Reviewer's report

Title: Novel SYBR-based duplex qPCR for the detection of gene dosage: Detection of an APC large deletion in a Familial adenomatous polyposis patient with unusual phenotype.

Version: 2 Date: 11 April 2012

Reviewer: ROSSELLA TRICARICO

Reviewer's report:

MAJOR ESSENTIAL REVISIONS:

Background

1. Second paragraph, first sentence: The authors state that the main aim of the study is to characterize the genomic alteration in a FAP patient. However, the title and discussion deal mostly with technical aspects, that are not fully novel, while the characterization of the genomic alteration is marginally discussed.

Methods

1. Somatic mosaicism has been described in some FAP patients (Aretz et al., 2007). It would be useful to provide information about the sensitivity (i.e. set up a serial dilution experiments) and the specificity of the method.

Discussion

1. Third paragraph, eighth sentence: As reported in the abstract “This paper presents the description and validation of this novel gene dosage method, as well as discusses the phenotype of patients presenting with large genomic deletions encompassing the APC gene”. The discussion of the phenotype should be expanded by adding information on developmental milestones and current performance. Has a dysmorphologic evaluation been performed?

MINOR ESSENTIAL REVISIONS:

Abstract

1. Methods, last sentence: “The reliability of the herein described qPCR method was validated for additional genes (HPRT1, ATM, PTEN and BRCA1)”: is not clear why (HPRT1, ATM, PTEN and BRCA1) are considered additional genes, since the test has been set up for all genes listed within brackets.

Background

1. First paragraph, sixth sentence: a link to the LOVD website should be included.
2. First paragraph, third sentence: references 1 and 2 should be replaced with more recent ones.

Methods

1. Samples, first paragraph, fourth sentence: Since the title refers to a case with unusual FAP phenotype, a more detailed description of the patient should be given. Please move here from results (fourth paragraph), discussion (second paragraph) and supplementary figure 1 legend, information about the clinical and family history of the patient. If available, provide more detailed data including the age at diagnosis of the polyps (are polyps and rectal cancer synchronous or metacronous?), the tumour histology, the description of any treatment or intervention, the clinical history of all affected proband's relatives.

2. Samples, first paragraph, fourth sentence: Please move here information about healthy and mutated tested controls from “SYBR-based duplex PCR” section, fourth paragraph. In addition, describe also here the deletions and duplications of mutated controls used.

3. Samples, first paragraph, last sentence: Provide information about the initial concentration of samples used to set up and perform the experiments. The novel gene dosage method developed is a quantitative test, thus it should be specified that all samples (controls and patients) had the same initial concentration.

4. SYBR-based duplex PCR. It would be better to describe the set up experiments for HPRT1 and validation experiments for ATM, PTEN and BRCA1 before performing experiments for APC.

5. SYBR-based duplex PCR, first paragraph, last sentence: Are also healthy controls tested in duplicate?

6. SYBR-based duplex PCR, last paragraph: Please specify that male and female controls are used to validate the novel test and to identify the value ranges corresponding to wild-type, hemideleted and hemiduplicated samples.

7. aCGH experiments and data analysis, eight sentence: Please, include information about ADM-2 software (i.e. manufacturer etc)

Results

1. Results of set up and validation experiments should be described before APC experiments.

2. First paragraph, first sentence: Healthy controls should be tested to verify the frequency of the novel missense variant.

3. First paragraph, third sentence: Please include link to of LOVD, dbSNP and 1000Genomes websites.

4. Second paragraph, third sentence: “The duplex qPCR showed PMc ratios of 0.67 and 0.63 for APC exons 2 and 15 respectively (figure 1B), revealing a haploidy for these APC exons in the FAP patient’s sample”. It is necessary to provide here information on the range of normal, deleted and duplicated PMc ratios (with the percentage of error) and validation of the test before asserting the
presence of APC haploidy.

5. Please report the APC sequence deletion at the genomic level as recommended by HGVS (http://www.hgvs.org/rec.html).

Discussion

1. Second paragraph, last sentence: See comment above on healthy controls.
2. Third paragraph, fourth sentence: Please, include link to Decipher Database website.
3. Third paragraph, last sentence: The statement “In our study, the identified gross deletion encompasses the entire APC gene and 19 additional genes and is likely to have been present in this family for at least three generations, with a quite unusual phenotype of absence of mental impairment and dysmorphic features” should be followed by discussion (i.e. influences of modifier genes or endogenous or exogeneous factors etc). Further reports of 5q deletion encompassing APC should be discussed (Lindgren et al., 1992, Raedle et al. 2001). Please, refer to this data.

Legends

1. Supplementary Figure 1, It is not clear if subject II:1 has been tested and his genotype. Please, clarify. In addition, indicate what the symbol “?” means.
2. Supplementary Figure 2: “Supplementary figure 2A” should be “A”. The same correction should be done for “Supplementary figure 2B, 2C, 2E and 2F.

Figures

1. Figure 1B: The peak heights of the melting curves of all genes tested (figure 2B and supplementary figure 2) show a reduction or increasing of target gene’s peak height for exon deletion or duplication, respectively. Please detail the melting curve peak heights observed in Figure 1B: why is reduction of APC peak heights not observed in patient FAP02?
2. Supplementary Figure 1, section D: Please add the minus symbol near subject III:4, and replace the plus symbol with minus near subject IV:1.

DISCRETIONARY REVISIONS

1. The paper would benefit from the following corrections in language:
   - Background, fourth sentence and Methods, second paragraph, first sentence: “NM_000038” should be replaced with the current accession number “NM_000038.5”
   - Results, first paragraph, fourth sentence: “lost” should be replaced with “loss”
   - Results, third paragraph, fifth sentence and supplementary figure 2, last sentence: “Hg18” should be replaced with the current chromosome build (e.g. NCBI Build 36.1 or UCSC Feb. 2009 (GRCh37/hg19) assembly)
   - Figure Captions, Figure 1; eight sentence: “hg18, Build36” should be replaced
with the current chromosome build (e.g. NCBI Build 36.1 or UCSC Feb. 2009 (GRCh37/hg19) assembly)

Supplementary Figure 1, first sentence: “family tree” should be replaced with “Pedigree”

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.