Mediterranean Fever (MEFV) Gene Variants in Kawasaki Disease in Turkish Children: Do They Influence the Disease Course?

Serkan Türkuçar, 1 Tayfun Çinleti, 2 Kaan Yıldız, 3 Ceren Yılmaz, 2 Balahan Bora, 1 Mustafa Kır, 2 Altuğ Koç, 4 Murat Derya Erçal, 2 Erbil Ünsal 1

1Department of Pediatric Rheumatology, Dokuz Eylül University, Izmir, Türkiye
2Department of Pediatric Genetics, Dokuz Eylül University, Izmir, Türkiye
3Department of Pediatric Cardiology, Dokuz Eylül University, Izmir, Türkiye
4Department of Genetics, Dokuz Eylül University, Izmir, Türkiye

Objective: Kawasaki Disease (KD) is a type of systemic vasculitis mediated by an abnormal immune response, similar to autoinflammatory disorders, due to increased release of proinflammatory cytokines. This cross-sectional study aims to evaluate the relationship between the frequency and severity of KD and the presence of Mediterranean Fever (MEFV) gene variants.

Materials and Methods: Thirty-eight children diagnosed with KD were included in the study. Demographic and clinical data such as age at diagnosis, gender, and duration of fever prior to intravenous immunoglobulin (IVIG) treatment were recorded. Laboratory and echocardiographic data at the time of diagnosis were also retrospectively reviewed. MEFV gene analysis was performed for all patients.

Results: The study comprised 38 patients, including 25 boys (65.8%) and 13 girls (34.2%), with a median age of 3.4 years (range 1.8–6.3 years). Coronary arterial lesions were observed in 11 patients (28.9%), and 13 patients (34.2%) were resistant to initial IVIG treatment. Twenty-two patients (57.9%) had at least one allele with MEFV variants. Of these, eight patients (36% of MEFV-positive patients) had at least one exon-10 variant, while 14 patients had only exon-2 variants. No statistically significant differences were found in clinical, demographic, or echocardiographic parameters between MEFV-positive and MEFV-negative patients.

Conclusion: This study found that the presence of MEFV gene variants in KD patients is almost three times higher compared to the general Turkish population. This relationship warrants further clarification through nationwide studies, especially in countries where Familial Mediterranean Fever (FMF) is a significant concern.

Keywords: Hyper-inflammation, Kawasaki, mediterranean fever (MEFV) gene, R202Q, Turkish children.
INTRODUCTION

Kawasaki Disease (KD) is a systemic febrile vasculitis occurring in childhood that primarily affects medium-sized vessels, especially the coronary arteries. Despite its generally self-limiting clinical course, which includes persistent fever along with mucocutaneous and lymphatic manifestations, coronary arterial lesions (CALs) can cause significant mortality and morbidity in approximately a quarter of untreated cases. To date, the etiopathogenesis of KD remains largely unexplained; however, it is widely believed to be due to an abnormal immune response triggered by environmental factors in genetically susceptible individuals. Recent molecular studies suggest that this exaggerated immune response is mediated by increased release of proinflammatory cytokines, similar to other autoinflammatory disorders. Although various autoinflammatory disease mutations, including Nucleotide-binding Oligomerization Domain-containing protein 1 (NOD1), Tumor Necrosis Factor Receptor 1 (TNFR1), and several cytokine-related genes, have been identified in Kawasaki Disease, there is no consensus on their impact on disease frequency or severity.

Familial Mediterranean Fever (FMF) is the most common hereditary auto-inflammatory disorder in the Mediterranean region, especially in Türkiye. Variants in the Mediterranean Fever (MEFV) gene lead to typical hyperinflammatory febrile polyserositis attacks via the interleukin 1-β (IL 1-β) pathway. There is also ongoing subclinical inflammation during attack-free periods, making patients susceptible to other hyperinflammatory disorders, such as Behçet’s Disease (BD), polyarteritis nodosa (PAN), and Immunoglobulin A vasculitis (IgAV). In many cases of related vasculitis, a more severe course due to a hyperinflammatory response could be attributed to accompanying MEFV variants. While studies have explored the association between Kawasaki Disease and MEFV, they have yet to establish a link concerning the frequency and severity of the disease.

This cross-sectional study aims to evaluate the relationship between the frequency and severity of KD and accompanying MEFV gene variants.

MATERIALS AND METHODS

Ethical approval for this study was obtained from Dokuz Eylül University Non-Interventional Research Ethics Committee under the number 2019/31-31 prior to commencement.

Patients and Definitions

This was a cross-sectional study involving 38 children diagnosed with KD between the years 2000 and 2018. None of the patients included had clinical features of FMF or were undergoing colchicine treatment. Diagnosis was based on the presence of six clinical criteria, with prolonged fever (over five days) (i) being mandatory, accompanied by oral mucosal changes (ii), conjunctival injection (iii), changes in the extremities (iv), polymorphous exanthema (v), and cervical lymphadenitis (vi) (1). Patients meeting five of these criteria were diagnosed with complete KD (cKD), while those presenting four or fewer criteria, alongside supporting findings such as leukocytosis, anemia, sterile pyuria, and elevated acute phase reactants and liver function tests, were diagnosed with incomplete KD (iKD). Demographic and clinical data including age at diagnosis, gender, and duration of fever prior to Intravenous Immunoglobulin (IVIG) treatment were recorded.

All patients were treated with 2 mg/kg of IVIG and 80–100 mg/kg/day of acetylsalicylic acid at diagnosis. Patients who continued to exhibit fever 48 hours post-initial IVIG treatment were identified as IVIG-resistant. Thirteen patients (34.2%) were resistant to the initial IVIG treatment and received a second dose of IVIG and corticosteroids, where necessary.

Laboratory Assessments

Laboratory results prior to the first IVIG treatment were reviewed retrospectively. Parameters noted included hemogram, levels of acute phase reactants such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), and biochemical parameters including liver and renal function tests, albumin levels, and serum electrolyte levels.

Echocardiographic Assessments

Patients were examined by pediatric cardiologists at the time of diagnosis, and echocardiographic assessments were performed initially and two weeks after the first IVIG treatment. Perivascular echogenicity, ectasia/dilation, and aneurysms of the coronary arteries were identified as coronary arterial involvement, classified according to the severity of the lesions. The Japanese Ministry of Health and Welfare guidelines were followed for performing echocardiographic assessments and measurements.

Assessment of MEFV Gene Variants

Molecular analysis of the MEFV gene was conducted for all patients. A minimum of 4 ml of peripheral blood was collected in tubes coated with Ethylenediaminetetraacetic acid (EDTA) from each patient. Total genomic DNA was isolated using the spin column DNA isolation technique, according to the manufacturer’s protocol. For this purpose, the Qiacube® automated DNA isolation device (Qiagen, Germany) and QIAamp® DNA Blood Mini kit (Qiagen, Germany) were used. Extracted DNA was then stored at -20 °C before the Real-Time Polymerase Chain Reaction (PCR) study. Real-Time PCR processes were carried out using the Cobas® z480 Real-Time PCR device (Roche Diagnostics, Switzerland) and LightSNiP® MEFV kit (TIB Molbiol, Germany), following the manufacturer’s
protocols. The eight most common MEFV variants in Türkiye (E148Q, R202Q, M680I, M694V, M694I, V726A, K695R, and A744S) were analyzed using this method.

Statistical Analysis
Statistical analysis was conducted using IBM Statistical Package for the Social Sciences (SPSS) Statistics version 22 software. A post-hoc power analysis of the study yielded a value of 0.83, with an effect size of 0.6, as calculated using the G-Power 3.1.9.4 program. The Kolmogorov-Smirnov test and histogram graphs were used to evaluate the normality of the variables. Mean±standard deviation (SD) values were reported for normally distributed variables, while heterogeneously distributed values were expressed as medians and interquartile ranges (IQR 25–75). The Chi-Square test was used to analyze differences between categorical variables. The Independent T-test was used for comparing normally distributed measurable variables, while the Mann-Whitney U test was applied to non-normally distributed variables. A p-value <0.05 was considered statistically significant.

RESULTS
Demographic and Clinical Features
This study included 38 patients, comprising 25 boys (65.8%) and 13 girls (34.2%), with a median age of 3.4 years (range: 1.8–6.3 years). Clinically, 22 patients (58%) were diagnosed with cKD, while 16 (42%) had iKD. Coronary arterial lesions were observed in 11 patients (28.9%), and 13 patients (34.2%) were resistant to initial IVIG treatment (Table 1).

MEFV Gene Analysis
Genetic analysis revealed that 22 patients (57.9%) had at least one MEFV variant allele. Eight of these patients (36% of MEFV (+) patients) had at least one variant in exon 10 (M680I, M694V, M694I, V726A, K695R, and A744S), while 14 patients present only with variants in exon-2 (R202Q and E148Q). The most common variant was the heterozygous R202Q, found in nine patients (41% of MEFV (+) patients). Detailed results of the MEFV gene molecular analysis are presented in Figure 1.

Comparison of Patients Based on MEFV Gene Variants
When comparing patients with at least one allele variant in the MEFV gene to those without any variants, no statistical differences were observed in demographic and clinical parameters such as age at diagnosis, clinical type, duration of fever before initial IVIG treatment, rates of CALs, and IVIG resistance (p>0.05 for all parameters). Laboratory parameters including acute phase reactant levels, complete blood count (except for hemoglobin (Hb) levels), and biochemical parameters also showed no differences between the two groups. The mean Hb level was 11.74±1.46 g/dL in the MEFV positive group, compared to 10.65±1.16 g/dL in the MEFV negative group (p=0.040) (Table 2). Similar results were obtained when grouping the patients by exon-10 variant presence, with no statistical differences in any demographic, clinical, or laboratory parameters between exon-10 carriers and non-carriers (p=0.05 for all parameters) (Table 3).
DISCUSSION

Kawasaki Disease is a highly variable childhood vasculitis worldwide due to differences in environmental and genetic factors. The incidence of the disease, the risk of developing CALs, and the rates of IVIG resistance vary between nations.9,11–13 Given the disease's hyper-inflammatory nature and the unclarified role of provoking genes, it is crucial to define the role of \textit{MEFV} variants. As FMF is the most common autoinflammatory disease in Türkiye, the influence of the \textit{MEFV} gene on the frequency and severity of KD was investigated. This study found a carrier state in approximately 60% of all included patients, nearly three times higher than the general Turkish population according to current epidemiological studies.14–16

Proinflammatory cytokines, regulated and driven by regulatory T-cells, play major roles in the etiopathogenesis of KD.16 Recent studies have indicated that serum interleukin (IL) levels—IL-1, IL-6, and IL-18—and Tumor Necrosis Factor Alpha (TNF-α) levels are elevated during the acute phase of KD and decrease following IVIG treatment.17–21 Furukawa et al.22 also demonstrated the pivotal role of nuclear factor kappa B (NF-κB) in the acute phase of KD, similar to autoinflammatory diseases. These findings have prompted researchers to investigate the link between autoinflammatory genes and KD. They identified several responsible genes, such as Nucleotide-binding Oligomerization Domain-like receptor Pyrin domain-containing protein 1 (NLRP1), Tumor Necrosis Factor Receptor Superfamily Member 1A

| Table 2. Comparison of patients based on \textit{MEFV} gene variants |
|--------------------------------------------------|-----------------------------------------------|------------------|
| **MEFV positive group (n=22)** | **MEFV negative group (n=16)** | \( p \) |
| **Clinical parameters** | | |
| Gender (boys/girls) | 15/7 | 10/6 | 0.490 |
| Age at time of diagnosis (years) | 2.3 (1.7–5.8) | 4.75 (2.9–6.5) | 0.849 |
| Type of KD (cKD/iKD) | 11/11 | 11/5 | 0.206 |
| Fever before first IVIG treatment (days) | 8 (6–12.8) | 11 (5–14) | 0.759 |
| Rate of IVIG resistant patients, n (%) | 7 (18%) | 6 (16%) | 0.490 |
| Rate of CAL positive patients, n (%) | 6 (15%) | 5 (13%) | 0.534 |
| **Laboratory parameters** | | |
| WBC (10\(^3\)/uL) | 13.9 (9.05–14.5) | 13.9 (9.2–19.1) | 0.185 |
| Neu (10\(^3\)/uL) | 8.2 (4.3–11.3) | 10.4 (2.8–15.9) | 0.159 |
| Lym (10\(^3\)/uL) | 3 (1.9–4.3) | 2 (1.3–3.9) | 0.842 |
| Plt (10\(^3\)/uL) | 470.5 (295.5–539) | 308.5 (208.5–521.3) | 0.491 |
| Hb (g/dL) | **11.74±1.46** | **10.65±1.16** | **0.040** |
| NLR (Neu/Lym) | 2.74 (1.14–5.3) | 3.7 (2.9–18.8) | 0.146 |
| PLR (Plt/Lym) | 121 (62.5–283.7) | 132.3 (11.6–241.3) | 0.408 |
| MPV (fL) | 7.6±0.9* | 7.3±0.7* | 0.198 |
| ESR (mm/h) | 64 (48.5–100) | 52.5 (29–79.5) | 0.842 |
| CRP (mg/L) | 85.5 (45–113) | 66.4 (40–185) | 0.325 |
| ALT (U/L) | 36 (21.5–48) | 35.5 (27–93) | 0.439 |
| AST (U/L) | 41 (32.3–60) | 53 (29–91) | 0.518 |
| T. Bil (mg/dL) | 0.5 (0.26–0.75) | 0.46 (0.24–0.6) | 0.651 |
| Alb (g/dL) | 3.46±0.58* | 3.66±0.45* | 0.769 |
| Na (mmol/L) | 135±3.1* | 134.5±3* | 0.884 |
| K (mmol/L) | 4.2±0.6* | 4.5±0.5* | 0.502 |
| Ca (mg/dL) | 9.2±0.6* | 9.3±0.7* | 0.470 |

\( P<0.05; \) Median (interquartile ranges 25–75), *: Mean±Standard deviation; cKD: Complete Kawasaki disease; iKD: Incomplete Kawasaki disease; IVIG: Intravenous immunoglobulin; CAL: Coronary arterial lesion; \textit{MEFV}: Mediterranean fever gene; WBC: White blood cell; Neu: Neutrophil count; Lym: Lymphocyte count; Plt: Platelet count; Hb: Hemoglobin; NLR: Neutrophil/Lymphocyte ratio; PLR: Platelet/Lymphocyte ratio; ESR: Erythrocyte sedimentation rate; CRP: C-Reactive protein; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; T. Bil: Total bilirubin; Alb: Albumin; Na: Sodium; K: Potassium; Ca: Calcium.
Variations in the MEFV gene also cause hyper-inflammation due to the activation of the NF-κB pathway and elevated IL-1 levels, contributing to the course of several rheumatological disorders. Abbara et al. reported a higher incidence of vasculitis such as IGAV, PAN, and BD in FMF patients compared to the healthy population. Similarly, Ozen et al. reported that 21.4% of FMF patients developed other rheumatological disorders or vasculitis, and 30.5% of patients with many rheumatological disorders also had MEFV variants. Furthermore, Altug et al. reported that IGAV patients with MEFV variants exhibited more gastrointestinal and joint complaints with higher CRP levels, emphasizing the role of MEFV variants in the severity of IGAV vasculitis. Yamaguchi et al. researched the effects of E148Q variants (the most common in Japan) on Kawasaki Disease but could not determine any influence on disease severity. Similarly, Salehzadeh et al. from Iran studied the frequency of MEFV variants among KD patients and reported that 10% of their patients had MEFV variants; however, this was found to be lower compared to the healthy population. This study showed that the frequency of MEFV variants was more common in KD patients compared to the healthy Turkish population. However, it did not seem to affect the disease severity in terms of IVIG resistance and CAL formation.
A remarkable finding of this study revealed that one-third of patients had the R202Q variant, which was significantly higher than in the healthy population.\textsuperscript{26,27} Although it is controversial whether the R202Q variant has a pathogenic effect, several recent studies have suggested that it may be responsible for both FMF attacks and hyper-inflammatory complications.\textsuperscript{28–30} Further genetic studies with a larger case series will perhaps enable us to understand the link between R202Q and KD more clearly.

**CONCLUSION**

In conclusion, we found a carrier state for MEFV variants nearly three times higher in KD patients compared to the general Turkish population, according to current epidemiological studies. These findings contribute to the existing medical literature by highlighting the potential role of these variants in inflammatory processes involved in the pathogenesis of Kawasaki Disease, particularly in regions where Familial Mediterranean Fever is prevalent.

**Ethics Committee Approval:** The Dokuz Eylül University Non-Interventional Research Ethics Committee granted approval for this study (date: 16.12.2019, number: 2019/31-31).

**Author Contributions:** Concept – ST, BB, MDE; Design – ST, EU, MDE; Supervision – ST, BB, MDE, EU; Resource – TC, KY, CY, MK; Materials – KY, MK, AK; Data Collection and/or Processing – TC, KY, ST, MK, KY, AK; Analysis and/or Interpretation – ST, EU, MK, MDE; Literature Search – ST, EU; Writing – ST, BB, EU, MDE; Critical Reviews – EU, MDE, BB.

**Conflict of Interest:** The authors have no conflict of interest to declare.

**Informed Consent:** Written informed consent was obtained from patients who participated in this study.

**Use of AI for Writing Assistance:** Not declared.

**Financial Disclosure:** This study was funded by Dokuz Eylül University Department of Scientific Research Project.

**Peer-review:** Externally peer-reviewed.

**REFERENCES**

15. Özen S. Update on the epidemiology and disease outcome of Familial Mediterranean fever. Best Pract Res Clin Rheumatol 2018; 32(2): 254–60. [CrossRef]


