Reviewer's report

Title: Disruption of exonic splicing motifs in NF1 exon 7 causes neurofibromatosis type 1

Version: 1 Date: 30 October 2006

Reviewer: Dieter Kaufmann

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General

The paper is related to the interesting topic of unexpected functional consequences of nucleotide exchanges in NF1 exons. The idea behind the paper is that each nucleotide substitution in an NF1 exon should be tested for its function as exonic splice enhancer or silencer. There are several nucleotide exchanges known in exon 7. The sample investigated is a little bit arbitrary and not complete.

It is irritating that the interesting investigation is mixed with a NF1 mutation report. The authors investigated NF1 exon 7 known to be skipped by mutations or by treating the tested cells under unphysiological conditions. The sample of the study is very small. It includes three previously characterized mutations and one novel silent change in NF1 exon 7. The first mutation was found by PTT by others and was suggested to result in a truncated neurofibromin. The second and third mutation together result in an exon 7 skip in the cells of the corresponding NF1 patients as published before by two of the authors. The fourth nucleotide exchange is new and suggested to be a silent change. Several other published mutations in exon 7 were not investigated by the group, also mutations known to affect exon 7 splicing.

It is often not clear in the text that two of the mutations act together to cause NF1. This should be clarified more.

The discussion needs revision as it appears not very well structured to the reader. A discussion of the splice isoform lacking part of exon 7 is completely missing. It is not very well pointed out that two of the mutations investigated act together to cause NF1. Also, it should be emphasized more that one mutation leads to a stop codon and the consequences this implies, i.e. possible degradation of exon 7 containing transcript by nonsense-mediated decay.

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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)

* Page 1: The title is not adequate as the general proposition only fits the two mutations previously described by the group (Colapietro et al., 2004). The only two additional mutations investigated are a stop mutation not disrupting an ESE/ESS and a silent change presumably not resulting in NF1.
* Page 1 (also page 10): “exon 7 was spliced in three alternative isoforms, including a transcript lacking NF1 exon 7”: how can an exon be spliced into a transcript lacking this exon? Better: The wild type minigene assay resulted in three alternative isoforms...
* Page 2: please use one spelling consistently throughout the paper: …wild type or wild-type....
* Page 2: ESE and ESS are not explained in the abstract
* Page 3: typing error: one of the most common autosomal dominant disorders.
* Page 4: please use one spelling consistently throughout the paper: NF1-gene or NF1 gene
* Page 4: “low expression of NF1 splice variants...”
* Page 4: “…the functional consequences of a number of nucleotide variants identified in exon 7 of the NF1 gene on the expression of this transcript, using...
* Page 4: typing error: “These include three previously reported...”
* Page 5: typing error: “…PCR settings, amplicon lengths, ...”
* Page 5: “Both sequence changes were found..” This section is confusing for the reader, because the “subject” is the father of a member of family NF-01 as shown later in figure 1.
* Page 5: The exchange N335N was found in the father of an NF1 patient. This father was affected by only three cutaneous neurofibromas but no other sings of NF1. In cells of his daughter an additional NF1 mutation was found in exon 4a. The findings of the paper will be ameliorated by investigating mRNA exon 7 skip in cells of the father and the daughter.
SR proteins. The abbreviation is not explained.

"...were amplified from DNA of a healthy subject using..."

"Plasmid with the minigene constructs were isolated using..."

"...cultured in T75 flasks at 37°C..."

"...performed as follows:..." the amount of the template is missing.

"...evaluated the effect of four nucleotide variants [...] on the NF1 pre-mRNA splicing process."

"...exonic sequences are summarized..."

"...does not appear to add...."

"To confirm this hypothesis..." : unclear which hypothesis exactly is meant. Better: “these predictions”

"The 435 bp PCR product contained exon 7..."

"in silico" and “in vitro” should be typed in italics (please correct throughout the paper)

"...under normal conditions..."

"induced by stressful factors as incubation of blood at room temperature" + citation [18]

"recognise": please use either –ise or –ize consistently throughout the paper

"...shifts the equilibrium between the three messengers [...] which is the only detectable one."  

Page 11: here the authors suggest destruction of an ESS around nucleotide 1007T while before they wrote of an creation of an ESE

"doesn’t", “didn’t” : should be “does not”, “did not"

"the authors speculate on the role of NF1DE7 but give no background for this (i.e. how could this transcript regulate neurofibromin levels)

"...undetectable mosaicism for the c.476delC mutation..."

"...proband’s daughter and nephew, both displaying the full-blown NF1 phenotype.”

unnecessary, because it was mentioned in the sentence before.

"...On the contrary, ..." should appear where both mutation were discussed before, and shortened. Also, the suggestion that each one of the mutations alone could cause NF1 seems to be a little far-fetched.

"...affect pre-mRNA splicing..."

"...role in NF1."

"Author’s contributions can be shortened where two individual’s contributions were identical."

"...by a blackened symbol, unaffected...

"...role in NF1."

"Quantification of the bands shown on the top by Quantity One software."  

Figure 3: the text in the boxes showing the exons is too small.

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: Needs some language corrections before being published

Statistical review: No

Declaration of competing interests:

I declare that I have no competing interest