Author's response to reviews

Title: BAC array CGH in patients with VCFS-like features reveals genomic aberrations on chromosome region 1q21.1

Authors:

Anna Brunet Jr (anna.brunet@crg.es)
Lluís Armengol (lluis.armengol@qgenomics.com)
Damià Heine (dheine@hsd.es)
Jordi Rosell (jrosell@hsd.es)
Manel García-Aragonés Jr (manel.garcia@qgenomics.com)
Elisabeth Gabau (egabau@tauli.cat)
Xavier Estivill (xavier.estivill@cr.cat)
Miriam Guitart (mguitart@tauli.cat)

Version: 4 Date: 9 July 2009

Author's response to reviews: see over
We enclose the comments of the reviewers and our reply to their points.

Reviewer 1: Cédric Le Caignec

In the present manuscript, Brunet et al. have analyzed 18 patients with VCFS-like features by array comparative genomic hybridisation (aCGH) using two different arrays: a whole genome and a chromosome 22-specific BAC array. They identified a patient carrying a combination of constitutional chromosome rearrangements on 1q21.1, consisting in a microduplication of 212 kb, reciprocal to the congenital thrombocytopenia-absent radius (TAR) syndrome microdeletion, and a close microdeletion of 1.15 Mb, previously reported in patients with mental retardation, congenital heart disease (CHD), and also associated to schizophrenia. This patient has already been included in a recent publication that reported 22 probands with the 1q21.1 microdeletion (Mefford et al. NEJM 2008). This rearrangement occurred de novo in the patient and was confirmed by FISH and MLPA. To determine whether copy number aberrations in this region could be associated with CHD, they screened 73 unrelated CHD cases and 326 control samples by MLPA covering the 1q21.1 region. While all control samples were negative for both 1q21.1 rearrangements, one patient was found to carry the same 212 kb microduplication. This duplication was also present in his unaffected father. The authors suggest the involvement of the 212 kb duplication on 1q21.1 in CHD. In addition, they suggest that screening of both regions might be considered in VCFS-like patients of unknown aetiology.

Major comments:

The message of the manuscript is confusing. Previous articles suggest that the 1.15 Mb 1q21.1 deletion is associated with CHD. Regarding the conclusion, Brunet et al. suggest the involvement of the 212 kb duplication on 1q21.1 in CHD. However, they also suggest that screening of both 1q21.1 regions (i.e. the microduplication and microdeletion) might be considered in VCFS-like patients of unknown aetiology. If the authors are convinced that this is the 212 kb microduplication that predispose to CHD, they should state it clearly. Even the Title is vague ?Submicroscopic aberrations on 1q21.1 in patients with CHD and VCFS-like features?.

We agree with the reviewer and we have modified the text and the title. The current view of these genomic changes is that they have a partial penetrance, so that only in some subjects have phenotypic consequences. We have clarified this point (second paragraph of the discussion) and we have changed the title.

In the second statement of the conclusion, the authors suggest that screening of both 1q21.1 regions might be considered in VCFS-like patients of unknown aetiology. Patients with VCFS, and a fortiori with VCFS-like, present with highly variable phenotypic expressivity. I think aCGH should be proposed to such patients but I?m not convinced that this requires to specifically checking this region before aCGH.

We agree that specific checking on 1q21.1 region is not required before aCGH, but it can be considered in other screening methods such as MLPA, especially for patients with VCFS-like phenotype and psychiatric disease. We have clarified this point in the conclusion section.

In the patient carrying a combination of constitutional chromosome rearrangements on 1q21.1 consisting in a microduplication of 212 kb, and a close microdeletion of 1.15 Mb, FISH has been performed to confirm both rearrangements. It would be interesting to know if both
the microdeletion and the microduplication occurred on the same allele. Metaphase and interphase FISH analyses using co-hybridization of two probes, one in the deletion and one in the duplication, or another assay may be performed to clarify this point.

**We agree also that these would be interesting experiments to perform, but unfortunately we do not have cytogenetic material to perform additional FISH analyses and insufficient DNA was available for microsatellite testing.**

Minor comments:

In the Abstract section, the 1q21.1 microdeletion has been reported in patients with CHD, and associated to schizophrenia. It has been also reported in patients with mental retardation.

**We have corrected this point.**

The custom array consisting of 130,000 isothermal probes is not described in the Methods section. A very brief description would be welcome.

**We have included the description of this array in the Methods section.**

The English should be corrected. For instance, barquicephaly instead of barchycephaly.

**We have corrected this.**

Please, write 22q11.2 in the text. Sometimes, the authors write 22q11.

**We have corrected this.**

Figure 2 legend: panel B is FISH and not panel A. The FISH legend should be clarified. The chromosomal location of the different probes used for FISH should be mentioned.

**We have corrected this legend.**

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, the manuscript does not need to be seen by a statistician.

--------

**Reviewer 2: Andrew Sharp**

Submicroscopic aberrations on 1q21.1 in patients with congenital heart defects and velocardiofacial-like features by Bruneti et al.

This manuscript reports a small study of CNVs in a cohort of patients presenting with congenital heart defects. In an initial discovery cohort of 18 cases, the authors use array CGH genome-wide, and detect one case with a duplication reciprocal to the TAR syndrome deletion on chromosome 1, in addition to a number of other inherited CNVs. They then study a second replication cohort using MLPA with probes in 1q21.1 and identify one further CHD patient with an apparently identical duplication of this same TAR locus.

Overall the manuscript is well written, and the conclusions, with some exceptions, are generally valid. The possible association of this 1q21.1 duplication with heart defects is novel and worthy of publication. However, this study does not have sufficient power to determine if this CNV really is a cause of CHD, or if the identification of these two cases in their CHD patients is merely chance, making its impact questionable.
I have the following comments:

In the abstract the authors state that ?We have analyzed 18 patients with VCFS-like features by array comparative genomic hybridisation (aCGH). We performed a sandwich-like hybridization of the sample and reference DNAs onto two different arrays: a whole genome and a chromosome 22-specific BAC array.? The phrase ?sandwich-like hybridisation? is not by any means a standard phrase and stated in the abstract in this way is rather opaque. On further reading the manuscript it becomes clear that the authors have in fact used a novel method to hybridise two microarrays simultaneously face-to-face using a single mix of labelled genomic DNA in between. I find this novel method of practical interest that I believe is worthy of additional explanation, as it may be of use to other investigators. I think that if the authors were to expand on their description of this technique, adding some technical details and a figure, it would add a new dimension to the manuscript, making it of broader appeal to readers. Can the authors comment on technical performance of this methodology compared to performing single hybridisations? For example it would be nice to know if data reproducability is comparable to single hybs, and do the authors make any technical modifications, eg. to the amount of DNA used, the amount of COT DNA added, and the practical details of how they construct the ?hybridisation chamber?. Such details would be a useful addition to this manuscript.

The phrase sandwich-like hybridization has replaced by face-to-face slide hybridization and we provide a more detailed description of the technique page 8 line 13. We adapted the protocol of Microarray Centre d’University Health Network, Ontario Cancer Institute (OCI), Toronto used to perform cDNA arrays and applied for BAC arrays. The methods basically consists in uses another slide in place of a coverslip and a chamber with increased depth.

The authors should make it clear that their case of 1q21.1 rearrangement was previously published by Mefford et al. NEJM 2008. Although this was already explained in the previous version of the manuscript, we now provide a clearer statement on this.

I find the discussion of the additional CNVs detected in some patients a little misleading. While it is possible that some of these CNVs contribute towards the phenotype of the patients, at least one of them is a frequently observed variation reported in many studies of normal individuals, making it highly unlikely that it is related to heart defects. Further the somewhat detailed discussions of potential candidate genes that are located in these CNVs (page 12 and 13) is over-interpreting the data, as the authors have no data to say if these genes (or exons) are even included in the CNV detected. We are in agreement that the size of the CNVs identified by BAC arrays is likely overestimated, we have added this point in the discussion. We have removed some of the speculative discussion on the candidate genes, as suggested.

Table 1 presents a list of CNVs identified by BAC array CGH in their initial discovery cohort. For this list of CNVs, the authors state the coordinates of the BAC clone that was variant, accompanied by a list of genes within that BAC. However, BAC array CGH is notorious for over-estimating the size of CNVs, and it should be made clear that because of this it is not possible to state if these CNVs include all of the genes listed or just a subset of them. Furthermore, because only BAC array CGH was used, the size estimates given for these CNVs are VERY approximate, which again should be made clear. This point is particularly relevant to a statement on page12 of the manuscript:
Finally, a gain of BAC RP11-138G4, located on 22q13.2, which was maternally inherited, was also identified with this chromosome-specific array. This region has not been reported in the literature as a CNV and spans the SLC25A17 gene that encodes a peroxisomal membrane protein belonging to the family of mitochondrial solute carriers [OMIM *606795]. Without breakpoint characterisation, given that the CNV is defined by only a single BAC clone, I do not think that the authors can know whether this gene is actually included in the CNV identified or not.

We have modified the statements on the size estimations. In the case of SLC25A17, the MLPA probe is on exon 2, which means that we could reasonably especulate that the gene or part of it is duplicated.

Reviewer 3: Stefania Gimelli

The manuscript deals with a CNV analysis of 18 VCFS-like patients, not presenting the typical 22q11 microdeletion, using a whole genome and a chromosome 22-specific BAC array. Various CNVs have been detected; among them the authors focused especially on a complex 1q21.1 rearrangement, consisting of a microduplication reciprocal to the TAR syndrome and a 1.15Mb deletion previously reported as associated with CHD and schizophrenia. This case, previously published by Mefford and Sharp., 2008, is here studied more in details, in relation to his hypothetical association with CHD. Moreover the same ?TAR-duplication? was found in one out of 73 unrelated CHD cases that have been screened to determine whether rearrangements within the 1q21.1 region could be associated with CHD. Furthermore, the authors describe some selected inherited CNVs identified in other loci throughout the genome.

The applied methods as the idea to use a sandwich-like hybridization and a dye-swap approach are appropriated, innovative and well described. The patient carrying the complex 1q21.1 rearrangement has been already previously published and the hypothetical correlation between the 1q21.1 microdeletion and CHD has been already described. However, the results and the discussion of the manuscript, with the additional screening of 73 CHD cases, looks enough interesting and exhaustive.

Therefore I have some comments:

1) Major Compulsory Revisions:
In results and discussion: the section concerning the CNVs detection throughout the genome (excluding the 1q21.1 rearrangements), and their hypothetical pathological relevance, doesn?t fit completely with the title and it should be better explained or revisited.

We have changed the title as suggested.

The results chapter should be more exhaustive especially with regard to the basis for the selection of the ?interesting? loci to be validated: why some CNVs have been excluded and not others? Why the validation has been done only for few of them? Four out of five of the validated CNVs are inherited and present in the Database of genomic variants, however they have been considered potentially pathological. While the others, that overlap with benign CNVs present in the same Database, have been previously eliminated?
This was already explained in the text but perhaps it was not clear enough. We have modified this point on page 10, first paragraph.

Page 10 line 10. Among the eleven selected loci, it is not clear which ones were not present in control, not previously described or previously reported. Could be useful to describe this point more in detail; perhaps this could be summarized in a table. This point have been changed and rewritten.

Supplementary table 1 needs a more detailed legend, and the eleven selected cases have to be better highlighted (in bold?). (For example it is not clear what do the numbers inside the columns ?GAIN/LOSES? mean).

We provide a detailed Table legend and highlighted selected CNVs.

The unbalances? extension and BAC content should be indicated in the results chapter (from which position/BAC to which position/BAC they extent)

In the table 1 we have added start and end positions of altered loci.

Page 13 line 14/15. By doing a dye-swap experiment, the authors should have seen twice the positive calls. Although, when confirming the result with the MLPA approach, they could not confirm them. This doesn?t necessarily mean that they are false positive. This point should be better discussed or revisited. This point is better discussed.

2) Minor Essential Revisions

Page 5 line 20 and 21. I suggest to use: BOTH in children with mental retardation associated with dysmorphic features AND in patients with CHD

We have corrected

Page 12 lines 7 and 8. Maybe should be better to give this information before

This has been rewritten to improve understanding.

Page 10 line 6. Perhaps is better to indicate the Human genome Build version instead of ? variants as in December 2008?

We have corrected this point.

Page 11 line 5. Patient V5 instead of patients V5

We have corrected this.

Page 11 lines 16/17. To screen the 1q21.1 region, on 73 additional cases with congenital heart defects have been screened using , we used the same MLPA mix designed for the validation of case V5.?

This has been modified.

Page 12 line 1. Is this a whole-genome array? Which is the coverage resolution within the 1q21.1 region? Is it a BAC or oligo-array?

We have added this information

Supplementary tables 2 and 3. The authors should use a smaller type dimension in the ?oligos sequences? Cells

This has been modified.
3) Discretionary Revisions

Considering that this is a paper concerning the finding of a complex rearrangement 1q21.1 and his hypothetical association with CHD, the authors might consider the option to modify the "inherited CNVs" section and just summarize all the CNVs findings (without making a selection of the most/less interesting) in a exhaustive table (perhaps using the one already done with some minor revisions). This means the paper could be more focus on the 1q21.1 region and on its possible correlation with the CHD as the title conveys. Moreover, the CNVs described in this section are found in single individuals, inherited from an unaffected parent and present in the Database of genomic variants, meaning that their pathogenicity is hard to discuss. In addition, none of them have been discussed in details regarding their gene contents and potentially pathological role.

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, the manuscript does not need to be seen by a statistician. Declaration of competing interests I declare that I have no competing interests