Author's response to reviews

Title: Detection of large deletions in the LDL receptor gene with quantitative PCR methods

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Author's response to reviews: see over
Firstly, we want to thank the three reviewers for reviewing our manuscript.

Point-by-point response to comments from reviewers:

Referee 1:

1) It is correct that FH has been synonymous with mutations in the LDL receptor gene, but now it turns out that mutations in at least two genes (apoB and PCSK9) can cause a phenotype indistinguishable from the phenotype caused by mutations in the LDL receptor gene. Therefore we have chosen to define FH clinically.

2) We agree that the evaluation of the Dutch Lipid Clinic Network Group diagnosis could be deleted from this manuscript. P 4, § 1, line 8: The following sentence has been deleted: Finally we wanted to test whether the Dutch Lipid Clinic Network Group criteria could be used for selection of patients for screening of the LDL receptor gene for major structural rearrangements. P 4, § 3 and P 5, § 1 have been changed to: To expand our assessment of the contribution of major structural rearrangements in the LDL receptor gene to the spectrum of mutations causing FH [8], we further studied, by MLPA analysis, 318 patients with an FH phenotype referred for molecular genetic analysis to Aarhus Sygehus, Aarhus University Hospital in the period January 1995 to June 2004, in whom no mutations in the LDL receptor gene had been detected by SSCP analysis and in whom the apoB R3500Q mutation also had been excluded. Two-hundred and seventy-six of these 318 patients have been described previously [11]. P 8, § 4, line 3: The following sentence has been deleted: Four of five deletions were found in the group of 37 patients fulfilling the criteria of clinically “definite FH”, and one was found in a patient, referred from another lipid clinic, who could not be categorised according to the Dutch Lipid Network Group criteria due to lack of data. P 10, § 4 has been deleted: Four of five large deletions of the LDL receptor gene studied here were found in patients fulfilling the clinical criterion of “definite FH”, not a surprising finding, since deletion of one or more exons almost certainly abolishes LDL receptor function. Accordingly, a screening strategy could involve testing for major structural rearrangements only in patients fulfilling the criterion of “definite FH”. The results of a previous study showed that 26.3% and 31.6% of single base mutations were detected in patients fulfilling the criteria of “probable FH” and “possible FH”, respectively [11]. We therefore suggest that a strategy for molecular genetic analysis of FH patients should include testing for single base mutations in patients both with strong and mild phenotypes, but that screening for large deletions could be reserved for patients with strong phenotypes, i.e. clinically “definite FH”.

3) It is correct that a deletion was found in 5 of 318 individuals tested. The 318 patients were recruited from a group of 318 + 157 patients with a clinical diagnosis of FH that were referred to molecular genetic analysis. In those an LDL receptor gene mutation was found in 162 patients (157 patients had either a single base mutation or small deletions/insertions and 5 patients were found to have a large deletion in this present study), meaning that the 5 deletions accounted for 3.1% (5 of 162) of all identified mutations in the LDL receptor gene in the period January 1995 to June 2004. The following changes have been made: P 10, § 3, line 5: In the period January 1995 to June 2004, a mutation in the LDL receptor gene was identified in 162 patients (data not shown) and major deletions accounted for 3.1% (5 of 162
patients) of these LDL receptor gene mutation carriers, a finding similar to that obtained in other genetically heterogeneous populations [1,4,5].

4) P. 8, § 2 line 2: The following sentence has been inserted: With both Real-Time PCR and MLPA analysis, it was possible to distinguish between one and two copies of the LDL receptor gene exon 5, but the coefficients of variation were larger for the Real-Time PCR method than for the MLPA analysis.

5) We agree that the results of the cost analysis could be clearer. Therefore we have calculated the price of reagents and laboratory technician time for a batch of 10 samples. Table 2 has been changed.

6) This present study and the study described in reference 11 were run in parallel. It is correct that three of the five deletions described in this manuscript have now been published (reference 11). Therefore the following sentence (P. 10, § 3, line 7) has been deleted: Four of five deletions identified in this study are novel in Denmark.

7) Table 3 has been omitted (see answers to referee 2 point 2). Therefore we chose to keep Figure 3.

8) The paragraph containing the term strong phenotype has been deleted. See point 2.

Referee 2

1) In figure 2 the mean relative copy numbers and standard deviations for 12 subjects without a LDL receptor gene deletions have been included as well as for 5’ and 3’ flanking control fragments.

2) The results presented in Table 3 have been put into the results section: P. 8, § 4, line 2: The five deletions were a 9.3 kb deletion of the promoter and exon 1 [11], a 1 kb deletion of exon 5 [6], a 3 kb deletion of exons 7-8 [11,18], a 9.5 kb deletion of exons 9-14 [11] and 5 kb deletion of exons 13-15 [18]. The mean and variation of relative copy numbers of normal individuals for all exons and flanking control fragments have been included in figure 2. Therefore we chose not to include a table presenting in principle the same data, but we are certainly willing to do so or alternatively provide such a table as additional information if editor and/or reviewers request that.

3) P 10, § 3, line 7: the following sentence has been inserted: In a study of Wang et al [22] eight different abnormal patterns of MLPA analysis were found in 12 of 21 patients with a clinical diagnosis of FH in whom sequencing of the LDL receptor gene had not revealed any mutations. In five patients with the same abnormal pattern, this was confirmed as a deletion with another method.

Referee 3

1) We agree that the use of different numbers of subjects in the manuscript is not clear. Please refer to the answers to point 2 and 3 raised by reviewer 1.
2) P. 7, § 2 line 3: the following sentence has been inserted: \textit{The sizes of deletions were estimated from the difference in size of the amplified allele with the deletion and the normal allele with long range PCR.}