The polymorphism C5507G of complement receptor 1 does not explain idiopathic pulmonary fibrosis among the Finns

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Summary
Idiopathic pulmonary fibrosis is the most common of the idiopathic interstitial lung diseases referring to the histo-pathological entity of usual interstitial pneumonia. It has been hypothesized that inflammation may trigger the multiformic fibrotic lesions found in the affected lung, and defects in the innate immune defense, including the complement, can predispose to pulmonary fibrosis. The polymorphism C5507G in the Complement Receptor 1 gene has been recently associated with idiopathic pulmonary fibrosis. C5507G causes an amino acid change from proline to arginine, and opens a potential cleavage site for trypsin-like enzymes and, therefore, a potential mechanism for increased shedding of the molecule from the cell surface. We studied the polymorphism in 96 Finnish patients with idiopathic pulmonary fibrosis and 164 population based controls. All the patients and controls were C5507 homozygous suggesting that either the Finns do not carry the G5507 polymorphism or it is extremely rare. We conclude that G5507 is not a susceptibility allele for idiopathic pulmonary fibrosis among Finnish patients.

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Introduction
Complement Receptor 1 (CR1) is located on the chromosome 1q32. The gene encodes a single-chain cell surface membrane glycoprotein, named CD35 (complement component 3b/4b receptor, C3-binding protein). CD35 is mainly expressed on the surface of peripheral blood cells, especially on erythrocytes (http://www.ncbi.nlm.nih.gov/prow). CD35 binds to circulating antigen–antibody complexes in the presence of the complement components C3b and C4b. By the action of erythrocytes, immune complexes are then transferred...
to the reticuloendothelial system and destroyed.\textsuperscript{1,2} Immune complexes that escape the reticuloendothelial system can in turn increase the kind of tissue damage associated with many immune diseases.

A total of 11 amino acid changing polymorphisms of the coding sequence of CR1 have been reported (http://www.ncbi.nlm.nih.gov/SNP). The substitution of C to G in exon 33 (rs3811381) causes an amino acid change from proline to arginine, named Pro1827Arg. The polymorphism opens a potential cleavage site for trypsin-like proteases that can increase shedding of the receptor expressed on the cell surface and, in return, decrease the activity of the complement.\textsuperscript{3} C5507G has been associated previously with idiopathic pulmonary fibrosis (IPF) among Italian patients.\textsuperscript{4} G5507 homozygosity was significantly more common in patients with IPF than in control subjects or COPD patients. In this study we report the results among the Finnish IPF patients and population-based controls.

Methods

Patients

The study population consists of 96 Finnish IPF patients (42 males and 54 females). Thirty-six of the patients were recruited at the Pulmonary Clinic of the Helsinki University Hospital, 30 at the Southern and Eastern Savo Central Hospitals, and 30 at the other Pulmonary Clinics in Finland. The diagnosis was made according to the ATS/ERS diagnostic criteria.\textsuperscript{5,6} At the time of diagnosis, the age of the patients was on average 62 yrs (ranging from 26 to 83 yr), patients’ lung vital capacity was 74% (ranging from 35% to 102%), and diffusing capacity for carbon monoxide 58% (ranging from 28% to 91%) of predicted.

The control population consisted of 96 voluntary blood donors across Finland and 68 regional health controls from the Savo region.\textsuperscript{7}

Genotyping and sequencing

DNA was extracted from peripheral blood leucocytes. We amplified the regions of genomic DNA comprising the C5507G polymorphism using two primer pairs: (A) 5’CTTTTGTCACACACACACACAG and 3’AAAGTTAAGCTCACAACACAAATACCA; and (B) 5’TTCACACCTATTGGGGAGAG and 3’GGCAGGGCTGCTCCAA. The polymorphism was studied using two restriction enzymes HpyCH4III (amplicon A) and MnlI (amplicon B) (New England Bio Labs, MA). The length of the PCR product A (HpyCH4III specific cleavage site) for the major allele (C5507) was 328 bp, and in the presence of the minor allele (G5507) 164 bp + 164 bp. The lengths of the PCR product B (MnlI specific cleavage site) for the major allele (C5507) were 37 + 29 + 9 bp, and in the presence of the minor allele (G5507) 66 + 9 bp.

Sequencing of the genomic DNA was done on the 96-capillary automated sequencer (ABI3730 Automatic DNA sequencer, Applied Biosystems).

Results

We studied C5507G among a total of 96 IPF patients and 164 controls. One third of the patients originated from a regional enrichment of familial IPF in South eastern Finland.\textsuperscript{6} The rest of the patients represented the sporadic IPF cases across Finland. The diagnostic criteria and clinical outcome of the disease were verified from the patients’ medical records. For 17 patients expressing atypical clinical features of IPF, such as early onset of the disease, the surgical biopsy with the UIP pattern further confirmed the diagnosis.

For genotyping we used two restriction enzymes showing altered restriction sites for the studied polymorphism. First, when HpyCH4III was used, none of the PCR fragments were digested, suggesting that all the study subjects were C5507 homozygous. In the absence of positive controls, we chose to confirm the genotyping results with another restriction enzyme, MnlI. Consistent with the previous results, again, only the major allele (C5507) was recognized. Since the results were somewhat unexpected, we then verified by sequencing that all the patient were C5507 homozygous.

Discussion

Results based on 520 studied chromosomes strongly suggest that the Finns do not carry the G5507 allele at all or it is extremely rare. Not finding the G5507 polymorphism was to some extent surprising, since previous studies among other Caucasian populations have estimated the frequency of the carrier-ship of G5507 at around 20%.\textsuperscript{4,8} Single allele frequencies, however, are known to vary in several standard deviations among European populations.\textsuperscript{9} The Finns have lived in isolation and reminded a small population for centuries, which also increases the possibility of losing some alleles simply because of random drift.\textsuperscript{10} Random drift has the strongest effect on rare alleles (frequency < 5%), as it has
been reported for some blood group antigens and the ΔF508 mutation in cystic fibrosis, but the loss of a common allele seldom occurs. Our study group included a genetically isolated subgroup of IPF patients with strong familial background and likely related to each other within a couple of generations. This, however, does not explain our findings, since polymorphism was found neither among sporadic IPF patients nor controls representing the Finns across the country.

Recent epidemiological studies in Finland show that IPF is not less common in Finland than in other European populations. Based on international diagnostic criteria, prevalence of IPF in Finland, 16–18 per 100,000, is concordant with reports from other populations. According to present knowledge, the clinical entities of IPF do not differ among populations. The absence of G5507 polymorphism allows us to assume that molecular genetic mechanisms other than C5507G explain the etiology to IPF among the Finnish patients.

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