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: Uta Francke

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General
This is a well-written, well-organized paper reporting a large study that tests a reasonable hypothesis that genetic variation in the myostatin gene may modify progression and phenotype of DMD. The strength of the paper is a clinically and genetically well-characterized study group of 102 DMD individuals. They all have premature stop codons in the dystrophin mRNA. The clinical parameters include standardized quantitative testing for muscle strength. The authors identified two novel missense mutations in addition to one that was already known in other populations. These mutations had no effect on the DMD phenotype, which is not surprising because they were only present in the heterozygous state.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)
1. The mouse work cited as rationale for this study used homozygous myostatin knockout animals for document improvement in the phenotype of the mdx mouse that has a mutation in the dystrophin gene. The relevant paragraph in the introduction should make this difference clear.
2. The authors' findings are also consistent with the observation that heterozygous myostatin mutations in humans do not cause muscle hypertrophy. Furthermore, reported literature studies showing that homozygous missense mutation in myostatin do not cause changes in muscle volume or strength in humans speak against the original hypothesis.
3. It is not clear what is meant with the sentence on page 9 that “it is supposed that these 5 cases, particularly the case with 2 different mutations, are phenotypically modified with proper muscle rehabilitation”? This sentence suggests a hypothesis that myostatin mutations would make DMD patients respond better to physical therapy. The rationale for such a hypothesis should be explained.
4. The sentence that occurs in the abstract and the conclusions “even in the patient carrying two missense mutations…” may mislead readers to think that this patient was a compound heterozygote for these mutations. It should be made clear in this sentence that the two missense mutations affect the same allele.
5. The authors are very consistent in calling these amino acid changes “mutations”. But some of them occur in the population in frequencies greater than 1%, and since those do not cause any abnormal phenotype, they would fall into the category of “polymorphisms”. In fact, other reports in the literature call them “myostatin polymorphisms”.

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. The title of reference 2 should not be in bold and should be in the same font as the rest of the paper.
2. In table 1A, the abbreviation “NA” (?not available?) should probably be changed to “ND” for “not detected”. NA would indicate that the mutation had not been looked for.
3. Table 1: “common mutations” should be changed to “polymorphisms”. Italia should be changed to Italy
4. Legend to Figure 2: wild sequence should be wild-type sequence.

Discretionary Revisions (which the author can choose to ignore)
1. The authors should state somewhere in the introduction or discussion that myostatin is also called GDF8 and is a member of the TGFbeta superfamily of growth and differentiation factors.
2. The authors could also address the possible functional consequences of the mutations they identified. Even though conserved amino acids are replaced, they are in the pre-protein part of the molecule, not in the biologically active carboxy terminal peptide. Therefore, if the mutations are not interfering with the proteolytic cleavage of the pre-peptide, one would not expect them have a major functional significance.
3. The authors only included 112 base pairs of the 3’ UTR. A recent report in Nature Genetics (Clop et al., July 2006) revealed single nucleotide changes further downstream in the 3’ UTR of the sheep myostatin gene that caused muscle hypertrophy. The nucleotide change created an illegitimate microRNA target site, suggesting a mutational mechanism that could involve microRNA binding and altered RNA
stability. Therefore, the authors may wish to extend their studies by sequencing more of the 3'UTR.

**What next?:** Accept after minor essential 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