Author's response to reviews

Title: Mutation screening of ASMT, the last enzyme of the melatonin pathway, in a large sample of patients with Intellectual Disability

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Version: 4 Date: 15 December 2010

Author's response to reviews: see over
Dear Sir or Madam,

Please find enclosed the revised version of our manuscript entitled “Mutation screening of ASMT, the last enzyme of the melatonin pathway, in a large sample of patients with Intellectual Disability” by Pagan et al. that we have submitted for publication in *BMC Medical Genetics*. The manuscript was revised according to the referees’ comments, as follows.

**Reviewer:** Derek Morris  
**Reviewer's report:**

Pagan and colleagues investigate the potential contribution of genetic mutations in the melatonin pathway to ID risk by screening the ASMT gene for rare coding or functional mutations in a sample of 361 ID cases and 440 controls. The authors set out a reasonable hypothesis for investigating this gene in an ID sample. The authors do not succeed in identifying a significant excess of rare mutations in cases versus controls, but do report that the activity of ASMT is reduced in case mutation carriers compared to controls.

**Comments**

1. It is not definitively stated in the methods section that all case samples are male. Is this the case? If yes, and because the control sample is part female, the authors should comment on whether this introduces any bias into the study.

   Indeed, all cases are males (this is now stated in the methods section), while the control sample includes males and females. However, considering that ASMT gene is located on the pseudo-autosomal region 1 (PAR1) of the heterochromosome, both males and females carry two copies of the gene, and it segregates as an autosomal gene. Thus, the difference in sex-ratio between case and control samples should not introduce any genetic or functional bias. This is now addressed in the discussion section of the manuscript.

2. ASMT activity in cell lines: the authors compare case mutation carriers to controls. Do any of the 31 controls carry functional mutations? Is it possible to compare either case versus control mutation carriers, or case mutation carriers versus case non-mutation carriers to explore this finding further? Is it possible that the reduced ASMT activity in the case mutation carriers is in fact not due to the mutation but due to some other element of the ID pathophysiology?
The 31 controls for which ASMT activity has been determined in BLCL are controls without mutations. This is now stated in the manuscript. Cell lines were not available for control individuals carrying an ASMT mutation. In this study, we measured ASMT activity in cultured cell lines derived from blood cells. Thus, the reduction observed in ASMT activity appears to be cell-autonomous, and it is unlikely that it results from abnormal central regulations.

3. Methods, Subjects section – there are references to “A1” and “A2” – please correct.

These references were not in the original text file (a pdf artifact?). There are no references in this section. The pdf file of the revised version will be checked.

4. Have the new mutations been deposited in dbSNP?

Yes, all variations have been submitted to dbSNP. The submission has been validated and is currently being loaded into dbSNP. The RefSNP IDs will be assigned soon.

Reviewer: Wiebe Braam
Reviewer’s report:
Although this study did not support the hypothesis that deleterious mutations as well as decreased ASMT activity play a major role in the development of ID, this is an interesting study. It is conducted in a relatively large group of patients with a suspected X-linked ID and controls.

At this point there is a discrepancy between the group of patients who were investigated in this study (ID) and the study by Melke et al. (2008) finding low ASMT activity in individuals with ASD and low melatonin levels. Because mutations in ASMT gene have been reported as a risk factor for autism spectrum disorders (Melke et al, 2008) it is of interest to know how many ID patients in this study have a diagnosis of autism. However, data on this subject are only provided for the 8 ID patients with ASMT mutations, of whom only 2 have ‘some autistic symptoms’.

The patients were initially recruited for X-linked intellectual disability, which implies a family history of intellectual disability among males. It is most likely that a subgroup of these patients might meet the full diagnostic for ASD or at least display autistic features. Unfortunately, this information was not available for all 361 patients. This is partly due to the difficulty to diagnose ASD without using specific instruments such as ADI-R, ADOS, DISCO or 3DI.

It is curious that the patient with the MECP2 duplication was reported not to show autistic features, as this syndrome is associated with autism or autistic features (Ramocki et al. 2010).

The patient with the MECP2 duplication does not specifically display impairments in social interactions (this information was double-checked). However, as already indicated in table 2 of the manuscript, he is reported to have severe language delay, which is one of the DSM-IV items for ASD.

On average, ASMT activity in BLCL of ID patients carrying ASMT variants was much lower as compared to control subjects. It is not clear to me why authors did not compare ASMT activity between the 8 ID patients and the 8 controls with ASMT variants, but with a larger group (n=31) of healthy controls (including those with ASMT mutations?). Figure 1 shows however 10 control subjects with low
ASMT activity compared to the 8 patients with ASMT mutations, so 2 control subjects without a ASMT mutation also showed low ASMT activity?

None of the 31 controls for which ASMT activity was determined was carrier of an ASMT mutation (this is now stated in the manuscript). Melatonin synthesis is known to display a high inter-individual variability in the general population (Burgess 2008). Genetic factors other than mutations have been described to account for variations in ASMT activity, including the promoter genotype (Melke 2008, Galecki 2010). Thus, low ASMT activity can be observed even in the absence of coding mutations. This is now mentioned in the discussion section of the manuscript.

It would be of interest to know whether the 8 ID patients with ASMT mutations have low melatonin levels (with or without a sleep-wake problems), and their response to melatonin treatment. This would indeed support their conclusion that melatonin treatment might be beneficial in patients with ID and sleep problems.

We agree with both reviewers that the absence of complete clinical data on autistic features and sleep in our sample of patients is a strong limitation of our study. Both symptoms were unfortunately not part of the main clinical description of the patients when European X-linked Mental Retardation Consortium (XLMR) has collected the patients. It is fortunate that nowadays the presence of autistic features and sleep problems are now considered as relevant clinical descriptions for patients with ID.

We hope you will find our this revised version of our article suitable for publication in BMC Medical Genetics.

Yours sincerely,
Prof. Thomas Bourgeron