Reviewer's report:

**Title:** Two novel missense mutations in the myostatin gene identified in Japanese patients with Duchenne muscular dystrophy

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**Reviewer:** Jean-Claude Kaplan

**Reviewer's report:**

General

The authors address the important question of the absence of direct correlation between a given mutation and the ensuing clinical phenotype, a situation which is the rule more than the exception in monogenic diseases. So-called « modifier » genes might be involved in the observed discrepancies. In this paper the authors make the sensible hypothesis that loss-of-function mutations in the myostatin (GDF8) gene may mitigate the severity of Duchenne muscular dystrophy patients due to nul mutations in the DMD gene. This assumption is based on the well documented inhibitory effect of myostatin on postnatal muscle growth, and on the fact that blocking the endogenous production of myostatin in mdx mice improves the dystrophic phenotype.

In a collection of 102 Japanese DMD patients (age range : 1 to 31y) carrying various types of mutations in the DMD gene, all introducing directly or indirectly a stop codon in the coding sequence of dystrophin, the authors searched for mutations in the GDF8 gene by sequence analysis of the 3 exons and flanking regions. The following sequence variations were found, all in the coding sequence : 1) in exon 1 : one missense mutation, found once (Asp→His at position 95) 2) in exon 2 one missense mutation (Glu→Lys at position 164) found in 4 patients, one of which also carried in cis another missense mutation (Leu→Ile at position 156) ; in exon 3 : no mutation was found. The frequency of nucleotide variation in the GDF8 gene in the Japanese population was found to differ substantially from the incidence previously reported in other populations (USA, Belgium, Italy). Because all the missense mutations reported here involved conserved residues, the authors assume that they have a loss-of-function effect. Since the patients carrying these mutations did not exhibit any increase on muscle volume or strength , the authors conclude that the observed missense mutations in the Japanese population of DMD patients have no clinical impact, even the double-mutation on a same allele.

These results are interesting, but are very preliminary because

(i) the GFD8 gene was insufficiently covered by the analysis (about 1.6 kb) . The total sequence of this short gene (about 7.2 kb) should have been scanned, particularly now that we know the relevance of mutations in non coding regions (as illustrated by the Texel sheep in which muscle hypertrophy is due to a mutation in the 3'UTR, Clop et al, Nature Genet, July 2006)

(ii) the actual biological impact of the missense mutations cannot just be inferred from the conservation of the changed residue. Other parameters must be considered, investigated or at least discussed , mainly the change of polarity of the missense and the precise location of the mutation, that may either affect the amount or the splicing pattern of the primary transcript, or affect the proteolytic processing of the immature protein. Also the variation, even if it is a missense mutation, may remain neutral, which is difficult to test. In the discussion the authors, say that the unique case of nul mutation of the GFD8 gene reported to date in humans (Schuelke, 2004) is phenotypically silent in heterozygous carriers . In fact in this paper heterozygotes are reported to be overtly athletic. This is an important point , because it indicates that haplo-insufficiency is not clinically silent.

My conclusion is that the appealing hypothesis that was at the origin of this work did not receive the treatment it deserved. In fact this paper is modestly entitled « Two novel missense mutations in the myostatin gene identified in Japanese patients with Duchenne muscular dystrophy » . These results per se are of value and could be published, after considerable shortening, as a mutation report in the GDF8 gene in DMD patients, without obvious effect on the clinical status.

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**Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)**
Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

Discretionary Revisions (which the author can choose to ignore)

Declaration of competing interests:

'I declare that I have no competing interests'