Genetic Polymorphism and Intravenous Immunoglobulin Resistance Relationship in Kawasaki Disease

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Abstract

Objective

Kawasaki disease (KD) is an acute febrile systemic vasculitis and the most common cause of coronary artery aneurysm (CAA) in children. Intravenous immunoglobulin (IVIG) therapy is used to prevent fever and systemic inflammation. However, IVIG resistance is the most important risk factor of morbidity and mortality. It has been identified several single nucleotide polymorphisms (SNPs) related to IVIG resistance and this research aims to analyze these polymorphisms in our study population.

Methods

Patients diagnosed with KD (n:259) were analyzed retrospectively. Blood samples were taken from a randomized subgroup (n:97). Previously reported IVIG resistance related exonic SNPs at five different gene loci *(IL16, TNFSF14, NFATC2, DERL3, SAMD9L)* were evaluated by whole exome sequencing (WES).

Results

Between 2010–2019, 259 patients (male/female: 1,67) with KD were submitted to our clinic. CAA and IVIG resistance rates were 11.6% and 21.6%, respectively. The risk of developing CAA was significantly increased in patients with IVIG resistance ($p < 0.001$). As a result, IVIG resistance frequency increased in the presence of three SNPs. These are "rs11556218"(p.Asn1147Lys), "rs344560"(p.Lys214Glu), "rs12479626"(p.His446Arg), and are located in *IL16, TNFSF14, NFATC2* genes, respectively.

Conclusions

Until now, KD-related genetic data mostly obtained from studies involving large cohorts from Northeast Asian countries. In the analysis of this largest Turkish cohort in the literature, we found that, similar with previous studies, the *IL16* gene may be plays important role in the IVIG resistance mechanism.

Introduction

Kawasaki disease (KD) is an acute febrile systemic vasculitis of childhood, characterized by fever, bilateral nonexudative conjunctivitis, erythema in the lips and oral mucosa, changes in the peripheral periphery, rash and cervical lymphadenopathy. [1–3]

It is usually seen under five years of age and when not treated, it is reported that it develops 15–25% coronary artery aneurysm (CAA) or ectasia.[2,3]

It causes myocarditis and arrhythmia during the acute period. In subacute and chronic period, it causes myocardial infarction and sudden cardiac deaths due to CAA. Early diagnosis and treatment reduces the risk of coronary complications, morbidity and mortality rates significantly.[1–4]

The state-of-the-art treatment is high-dose intravenous immunoglobulin (IVIG) together with acetylsalicylic acid (ASA).[2] Since resistance to intravenous immunoglobulin (IVIG) is associated with coronary artery lesions (CALs) in KD, it is crucial to identify patients at risk group to protect them from coronary involvement.

The definition of IVIG resistance is used to prevent the fever from falling or recurrence 36 hours after the end of treatment and its mechanism has not been fully elucidated.[5] Unresponsiveness to IVIG, which is 10–20% in the literature, significantly increases the risk of developing coronary complications, and more aggressive treatments are needed in these patients. The researchers have developed different risk scoring systems over the years for various clinical and laboratory parameters for predicting the development of CAA with IVIG resistance in patients.[6–9] Risk scoring systems used to predict IVIG resistance gave different results in different races, therefore, the effects of genetic factors on IVIG resistance development were investigated.

In recent years, thanks to the rapidly developing molecular genetic techniques, studies on detection of candidate genes and polymorphisms are continuing in terms of susceptibility to many diseases or response to treatment. Here, selected SNPs and thier associations with IVIG resistance in KD is investigated.

Materials And Methods

Patients

The study protocol was approved by the University of Health Science, Kanuni Sultan Süleyman Research and Training Hospital Human Investigation Committee (HIC) (protocol number 48865165-302.14.01). Informed consent forms were signed by the parents.

Between 2010 and 2019, patients were recruited in our clinic based on the diagnosis criterias of the American Heart Association (AHA) guidelines.[2,3] A fever requirement of ≥5 days for the diagnosis of typical KD and the presence of at least four of the five basic clinical findings were sought.[2] Incomplete KD; defined for cases with fever lasting ≥4 days and less than four criteria were met, with suspected laboratory and echocardiographic findings.[2]

The patients had an echocardiographic examination at the time of diagnosis and in the subacute period for the presence of coronary involvement and the risk of complications. Myocardial wall mobility, ejection fraction (%), diameters of coronary arteries, and Z scores (calculated according to AHA guidelines) were recorded. The following findings were indicated by the Z score calculation: dilation, 2< Z score <2.5 or if initially <2 and a decrease in Z score during follow-up ≥1; small aneurysm, 2.5≤ Z score <5; moderate aneurysm, 5≤ Z score <10; large or giant aneurysm, Z score was defined as ≥10.[2] The examinations were performed by the same pediatric cardiologist as 2D, M mode, CW, and PW Doppler methods. A Vivid S5 with GE 3S and 6S probes (General Medical Electric Systems, Milwaukee, WI, USA) echocardiogram device was used.

According to the Z score calculation, the following definitions were applied: dilation, 2 < Z score < 2.5 or if initially < 2 and a decrease in Z score during follow-up ≥1; small aneurysm, 2.5 ≤ Z score < 5; moderate aneurysm, 5 ≤ Z score < 10; and large or giant aneurysm, Z score was defined as ≥ 10.[2]

IVIG and aspirin were given in appropriate doses to all patients hospitalized with a diagnosis of KD according to AHA criteria as the standard primary treatment.[2] IVIG resistance is defined as recurrent or permanent fever development at least 36 h after the end of the IVIG infusion.[2]

Gender, age at diagnosis, presence of diagnostic criteria, number of days with fever, and personal and family histories were recorded. The cases were grouped in terms of responses to IVIG treatment. Retrospective statistical comparisons were made between the groups.

METHODS

DNA extraction

Peripheral blood was collected from all participants in the subgroup of 97 cases and stored in PAXgene tubes. DNA isolation was performed using DNeasy Blood & Tissue Kit (Cat No./ID: 69506; QIAGEN, Germany) from lymphocyte cells from blood samples taken from patients in accordance with the manufacturer' protocol.

Whole Exome Sequencing

After genomic DNA is sheared to a mean fragment length of about 220 bp using focused acoustic energy (Covaris E220), purification and then PCR amplification are achieved using custom-made primers (IDT). The DNA library was created according to manufacturer's protocol by IDT (xGen Exome Panel). Samples were sequenced on the S4 flow cells using Illumina NovaSeq6000. Primary analysis is performed using Illumina's CASAVA 1.8.2 software suite. 10x target coverage was > 95%.

SNPs in five different gene loci (IL16, TNFSF14, NFATC2, DERL3, SAMD9L) identified by recent study related to IVIG resistance were examined.[10]

Data were analyzed using (SPSS Statistics for Macintosh, version 24.0; IBM Corp., Armonk, NY, USA). Categorical variables were summarized using frequencies (percentage [%]). The mean ± standard deviation was used for continuous variables. Normality was assessed for continuous data using the Kolmogorov–Smirnov test. An independent samples t-test was used for continuous variables. Categorical variables were compared using a chi-squared test. A p-value < 0.05 was taken as an indicator of statistical significance. Results and significance values are summarized in the relevant tables.

Results

During the study, 259 patients (Male / Female = 1.67) were recruited between 3 and 117 months of age. The clinical and demographic data of the patients in terms of responsiveness to IVIG treatment are summarized in the Table 1. IVIG resistance was observed in 56 cases (21.6%). The mean age of patients with IVIG resistance was significantly younger than that of patients with IVIG response (Table 1). While IVIG unresponsiveness was significantly increased in the age group of infants, there was no difference in gender and type of diagnosis. There was a significant correlation between IVIG resistance and frequency of CAA development and hospitalization time (p < 0,001). The risk of developing coronary artery lesions especially in IVIG resistant patients appears to be significantly increased compared to those who are sensitive. (Table 1).

Table 1
Demographic data of patients according to response to IVIG treatment

Variable	IVIG responsiveness		*p* value
	Responders (n = 203)	Non-responders (n = 56)	
Sex			0.762[a]
Male	126 (77.8%)	36 (22.2%)	
Female	77 (79.4%)	20 (20.6%)	
Age range (months)	34.6 (1.9–118.8)[d]	22.2 (1.3–117.4)[d]	**0.045[b]**
Age group			**0.035[c]**
< 1 year	31 (64.6%)	17 (35.4%)	
1 ≤ and < 5 years old	123 (82%)	27 (18%)	
≥ 5 years old	49 (80.3%)	12 (19.7%)	
Total fever days	5 (1–20)[d]	6 (1–20)[d]	0.174[b]
Hospitalization time	10 (3–34)[d]	15 (5–35)[d]	**< 0.001[b]**
Coronary involvement	37 (17.3%)	19 (42.2%)	**< 0.001[a]**
Type of diagnosis			0.796[a]
Typical KD	69 (79.3%)	18 (20.7%)	
Incomplete KD	134 (77.9%)	38 (22.1%)	

a Pearson Chi-Square analysis was used.

b Mann-Whitney U test was used.

c significant difference was observed in the < 1 years age group.

d "Median" and "lowest - highest values" in parentheses were specified for non-normally distributed data.

IVIG: intravenous immunoglobulin.

Laboratory findings of the patients were evaluated according to the IVIG response. The data showed statistically significant, elevated levels of white blood cells, C-reactive protein, aspartate aminotransferase, lactate dehydrogenase, troponin I, N-terminal (NT)-pro hormone BNP (NT-proBNP), triglycerid, and decreased levels of hemoglobin, hematocrit and sodium in IVIG resistant patients (Table 2).

Table 2
Central tendency measures of laboratory findings according to IVIG treatment responsiveness

Variable	IVIG responders (n = 203)	IVIG non-responders (n = 56)	*p* value
Sedimentation	65.5 (28.1)[c]	71.6 (27.9)[c]	0.536[a]
WBC	12 800 (1 000–49 570)[d]	15 850 (3 800–33 230)[d]	**0.005[b]**
NEU (%)	60.09 (17.17)[c]	65,44 (16.31)[c]	0.813[a]
Hb	10.64 (1.49)[c]	10.47 (1.44)[c]	**0.005[a]**
Hematocrit	32.46 (4.25)[c]	31.63 (4.02)[c]	**0.002[a]**
PLT	381 300 (95 000–2 099 000)[d]	384 500 (51 000–1 356 000)[d]	0.783[b]
CRP	65 (1–431)[d]	88.8 (3.1–369)[d]	**0.012[b]**
Albumin	3.7 (2.1–4.5)[d]	3.7 (2.3–4.9)[d]	0.846[b]
AST	32 (11–604)[d]	40.9 (4–628)[d]	**0.038[b]**
ALT	22 (4–527)[d]	24 (5–705)[d]	0.156[b]
LDH	322 (171–686)[d]	383 (170–944)[d]	**0.022[b]**
Total Bilirubin	0.22 (0.03–2.79)[d]	0.26 (0.09–6.54)[d]	0.363[b]
Direct Bilirubin	0.09 (0.01–2.66)[d]	0.13 (0.01–6.34)[d]	0.192[b]
Na	136 (121–143)[d]	134 (127–141)[d]	**0.014[b]**
Troponin I	0.003 (0.003–0.03)[d]	0.007 (0.003–0.127)[d]	**0.028[b]**
NT-proBNP	253 (55 – 8 149)[d]	2 925 (277–35 000)[d]	**0.004[b]**
Total Cholesterol	142 (77–296)[d]	151 (55–245)[d]	0.744[b]
HDL	21.9 (10.5)[c]	20.7 (14.6)[c]	0.704[a]
LDL	84.5 (28–224)[d]	81 (11–172)[d]	0.473[b]
Triglycerid	167 (57–440)[d]	219 (69–550)[d]	**0.016[b]**

Variable	IVIG responders (n = 203)	IVIG non-responders (n = 56)	*p* value

[a] *Student's t test was used from parametric tests.*

[b] *Mann-Whitney U test was used from non-parametric test.*

[c] *"Mean" and "standard deviation" values in parentheses were specified for the data that fit the normal distribution.*

[d] *"Median" and "lowest - highest values" in parentheses were specified for data that do not fit the normal distribution.*

ALT: alanine transaminase; AST: aspartate aminotransferase; CRP: C-reactive protein; Hb: hemoglobin; HDL: high density lipoprotein; LDH: lactate dehydrogenase; LDL: low density lipoprotein; Na: sodium; NEU (%): neutrophil / leukocyte percentage; PLT: platelet count; NT-proBNP: N-terminal brain natriuretic peptide precursor; WBC: White blood cell count / mm^3.

While coronary artery aneurysm (Z > 2.5) was detected in 30 (11.6%) of the cases, coronary ectasia / dilatation was detected in 15 patients (6%) (2 < Z score < 2.5), and 214 (83% coronaries were completely normal. According to the diagnostic criteria, 33.6% were evaluated as typical KD and 66.4% as atypical (incomplete) KD.

While IVIG and aspirin, which are the standard primary treatment, are given to all patients in appropriate doses, aspirin was discontinued in 29 patients (11%) because salicylic findings developed. Eight patients were needed intensive care due to left ventricular dysfunction secondary to myocarditis during the acute phase, while one underwent therapeutic plasmapheresis.

61 patients with IVIG response and 37 patients with IVIG resistance evaluated by all exom analysis. Based on SNP frequency, although statistically not significant, IVIG resistance risk increased with three exonic variants including "rs11556218" [OR = 2.34] in the *IL16* gene, "rs344560" [OR = 2.32] in the *TNFSF14* gene and "rs12479626" [OR = 1.67] located in the *NFATC2* gene; respectively (Table 3).

Table 3
Analysis of the association of five candidate SNVs with IVIG resistance

Gene	SNV	Position	Allele	No. of samples	RAF		Association	
					IVIG non-responder	IVIG resp.	OR (%95 CI)	P
IL16	rs11556218	p.Asn1147Lys	T/G	7	0.108	0.049	**2.343** (0.494–11.116)	0.421[a]
TNFSF14	rs344560	p.Lys214Glu	A/G	13	0.189	0.098	**2.319** (0.659–6.945)	0.229[a]
NFATC2	rs12479626	p.His446Arg	C/T	2	0.027	0.016	**1.667** (0.202–37.475)	1[a]
DERL3	rs1128127	p.Ala211Val	A/G	28	0.189	0.344	0.444 (0.167–1.181)	0.099[b]
SAMD9L	rs10488532	p.Val266Ile	T/C	20	0.135	0.246	0.246 (0.158–1.451)	0.187[b]

95% CI: 95% confidence interval; A: adenine; C: cytosine; G: guanine; IVIG: intravenous immunoglobulin; RAF: risk allele frequency; SNV: single nucleotide variant; T: thymine; OR: Odds ratio;

[a] *Fisher's exact test used*

[b] *Pearson Chi-square test was used.*

Discussion

Kawasaki disease is a self-limiting acute febrile systemic vasculitis that usually involves small and medium vessels, particularly coronary arteries.[11] The most serious complication, CALs (coronary artery involvement), is reported in 15–25% of untreated cases.[12] CALs coronary involvement rate was found 17.6% in our study group.

It has been reported that the probability of developing coronary artery lesions decreases with high dose IVIG treatment administered in the acute period of the disease. Therefore, the response of patients to standard therapy is an important factor that determines the risk of complications. In their study, Fabi et al. showed the frequency of CALs in the patients with IVIG response and resistance in 19.6% and 37.2%, respectively ($p = 0.01$).[13] In our study, this rate was found to be 17.3% and 42.2% in the resistant group with IVIG response, respectively ($p < 0.001$).

The incidence of resistance to IVIG is generally reported in the literature between 10–20%.[14–16] Classification of patients according to the risk of IVIG resistance could inform decisions to administer more aggressive initial treatment, with the aim of reducing the risk of coronary artery lesion development.[10]

Many risk scoring systems have been developed so far with the idea of coronary complications can be reduced by using aggressive treatment regimens in the early period if the response to treatment can be predicted at the beginning of the disease.[6–9] These scoring systems, which were developed based on clinical findings and laboratory parameters, have not been widely used worldwide since their prediction power varies according to the population they are applied to.[17–21] In the studies conducted in Northern American Cohort,[18] US Midwest,[19] Spain,[20] The UK,[21] German,[22] France[23] populations, IVIG resistance was found at different rates. The determination of IVIG resistance at different rates in different races led to genetic studies.

In the last ten years, with the rapid advancement of next generation sequencing techniques reserachers identified variants related to responsiveness to treatment, the risk of complications and mortality in multifactorial diseases including KD. In a recent study, Kim et al. performed WES to 296 KH patients, in which 101 cases were IVIG resistant, and identified different SNP variants in five different gene loci related to immune response.[10] They obtained a broad-based risk analysis by adding the results of replication studies of two separate cohorts with 903 with IVIG response and 352 with IVIG resistance. In GWAS analysis of these variants were: "rs11556218" (p.Asn1147Lys) [OR = 1.89 p = 0.0042] in the *IL16* gene; "rs344560" (p.Lys214Glu) [OR = 2.26 p = 0.0096] in the *TNFSF14* gene and "rs12479626" (p.His446Arg) [OR = 2.79 p = 0.0035] located in the *NFATC2* gene; “rs1128127” (p.Ala211Val) in the *DERL3* gene [OR = 2.45 p = 0.0109]; “rs10488532” (p.Val266 with) located in the *SAMD9L* gene [OR = 3.46 p = 0.0067].

Here we report only three of them had an estimated relative risk (OR) greater than 1.
These were localized polymorphisms in the *IL16* gene (rs11556218), the *TNFSF14* gene (rs344560) and the *NFATC2* gene (rs12479626), respectively. Since *p* values > 0.05 were not statistically significant this may be related to patient number whic is lower than Kim et al.’ study.

In general, KD develops as a result of irregularity in the immune response. It has been suggested that there is a similarity between KD and the pathogenesis of autoimmune diseases.[24] T lymphocytes are predominant in the immunopathogenesis of KD. Increased T lymphocyte activation leads to the production of cytokines responsible for the pathogenesis of the disease.

Interleukin-16 (IL-16) encoded by the *IL16* gene is a pileiotropic cytokine that acts as a modulator in T cell activation and was first described in 1982.[25] IL-16, which shows chemoattractant function mainly on CD4 + T lymphocytes, has an effect on CD4 + / CD8 + ratio. IL-16, which showed an inhibitory role in HIV replication in the 1990s[26], has been associated with a number of diseases that progress with inflammation or autoimmunity in different studies. Disorders in the *IL16* gene play an important role in the pathophysiology of these diseases with CD4 + / CD8 + ratio and CD8 + T cell activation. The diseases associated with IL-16 are bronchial asthma,[27] Crohn's disease[28], systemic lupus erythematosus,[29] rheumatoid arthritis,[30] and multiple myeloma.[31] *IL16* variant of "rs11556218 (T / G)" identified by Kim et al.[10] On IVIG resistance in IL16 gene in KD was associated with a predisposition to multiple sclerosis.[32]

TNFSF14 gene encodes one of the tumor necrosis factor (TNF) ligands. The receptor to which it binds is a member of the tumor necrosis factor receptor superfamily (TNFRSF14) and is also known as the herpesvirus entry mediator (HVEM). The encoded protein provides co-stimulation for T cell activation. In this way, it plays a

preventive role in herpesvirus infections. It has also been shown to stimulate T cell proliferation and trigger apoptosis of various tumor cells.[33]

NFATC2 gene is a member of the nuclear factor (NFAT: Nuclear factor of activated T-cells) family of activated T cells, and the product it encodes is a DNA-binding protein. The protein contained in the T cell cytosol induces nuclear transcription complexes by binding to the nucleus when the T cell receptor (TCR) is activated.[34] Nuclear Factor of Activated T cells (NFAT) family members are known for their roles in T cell development and activation but still largely undetermined in CD8 + T cell differentiation in vivo. NFAT1 and NFAT2 in T cells display a significant increase in KLRG1hi CD127hi population and are unable to clear an acute viral infection.[35] NFAT-deficient CTLs showed different degrees of impaired IFN-γ and TNF-α expression with NFAT1 being mainly responsible for IFN-γ production upon ex-vivo stimulation as well as for antigen-specific cytotoxicity.

SAMD9L is adjacent to its close paralog sterile alpha motif domain-containing protein 9 (SAMD9) on chromosome 7q21.2 and encodes a cytoplasmic protein that has important roles in multiple cellular processes such as cell proliferation (most likely as a tumor suppressor), the neoplastic phenotype, and the innate immune response to viral infection.[36,37] The physiological functions of SAMD9L currently remain poorly understood, but its importance has recently been emphasized during the discovery of the genetic cause of a rare, life-threatening human disease.[38] In the study Kim at al., although associations between SAMD9L variants and KD susceptibility or IVIG resistance have not been reported, it is most likely that the role of SAMD9L in IVIG resistance could be mediated through various immune signaling pathways in which SAMD9L plays important roles in immune-related diseases.[39]

Conclusion

Our study is associated with three genes related to T cell activation (IL16, TNFSF14, NFATC). From this point of view, it can be said that T cell activation has an important role in IVIG resistance pathogenesis. Determination of factors that increase the risk of developing coronary artery aneurysm and IVIG resistance with all exom gene analysis will be helpful in determining the diagnosis, treatment and complications of the disease.

Abbreviations

AHA – American Heart Association

CAA – coronary artery aneurysm

IVIG – intravenous immunoglobulin

GWAS – genome-wide association studies

KD – Kawasaki disease

SNPs – single nucleotide polymorphisms

Declarations

Ethical Approval and Consent to participate

The study protocol was approved by the University of Health Science, Kanuni Sultan Süleyman Research and Training Hospital Human Investigation Committee (HIC) (protocol number 48865165-302.14.01). Informed consent forms were signed by the parents.

Consent for publication

Not applicable.

Availability of supporting data

The dataset used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors have no conflicts of interest to disclose.

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Authors' contributions

YZV reviewed the literature, contacted the parents of the patients and examined them, took blood samples, collected data about the disease, performed statistical analysis of the data of the cases and contributed to the writing of the article. KO and AOC conceptualized and designed the work, provided general direction and planning, encouraged YZV, reviewed and revised the article, supervised the project. MG and KB provided the physical and material requirements required to perform the whole exome analysis from the blood samples taken in the study. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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