Author’s response to reviews

Title: Whole exome sequencing in an Indian family links Coats plus syndrome and dextrocardia with a homozygous novel CTC1 and a rare HES7 variation.

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Author’s response to reviews:

Response to the Reviewer’s comments

We are sincerely thankful to both the reviewers for providing the suggestion related to our manuscript and the study. We have corrected text and incorporated all the suggested changes.

Referee 1

Comment: The paper has been now modified as previously required. The text is now clearer and more readable. It has been rewritten with accuracy and several mistakes were corrected. The case description has been improved and language is now more appropriate.

Reply: We sincerely thank the reviewer for accepting our correction in the manuscript.

We would like to mention to the reviewer about his earlier following comment at the first evaluation “Comment 1: Line 2: the terms “Cerebroretinal microangiopathy with calcifications and cysts (CRMCC)” and “Coats plus syndrome” are used as synonymous. It is not correct, and the Authors should preferably use the term CRMCC than Coats plus syndrome”. This problem is present in many part of the text.”

Although, in revision submission later, we had used term CRMCC uniformly
throughout the text instead of Coats plus. We would like to make a change with this overlapping and confusing terminology of CRMCC/Coats plus (which although not synonym) and we have preferably now changed it to Coats plus syndrome to describe the phenotype of our patient. Since, CRMCC was initially used to describe as an umbrella term to define both LCC and Coats plus, but since identification of CTC1 mutation in Coats plus but not in LCC warrants the use of term Coats plus to define the LCC with extraneurological features.

With reference from Livingston et al. 2014 Neuropediatrics.

“Coats plus, a multisystem disorder the most characteristic features of which are as follows: retinal telangiectasia and exudates (Coats disease), intracranial calcification with an associated leukoencephalopathy and brain cysts, osteopenia with a tendency to fractures and poor bone healing, and a high risk of life-limiting gastrointestinal bleeding and portal hypertension caused by the development of vascular ectasias in the stomach, small intestine, and liver. Mutations in CTC1 were not identified in any patient with a purely neurological disorder, indicating that LCC and CP are distinct entities. Polvi et al. also reported CTC1 mutations in 11 patients with what they describe as CRMCC. All of the patients had extraneurological features. In 10 patients there was retinal involvement, and in the remaining patient osteopenia and fractures. Again, they concluded that CTC1 mutations were not responsible for the LCC phenotype.”

“This purely neurological disorder (LCC) may present at any age from infancy to adulthood. Our clinical and mutation data allow us to distinguish LCC from Coats plus, leading us to suggest that the use of the “umbrella” term of CRMCC is no longer helpful. Further delineation of the genetic basis of LCC, and its relationship to Coats plus, must await the results of ongoing genetic studies.”


“Some patients diagnosed with LCC also have Coat’s retinopathy.[3] It has been proposed that Coat’s retinopathy is a part of the spectrum of this disorder—the combined presentation has been termed as “cerebroretinal microangiopathy with calcification and cysts” or Coat’s plus.[6, Briggs et al. Am J Med Genet A. 2008].”

We also understand that in the literature CRMCC and Coats plus are sometimes referred as synonym. In the first two publications reporting CTC1 mutations, one described it with CRMCC and other with Coats plus while the spectrum of the abnormality were the same that is Cerebroretinal microangiopathy with extraneurological manifestation skin/hair pigmentation changes, GI bleeding and osteopenia.

We also believe that use of Coats plus syndrome will be more appropriate which can be used to describe Coats disease of eyes with presence of other non-neurological abnormalities.

We hope that it would be a justified use of Coats plus term than CRMCC to describe our case.
Referee 2
Reviewer's report:
Since my original review of this manuscript there have been significant changes to the text.
I have made some specific recommendations below but I feel that overall the paper would now benefit from review by a native English speaker.
The authors have addressed all of the comments from my previous review. My main concern was the need to increase the number of ethnically matched controls that were screened for the CTC1 variant. I am now happy with the number of controls that have been screened.
However, since my previous review a new database of genetic variation has been launched, the ExAC Browser. I recommend that the authors replace the frequency data cited in the text from 1000Genomes with the more comprehensive data available on this database.
http://exac.broadinstitute.org/
Response: We are extremely thankful to the reviewer for the appreciating our amendments in the manuscript as advised and for providing the suggestions. Below are the detailed response to the issues pointed.

MAJOR COMPULSORY REVISIONS
Comment 1. Include variant frequency data from the ExAC database.
We have included the frequency data from ExAC database both in the main text and the supplementary file wherever required.

Comment 2. I notice that the authors have altered the manuscript to describe the phenotype as CRMCC throughout the text. I believe that this is incorrect terminology.
CRMCC is an umbrella term which encompasses two separate but similar diseases, Coats Plus syndrome and LCC. This is clearly a case of Coats plus syndrome (and not LCC) and I believe that the phenotype should be described as Coats plus throughout the text. CRMCC is no longer a useful umbrella term as it is now known that Coats Plus and LCC are not allelic. Please see Livingston et al. Neuropediatrics 2014 Jun;45(3):175-82 for further clarification of this point.
We have followed the suggestion and used the correct terminology of Coats plus to define the phenotype of our patient.

3. The authors have amended the text to include information on another variant identified in the WES sequencing in the gene TEK. This variant/gene was not discussed at length in the original manuscript. It is unclear to me why the authors have suddenly decided to include a discussion of this variant. Do they believe that this variant is relevant to the disease phenotype? Have mutations in this gene previously been implicated in dextrocardia or Coats plus syndrome? Please clarify the relevance of this variant to the phenotype described here and comment clearly on the likely pathogenicity if any.
We would like to clarify that in our first version of the manuscript in Table 1 we had shown that following a variation filter protocol, we had obtained four other variations than CTC1 which included TEK variations too. A revisit of the data from the perspective of Notch signalling since HES7 is the target of Notch signalling, we found it worth mentioning about TEK variation (p.E103D). This variation is even reported with a very less frequency (0.08948\%) in ExAC data base that too as a heterozygous genotype. We have obtained this variation as homozygous variation. We have added comments about this variation in this discussion as following “TEK a receptor protein with its ligand ANGPT1, promotes vasculogenesis through Notch signaling [29]. Missense mutations in TEK have been reported to cause autosomal dominantly inherited cutaneomucosal venous malformation (VMCM). Some patients with TEK mutation manifests ventricular septal defect suggesting its (TEK) role in cardiac development.” At this point we cannot ascertain whether the phenotype in our patient is independent of this TEK variation or it is also possible that a concerted effect of CTC1, HES7 and TEK would be responsible for overall phenotype but that is very speculative.

Comment 4. This manuscript requires review by a native English speaker. Minor alterations will be required throughout the text however I have noted some sections below which I feel need the most attention.

Background, 1st paragraph, delete the word abnormality

Background, 2nd paragraph, delete ‘with other system impairment’

We appreciate the reviewer’s suggestions and we have now our manuscript proof read by a proficient English Speaker and we have amended the sentences accordingly throughout the text.

We are once again thanking the reviewer for providing us the invaluable suggestions.