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Familial Mediterranean fever gene variations could trigger *VPS16*-associated early-onset dystonia and diabetes mellitus: clinical identification of a family with *MEFV* and *VPS16* genetic variation association

Yagmur Inalkac Gemici[1,*], Cemal Ekici[2], Melike Batum[1], Cenk Akbostanci[3], Ahmet Koc[2], Hatice Mavioglu[1]

[1]Department of Neurology, Manisa Celal Bayar University School of Medicine, Manisa, Turkey
[2]Department of Medical Genetics, Inonu University School of Medicine, Malatya, Turkey
[3]Department of Neurology, Ankara University School of Medicine, Ankara, Turkey

*Correspondence to: Yagmur Inalkac Gemici, Department of Neurology, Manisa Celal Bayar University School of Medicine, Uncubozkoy mah 5524.sok No: 11/1 Bozkoy Manzara Evleri B blok kat: 4 Daire: 13 Yunusemre/Manisa, Turkey. E-mail: yagmur.gemici@cbu.edu.tr

Abstract

Objectives: We describe the clinical pictures of an index case with dystonia and his family resulting from *VPS16* and *MEFV* genetic variations based on previously published data and discuss the mechanisms that may have brought out the clinical findings.

Methods: A 17-year-old male had generalized dystonia that started at age 6 years, non-febrile abdominal pain attacks and was diagnosed with type 1 diabetes at age 14 years. Meanwhile, his 13-year-old sister had the same clinical presentation. His father was diabetic and his mother was asymptomatic. There was no consanguinity between the parents. Genetic variations were detected with whole exome sequencing.

Results: *VPS16* c.1513C>T/p.Arg505* (likely pathogenic), *MEFV* c.2080A>G p.Met694val (pathogenic) and *MEFV* c.1772T>C p.Ile591Thr (unknown significance) heterozygous variants were detected in his siblings. The father had *VPS16* c.1513C>T/p.Arg505* and *MEFV* c.2080A>G p Met694val variations and the mother had *MEFV* c.1772T>C p.Ile591Thr variations.

Conclusions: The occurrence of these diseases in siblings but their absence in the parents suggests the idea that the coexistence of two separate variations in the *VPS16* and *MEFV* genes determines the phenotype. In addition, the increase in *MEFV* variation load in this family and the fact that DM occurs at an earlier age suggest that inflammation may cause an early diabetic clinical presentation.

Lay Summary

What does this mean for patients?

Familial Mediterranean fever (FMF) is an inherited autoinflammatory disorder that causes recurrent fever and pain in the joints, chest and abdomen. However, it is not just a rheumatological disorder. FMF can also aggravate the patient's clinical condition or trigger other underlying diseases. We described a 17-year-old male patient with FMF, diabetes and dystonia (uncontrolled muscle movements). Dystonia is associated with variations in the *VPS16* gene, while FMF is associated with variations in the *MEFV* gene. Due to the genetic nature of FMF and dystonia, we also described the patient's family. We found that the patient's sister also had FMF, diabetes and dystonia. This suggests that if a person has specific variations of both the *VPS16* and *MEFV* genes, they may have FMF, dystonia and diabetes from a young age.

Keywords: dystonia, diabetes mellitus, FMF, MEFV, VPS16.

Key messages

- In patients with the *VPS16* possible pathogenic variation, addition of the *MEFV* variant may result in the occurrence of the dystonia phenotype.
- *VPS16* and *MEFV* variation phenotype coexistence could be dystonia, diabetes mellitus and familial Mediterranean fever.
- Age of diabetes onset could become earlier with the addition of other *MEFV* variants of uncertain significance.

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Introduction

Dystonia makes up ≈20% of all movement disorders and can be classified according to aetiology, localization, age of onset and accompanying symptoms. Neurodegenerative and structural lesions must be investigated first with cranial imaging for aetiological diagnosis after clinical diagnosis [1]. Dystonia 30 has recently been included in the idiopathic isolated dystonia classification. Dystonia 30 is an autosomal dominant genetic disease causing dystonic symptoms in individuals in the first decade of life, affecting the autophagy–endolysosomal system, and is caused by heterozygous and homozygous variations in the vacuolar protein sorting 16 (*VPS16*) gene [2].

FMF is a monogenic, autoinflammatory syndromic disease characterized by recurrent fever and abdominal pain associated with *MEFV* variations. The diagnosis can be made by clinically recurrent abdominal or chest pain, arthritis and the presence of at least 1–3 days of these symptoms and at least two of these criteria, and a single clinical episode is sufficient for the diagnosis in case of confirmed genetic anomaly [3]. The genetic aetiology of FMF is complex, as epigenetic factors and interactions with other genes occur [4]. It was reported that neurological disorders are also detected as well as the symptoms of FMF in patients with *MEFV* variations, but dystonia is not among these symptoms [5]. In a large-scale review [6], genes with risk factors for dystonia were identified, but *MEFV* was not included in this list. In addition, when all *VPS16* gene variations described in the literature were examined, it was found that the comorbidity of dystonia and diabetes mellitus (DM) was not reported.

In this article, we discuss a male index case who was diagnosed with generalized dystonia, DM and FMF as well as his family phenotype and genotype relationship.

Methods

Approval was obtained from the Manisa Celal Bayar University Ethics Committee for the processing of the index family data. Permission was obtained from the family with an informed consent form. No financial support was received for this article.

Genetic analysis method

DNA samples of the patient and parents were isolated from peripheral blood. Next-generation sequencing technology was used to determine the sequence of all genes using the whole exome sequencing (WES) technique. All replicated FASTQ files and bioinformatics analyses were processed using (Qiagen QCI Analyse Universal 1.5.0, Hilden, Germany). Secondary analyses were performed using QCI Analyze Universal 1.5.0 (Qiagen) and tertiary analyses and interpretations were made using Clinical Insight Interpret (Qiagen). The variants was confirmed by the Sanger sequence analysis method and the results were analysed using the Variation Surveyor program (Philadelphia, USA).

Results

Patient 1 (index case)

Patient 1 was a 17-year-old male diagnosed with generalized dystonia prominent in the cervical region at 10 years of age and type 1 DM at 14 years of age. FMF was diagnosed by the Rheumatology Unit. Examinations included cranial MRI, laboratory examinations of metabolic diseases, echocardiography, thoracic and abdominal CT, electromyography (EMG) and cognitive tests. All results were found to be normal.

Genetic testing included *DYT-1* gene variation analysis, which was normal. Exon scanning was performed with WES and *VPS16* c.1513C>T/ Arg505* heterozygous class II (possibly pathogenic for dystonia), *MEFV* c.2080A>G p.Met694val heterozygous class I (pathogenic for FMF) and *MEFV* c.1772T>C p.Ile591Thr (with an unknown meaning for FMF) heterozygous class III variations were detected.

For dystonia, the patient was started on levodopa, and when there was no response, an appropriate dose of trihexyphenidyl was initiated. When the response was not sufficient, repetitive botulinum toxin injections were administered. He had fever and acute abdominal pain at the age of 11 years and an appendectomy was performed. Insulin therapy was started for type 1 DM. Colchicine was added to the treatment for FMF.

Patient 2

Patient 2 was a 14-year-old female (sister of index case) diagnosed with type 1 DM at 12 years of age and writer's cramp at 14 years of age. Examinations included cranial MRI, abdomen and thorax CT, EMG, echocardiography and cognitive tests. All results were normal.

Genetic testing showed the same variations on WES as in the index case.

Botulinum toxin injections were administered for writer's cramp. She also takes insulin therapy for type 1 DM.

Patient 3

Patient 3 was a 55-year-old male (father of index case) with type 1 DM, but no symptoms related to FMF or movement disorder. His haemoglobin A1c was 7.5%.

Genetic testing showed heterozygous *VPS16* c.1513C>T/ Arg505* and *MEFV* c.2080A>G p.Met694val variations.

He did not use the recommended oral antidiabetic and did not go for follow-ups.

Patient 4

Patient 4 was a 44-year-old female (mother of index case) diagnosed with hypothyroidism. No symptoms related to FMF or movement disorder were in her history and no DM. Laboratory examinations did not reveal any findings suggestive of inflammation or diabetes.

Genetic testing showed a heterozygous MEFV c.1772T>C Ile591Thr variation.

She had been on levothyroxine for a long time to treat hypothyroidism.

The clinical and genetic variations of the family are summarized in Fig. 1. The pedigree of the family is shown in Fig. 2.

Discussion

This article describes a Turkish family with different phenotypes associated with two genetic variations not previously described together. The father had *VPS16* PPV (Possible Pathogenic Variation) and *MEFV* c.2080A>G p.Met694val PV (Pathogenic Variation) but only had diabetes, while the siblings had additional *MEFV* c.1772T>C p.Ile591Thr VUS (Variation of Unknown Significance) for FMF along with dystonia and type 1 DM. The mother, with only the *MEFV* VUS, did not have dystonia, FMF or diabetes. These

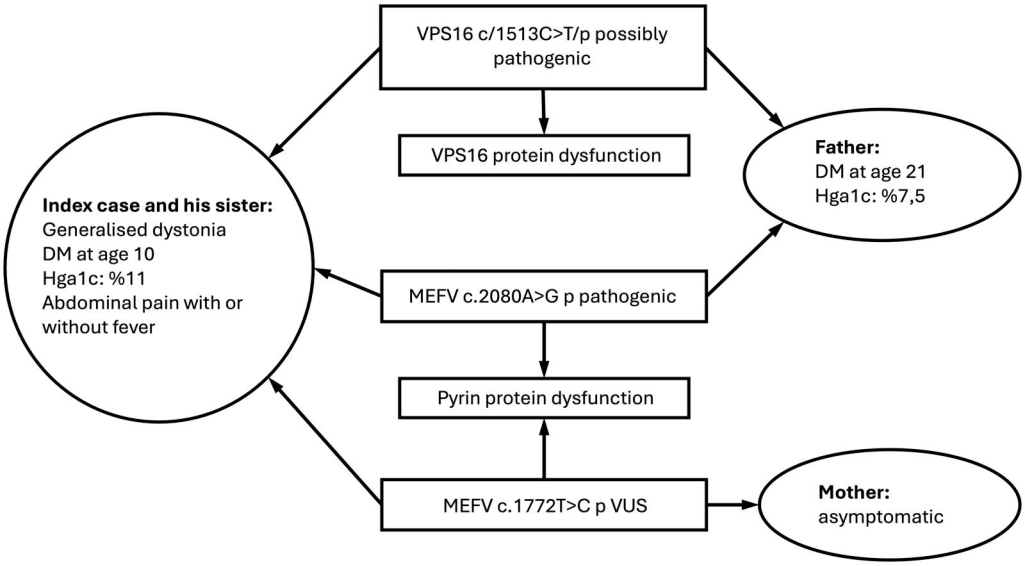

Figure 1. The genetic and phenotypic characteristics of cases

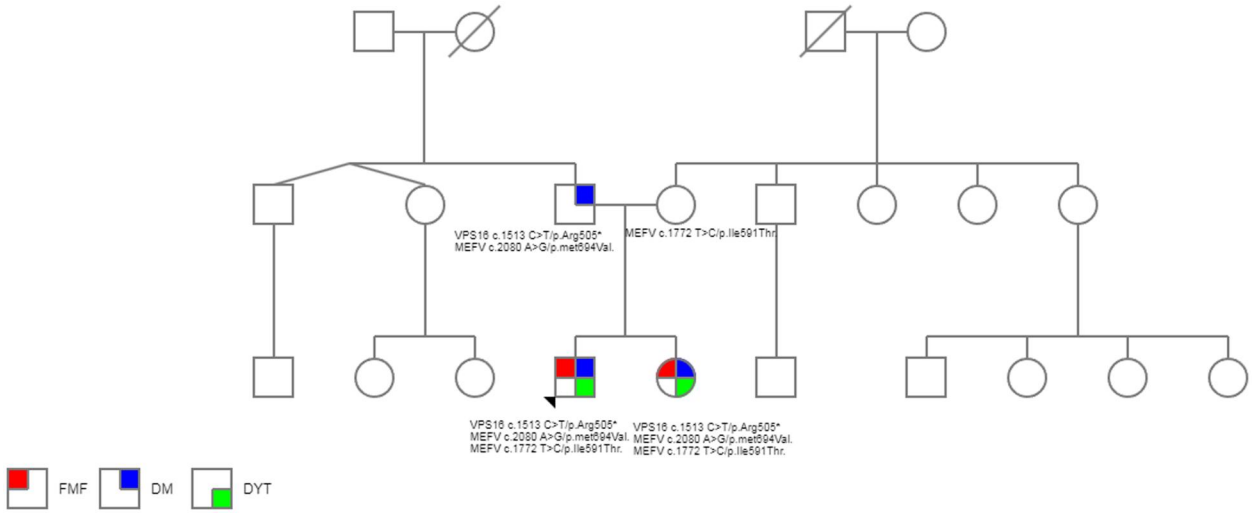

Figure 2. Pedigree of the index case and his family

differences suggest a potential interaction of genetic variations in dystonia, diabetes and FMF.

The *VPS16* gene is located on chromosome 20, which encodes the VPS16 protein. The VPS16 protein is involved in cellular processes such as autophagosome maturation, endosomal transport, endosomal vesicular fusion, endosome-to-lysosome transport, intracellular protein transport, regulation of SNARE complex assembly and non-autophagic vacuolar fusion [7]. Cai *et al.* [3] described a disease with autosomal recessive dystonia caused by homozygous variations in the *VPS16* c.156 C> gene in five individuals with adolescent-onset primary dystonia secondary to this variation in 2016, then named 'dystonia 30'. Steel *et al.* [8] described the clinical and demographic characteristics of 19 patients who were diagnosed with dystonia 30. In clinical terms, patients have abnormal dystonic contractions. These contractions can be generalized and progress slowly. Besides dystonia, psychiatric symptoms were also reported in these patients and the frequency of autosomal dominant transmission with incomplete penetration of *VPS16* was found to be higher. In cell lines obtained from patients, microscopic vacuolar changes resulted in lysosomal dysfunction and abnormalities in autophagosome–lysosome fusion after the pathologies in endosomal–lysosomal fusion [8]. The genetic variations in this study involve different regions in the *VPS16* gene PPV; however, the *VPS16* gene c.1513C>T/p.Arg505* detected in the family presented was not reported. Although the father in the family had a PPV of *VPS16*, the absence of dystonia could be explained by incomplete penetrance or the absence of *MEFV* VUS. Further studies are needed to determine these possibilities. However, although no diabetes-related gene variation was detected in the family, the father and his two children were diagnosed with diabetes. Moreover, the children were diagnosed with diabetes 10 years earlier than the father's age at diagnosis.

The *MEFV* gene encodes the pyrin, located on chromosome 16 and causes FMF disease in its pathologies [3, 9]. This protein acts as an associative protein, ensuring the binding of the main proteins needed for the formation of the inflammasome complex, inhibiting autophagosome and lysosome assembly in its deficiency [10, 11]. FMF clinical

diagnosis can be made if at least two of the related criteria exist (recurrent abdominal or chest pain and arthritis) and persist for at least 1–3 days. In the case of a confirmed pathogenic or possibly pathogenic genetic anomaly, a single clinical attack is sufficient for the diagnosis of FMF [3]. However, the genetic aetiology of FMF is complex; it is not only caused by pathogenic variations, PPVs and VUSs, but also by the interaction with epigenetic factors and other genes [9]. Neurological symptoms defined in FMF are headache, myalgia, syncope, recurrent meningitis, vertigo, seizures, paraesthesia, demyelinating diseases, cerebrovascular diseases, posterior encephalopathy, prolongation of subclinical visual evoked potentials, pseudotumor cerebri and optic neuritis [5]. In a previous study reviewing risky genes for dystonia, the *MEFV* gene was not listed [6]. The fact that appendectomy was performed in the index case here because of fever and abdominal pain and that the pathological examination was reported as normal strongly supports that this was an FMF attack. This is frequently reported in undiagnosed patients with fever and abdominal pain, such as FMF [12]. The index case and the sibling were evaluated as FMF and investigated in terms of neurological symptoms reported to be associated with FMF.

Type 1 DM is caused by an autoimmune reaction against insulin-secreting pancreatic β cells [13]. IL-1β is released and insulin secretion is decreased with the activation of NLRP3 inflammasome, which may cause the death of pancreatic β cells by causing activation of pro-apoptotic signalling pathways [13]. Dysfunction of pyrin leads to an impaired inflammasome, resulting in the upregulation of IL-1β [14]. Similar to IL-6, IL-1 is a pro-inflammatory cytokine that facilitates the differentiation of T cells into Th17 cells. Kumar *et al.* [15] demonstrated that Th17 cells play a significant role in the pathogenesis of type 1 DM. In addition to IL-1, NF-κB, NLRP3, impaired mitophagy, reactive oxygen species and chronic inflammation are also implicated in the pathogenesis of type 1 DM [13]. Notably, the NF-κB and NLRP3 pathways are involved in the inflammatory process of FMF as well [14]. IL-1 monoclonal antibodies are effective in the treatment of both DM and FMF [11, 16]. Thus it can be asserted that the pathogenesis of type 1 DM and FMF involves numerous intersecting inflammatory pathways. There are three documented cases of patients with both type 1 DM and FMF [17–19]. One of these diabetic patients had undergone an appendectomy, similar to our index case [17], while another had additional autoinflammatory conditions [18]. However, these reports did not indicate whether there was a family history of diabetes.

Anwar *et al.* [20] screened FMF gene variations in children with type 1 DM and found a higher rate of compound heterozygous mutations in these children. According to the literature, no information is available regarding the onset of diabetes, which may occur earlier as the FMF mutation burden increases. Therefore, the family described in this report is of particular significance. The co-occurrence of type 1 DM and FMF in this family, along with the early onset and more severe clinical presentation of DM in the siblings, suggests that additional *MEFV* variations contribute to increased autoinflammation and play a crucial role in this deterioration. However, no report was detected on the relationship of VPS proteins with diabetes.

The two siblings reported in this article have a fairly homogeneous clinical manifestation, presenting at similar ages and with the same variations: dystonia PPV, FMF PV and VUS. In the father, who had possible pathogenic dystonia-associated and FMF-associated pathogen gene variations, there was only diabetes and no clinical signs compatible with dystonia and FMF. Interestingly, no diabetes-associated variations were detected in any of the three. Did the coexistence of these two variations cause diabetes? Is the absence of dystonia and the FMF phenotype related to incomplete penetrance or the absence of a third VUS for FMF? The absence of signs and symptoms of these three diseases in the mother, who had an *MEFV* VUS, suggests that this variation alone is not effective. Did the coexistence of this unknown variation with the other two variations cause clinical findings of dystonia, diabetes and FMF? This needs to be investigated.

In conclusion, although these two variations were seen coincidentally in this family, the increased FMF variation load and the earlier age of phenotype formation and clinical presentation suggest that the interaction between these two genes and the accumulation of FMF gene variations could determine phenotype presentation.

Data availability

The data underlying this article were provided by Manisa Celal Bayar University (MCBU) Hafsa Sultan Hospital by permission. Data will be shared upon request to the corresponding author with permission of MCBU Hafsa Sultan Hospital.

Authors' contributions

Conceptualisation: Yagmur Inalkac Gemici, Cemal Ekici, Ahmet Koc, Hatice Mavioglu; Design analysis: Yagmur Inalkac Gemici, Melike Batum, Cenk Akbostancı, Hatice Mavioglu; Planning: Yagmur Inalkac Gemici, Cemal Ekici, Ahmet Koc, Hatice Mavioglu; Conduct: Yagmur Inalkac Gemici, Cemal Ekici, Melike Batum, Cenk Akbostancı, Ahmet Koc, Hatice Mavioglu; Data analysis: Yagmur Inalkac Gemici, Cemal Ekici, Melike Batum, Cenk Akbostancı, Ahmet Koc, Hatice Mavioglu; Manuscript preparation: Yagmur Inalkac Gemici, Cemal Ekici, Melike Batum, Cenk Akbostancı, Ahmet Koc, Hatice Mavioglu.

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