Author's response to reviews

Title: Sequence diversity within the HA-1 gene as detected by melting temperature assay without oligonucleotide probes

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Author's response to reviews:

Goulmy

major point) The original contribution of Arostegui et al. (ref. 8) has been acknowledged on pag 4.

minor point) The suggested comment on the nature of the novel polymorphisms has been added in the conclusion section on pag 6.

Hess

Major points:

1.1 We apologize for having misled this reviewer; he correctly pointed out that the text was in contradiction with the title: we have changed the latter accordingly.

1.2 We typed 131 normal subjects from the Italian population using SSP-PCR according to Wilke et al. (ref. 6); allele frequencies based on this sample size are associated with a standard error of 0.03 and this information is now provided in the first paragraph of pag 4; sequencing the entire (486bp) genomic region defined by the external primers A(1) and B(4) according to Wilke et al. (ref. 6) on 72 random chromosomes was demanding but enough to estimate the frequencies of the common haplotypes A and B (0.54±/0.06 and 0.38+/0.06, respectively).

2 We agree with this reviewer that other, more powerful tools are available; however, one of the aims of this work was to challenge the Tm assay under different experimental conditions. More information concerning the way with which they were carried out and validated is now given in the last paragraph of pag 5.

Minor points:

1 Possible clinical correlates were beyond the scope of the present work, which rests on descriptive methodological issues.

2 We hope that the last paragraph of pag 5 now reads better than in the previous version: here we state that replicate analyses gave consistent results.

3 We conformed to the nomenclature rules for intronic nucleotide numbering by replacing the term IVS with c.509+ throughout pag 4.

4 See both major and minor point 2.

Discretionary points:

1 We tried to improve readability of table 1 by shadowing the two positions defining H/R alleles.

2 The results and discussion section has been re-edited to include suggestions and to address the
criticisms raised by both reviewers.

3 The second paragraph of the background section has been modified according to the suggestion of this reviewer.