LETTER TO THE EDITOR

CR1 gene polymorphism in Finland

We read with interest the recently published article by Hodgson et al. reporting a lack of association between idiopathic pulmonary fibrosis and the complement receptor 1 (CR1) gene C5507G polymorphism among a series of Finnish patients. This evidence contrasts with our research group’s previous report, showing an association between polymorphisms and this disease in an Italian series of patients. Moreover, based on their results, Dr. Hodgson and coworkers stated that the CR1 G5507 is not a polymorphic site in the Finnish, since the C allele was never found, either among patients or among controls. Since we noticed that Dr. Hodgson and coworkers used a markedly different methodology than that described by Zorzetto et al. we decided to conduct a comparative experiment.

To this aim, we investigated six individuals already assessed for the CR1 C5507G by sequencing. We used three pairs of primers: Zorzetto F and R, Hodgson 1 F and R and Hodgson 2 F and R: (Table 1), and two restriction enzymes: MnlI and HpyCH4III, showing altered restriction sites for the C5507G polymorphism. PCR fragments amplified with Zorzetto and Hodgson 1 primers, were digested with the MnlI and HpyCH4III enzymes, while PCR fragments amplified with Hodgson 2 primers was digested only with MnlI. Figs. 1A and B illustrate the digestion of the amplimers obtained with Zorzetto primers, with MnlI and HpyCH4III, respectively; Figs. 1C and D show the digestion of amplimers obtained with Hodgson primers 1 and digested with MnlI and HpyCH4III, respectively. Fig. 1E shows the digestion of amplimers obtained with Hodgson primers 2 and digested with MnlI. The figure clearly shows that amplification of the six samples with Zorzetto primers, followed by digestion with either MnlI (Fig 1A) or HpyCH4III (Fig 1B), differentiates the three genotypes determined by the C5507G polymorphism. In contrast, amplification of the same samples with Hodgson 1 and 2 primers, followed by the digestion with MnlI (Fig 1C and E) or HpyCH4III (Fig 1D) was not able to discriminate the three genotypes determined by the polymorphism.

Further to this, we aligned the sequences of the three pairs of primers with a sequence (AL137789.11) on chromosome 1 containing the CR1 gene. The blast result of our primers with sequence AL137789.11 shows a unique possible amplimer in our PCR conditions (F: 44674 > 44694 and R: 44978 > 44957). We well know that the R primer also recognizes the AL137789.11 sequence from 13052 to 13031, but the putative PCR product is too long to be amplified by Taq Polymerase in these conditions.

The first pair of primers described in Hodgson’s paper (Hodgson1) aligned with a AL137789.11 sequence (F: 44761 > 4470 or 12835 > 12854 and R: 13162 > 13137) does not amplify the same sequence of our primers, therefore with this pair of primers it is not possible to amplify the region containing the C5507G polymorphism.

When we aligned the second pair of Hodgson primers with sequence AL137789.11, we found at least two different possible PCR fragments of the CR1 gene (F: 44882 > 44901 or 12956 > 12975 and R: 44956 > 44940 or 13030 > 13014). Only one of these contains the C5507G polymorphic site, thus, using this couple, amplification of only the region of interest is not guaranteed.

The human CR1 molecule is a large transmembrane glycoprotein that is encoded in a cluster on chromosome 1q32 together with genes for five other closely related C3b/C4b-binding proteins, known together as the regulators of complement activation (RCA). The RCA proteins are structurally similar in that they are predominantly composed of repeated motifs of approximately 60–70 amino acids, termed short consensus repeats (SCRs), or complement control protein repeats (CCPs). Each SCR has a number of highly conserved residues in common, including four invariant cysteines that form two disulfide bonds. These characteristics of the CR1 gene cause problems for anyone trying to design primers for this region. In fact a pair of primers could recognize various regions inside the CR1 that could be amplified along with the region of interest.
Given the unique genetic make-up of the Finnish population, due to its genetic isolation, we cannot exclude that the CR1 G5507 might indeed be a monomorphic site in the Finnish, despite being polymorphic in the other populations investigated, but we would like to emphasize that the conclusions drawn by Dr. Hodgson are dependent on the investigative methodology used, which could be flawed.

References


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