Author's response to reviews

Title: Clinical and molecular characterization of a cohort of patients with novel nucleotide alterations of the Dystrophin gene detected by direct sequencing

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Author's response to reviews: see over
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Dr. Esther van de Vosse,
Editor, BMC Medical Genetics

Re: Manuscript ID: 941736970473694
Title: Clinical and molecular characterization of a cohort of patients with novel nucleotide alterations of the Dystrophin gene detected by direct sequencing

Dear Dr. van de Vosse:

Please find enclosed the revised version of the manuscript entitled “Clinical and molecular characterization of a cohort of patients with novel nucleotide alterations of the Dystrophin gene detected by direct sequencing”.

The manuscript has been extensively revised, taking into account the critiques and suggestions of reviewers. Major changes are highlighted in red in the revised manuscript. We have also specified the institutional connections and ties of the local ethics committee.

Figure 3 has been changed from the original manuscript version. As suggested by Reviewer 2, we have added a new figure (Fig. 1) that depicts the distribution of the mutations along the DMD gene, and the numbering of the subsequent figures has been modified accordingly. In addition, Supplementary Table 1 has been changed as suggested by Reviewer 2.

We have also included a point-by-point response to the reviewers’ comments.

I hope that you will find the revised version of the manuscript suitable for publication in BMC Medical Genetics.

Yours sincerely,

Giacomo P. Comi
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Response to Reviewers

We wish to thank the reviewers for their interest in our work. In accordance with the suggestions of both reviewers, we have revised the entire manuscript. The English has been improved. A point-by-point response to the reviewers’ comments is provided below.

Reviewer #1:

1. We used the revised DMD gene reference sequence, based on GenBank file NM_004006.1, as reported in the Leiden Muscular Dystrophy database. The ATG corresponds to nucleotide +1.

2. As suggested by the reviewer, we have changed the nomenclature of the mutation for patient I, substituting “duplication of C” with “insertion of C” throughout the manuscript.

3. We have added details to the figure legends, making them more explanatory of each item. In particular, we have modified Figure 3, adding numbers and rewriting the legend.

Reviewer #2:

1. We thank the reviewer for his perceptive comment regarding the novelty of the present work: indeed, a relatively small number of deep intronic mutations and other rare mutations affecting dystrophin pre-mRNA maturation have been reported in detail. The conclusion has been modified accordingly. We have also acknowledged in the Introduction the larger knowledge regarding single nucleotide substitutions and other small nucleotide changes within the DMD coding exons listed in the Leiden reference database, quoting several major contributions in this field.

2. In accordance with the reviewer’s suggestion, we have detailed in the Methods section the materials used for transcript analysis.

3. We have added a section to the Methods that specifically describes the bioinformatics prediction tools used to analyze splicing parameters. To estimate the effect of the T/G mutation in intron 65 of
the DMD gene, we used the splice site models introduced by Yeo and Burge [J Comput Biol 2004; 11: 377-94] and the software available at: http://genes.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq_acc.html. The sequences CTGGTAATT and CTGGTAAGT, which correspond respectively to the wild-type and mutated 5' splice site of the included exon were scored. We obtained maxENT scores, that represents the probability that a sequence is a functional splice site.

4. Transcript analysis was performed using primers previously published by Roberts et al. (Am.J.Hum.Genet. 49: 298-310, 1991). New primers were designed for some specific cases. All primers will be provided if required.

5. We have modified Table 1 as suggested: "kind of mutation" has been changed to "Mutation" and “Spl?” has been substituted with “cDNA analysis NOT done”.

6. We have added a new figure (Fig. 1) that depicts the distribution of the mutations identified in both BMD and DMD populations along the DMD gene, as suggested by the reviewer.

7. Following the reviewer’s suggestion, the English text has been revised and grammatical and spelling errors have b