Author's response to reviews

Title: Reliable and rapid characterization of functional SNPs in human FCN2 gene reveal diverse geographical patterns.

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Author's response to reviews: see over
Reviewer's report
Title: Reliable and rapid characterization of functional SNPs in human FCN2 gene reveal diverse geographical patterns.
Version: 1 Date: 17 February 2012
Reviewer: Kabilan Velliyagounder
Reviewer's report:
Commons for the authors:
I have read this manuscript and submit these recommendations to you.

Ojurongbe et al present a study of “Reliable rapid characterization of functional SNPs in human FCN2 gene reveal diverse geographical patterns”. The title of this manuscript appropriately reflects the purpose of this study. The abstract, introduction and research hypothesis is clear and concise. The authors observed genotype distribution of three functional FCN2 SNPs from different ethnic groups. The results also showed that the distribution of haplotypes revealed distinct geographical patterns. In addition to this, the authors showed a simple and reliable FRET based real-time PCR to analysis for detection of SNPs. Based on these and previous study, the authors believed that these SNPs play an important role in the level of ficolins concentration in the serum. This is an interesting study. However, I would like to make some points regarding the manuscript.

Some comments (in no particular order):

The authors have based their conclusions; the SNPs from promoter region (-986G>A, -602G>A and -4A>G) of FCN2 gene may associate with altered the serum level of ficolins2. Did the authors measure the levels of serum ficolins in the studied populations? This would strengthen the conclusion that there are changes in the levels of ficolins in the studied populations and between the SNPs.

The authors believe that FRET based real-time PCR technique can be used for efficient genotyping in other world population based on their tested population for FCN2 SNPs. Was there any other ethnic samples tested other than those used in this study demonstrating no need for assay optimizations?

As suggested added to the text
These samples were tested across different ethnicities, irrespective of world populations. These samples belong to Viet Ethnicity of Vietnamese origin, Yoruba ethnicity of Nigerian origin, Kanigang ethnicity of Brazilian population and Caucasians are admixture from European population.

Minor comments:
Line 2 and 44: FCN2 gene name should be in italics.
Changed as suggested
Line 274, 280: there should be a space between Figure and number.
Changed as suggested
Line140, 141,142 and 149, there should be a space between Table and the number.
Changed as suggested
Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
"I declare that I have no competing interest’

Reviewer's report
Title: Reliable and rapid characterization of functional SNPs in human FCN2 gene reveal diverse geographical patterns.
Version: 1 Date: 22 March 2012
Reviewer: Jamal Mohamed
Reviewer's report: 03/22/2012

BMC Medical Genetics: Reliable and rapid characterization of functional SNPs in human FCN2 gene reveal diverse geographical patterns

Ojurongbe et al reported on use of real time PCR based FRET technique for the detection of SNPs and distribution of FCN2 SNPs (-986G>A, -602G>A, -4A>G and +6424G>T) among Brazilian, Nigerian, Vietnamese and European Caucasian ethnicity. The authors demonstrated that the distribution of genotype of three SNPs (-986G>A, -602G>A, -4A>G) vary significantly between the populations and highly linked to each other with significant patterns in populations studied. Based on the results obtained, the authors have concluded that (1) ethnic differences in FCN2 functional SNP variants observed is believed to affect the concentration and the function of the ficolins, (2) the FRET based real-time PCR for FCN2 gene is rapid, reliable method for FCN2 genotyping that benefit a larger scientific community who extensively depend on genotyping. I feel that the authors’ first conclusion could be as RT-PCR based FRET is rapid and reliable method to characterize SNPs of FCN2 and reveals diverse geo pattern. These are my comments/suggestions;

Title: SNP should be expanded as single nucleotide polymorphisms changed

Abstract:
1. The authors should provide rs# number for all the four SNPs studied here.

Provided as genotype four functional SNPs including -986G>A (#rs3124952), -602G>A (#rs3124953), -4A>G (#rs17514136) and +6424G>T (#rs7851696) in the ficolin-2 (FCN2) gene

2. Conclusions should be rewritten. The author have NOT done any phenotypic association study (for instance, looking at the levels of ficolin-2 in the serum among individuals) to link the genotypes, levels and functions of ficolin-2. I feel that the authors’ primary conclusion should be the use of this method for genotyping of FCN2.

Restructured as
The observed distribution of the FCN2 functional SNP variants may likely contribute to altered serum ficolin levels and this may depend on the different disease settings in world populations.
To conclude, the use of FRET based real-time PCR especially for FCN2 gene will benefit a larger scientific community who extensively depend on rapid, reliable method for FCN2 genotyping.

Introduction/Background:
3. Line 57-58 is repeated and should be deleted.
   Deleted as suggested

4. Line 61: the authors should clearly state that gram positive bacteria as the presence of LTA is characteristics of gram positive bacterial system.
   Amended as suggested

5. Line 68: why exon8 is so important?? Why not other SNPs in exons 1-7?? Of course, rationale other than literature available/published.
   Included in the text

Exon 1 of the FCN2 encodes the 5’ UTR, the leading peptide and a small segment of N-terminal amino acids. Exons 2 and 3 encode the collagen-like domain. The fourth exon encodes the linker region and exons 5–8 encode the FBG domain. The last exon also encodes the 3’ UTR . The observed SNPs at exon 8 are likely to cause specific alterations of the FBG domain, thus affecting the binding to ligands, but they are not located in the observed FBG binding sites that have been deduced based on crystallographic observations.

In addition, When serum from individuals homozygous for the wild type and the Ala258Ser variants, was incubated with solid phase or soluble GlcNAc, great differences in their ability to bind to GlcNAc were observed.

6. Line 71: what are the three populations? Citation needed here.
   Cited as 10

Materials & Methods:
7. The statement about informed consent should be dedicated here.
   Informed written consent was obtained from the study volunteers.

8. Line 99-100: should be rewritten for clarity.
   Added for clarity as

   Both anchor and sensor probes were labeled with either fluorescein or cyanine dye as applicable to the orientation of the SNP detection. The region of interest is amplified by forward and reverse primer pairs. The SNP specific sensor probes were designed one nucleotide apart from the anchor probe to facilitate the energy transfer between the two fluorescent dyes in proximity. Both probes were designed to be localized on the same DNA strand so that they could anneal on the target sequence in a head-to-tail arrangement. During the melting phase, energy transfer referred to as FRET occurs. This excitation energy is transferred from the anchor to the sensor probe and the emitted fluorescence is detected at 660hp wavelength during the melting phase. Gradual increase in temperature decreases the fluorescence intensity as one of the probes melt off leaving the two fluorescent dyes apart. The sensor probe with a clear match can still anneal to the target SNP but it melts off at a higher temperature contrary to the mismatch that melts off at a lower melting temperature. Therefore the difference in the melting temperature remains as a basis to differentiate the genotypes.
9. All PCR primer concs. should be moved to table 1.
Shifted to table 1 as suggested

10. Direct sequencing method is one of the established protocol; the authors have deliberately elaborated methods in order to present as a full-text article.
Is precisely described to the point

Results:
11. Line 148: The authors should present rs# numbers in the text as Haploview plot (Fig 2) was shown with rs#s.
Provided as suggested all three SNP variants -986G>A (#rs3124952), -602G>A (#rs3124953), -4A>G (#rs17514136)) in the promoter region

12. Again, the authors have deliberately presented the results both in tables and figure format. Overall, ALL the figures SHOULD BE DELETED except Fig 2.
We retained figures of haploview plot and for FCN2 haplotypes world distribution image, and deleted the rest of figures as suggested.

Discussion:
14. Line 154-156: The statement does not make sense; should be rewritten.
Changed as
Ficolins are able to bind to specific ligand such as pathogen-associated molecular patterns (PAMPs) expressed on the surface of pathogens and trigger the complement cascade by interacting with mannose binding lectin associated serine proteases (MASPs) to swiftly contain the early infection.

15. Line 165: limited turn over time?? The authors failed to mention how long the turn over time is.
Rectified as
Moreover the employed methodology utilizes simple data analysis for accurate genotype calling with a limited turn over time of three hours for 72 samples for one single run.

16. Line 174-175: I take an issue with the authors’ statement that “Since this methodology is consistent with different ethnicities tested, we strongly believe that there arises no necessary optimization for an efficient genotyping in other world populations”. The authors have shown ONLY SMALL NUMBER of samples among different populations. This statement should be withdrawn.
Withdrawn

17. Line 181: AA property should be mentioned; acidic to basic or polar to non-polar.
Included non-polar to polar (Ala258Ser)

18. Have the authors performed serum ficolin levels and looked for association between genotypes and levels of ficolin? I feel that such a study would have strengthened the conclusions as well as discussion. Generally, the discussion focuses on speculation about serum ficolin levels and genotypes /homozygocity. The discussion would be much more succinct if the speculation was removed and the authors focused only on their results and direct implications of these results.
Yes we do looked at the distribution of serum ficolin levels in three (Brazil (unpublished), Vietnam (PLosone 2011) and Nigeria (JID_In press) and observed differential distribution between genotypes and serum ficolin levels but this distribution remained insignificant in controls. However when looked as case –control study the distribution of serum ficolins between cases and control were significantly distributed.

However, we have deleted large part of text in discussion that remains of speculation.

19. Of course, I do see lot of typos across the manuscript. Corrected as suggested

Level of interest: An article of importance in its field
Quality of written English: Needs some language corrections before being published
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
'I declare that I have no competing interests'