Reviewer's report

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

Version: 1 Date: 9 February 2005

Reviewer: Trond Leren

Reviewer's report:

General
The authors have compared MLPA and real-time PCR to detect deletion of exon 5 in the LDL receptor gene. They found that both methods discriminate between carriers and non-carriers of this deletion, although CV of real-time PCR was higher than that of MLPA. However, the CV of real-time PCR could possibly be lower by optimization of the method. Generally, the results and the discussion could be more stringent and more clearly written.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1) Familial hypercholesterolemia is caused by mutations in the LDL receptor gene only. Mutations in other genes (apoB, PCSK) underlie other forms of autosomal dominant hypercholesterolemia (p.3)

2) That one of the aims of the study is to evaluate the Dutch criteria for a clinical diagnosis of FH for selecting patients to be screened for structural rearrangements, should be deleted (p 4). It goes without saying that the more stringent criteria used for selecting patients for mutation screening, the higher proportion of the patients tested will possess a mutation. Four of the five patients possessing a deletion, had a clinical diagnosis of Definite FH. If one assumes that the fifth patient had a diagnosis of Possible or Probable FH, then 20% of patients possessing a deletion, had a diagnosis of Possible or Probable FH. This figure is comparable to that of the percentage of single base mutations of 26.3% and 31.6%, found in the same group of patients (p 11).

3) On page 5, 318 patients were included in the screening for deletions/duplications by MLPA. Based upon p 5 of results, five patients possessed a deletion. It is assumed that these were five of the 318. However, on page 10 it is concluded that 3.1% (5/162) patients possessed a deletion. This should probably be 1.6% (5/318)?

4) A sentence or two interpreting the main findings of the comparison of MLPA and real-time PCR (Table1/Figure1) should be included in the third paragraph on page 8. Moreover, either Table 1 or Figure 1 may be deleted. They give essentially the same information.

5) It is a little hard to interpret and understand the comparison of costs in Table 2. For real-time PCR the cost of testing one sample for 16 exons (cost of testing for exon 5 multiplied by 16?) as a part of testing 10 samples together, has been compared to the cost of testing 30 samples by MLPA. Rather, the cost of testing the same number of patients for 16 exons by the two methods should be compared. Only half an hour of work to analyze 30 samples by MLPA, possibly is an underestimate (aneal, ligate, PCR, pouring and loading an ABI 370 gel)
6) From the last sentence of the third paragraph on page 10, one gets the impression that four of the five deletions are first detected in Denmark in this study. However, in Table 3 it is evident that three of these deletions previously have been detected and will be appear in a paper which is in press and which will have been published before this paper is published. Thus, at the time this paper is published the three deletions have already been published and can therefore not be considered novel.

7) Figure 3 may be omitted.

8) Substitute the term strong phenotype with severe phenotype (p 11)

Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:
I declare that I have no competing interests