Case Report

M680I(Arm2)/M694V(Med) mutations in a patient with familial Mediterranean fever and polyarteritis nodosa

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Introduction

Familial Mediterranean fever (FMF) is an autosomal recessive disease, characterized by recurrent and self-limited attacks of fever usually accompanied by polyserositis [1]. The gene responsible for FMF, localized on chromosome 16, has recently been identified [2,3]. Vasculitic features have been reported in FMF and there are rare case reports associated with polyarteritis nodosa (PAN) [4–8]. In this report, we present genetic mutation analysis of a patient with FMF and PAN.

Case

A 20 year-old man was hospitalized because of fever, testicular pain, hypertension, epistaxis, weight loss, myalgia and arthralgia of the lower extremities 2 weeks prior to admission in February 1998. His one paternal aunt and grandfather were known to suffer from FMF. His recurrent abdominal pain accompanied by fever had started at the age of 13 years. The attacks subsided within 2–3 days. He was first seen in our hospital in 1993. At that time, samples obtained during an attack revealed an erythrocyte sedimentation rate of 60 mm/h, blood leukocytes of 11 800/m³, serum fibrinogen of 624 mg/dl and a positive C-reactive protein. With this medical history and laboratory findings he was diagnosed as FMF and colchicine treatment was initiated.

On his admission in 1998, his blood pressure was 190/110 mmHg, heart rate 88 bpm, ventilation rate 20/min and temperature 38.5°C. The pathological physical examination findings were pale conjunctivae, hepatomegaly, left costovertebral tenderness and muscle atrophy. Laboratory studies disclosed the following results: hemoglobin 5.5 mmol/l, leukocytes 12 400/m³, hematocrit 32.8%, thrombocytes 463 000/m³, erythrocyte sedimentation rate 76 mm/h, blood urea nitrogen 4.9 mmol/l, serum creatinine 70 μmol/l, total protein 76 g/l, albumin 29 g/l, calcium 2.0 mmol/l, phosphorus 1.6 mmol/l, alanine aminotransferase 1.2 μkat/l, asparate aminotransferase 1.5 μkat/l, alkaline phosphatase 6.1 nkat/l, iron 2 μmol/l, transferrin 49 μmol/l, IgG 17 g/l, IgA 2.75 g/l, IgM 1.62 g/l, lactate dehydrogenase 5.7 μkat/l, creatine kinase 23.7 μkat/l, creatine kinase-MB 0.25 μkat/l, fibrinogen 1304 mg/dl, C₃ 1.0 g/l, C₄ 0.3 g/l, Antistreptolysin O 1600 U, C-reactive protein 0.11 mg/l, rheumatoid factor negative, ECG normal, EMG compatible with axonal neuropathy, hepatitis B surface antigen negative, antibody to hepatitis C virus negative, brucella agglutination test negative, blood and urine cultures negative and chest X-ray normal. Echocardiography revealed mild tricuspid regurgitation. Urinalysis showed pH 6.0, specific density 1020, protein 100 mg/dl, 4–6 leukocytes and abundant erythrocytes. Antineutrophilic cytoplasmic antibody could not be studied. Abdominal ultrasonography demonstrated hepatomegaly and slightly enlarged kidneys with increased echogenity. Renal biopsy showed proliferative glomerulonephritis. Congo Red staining was negative.

Selective right and left arteriograms showed multiple saccular aneurysms originating from interlobar, lobular and arcuate arteries (Figure 1).

Polyarteritis nodosa was diagnosed and prednisolone (250 mg/day intravenous for 3 days followed by 1 mg/kg/day p.o.) and cyclophosphamide (2 mg/kg/day p.o.) was started in addition to colchicine. Other medications were nifedipine GITS, prazosin, lisinopril, hydrochlorothiazide, pindolol and famotidine. At the end of first month, the patient was afebrile and had improved markedly, with resolution of fever, myalgia and weight loss. Blood pressure and serum liver enzymes were normal.

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The genetic saccular aneurysms of both kidneys by arteriography.

**Genetic analysis**

DNA was extracted from peripheral blood using a standard method. Polymerase chain reaction (PCR) was carried out in 25 µl reaction volumes containing 100 ng genomic DNA, 25 pmol primers, 0.2 mM dNTP, 2.5 ml reaction buffer (100 mM Tris pH 8.3, 500 mM KCl, 15 mM MgCl₂ 0.01% gelatin) and 1 U Taq DNA polymerase (Biotools). Cycling parameters were 94°C 2 min., followed by 35 cycles of 61°C 30s, 72°C 30s, 94°C 30s and a final step of 72°C 5 min. PCR products which amplified with primer p12.2 (5’ TAT CAT TGT TCT GGG CTC 3’) and p10.1 (5’ CTC CGT ACT TCC TCT TCT 3’) were digested as suggested by the manufacturer. *Hinf I* restriction endonuclease (Promega) was used to the screen M680I mutation. Digestion products were analysed on 3% agarose gel. The M694V mutation analysis was performed on genomic DNAs with the aid of the amplification refractory mutation system (ARMS). The MED (M694V) mutation was distinguished with med I (5’ TGG TAC TCA TTT TCC TTC AT 3’) and p12.2 and with val I (5’ TGG TAC TCA TTT TTC AC 3’) and p12.2 primers. PCR products were analyzed on 2% agarose gel. The mutation analyses of the patient and his family are shown in Table 1.

**Table 1. Results of mutation analyses for FMF in the family**

<table>
<thead>
<tr>
<th>Subject (age)</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient (20)</td>
<td>M680I(Arm2)/M694V(Med) mutations</td>
</tr>
<tr>
<td>Father (50)</td>
<td>Carrier for M680I mutation</td>
</tr>
<tr>
<td>Mother (40)</td>
<td>Carrier for M694V mutation</td>
</tr>
<tr>
<td>Brother (18)</td>
<td>Carrier for M694V mutation</td>
</tr>
<tr>
<td>Sister 1 (15)</td>
<td>Carrier for M680I mutation</td>
</tr>
<tr>
<td>Sister 2 (23)</td>
<td>Normal</td>
</tr>
</tbody>
</table>

**Discussion**

The patient had six (weight loss, testicular pain, myalgia, polyneuropathy, hypertension and renal aneurysms) of the 1990 American College of Rheumatology criteria for diagnosis of PAN [9]. Recently, a set of diagnostic criteria was derived by Livneh et al. [10] for FMF and the patient fulfilled these criteria as well. Proliferative glomerulonephritis in this patient is an interesting finding as previously reported by Tinaztepe et al. [4].

There have been many reports of vasculitic diseases such as PAN and Henoch–Schönlein syndrome associated with FMF [4–8]. Familial Mediterranean fever is a self-limited form of inflammatory attack, whereas PAN presents a persistent and severe form of inflammation. The coexistence of these vasculitic diseases and FMF seem to be too frequent to be explained by chance. Streptococcal infections have also been implicated as triggering agents. The pathogenetic relationship of PAN and FMF is not clear.

Genetic analysis of patients with PAN and FMF may contribute to the understanding of the relationship between these two disorders. The FMF gene has recently been cloned and has been identified as coding a protein called pyrin [2]. The exact function of pyrin is currently unknown, however, it is expected to have some role in the inflammatory pathway of leukocytes.
The most common mutation is the substitution of valine for methionine at codon 694 (M694V) of the gene [2], which was also present in one allele of our patient. The other allele in our patient carried a substitution of isoleucine for methionine at codon 680, the M680I mutation, which has been associated with the Armenian 2 haplotype. In turn, these mutations are expected to cause a defective pyrin. Thus the patient had inherited two different mutations in the FMF gene from his parents. To our knowledge, genetic analysis of patients with PAN and FMF has not been reported previously. More studies are needed to clarify the relationship of these two diseases.

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References

2. The International FMF Consortium. Ancient missense mutations in a new member of the RoRet gene family are likely to cause familial Mediterranean fever. Cell 1997; 90: 797–807

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