Original Article

MEFV gene p.Met694Val variation is not associated with subacute thyroiditis in Turkish patients

Nurdan Gül¹, Özlem Soyluk¹, Melda Sarıman³, Neslihan Abacı³, Sema Sirma-Ekmekçi³, Ferihan Aral¹, Refik Tanakol¹, Ahmet Gül²

¹Division of Endocrinology and Metabolism, ²Division of Rheumatology, Department of Internal Medicine, Istanbul Faculty of Medicine Istanbul, Turkey; ³Department of Genetics, Institute for Experimental Medicine, Istanbul University, Istanbul, Turkey

Received February 21, 2016; Accepted May 15, 2016; Epub July 15, 2016; Published July 30, 2016

Abstract: Subacute thyroiditis (SAT) is a self-limited, granulomatous inflammatory thyroid disorder associated with neck pain and systemic inflammatory findings such as fever and acute phase response. Familial Mediterranean fever (FMF), an autosomal recessively inherited autoinflammatory disorder, is characterized by recurrent inflammatory attacks in serosal and synovial tissues. Heterozygous carrier state is quite prevalent in Eastern Mediterranean countries, especially the most penetrant p.Met694Val variant, has been found to be a risk factor for other inflammatory disorders due to a tendency to higher IL-1 production. We herein aimed to investigate the possible role of p.Met694Val variant in the pathogenesis of subacute thyroiditis in Turkish patients, in which the prevalence of the variation in healthy individuals is around 3%. We genotyped 58 SAT patients with typical clinical and laboratory features, and we could not identify any individual with p.Met694Val variant among them. On the other hand, 7 heterozygous individuals were found among healthy controls, who were matched to the study group according to the their birth places. Two of the patients were on anti-TNF agents for the treatment of rheumatoid arthritis and ankylosing spondylitis, and additional two patients, one with systemic lupus erythematosus and another with eosinophilic granulomatosis with polyangiitis (Churg-Strauss syndrome) were on immunosuppressive drugs. Additionally, another patient with ankylosing spondylitis was receiving sulphasalazine. In conclusion, we did not find an association between the MEFV gene p.Met694Val variant and subacute thyroiditis in Turkish patients, which may suggest no contribution of pyrin inflammasome in the pathogenesis. Increased proportion of patients on immunosuppressive agents supports further the triggering role of infections, and investigations of genetic polymorphisms associated with infection susceptibility are warranted.

Keywords: Subacute thyroiditis, familial Mediterranean fever, MEFV, interleukin-1

Introduction

Subacute thyroiditis (SAT), also known as granulomatous thyroiditis or de Quervain thyroiditis, is a self-limited, inflammatory thyroid disorder associated with neck pain and systemic inflammatory findings such as fever and acute phase response [1]. Mygind reported 18 cases of thyroiditis in a previously normal gland without abscess formation for the first time in 1895 and named the disorder as “thyroiditis akuta simplex” [2]. Later in 1904, a Swiss surgeon, Fritz de Quervain described the unique pathology of painful subacute thyroiditis [2]. The incidence of the disease is higher in women like many other thyroid disorders. Although mounting evidence suggests that SAT may be induced by viral infection in genetically predisposed individuals, the exact pathogenesis of this disease is still not well understood [1, 3]. Genetic tendency has mainly been associated with class I HLA molecules, such as HLA-B35 and -B67 [4, 5], but no strong non-HLA variation associated with SAT has yet been described. Familial Mediterranean fever (FMF) is an autosomal recessively inherited autoinflammatory disorder, which is characterized by recurrent inflammatory attacks in serosal and synovial tissues [6]. Variations of the MEFV gene encoding pyrin protein are responsible for the disease phenotype, and especially those affecting the C-terminal SPRY domain encoded by exon 10 are associated with deregulation of inflammasome and increased processing of interleukin-1.
No association of MEFV M694V variant with subacute thyroiditis

beta [6, 7]. FMF is considerably common in Eastern Mediterranean populations with Jewish, Turkish, Armenian and Arab ethnic background, and the prevalence of heterozygous carriers is high, ranging between 10-30% in the region [6]. Heterozygous carrier state, especially for the most penetrant p.Met694Val variant, was found to be a risk factor for other inflammatory disorders, especially in countries where FMF is prevalent [8]. We previously showed the association of p.Met694Val variant with ankylosing spondylitis and Behçet’s disease, possibly due to a tendency to higher IL-1 driven inflammatory response contributing to the development of underlying diseases [9, 10].

Since subacute thyroiditis is associated with a strong inflammatory response manifesting with fever, local tenderness and elevated acute phase reactants, we herein aimed to investigate the possible role of penetrant p.Met694Val variant in the pathogenesis of subacute thyroiditis in Turkish patients, in which the prevalence of the variation in healthy individuals is around 3% [10].

Materials and methods

Patients

We screened our medical records for patients with SAT referred to our Endocrinology and Metabolism outpatient clinic in the last 10 years. We reviewed their records to confirm their diagnosis according to the clinical features like painful and tender thyroid gland, laboratory findings of elevated erythrocyte sedimentation rate or elevated C-reactive protein (CRP), elevated serum free thyroxine (FT4) and decreased serum thyroid stimulating hormone (TSH), or scintigraphy findings and/or low radioiodine uptake results. Patients who were diagnosed pathologically (either after biopsy or surgery) were also included in the study group. We then tried to reach all patients with confirmed diagnosis by phone for genetic testing.

We selected 200 previously genotyped healthy individuals [10] according to the birth place of the study group for comparison.

Genotyping

Genomic DNA was isolated from venous blood using the MagNA Pure Instrument with the MagNA Pure Compact DNA Isolation Kit (Roche Diagnostics). All patients were genotyped for the MEFV gene p.Met694Val variant (rs6175-2717; c.2080A>G) using the restriction fragment length polymorphism method as described previously [11]. Briefly, we amplified a 194-bp genomic segment by using forward 5'-AGA ATG GCT ACT GGG TGG AGA T-3' and reverse 5'-AGA GAA AGA GCA GCT GGC GAA TGT AT-3' primers, and HphI restriction enzyme digested products were separated on EtBr stained 4% agarose gels for genotyping. We included homozygous and heterozygous p.Met694Val positive controls in each genotyping analysis.

Statistical analysis

We had enough power for detecting >20% increase of heterozygous individuals. We compared the p.Met694Val allele frequencies of SAT patients and healthy controls by chi-square test, and P values less than 0.05 were considered significant.

The local ethics committee approved the study protocol, and all patients gave written informed consent before blood collection for genotyping.

Results

We identified 91 patients from the medical records, and we could reach 59 of SAT patients with confirmed diagnosis by phone for inviting to the study. One of the patients was already diagnosed with FMF, and she was excluded from the genotyping. Demographic data and inflammatory parameters of the study group are given in Table 1.

Two of the patients were on anti-TNF agents for the treatment of rheumatoid arthritis and ankylosing spondylitis, and additional two patients, one with systemic lupus erythematosus and another with eosinophilic granulomatosis with polyangitis (Churg-Strauss syndrome) were on immunosuppressive drugs. Another patient with ankylosing spondylitis was receiving sulfasalazine.

We could not identify any individual with p.Met694Val variant among 58 patients with SAT. We found 7 heterozygous individuals among healthy controls, who were matched to
the study group according to their birth places. We could not identify any significant increase of the MEFV p.Met694Val variant in the SAT patients compared to matched Turkish healthy controls.

**Discussion**

SAT is a rare form of inflammatory thyroid disease manifesting with local tenderness as well as systemic findings such as fever and elevated acute phase response. Self limited granulomatous inflammatory response has usually been considered to be associated with viral infections, and increased frequency of HLA-B35 or HLA-B67 is suggested to be related to a susceptibility for certain infections.

Among the non-HLA genes, interleukin 1 receptor antagonist gene (IL1RN) polymorphisms were found to be associated with SAT and transient expression of anti-thyroid peroxidase antibodies (anti-TPO) [12]. Deregulated excessive IL-1 beta production following pathogen-associated or danger-associated molecular pattern signaling is an expected finding in MEFV variant carriers, and penetrant MEFV mutations such as p.Met694Val are considered as modifier factors for other diseases by either augmenting the inflammatory response or affecting the disease phenotype [8-10]. No association of the most penetrant MEFV variant with SAT in Turkey may suggest that pyrin inflammasome is probably not an important pathogenic pathway in the pathogenesis of SAT. Development of strong inflammatory findings despite anti-tumor necrosis factor (TNF) treatment in two SAT patients with accompanying rheumatic diseases may also suggest that TNF pathway is also not so critical in the pathogenesis of SAT. TNF is known to be associated with the maintenance of granuloma but not with the initial granuloma formation in tuberculosis immunity [13]. Anti-TNF agents seem to be not preventing development of granulomatous inflammation in SAT. Anti-TNF and other biologic agents are increasing the risk of infections, and considerably higher prevalence of patients receiving immunosuppressive agents in our cohort may either be due to Berkson bias observed in a tertiary referral center [14] or more possibly due to increased risk of infections, which may trigger SAT. Vassilopoulos and Canas previously reported patients with rheumatoid arthritis who developed SAT on etanercept treatment [15, 16], and Andre and colleagues recently reported a psoriatic arthritis patient developing cytomegalovirus associated SAT following infliximab treatment [17]. Also, Kawashima and colleagues reported a patient with amyloid goiter and Crohn’s disease, who developed SAT-like manifestations following anti-TNF treatment, showing the variability of clinical findings associated with anti-TNF agents [18]. Additionally, similar increased risk for SAT was previously reported in patients receiving alemtuzumab (anti-CD52) treatment, which targets lymphocytes [19].

This study has several limitations. First it has a small sample size, which may allow to rule out p.Met694Val associated increased risk, but it may not be enough to show a negative association, which was actually contrary to the original hypothesis. Also, contribution of other MEFV variants to SAT pathogenesis could not be ruled out with our approach. However, our previous works indicated that p.Met694Val is the major risk factor for other non-FMF inflammatory disorders, possibly due to its relatively higher functional impact in heterozygous state compared to other variants [8-10]. Therefore, we do not expect to see a significant contribution of other MEFV variants to the pathogenesis when there is no p.Met694Val association.

In conclusion, we did not find an association between the MEFV gene p.Met694Val and subacute thyroiditis in Turkish patients. Increased proportion of patients on immunosuppressive agents supports further the triggering role of infections, and investigations of genetic polymorphisms associated with a susceptibility to infections are warranted.

**Acknowledgements**

This study was supported by Istanbul University Research Fund.
Disclosure of conflict of interest

None.

Address correspondence to: Dr. Nurdan Gül, Division of Endocrinology and Metabolism, Department of Internal Medicine, Istanbul Faculty of Medicine, 34093, Fatih, Istanbul, Turkey. Tel: +90-212-414 2000/32735; Fax: +90-212-523 2891; E-mail: nurdangul26@gmail.com

References


