Author’s response to reviews

Title: Similar gene expression profiles of sporadic, PGL2-, and SDHD-linked paragangliomas suggest a common pathway to tumorigenesis

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Author’s response to reviews:

Dr. Scott Edmunds
In-house Editor
BMC Medical Genomics

Dear Dr. Edmunds,

Please find enclosed our revised manuscript MS:1846771182224897. We would like to thank the referees for their precise review of the article. Please find below the point-by-point response to the comments made by the referees. In accordance with the suggestions of referee 1, we have changed the title of this manuscript to “Similar gene expression profiles of sporadic, PGL2-, and SDHD-linked paragangliomas suggest a common pathway to tumorigenesis”.

Comments of referee 1:

Major points

1. We have altered table 1 and added the following clinical data regarding the paraganglioma patients included in this study: gender (‘sex’), age at onset (‘age at onset (yrs’) ), and whether or not the patient has suffered from multiple paragangliomas (‘multiple paragangliomas’). We have changed the legend of table 1 accordingly. Furthermore, we have added the following line to the ‘Methods’ section, paragraph ‘tumor specimens’:

‘No malignant paragangliomas were included in the study.’

2. We noticed that the patient description and mutation scanning paragraphs of the Method section contained errors with regard to the mutation scanning of VHL,
RET, and NF1. We thank the reviewer for noting this. This was a copy-paste error that was part of earlier draft versions of the manuscript that should have been removed from the submitted copy. These genes were not part of the DNA analysis procedures to exclude germline mutations because the prior probability of finding such mutations in these samples was considered too small. None of the sporadic cases, nor their family relatives, carried any of the typical clinical stigmata associated with these syndromes, nor did the PGL2-linked family. We apologize for this misunderstanding, but it does not affect in any way our overall conclusions of the manuscript. We have adapted the text in the Method section accordingly:

SDHB, SDHC, and SDHD genes were scanned for the presence of mutations at the laboratory for DNA diagnostics at the LUMC. All exonic regions of these genes were tested by direct sequencing using the Sanger method on an ABI 3177 Genetic Analyzer, starting with the exon containing the known Dutch founder mutations in SDHD followed by exons that had previously been found to contain pathogenic mutations in SDHD, SDHB, and SDHC (in that order) in the Dutch population[4,11]. If that remained negative, scanning was completed by analyzing the remainder of exons of these genes. More recently, the sporadic, mutation-negative cases were also examined by MLPA for the presence of large deletions in SDHB, SDHC, and SDHD[12]. MLPA was carried out with the P226 MLPA kit, containing probes for all exons and the promoter of each of these genes (27 different probes), according to the MRC Holland protocol[13].

We have added the following references to this section:


13. MRC Holland website. [http://www.mrc-holland.com]

In the most recent MLPA screening for large deletions SDHB, SDHC, and SDHD, two samples included in this study and classed as sporadic samples were shown to harbour mutations in SDHB. As this is very recent work, these results were unknown to the investigators at the time of the analysis of the microarray results and drafting of the manuscript. We have now excluded these samples from this study as they constitute too small a group for adequate analysis. We have performed all microarray analyses anew without these two samples, and as is to be expected, no novel differences in gene expression between the other samples is observed. Therefore, the outcome and conclusions of the study are not affected. However, it affects the sample size and sample size calculations. We have adapted these paragraphs in the methods and results sections:
Methods:
Eighteen paraganglioma cases were selected: 7 cases with a known D92Y founder mutation in the SDHD gene, 6 cases from the family with significant linkage to the PGL2 locus on 11q11, and 5 sporadic cases.

Results:
In all, 21 samples were hybridized including 3 duplicates. Four samples (1 SDHD-linked sample, 2 PGL2-linked samples and 1 duplicate experiment) were excluded because of poor RNA or hybridization quality, leaving 15 different tumors in the analysis (5 sporadic, 6 SDHD-linked and 4 PGL2-linked samples) (Table 1).

Calculations showed that with this sample set and assuming that at least 30 to 35 genes are truly differentially expressed between subgroups with a fold change of 2.0 or more, at least 10 differentially expressed genes would be detected with a false discovery rate of 0.1.

We have adapted Table 1 accordingly.

Furthermore, Figures are adapted accordingly.

4. The reviewer has an interesting point, which we are currently indeed analyzing. However, this analysis is more complex than suggested, as it requires copy-number and LOH analyses in 11p15, 11q13, and 11q23, in PGL2-linked and sporadic cases. This work is in progress and will be part of other studies. We have adapted the discussion to indicate this complexity more accurately:

As the relation between chromosome loss and gene expression alterations is complex, we must interpret the observed lack of gene expression differences between these groups cautiously in this context. It has been shown previously that all SDHD-linked HN-paragangliomas show loss of the entire copy of the wildtype maternal chromosome 11, and the same applies to PGL2-linked paragangliomas. Partial or entire chromosome 11 loss has also been observed in sporadic paragangliomas, although only in 2 out of 9 cases. Chromosome 11 loss could thus be an important step in paraganglioma formation irrespective of the genetic background.

Minor points
1. We have defined the paragangliomas and phaeochromocytomas more precisely. Indeed, not all paragangliomas consist of chromaffin cells. Furthermore, we have included the paragangliomas originating from paraganglia that are not situated in the adrenal medulla or head and neck region in the definition. The concerned paragraphs in the ‘Background’ section now read:

‘Paragangliomas are tumors originating in cells of neural crest origin in the extra-adrenal paraganglia associated with the autonomic nervous system. Most paragangliomas arise in the parasympathetic paraganglia of the head and neck region, but they can also arise in the parasympathetic paraganglia of the
mediastinum or in the orthosympathetic para-aortic and retroperitoneal paraganglia.'

‘Mutations in SDHB, SDHC and SDHD are also implicated in the formation of phaeochromocytomas, tumors arising in cells derived from the neural crest in the adrenal medulla’.

2. The concerned paragraph in the ‘Discussion’ section now reads:
‘This correlates well with the observation that sporadic as well as SDHD-linked and PGL2-linked paragangliomas of the head and neck share important clinical characteristics like the age of onset of symptoms, the indolent growth pattern, and a usually benign behaviour of the tumor, although multiple paragangliomas are less often observed in sporadic cases.’

We have included the following references in this section:


These two articles describe clinical characteristics of SDHD and SDHB-linked paraganglioma syndromes, and are therefore helpful in understanding the clinical picture of SDHD-linked cases. We have also kept the original two references because these articles specifically refer to the clinical characteristics of the SDHD-linked paragangliomas found in the Netherlands, and PGL2-linked cases respectively.

3. We have altered the title of the manuscript to ‘Similar gene expression profiles of sporadic, PGL2-, and SDHD-linked paragangliomas suggest a common pathway to tumorigenesis’.

Comments of referee 2:

1. We would like to thank referee 2 for his kind appraisal of our manuscript.

2. Comparing gene-expression data of normal, sporadic, SDHD-linked and other hereditary phaeochromocytomas might indeed provide new insights into differences in phaeochromocytoma tumorigenesis. This has, in part, already been shown by Dahia et al. (Plos Genetics 2005, 1: 72-80.). In our study, we have evaluated the gene-expression of branchiomeric extra-adrenal paragangliomas arising in the head and neck region, and therefore phaeochromocytomas originating in the adrenal medulla are slightly out of the scope of this study. It is however a wonderful suggestion for future studies as it might overcome difficulties with the acquisition of normal paraganglion tissue from the head- and neck region.
Yours sincerely,

Erik F. Hensen, MD