable	Autoimmune hepatitis type-1 (n.=125)	Controls (n =125)	Z_{MWU} test	P
	Mean ± SD (Range)			
AST (IU/L)	234.4 ± 151.3 (57-960)	25.5 ± 8.4 (10-40)	13.6	<0.001
ALT (IU/L)	212.4 ± 136.6 (68-825)	32.3 ± 8 (7-34)	13.03	<0.001
Total bilirubin (mg/dl)	2.99 ± 0.62 (2-4)	0.57 ± 0.24 (0.1-1.0)	13.7	<0.001
Direct bilirubin (mg/dl)	1.02 ± 0.33 (0.4-2.0)	0.34 ± 0.14 (0.06-0.6)	13.4	<0.001
Serum albumin (g/dl)	3.27 ± 0.57 (1.9-4.8)	4.19 ± 0.46 (3.4-4.9)	10.9	<0.001
Serum protein (g/dl)	7 ± 1.69 (5-14)	7.8 ± 0.25 (6.7-8.2)	7.69	0.06
IgG (mg/dl)	3742.9 ± 1945.4 (1653-8320)	1062.9 ± 205.9 (758-1434)	13.7	<0.001
PT (sec)	16.2 ± 3.05 (10-28)	11.9 ± 0.82 (11-13)	12.2	<0.001
PTT(sec)	41.8 ± 7.20 (25-55)	29.9 ± 2.94 (25-35)	11.7	<0.001
INR	1.61 ± 0.40 (1-3.4)	1.00 ± 0.00 (1-1)	12.9	<0.001
ALP (IU/L)	225.8 ± 136.4 (55-666)	109.9 ± 31.0 (65-165)	9.01	<0.001
Serum creatinine (mg/dl)	0.61 ± 0.21 (0.1-1.0)	0.52 ± 0.25 (0.1-1.0)	2.83	0.06

AST: Aspartate transaminase, ALT: alanine transaminase, ALP: alkaline phosphatase, PT: Prothrombin time, PTT: partial thromboplastin time, IgG: immunoglobulin G, INR: international normalized ratio, ZMWU test: Z value of Man Whitney U test.

The baseline biochemical and histopathological characteristics of patients are listed in Tables 1 and 2.

By applying the scoring system for AIH diagnosis, we found that the frequency of definite diagnosis of AIH by the revised original scoring system of the IAIHG compared to the simplified scoring system was (63.2% vs. 80% respectively), and probable diagnosis of AIH was (36.8% vs. 20% respectively). All our patients received steroids as initial therapy and azathioprine was added to 54.4 patients while starting steroid withdrawal. A complete response to treatment [which was defined as normalization of liver enzymes and absence of clinical symptoms (normal ALT on a minimum of two occasions at least a month apart)] was observed in 79 (63.2%) patients and 46 (36.8%) patients had relapses [which was defined as a least two-fold increase in AST or ALT in isolation or in combination with histological evidence of disease activity].

STAT4 (rs7574865) genotype and allele frequencies in AIH-1 patients and controls

STAT4 (rs7574865) GT genotypes were two folds increased risk of AIH development than GG individuals (30.4% vs. 21.6%). Also, those with TT genotype were about four folds increased risk of AIH development than GG ones (20.8% vs. 8.0%). All these associations were statistically significant (p = 0.019 and =0.001 respectively). Individuals with the T allele were about 2.5 folds increased risk of AIH development than those with G allele (36% vs. 18.8%). This association was statistically significant (p < 0.001). The G allele is protective, but the T allele is an independent risk factor for AIH (Table 3).

When analyzing the dominant model of inheritance, it was found that GT+TT genotypes were significantly associated with AIH patients than healthy controls (51.2% vs. 29.6%), with about two and a half-fold increased risk of AIH development (p = 0.001, OR = 2.49). When analyzing the recessive model of inheritance, it was found that the TT genotype was significantly associated with AIH patients than healthy control (20.8% vs. 8.0%) with a three-fold increased risk of AIH development (p = 0.004, OR = 3.0) (Table 3).

STAT4 (rs7582694) genotype and allele frequencies in AIH-1 patients and controls

STAT4 (rs7582694) *GC* genotypes were 1.76 folds increased risk of AIH type-1 development than GG individuals (41.6% vs. 32%). Also, those with CC genotype showed 3.8 folds increased risk of AIH development than GG ones (11.2% vs. 4%). All these associations were statistically significant (p = 0.037 and =0.015, respectively). Individuals with the C allele were about 1.88 folds increased risk of AIH development than those with the G allele (32% vs. 20%). This association was statistically significant (p = 0.002). The G allele is protective but the C allele is an independent risk factor for AIH (Table 3).

When analyzing the dominant model of inheritance, it was found that GC+CC genotypes were significantly associated with AIH patients than healthy control (52.8% vs. 36%) with a 1.99 fold increased risk of AIH development (p = 0.008, OR = 1.99). When analyzing the recessive model of inheritance, it was found that CC genotype was significantly associated with AIH patients than healthy control (11.2% vs. 4%) with a 3.03 fold increased risk of AIH development (p = 0.032, OR=3.3) (Table 3).

Binary logistic regression analysis for predictors of AIH type-1

By applying binary logistic regression analysis for predictors of AIH, regarding STAT4 (rs7574865), both dominant and recessive models

Table 2 Histopathological characters of the autoimmune hepatitis type-1 group according to METVAR score.

Variable		Autoimmune hepatitis type-1 (n=125)	
		n.	%
Histological Activity Index	A1 (Mild Activity)	78	62.4
	A2 (Moderate Activity)	35	28
Fibrosis stage	A3(Severe Activity)	12	9.6
	F1 (mild fibrosis)	30	24
	F2 (moderate fibrosis)	31	24.8
	F3 (sever fibrosis)	40	32
Interface Hepatitis score	F4 (cirrhosis)	24	19.2
	1	51	40.8
	2	38	30.4
	3	29	23.2
Cell type	4	7	5.6
	Plasma cells	82	65.4
	Lymphocytes	23	18.4
	Mononuclear	14	11.2
Type of Interface hepatitis	Eosinophils	6	4.8
	Piecemeal	101	80.8
	Rosette & ballooning	14	19.2

Table 3 Genotype and allele frequencies of rs7574865 and rs7582694 among the studied groups

STAT4 SNP	Autoimmune hepatitis type-1 (n = 125)	Control (n = 125)			
	n. (%)		P	OR	95% CI
rs7574865					
Additive genotypes					
GG	61 (48.8)	88 (70.4)	Ref		
GT	38 (30.4)	27 (21.6)	0.019	2.03	1.1-3.7
TT	26 (20.8)	10 (8.0)	0.001	3.75	1.68-8.3
Dominant model					
GG	61 (48.8)	88 (70.4)	Ref		
GT+TT	64 (51.2)	37 (29.6)	0.001	2.49	1.48-4.2
Recessive model					
GG+GT	99 (79.2)	115 (92.0)	Ref		
TT	26 (20.8)	10 (8.0)	0.004	3	1.38-6.6
Alleles					
G	160 (64.0)	203 (81.2)	Ref		
T	90 (36.0)	47 (18.8)	<0.001	2.43	1.6-3.6
rs7582694					
Additive genotypes					
GG	59 (47.2)	80 (64)	Ref		
GC	52 (41.6)	40 (32)	0.037	1.76	1.04-3
CC	14 (11.2)	5 (4)	0.015	3.8	1.3-11.1
Dominant model					
GG	59 (47.2)	80 (64)	Ref		
GC+CC	66 (52.8)	45 (36)	0.008	1.99	1.2-3.3
Recessive model					
GG+GC	111 (88.8)	120 (96)	Ref		
CC	14 (11.2)	5 (4)	0.032	3.03	1.05-8.7
Alleles					
G	170 (68)	200 (80)	Ref		
C	80 (32)	50 (20)	0.002	1.88	1.3-2.8

Ref: reference genotype or allele, OR: odd ratio, CI: confidence interval

are significant predictors of autoimmune hepatitis (the dominant model was 5.63 times more risk and the recessive model was 2.77 times more risk). Regarding STAT4 (rs7582694) only the dominant model was a significant predictor for autoimmune hepatitis (3.1 times more than normal), while the recessive model was not significant (Table 4).

There was no correlation between STAT4 (rs7574865 & rs7582694) gene and age, sex, other autoimmune disease and autoimmune disease in first degree relatives, liver span and spleen span, IgG, AST, ALT, T. bilirubin, D. bilirubin, S. protein, PT, PTT, INR, ALP, S. creatinine, autoantibodies (ANA, ASMA), pathological type of AIH, diagnostic scoring system (revised original scoring system and simplified scoring system) and regarding response to treatment.

Table 4 Binary logistic regression analysis for predictors of autoimmune hepatitis type-1.

Variable	β	Adjusted OR	95% CI	P
rs7574865				
GT+TT vs GG	2.5	5.63	1.2-28.6	0.014
TT vs GG+GT	1.73	2.77	1.13-19.5	0.037
rs7582694				
GC+CC vs GG	1.78	3.1	1.2-11.7	0.025
CC vs GG+GC	0.84	1.28	0.34-4.7	0.11

Vs: versus, S: significant, NS: nonsignificant; rs7574865: Wild form = GG; Mutant forms = GT and TT; Recessive model = TT vs GG+GT; Dominant model = GT+TT vs GG; rs7582694: Wild form = GG; Mutant forms = GC and CC; Recessive model = CC vs GG+GC; Dominant model = GC+CC vs GG.

DISCUSSION

Recent in vivo and in vitro studies have shown that STAT4 plays an important role in the pathogenesis of various human diseases, especially autoimmune and inflammatory diseases such as inflammatory bowel diseases, psoriasis, and systemic sclerosis. *STAT4*, activated by numerous cytokines through the signaling cascade of Janus kinase (JAK)-STAT, controls several aspects of innate and adaptive immune responses. The effect of SNPs on *the STAT4* gene is minimal and further study is required[15]. This is why our study focused on how the SNPs in *STAT4* (rs7574865 and rs7582694) contribute to immune dysregulation and autoimmunity in AIH.

In the present study, we found that rs7574865 and rs7582694 in *STAT4* gene minor alleles are associated with increased type 1 AIH risk, as the G allele is protective but T (rs7574865) & C alleles (rs7582694) are independent risk factors for AIH. These findings were in agreement with Li et al[9] who found that both genotypes in controls are distributed according to HWE (both p values > 0.05). The rs7574865 T allele and rs7582694 C allele frequencies were significantly higher in type-1 AIH cases than in the control group (30.6% vs 19.3%, 32.5% vs 20.0%). Logistic regression analysis indicated that type-1 AIH was significantly higher in carriers of T allele of rs7574865 than those with GG genotype (GT + TT vs GG), and higher in carriers of C allele of rs7582694 than those with GG genotype (GC + CC vs GG). In addition, they observed that participants with GC or CC of rs7582694 genotype had the highest type-1 AIH risk, compared to participants with GG of rs7582694 genotype. After covariates modification, they concluded that haplotype containing the rs7582694-C and rs7574865-T alleles within the *STAT4* gene was associated with a statistically increased type-1 AIH risk.

Moreover, Liang et al[16] meta-analysis found that the STAT4 rs7574865 T allele conferred susceptibility to many autoimmune diseases, indicating an association between the *STAT4* gene polymorphism and autoimmune diseases.

Migita et al[17] previously proposed that STAT4 polymorphism was positively associated with type-1 AIH risk. STAT4 was one forum of an essential transcription factor involved in the modulation of the cytokine balance of Th1/Th2[18]. In several previous studies the association between STAT4 and some autoimmune diseases, including rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE) has been reported[19-21]. The pathogenesis of AIH was complex suggesting that STAT4 is a transcription factor involved in the distinction between Th1 and Th17[22]. STAT4 was an important genetic factor in the synthesis of IL-22, which plays a pathological function in IL17- dependent hepatitis[23].

In the present study, according to binary logistic analysis for the predictor of AIH, both dominant and recessive models of STAT4 (rs7574865) are significant predictors of autoimmune hepatitis. While regarding STAT4 (rs7582694), only the dominant model was a significant predictor.

The activation of dendritic cells and macrophages, and signal transductions within activated peripheral blood monocytes play an important role in STAT4. TGFβ is a cytokine that plays an integral role in immune response regulation. Increased TGFβ1 expression levels and severe progression of AIH has been linked with functional polymorphisms affecting the coding region of the TGFβ1 gene (codon 10 and codon 25)[24]. The transcription factor *STAT4* would be an essential genetic factor inducing the production of IL-22, which by inducing IL-17 production and differentiation into effector cells would play a pathological role during autoimmune hepatitis[19]. The susceptibility SNP rs7574865 is located within the non-coding region of intron 3 of STAT4. It is hypothesized that at the stage of transcription or splicing variation, it can influence the gene expression of STAT4[25]. STAT4α and STAT4β are the main alternative spliced isoforms of *STAT4*. STAT4β is a shorter form of the full-length STAT4α and is not as efficient as STAT4α in the direct induction of IL-12 activated IFN-γ gene expression in Th1 cells[26].

Furthermore, de Boer et al[27] suggested that there is a close correlation between susceptibility to autoimmune hepatitis and genes encoding the immunoregulatory molecules, such as the transcription factor *STAT4*.

CONCLUSION

Minor alleles of both STAT4 SNPs (rs7574865 T allele, rs7582694 C allele) are associated with an increased risk of type-1 AIH. The disease could be predicted by the dominant model of either SNPs and by the recessive model of rs7574865. So we have confirmed an association of *STAT4* polymorphisms with susceptibility to type-1 AIH and our data suggest that *STAT4* may be an "autoimmune disease susceptibility gene" and support the concept of deregulated pathways across multiple autoimmune diseases.

ACKNOWLEDGEMENTS

Authors would like to thank all children and their parents who participated in this study.

REFERENCES

1. Pathak S, Deepak K. Autoimmune hepatitis in children. *Pediatric Annals*. 2018; **47(2)**: 81-86. [PMID: 29446799]; [DOI: 10.3928/19382359-20180126-01]
2 Sciveres M, Nastasio S, Maggiore G. Novel diagnostic and

therapeutic strategies in juvenile autoimmune hepatitis. Front Pediatr. 2019; **7(382)**: 1-18. [PMID: 31616649]; [PMCID: PMC6763601]; [DOI: 10.3389/fped.2019.00382]
3. Nunes MEG, Rosa DV, Fagundes EDT, Ferreira AR, Miranda DM, Ferri Liu PM. HLA-DRB1 gene polymorphisms in pediatric patients with type 1 autoimmune hepatitis and type 1 autoimmune hepatitis overlap syndrome with autoimmune cholangitis. *Arq Gastroenterol*. 2019; **56(2)**: 146-150. [PMID: 31460577]; [DOI: 10.1590/S0004-2803.201900000-29]
4. Baranzini SE. The genetics of autoimmune diseases: a networked perspective. *Curr Opin Immunol.* 2009; **21(6)**: 596-605. [PMID: 19896815]; [DOI: 10.1016/j.coi.2009.09.014]
5. Yamamoto K, Kobayashi H, Arai A, Miura O, Hirosawa S, Miyasaka N. cDNA cloning, expression and chromosome mapping of the human STAT4 gene: both STAT4 and STAT1 genes are mapped to 2q32.2-->q32.3. *Cytogenet Cell Genet.* 1997; **77(3-4)**: 207-10. [PMID: 9284918]; [DOI: 10.1159/000134578]
6. Wang Y, Qu A, Wang H. Signal transducer and activator of transcription 4 in liver diseases. *Int J Biol Sci.* 2015; **11(4)**: 448-55. [PMID: 25798064]; [PMCID: PMC4366643]; [DOI: 10.7150/ijbs.11164]
7. Kaplan MH. STAT4 a critical regulator of inflammation in vivo. *Immunol Res*. 2005; **32(1)**: 231-41. [PMID: 15888914]; [DOI: 10.1385/IR:31:3:231]
8. Bettelli E, Korn T, Kuchroo VK. Th17: the third member of the effector T cell trilogy. *Curr opin immunol.* 2007; **19(6)**: 652-7. [PMID: 17766098]; [PMCID: PMC2288775]; [DOI: 10.1016/j.coi.2007.07.020]
9. Li X, Chen H, Cai Y, Zhang P, Chen Z. Association of STAT4 and PTPN22 polymorphisms and their interactions with type-1 autoimmune hepatitis susceptibility in Chinese Han children. *Oncotarget*. 2017; **8(37)**: 60933-60940. [PMID: 28977835]; [PMCID: PMC5617395]; [DOI: 10.18632/oncotarget.17458]
10. Mieli-Vergani G, Vergani D, Baumann U, Czubkowski P, Debray D, Dezsofi A, et al. Diagnosis and Management of Pediatric Autoimmune Liver Disease: ESPGHAN Hepatology Committee Position Statement. *J Pediatr Gastroenterol Nutr.* 2018; **66(2)**: 345-360. [PMID: 29356770]; [DOI: 10.1097/MPG.0000000000001801]
11. World Medical Association. WMA Declaration of Helsinki - Ethical Principles for Scientific Requirements and Research Protocols. *World Med Assoc*. 2013; **(June 1964)**: 29-32.
12. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. *Hepatology* 1996; **24(2)**: 289-93. [PMID: 8690394]; [DOI: 10.1002/hep.510240201]
13. Wilfinger WW, Mackey K, Chomczynski P. Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. Biotechniques. 1997; **22(3)**:474-6, 478-81. [PMID: 9067025]; [DOI: 10.2144/97223st01]
14. El-Saadany HM, Amer WH, Khalil HS, Gaber RA, Elshweikh SA. Association of STAT4 polymorphism with susceptibility and severity of rheumatoid arthritis and systemic lupus erythematosus in Egyptian patients. *The Egyptian Rheumatologist.* 2016; **38(1)**: 21-7.
15. Yang C, Mai H, Peng J, Zhou B, Hou J, Jiang D. STAT4: an immunoregulator contributing to diverse human diseases. *Int J Biol Sci.* 2020; **16(9)**: 1575-1585. [PMID: 32226303]; [PMCID: PMC7097918]; [DOI: 10.7150/ijbs.41852]
16. Liang YL, Wu H, Shen X, Li PQ, Yang XQ, Liang L, et al. Association of STAT4 rs7574865 polymorphism with autoimmune diseases: a meta-analysis. *Mol biol rep*. 2012; **39(9)**: 8873-82. [PMID: 22714917]; [DOI: 10.1007/s11033-012-1754-1]
17. Migita K, Nakamura M, Abiru S, Jiuchi Y, Nagaoka S, Komori A, et al. Association of STAT4 polymorphisms with susceptibility to type-1 autoimmune hepatitis in the Japanese population. *PLoS One*. 2013; **8**: e71382. [PMID: 23990947]; [PMCID: PMC3750035]; [DOI: 10.1371/journal.pone.0071382]
18. Watford WT, Hissong BD, Bream JH, Kanno Y, Muul L, O'Shea JJ. Signaling by IL-12 and IL-23 and the immunoregulatory roles of STAT4. *Immunol Rev.* 2004; **202**: 139-56. [PMID: 15546391]; [DOI: 10.1111/j.0105-2896.2004.00211.x]
19. Remmers EF, Plenge RM, Lee AT, Graham RR, Hom G, Behrens TW, et al. STAT4 and the risk of rheumatoid arthritis and systemic lupus erythematosus. *N Engl J Med*. 2007; **357(10)**: 977-86. [PMID: 17804842]; [PMCID: PMC2630215]; [DOI: 10.1056/NEJMoa073003]
20. Sugiura T, Kawaguchi Y, Goto K, Hayashi Y, Tsuburaya R, Furuya T, et al. Positive association between STAT4 polymorphisms and polymyositis/ derma- tomyositis in a Japanese population. *Ann Rheum Dis.* 2012; **71(10)**: 1646-50.
21. Taylor KE, Remmers EF, Lee AT, Ortmann WA, Plenge RM, Tian C, et al. Specificity of the STAT4 genetic association for severe disease manifestations of systemic lupus erythematosus. *PLoS Genet.* 2008; **4(5)**:e1000084.
22. Murphy KM, Ouyang W, Szabo SJ, Jacobson NG, Guler ML, Gorham JD, et al. T helper differentiation proceeds through Stat1-dependent, Stat4- dependent and STAT4-independent phases. *Curr Top Microbiol Immunol.* 1999; **238**: 13-26. T helper differentiation proceeds through Stat1-dependent, Stat4-dependent and STAT4-independent phases. *Curr Top Microbiol Immunol.* 1999; **238**
23. Xu M, Morishima N, Mizoguchi I, Chiba Y, Fujita K, Kuroda M, et al. Regulation of the development of acute hepatitis by IL-23 through IL-22 and IL-17 production. *Eur J Immunol.* 2011; **41(10)**: 2828-39. [PMID: 21953641]; [DOI: 10.1002/eji.201141291]
24. Beltrán Ramírez O, Mendoza Rincón JF, Barbosa Cobos RE, Alemán Ávila I, Ramírez Bello J. STAT4 confers risk for rheumatoid arthritis and systemic lupus erythematosus in Mexican patients. *Immunology letters.* 2016; **175**: 40-3. [PMID: 27178308]; [DOI: 10.1016/j.imlet.2016.05.003]
25. Korman BD, Kastner DL, Gregersen PK, Remmers EF. STAT4: genetics, mechanisms, and implications for autoimmunity. *Curr Allergy Asthma Rep.* 2008; **8(5)**: 398-403. [PMID: 18682104]; [PMCID: PMC2562257]; [DOI: 10.1007/s11882-008-0077-8]
26. Hoey T, Zhang S, Schmidt N, Yu Q, Ramchandani S, Xu X, et al. Distinct requirements for the naturally occurring splice forms Stat4α and Stat4β in IL-12 responses. *The EMBO journal* 2003; **22(16)**: 4237-48. [PMID: 12912921]; [PMCID: PMC175783]; [DOI: 10.1093/emboj/cdg393]
27. de Boer YS, van Gerven NM, Zwiers A, Verwer BJ, van Hoek B, van Erpecum KJ, et al. Genome-wide association study identifies variants associated with autoimmune hepatitis type 1. *Gastroenterology*. 2014; **147(2)**: 443-52.e5. [PMID: 24768677]; [DOI: 10.1053/j.gastro.2014.04.022]