



Genetic and Clinical Study of Children with Familial Mediterranean Fever in Northeastern Iran

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Abstract

Background: Familial Mediterranean fever (FMF) is an autosomal recessive disorder caused by a mutation in the *MEFV* gene and characterized by recurrent episodes of fever and polyserositis.

Objectives: This study aimed to identify the frequency and distribution of *MEFV* mutations in children with FMF in northeastern Iran and determine clinical examinations.

Methods: Our study was a descriptive and analytical cross-sectional study conducted among 29 patients under the age of 18 who visited the pediatric rheumatologist between April 2014 and 2021. After clinical diagnosis, the patients underwent genetic evaluation. The mutations related to each patient were identified using Sanger sequencing of the entire *MEFV* gene sequence. The rest of the information was extracted from the checklist. Finally, the data were analyzed using SPSS v. 16.

Results: Fever was the most common symptom, followed by abdominal pain. During the acute attack period, laboratory inflammatory factors increased in all patients. None of the patients had complications. There was no significant relationship between the demographic variables in the groups (benign, variant of uncertain significance [VUS], pathogen) except for the rate of hospitalization (P-value = 0.039). Moreover, 34.5% of the studied patients had pathogenic mutations. The most common mutation was E148Q, which was reported to be benign, followed by pathogenic mutations (M680I), with a frequency of 10.2%.

Conclusions: The FMF is rare in Northeastern Iran, and the number of pathogenic mutations is lower compared to Northwest Iran and other studies. It is necessary to conduct a genetic examination and treatment of affected patients to control the course of the disease and its complications.

Keywords: Amyloidosis, ANOVA, Children, Familial Mediterranean Fever, Genetics, Iran, *MEFV* Gene

1. Background

Familial Mediterranean fever (FMF) is an autosomal recessive disease characterized by recurrent episodes of fever and symptoms of serous inflammation, ultimately leading to abdominal, joint, and chest pain (1, 2). It is most commonly observed in populations living in the Mediterranean region, including Jews, Arabs, Turks, and Armenians (1). The prevalence of FMF in the Middle East ranges from approximately 1 in 2000 to 1 in 100 (3). Diagnosis of FMF is primarily clinical, and one of the most significant complications is amyloidosis, which can result in kidney failure (4). Its symptoms usually manifest in childhood or adolescence in over 80% of patients (5).

Colchicine, an alkaloid derivative with anti-inflammatory effects, is the only known drug that can decrease the incidence and severity of disease episodes and prevent the development of amyloidosis (6). Studies have shown that the genetic basis of FMF is linked to mutations in the *MEFV* gene, located on the short arm of chromosome 16 at position 16P13.3 (7). The M694V gene mutation is the most prevalent among FMF patients (8). This mutation is associated with an earlier onset of the disease, a higher incidence of arthritis, and an increased risk of amyloidosis (9). Identifying mutations associated with this disease can significantly aid in early diagnosis and the prevention of affected births (10). Since FMF is relatively prevalent in the

Mediterranean region, most of the published research on this disease has been conducted in this area. A 2014 study conducted in Ardabil reported that the genetic profile of Iranian FMF patients is similar to that of Armenians and Arabs, with the M694V and V726A gene mutations being the most common among patients (11). However, only a few studies have explored the genetic and clinical profile of FMF in eastern Iran.

2. Objectives

This study aims to investigate the frequency, genetic, and clinical features of children with FMF in northeastern Iran.

3. Methods

3.1. Study Design and Participants

Our study was a descriptive and analytical cross-sectional study conducted among 29 patients under the age of 18 who visited the pediatric Rheumatology Department at Akbar Children's Subspecialty Hospital, a referral center in northeastern Iran, between April 2014 and April 2021. The inclusion criteria for the study were patients under 18 years of age with a confirmed diagnosis of FMF by a pediatric rheumatology subspecialist or approved by a genetic counselor. For the clinical diagnosis of FMF, we used Tel-Hashomer criteria, requiring at least 2 major criteria or 1 major and 2 minor criteria for diagnosis. The major criteria include fever with peritonitis, synovitis or pleurisy, recurrent febrile attacks, and AA amyloidosis. The minor criteria include erysipeloid erythema, response to colchicine, and family history of the periodic syndrome. For further details and explanations, please refer to (12). Exclusion criteria included parents not consenting to participate in the study, failure to attend the children's rheumatology clinic regularly for follow-up, arbitrary discontinuation of medication without coordination with the attending physician, lack of cooperation and participation of patients in providing information through phone calls, and incomplete medical records of patients.

3.2. Data Gathering

After the clinical diagnosis, the parents were provided with an explanation of the situation, and the patients were subsequently referred for genetic evaluation. This study focused on patients who had received a definitive diagnosis of FMF and were being treated with drugs such as colchicine and dapsone. DNA extraction was

performed using 3 mL of whole blood following the salting-out DNA extraction procedure. All exons of the *MEFV* gene were amplified using specific primers for each exon (Table 1). Sanger sequencing was conducted using an ABI 3130xl sequencer according to the manufacturer's instructions. Data were analyzed using the sequencer software, and the pathogenicity of candidate variants was assessed following the American College of Medical Genetics and Genomics (ACMG) guidelines, utilizing bioinformatics prediction tools such as Mutation Taster (<http://www.mutationtaster.org>), SIFT (sift.jcvi.org), ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar>), and PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2>). Sequencing results underwent a quality evaluation. In addition to the internal analysis performed by the sequencing equipment's software, a quality analysis review of the sequencing data was carried out by visually inspecting both strands. The data used in our study were obtained from the gold standard repositories mentioned above, indicating that data quality had already been assessed. Both forward and reverse sequencing were conducted to confirm detected mutations.

The variables considered in this study included demographic information such as patient age, age of disease onset, sex, height, weight, parental history, disease duration, rate of hospitalization, and clinical symptoms (fever, abdominal pain, chest pain). Laboratory tests conducted during attacks (erythrocyte sedimentation rate [ESR], C-reactive protein [CRP], white blood cells [WBC]) and complications (amyloidosis and intestinal obstruction) were also recorded, along with details of drug treatment and dosage (colchicine, dapsone, etc.). Clinical follow-up of patients was conducted through telephone calls and regular visits. The study identified mutations related to each patient and calculated the frequency of each mutation using SPSS v. 16 software (SPSS Inc., Chicago, IL, USA) to analyze the data extracted from the checklists.

3.3. Statistical Analysis

The data were analyzed using SPSS v. 16. Mean \pm standard deviation (SD) was calculated for quantitative variables, and frequencies and percentages were reported to describe qualitative variables. Specifically, the frequency, percent, and distributional measures were added. A cross-sectional analysis was conducted to classify variables into groups as benign, variant of uncertain significance (VUS), and pathogenic. Furthermore, analysis of variance (ANOVA), including mean differences and P-values, was conducted to compare group means for a

Table 1. Amplicon Size and Primer Sequences Used in the Study

Variables	Primers Sequence	Product Size
Exon 1		473
FMF-1F	TCCCCACCAAGACACAGAGC	
FMF-1R	CTTCCCACAAAGCAGCCAG	
Exon 2		739
FMF-2F	ATCTTGGGCCCTAAACGTGG	
FMF-2R	GCCATTCTTTCTCTGCAGCC	
Exon 3		469
FMF-3F	CCGCTGTGCTTTGTGATACC	
FMF-3R	AAGTGCCTGGCAGAGAAGAG	
Exon 4		239
FMF-4F	CAGCTAAAGATGGCAGGAGC	
FMF-4R	CTGCTGGTTACCTCTGTCC	
Exon 5		355
FMF-5F	GGTTCCTGGACATCCACGTC	
FMF-5R	CCTGAGGCATCTGATAGGC	
Exon 6		254
FMF-6F	GCCACTAGGAGCCTGGTAAC	
FMF-6R	CTGACCAGATGCCCTTCTCC	
Exon 7-8-9		852
FMF-7-8-9F	TTCCAGCTCACGGGTACTTG	
FMF-7-8-9R	AGAGCACAGGGATCCAGCAG	
Exon 10		839
FMF-10F	CCTGTCCCTGTTCTGCAG	
FMF-10R	ATGCTTCACCCGGATTGAC	

significant statistical analysis. Multiple comparisons were also included to specify variables with equal group means.

3.4. Ethical Considerations

This study was approved by the Vice-Chancellor of Research at Mashhad University of Medical Sciences (code: IR.MUMS.MEDICAL.REC.1401.112). Written informed consent letters were obtained from all parents/guardians.

4. Results

This study included 29 patients with FMF, consisting of 17 girls (58.6%) and 12 boys (41.4%). The average age of the patients was 9.34 ± 4.27 years, with the youngest being 3 years old and the oldest 18. The average age of disease onset was 3.29 ± 2.54 years, with the youngest starting at 1 year and the oldest at 13 years. Among the 17 female patients, 35.3%, 17.6%, and 47% were categorized as benign,

VUS, and pathogenic, respectively. Similarly, among the 12 male patients, 75%, 8.3%, and 16.6% were categorized as benign, VUS, and pathogenic, respectively. Overall, one-third (34.5%) of the studied patients were classified as pathogenic. Furthermore, 48.3% of the patients had parents who were related to each other. The majority of patients (79.3%) had FMF for more than 24 months. All patients had experienced at least 3 bouts of fever before participating in the study, and 51.7% had abdominal pain. However, other symptoms, such as rash and myalgia, were not observed among the patients. Arthritis was present in 13 patients (44.8%), with most cases involving the knee (37.9%). In terms of laboratory parameters, all patients had a WBC count higher than 15 000 (with an average of $18\,000.9 \pm 0.62$ mL), and during acute attacks, ESR (with an average of 64.10 ± 3.17 mm) and CRP (with an average of 59.82 ± 4.51 mg/L) levels were elevated. Refer to [Table 2](#) for a more detailed statistical analysis. Colchicine was used by 28 patients (96.6%). Regarding colchicine dosage and the treatment plan, patients were treated with a standard dose of colchicine. Young children were treated with 0.3 mg/kg, while pediatric patients received the same dose as adults, which is 1 - 2 mg/kg. Furthermore, none of the patients had comorbidities, and a few patients reported abdominal discomfort and diarrhea, which were managed by slowly titrating the drug. In total, 97% of patients responded to treatment. One patient with symptoms of pleural and pericardial effusion, dyspnea, and chest pain did not respond to colchicine and required dapsone for symptom control and to manage FMF attacks. In terms of patient zygosity, 23 patients (79.3%) were heterozygous, and 6 patients (20.6%) were homozygous (see [Table 3](#)). The classification of *MEFV* gene mutations showed that four patients (13.8%) had VUS variants, 10 patients (34.5%) had pathogenic mutations, and 15 patients (51.7%) had benign variants. The most common amino acid substitution among patients was P. Glu148Gln (E148Q), which was reported as benign, with a frequency of 41.3%, followed by pathogenic mutations P. Met680Ile (M680I), with a frequency of 10.2%. The most common nucleic acid alteration in this study was c.442G>C, with an abundance of 41.3%. Clinical diagnosis suggested FMF, as mutations in the *MEFV* gene are associated with this disease. To identify the causative mutation, we sequenced all ten exons of the *MEFV* gene with specific primers using the Sanger sequencing method performed by the ABI 3130xl sequencer according to the manufacturer's instructions. Data were analyzed using sequencer software, and the pathogenicity of candidate variants was evaluated using the VarSome tool following the ACMG guidelines. The

results are summarized in Table 1. For significant statistical analysis, we compared the means of age, age of onset, disease duration, rate of hospitalization, weight, and height using ANOVA at the significance level $\alpha = 0.05$. If μ_{benign} , μ_{vus} , and μ_{pathogen} denote the mean of any variables mentioned above for the 3 groups, i.e., benign, VUS, and pathogenic, respectively, then using P-values, we observed that for all variables except for the rate of hospitalization (with a P-value equal to 0.037), the null hypothesis $H_0: \mu_{\text{benign}} = \mu_{\text{vus}} = \mu_{\text{pathogen}}$ was accepted at the 0.05 significance level, indicating no significant difference between these groups. For the rate of hospitalization, the null hypothesis H_0 was accepted, and consequently, a post-hoc test was conducted to determine which groups had different means for this variable. Accordingly, the 2 groups, benign and pathogenic, had different means, with a P-value of 0.039 in the multiple comparisons based on the Tukey HSD (Tables 2 - 8 show the detailed results).

Table 2. Frequency Analysis of Categorical Variables

Variables	Mean \pm SD	Min	Max
Age	9.34 \pm 4.27	3	18
Height	130 \pm 20	95	171
Weight	30 \pm 13.7	14	65
Attack WBC (mCL)	18000.9 \pm 0.62	15000	28000
Attack ESR (mm/h)	64.10 \pm 3.17	37	95
Attack CRP (mg.L)	59.82 \pm 4.51	20	105
Variables	Frequency	Percent (%)	Cumulative P
Sex			
Male	13	44.8	100
Female	16	55.2	55.2
Consanguinity of parents			
Yes	15	51.7	51.7
No	14	48.3	100
Family history			
None	25	86.2	86.2
FMF	2	6.9	93.1
PFAPA	2	6.9	100
Colchicine			
Yes	28	3.4	3.4
No	1	96.6	100

Abbreviations: WBC, white blood cells; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein.

Table 3. Distribution of the MEFV Gene Mutations

Mutations and Genotypes	Number of Patients (%)
Heterozygote; N = 23 (79.3%)	
A744S	2 (6.8)
R408Q	2 (6.8)
R761H	1 (3.4)
E148Q	9 (31)
E148V	2 (6.8)
M680I	2 (6.8)
M694I	1 (3.4)
P369S	1 (3.4)
V726A	1 (3.4)
E148D	2 (6.8)
Homozygote; N = 6 (20.6%)	
E148Q	3 (10.3)
M694V	2 (6.8)
M680I	1 (3.4)

Table 4. Clinical Findings in Our Patients

Clinical Features	Number of Patients (%)
Fever	29 (100)
Abdominal pain	15 (51.7)
Chest pain	2 (6.9)
Arthritis	13 (44.8)
Pruritus	1 (3.4)
Rash (Erysipelas-like erythema)	0 (0)
Myalgia	0 (0)

5. Discussion

This study aimed to determine the genetic and clinical characteristics of children with FMF in northeastern Iran. A descriptive-analytical study was conducted, investigating 29 patients with FMF. This disease typically manifests at a young age, with 90% of patients experiencing their first attack before the age of 20. Although the onset of the disease can occur at an older age, it becomes rare after the age of 40 (13). The date of FMF onset is usually established through patient history; however, misinterpretation and misdiagnosis can sometimes occur (14). In this study, the average age of the first attack was 3.29 ± 2.54 years, with the oldest onset being at 13 years of age. These findings align with Tamir et al.'s (15) study, which reported that only 0.5% of patients experienced their first attack after reaching the age of 40. In contrast, Sayarlioglu et al. (16) reported that

Table 5. Results of *MEFV* Gene Mutations and Different Alleles Analysis ^a

Numbers	Variant	Amino Acid	Classification	No. (%)
1	c.2230G>T	A744S	Likely Benign	2 (6.8)
2	c.442G>C	E148Q	Benign	12 (41.3)
3	c.1105C>T	P369S	Benign	1 (3.4)
4	c.1223G>A	R408Q	VUS	2 (6.8)
5	c.443A>T	E148V	likely pathogenic	2 (6.8)
6	c.2177T>C	V726A	Likely pathogenic	1 (3.4)
7	c.2282G>A	R761H	Pathogenic	1 (3.4)
8	c.2082G>A	M694I	Pathogenic	1 (3.4)
9	c.2080A>G	M694V	Pathogenic	2 (6.8)
10	c.2040G>C	M680I	Pathogenic	2 (6.8)
11	c.2040G>A	M680I	Pathogenic	1 (3.4)
12	c.444G>C	E148D	VUS	2 (6.8)

Abbreviation: VUS, variant of uncertain significance

^a The frequency and percentage of nucleic acid types in patients with familial Mediterranean fever participating in the study are denoted by No. (%).**Table 6.** Frequency Table for the 3 Groups, Classified by Sex

Crosstab			
Classifications	Sex		Total
	Female	Male	
Benign			
Count	6	9	15
% Within classification	40.0	60.0	100.0
% Within sex	35.0	75.0	51.7
VUS (variant of uncertain significance)			
Count	3	1	4
% Within classification	75.0	25.0	100.0
% Within sex	17.6	8.3	13.8
Pathogen			
Count	8	2	10
% Within classification	80.0	20.0	100.0
% Within sex	47	16.6	34.5
Total			
Count	17	12	29
% Within classification	58.6	41.4	100.0
% Within sex	100.0	100.0	100.0

5 patients (8.8%) had their first attack after the age of 40. This difference may be attributed to variations in the study population, as our study exclusively included children.

While most studies have reported that FMF affects both sexes equally (13), in our patient cohort, the ratio of women to men was approximately 1.4, with the majority of patients (58.3%) being female. Similarly, Ureten et al.'s (17) study found that the majority of patients were female (female/male: 1.85). However, in a Turkish study (16), 80% of FMF patients were male. Another report from northwest Iran indicated a higher prevalence of this disease in males (1: 7: 1) (18). Familial Mediterranean fever is a disease characterized by recurrent acute fever episodes, along with the involvement of various body parts, including the abdomen, chest, joints, muscles, scrotum, and skin. Abdominal pain is present in approximately 95% of patients, and the clinical presentation often resembles acute peritonitis (19). In our study, we observed that abdominal pain (51.7%) and fever (100%) were the most common clinical findings in our patients, consistent with findings in most other studies (19, 20). However, Tsuchiya-Suzuki et al. (21) reported that only 55% of their patients experienced abdominal symptoms. Our study also revealed that 44.8% of FMF cases exhibited arthritis, and 6.9% had chest pain, findings in line with other studies (19, 20). These symptoms had a lower prevalence compared to fever and abdominal pain. Among patients with arthritis, the knee joint (37.9%) was the most commonly affected site. Notably, myalgia was not observed in any of the patients. Tunca et al. (19) similarly concluded

Table 7. Analysis of Variance for Comparing the Group Mean of Variables Under Study in Patients with Familial Mediterranean Fever

ANOVA					
Variables	Sum of Squares	df	Mean Square	F	P-Value
Age				0.435	0.652
Between groups	14.544	2	7.272		
Within groups	434.767	26	16.722		
Total	449.310	28			
Age of onset				0.024	0.976
Between groups	0.288	2	0.144		
Within groups	155.850	26	5.994		
Total	156.138	28			
Disease period				1.048	0.365
Between groups	1.235	2	0.618		
Within groups	15.317	26	0.589		
Total	16.552	28			
Rate of hospitalization				3.756	0.037
Between groups	10.357	2	5.178		
Within groups	35.850	26	1.379		
Total	46.207	28			
Weight				0.289	0.751
Between groups	114.873	2	57.436		
Within groups	5159.817	26	198.454		
Total	5274.690	28			
Height				0.602	0.555
Between groups	526.909	2	263.454		
Within groups	11369.850	26	437.302		
Total	11896.759	28			

Table 8. Post-Hoc Test for Checking the ANOVA Result of the Hospitalization Rate in Patients with Familial Mediterranean Fever

Multiple Comparisons; Tukey HSD							
Dependent Variable	(I) Classification	(J) Classification	Mean Difference (I-J)	Std. Error	P-Value	95% Confidence Interval	
						Lower Bound	Upper Bound
Rate of Hospitalization	Benign	VUS	1.267	0.884	0.339	-0.93	3.46
		Pathogen	1.183 ^a	0.455	0.039	0.05	2.31
	VUS	Benign	-1.267	0.884	0.339	-3.46	0.93
		Pathogen	-0.083	0.897	0.995	-2.31	2.15
	Pathogen	Benign	-1.183 ^a	0.455	0.039	-2.31	-0.05
		VUS	0.083	0.897	0.995	-2.15	2.31

Abbreviation: VUS, a variant of uncertain significance.

^a The mean difference is significant at the 0.05 level.

that arthritis, myalgia, and erythema erysipelas were significantly less common among adult-onset patients.

All patients who continued colchicine treatment exhibited a satisfactory response, with 75.7% achieving a complete response and 21.3% showing a partial response. These findings are consistent with other studies (15, 16), suggesting that colchicine is highly effective in treating FMF, and lower colchicine doses (1 mg/day) may be sufficient. Test results revealed elevated levels of acute phase reactants during FMF attacks, with all patients in our study experiencing an increase in the average number of white blood cells, ESR, and CRP. These results align with other reports (16, 22), which have demonstrated that ESR, CRP levels, and leukocyte counts significantly exceed the normal range during FMF attacks.

Concerning the genetic aspect of FMF, our study identified 23 patients as heterozygous, while 6 were homozygous for *MEFV* gene mutations. These results were consistent with Dundar et al.'s study (23), in which the majority of mutations were found to be heterozygous. In our study, the most prevalent heterozygous mutation was associated with the *E148Q* gene, with a frequency of 31%. However, in Salehzadeh et al.'s study (18), the most common heterozygous mutation was V726A-M694V, with a frequency of 10.46%. The predominant genotype in our study was *E148Q*, accounting for 41.3% of cases. This diverges from other studies where the most frequent genotypic mutation was M694V (12).

Among patients of Turkish and Armenian descent, M680I is typically the second most common mutation after M680V (24). Nevertheless, in our study, M680I emerged as the second most common mutation following *E148Q*, with a frequency of 10.2%, while the M694V mutation ranked third at 6.8%. In Jewish studies, M694V, V726A, and *E148Q* were more common than M680I (25).

In studies involving Jewish and Arab populations (26, 27), M680I was reported as a rare mutation, whereas V726A was found to be more common in Armenians than in other populations (24). Among our patients, the 3 most prevalent mutations were M694V, M680I, and *E148Q*, which also happen to be the most common mutations reported in Mediterranean populations but were absent in Japanese populations. Most Japanese patients carried M694I on at least one allele, whereas this mutation was present in only 1 of our patients (21). Furthermore, we identified rare mutations, such as R761H and R408Q, with a higher detection rate compared to other populations. This observation may be attributed to consanguineous marriages and a high prevalence of siblings among our patients.

Our study does have some limitations. One limitation is the lack of reliable and complete family history to construct the genealogy of patients, including factors such as parental separation or the death of a family member, which could hinder accurate genetic analysis. Additionally, a comprehensive examination of all genotypes, including heterozygous and compound heterozygous mutations, would provide a more accurate frequency of genotypes among affected patients.

Considering the prevalence of FMF in the western regions of Iran and the Mediterranean regions, and given the increasing number of studies conducted in these areas, our study results indicate a lower prevalence of this disease in the eastern part of Iran. Based on the data obtained from this study, we suggest that other regions, regardless of the prevalence of this disease in Mediterranean regions, should also conduct research in this field. Further investigations will yield more comprehensive and precise results regarding the genetic and clinical profile of this disease throughout the country.

Thus, we have demonstrated a lower prevalence of this disease in the eastern part of the country as well, contrary to expectations. Indeed, additional investigations are warranted to provide a more comprehensive and precise understanding of the genetic and clinical aspects of this disease nationwide. Therefore, according to our sample, there is a possibility of discovering more mutations in future studies, and other regions of Iran can gather comprehensive information about the state of the disease in the country through a registry study.

5.1. Conclusions

Our findings reveal that the most common genotype in our study was E148Q, which is considered benign. M680I and M694V were the most frequent pathogenic mutations, each occurring in 5 cases, indicating that FMF disease is rare in Northeastern Iran, and the number of pathogenic mutations is lower compared to Northwest Iran and other studies. Further investigations with a larger patient cohort and a comprehensive examination of the *MEFV* gene are necessary to establish the relationship between FMF symptoms and genotypes, which can aid in the treatment and management of the disease and its potential complications, such as amyloidosis.

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Footnotes

Authors' Contribution: N.V.: Data acquisition, manuscript drafting; M.R.A., T.Z.: Analysis and interpretation of data, statistical analysis; A.R.M.: Study concept and design, critical revision of the manuscript for important intellectual content; E.G.H.K., A.S.N.: Administrative, technical, and material support, study supervision. All the authors read and approved the final manuscript.

Conflict of Interests: The authors declare that they have no competing interests.

Data Availability: The mutations identified in the patients in our study have been previously reported and are publicly available in dbSNP with specific rs numbers.

Ethical Approval: This study was registered with the Research Ethics Committee of Mashhad University of

Medical Sciences and was approved under the ethical code [IR.MUMS.MEDICAL.REC.1401.112](https://doi.org/10.1186/1745-6215-14-112).

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