Reviewer's report

Title: Gene Expression in Lungs of Mice Lacking the 5-hydroxytryptamine Transporter Gene

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Reviewer: Luc Maroteaux

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Subject: Review of BMC Pulmonary Medicine manuscript
Gene Expression in Lungs of Mice Lacking the 5-hydroxytryptamine Transporter Gene
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BMC Pulmonary Medicine

Major

The goal of this study is to examine changes in gene expression in the mouse lung of 5-HTT/-/-, 5-HTT+/- compared to 5-HTT+/-+ + , in order to determine in vivo the pathways that are modified by 5-HTT in the lung.

Previous report have established that 5-HTT+/+ or +/- mice displayed no difference of 5-HTT expression assessed by 125I-RTI binding; by using synaptosomes prepared from brain stem and cortex no differences for the 5-HTT+/- and 5-HTT+/+ mice 5-HT uptake and no differences for the Vmax or Km were recorded; 5-HT uptake was absent in 5-HTT/-/- mutants; in addition, essentially normal levels of 5-HT were reported in the brain of 5-HTT+/+ and 5-HTT+/- mice. (Bengel et al., 1998, Mol Pharmacol 53: 649-655.). Similar information about differences between 5-HTT+/+ and +/- are missing in the lung.

The authors verified that under normoxic conditions, with ambient pressure and spontaneous ventilation, RSVP and heart rate were unchanged between 5-HTT+/+, 5-HTT+/- and 5-HTT/-/- mice. It is therefore somewhat hard to follow the rationale of this work looking for differences between three genotypes in basal conditions in the absence of characterized functional differences. It is even harder to understand why most of the observed changes appears between +/- and +/- and not between +/- and -/-, questioning the reliability of the data. Why the authors selected a threshold of 1.2 fold change for selectivity when usually it is 2 fold? In this later case, only 31 changes would be recorded and 3 between 5-HTT +/- and -/- mice. It is quite surprising to realize that these three major changes (Gdph1, Fbox39 and Msi2) have not even been analyzed in the present study. Recently, the profile of gene expressed by the brain of adult 5-HTT knockout mice was compared to wild type mice (Ichikawa et al., 2008, Biochem Biophys Res Commun 368: 43-49). It is also surprising to find very few common gene expression changes (only S100A9 seems common) and not to find in the present data the increase in 5-HTT know to be highly expressed in these mutant

In the introduction, the authors by selecting mostly their own work may generate misleading interpretation of the field:

- The authors report that "dexfenfluramine causes overexpression of the 5-HTT, which in turn promotes hypertensive process" (Eddahibi et al., 2001, JPET 297: 148-154.). If 5-HTT overexpression in hypoxic lung has been reported by different authors, the link with functional alterations has not been directly established: In hypoxic lungs, specific maximal initial rate of 5-HT uptake was reported to be reduced (Jeffery et al., 2000, Eur J Pharmacol 396: 137-140; Callebert et al., 2006, JPET 317: 724-731).

- The authors also said that "the L-allele variant of the 5-HTT promoter, causes increased expression of 5-HTT", which has been reported in lymphocytes (Lesch et al., 1996, Science 274: 1527-1531.) but never in lungs.

- The authors state that the L-allele variant of 5-HTT "is overrepresented in some patient groups with IPAH" (Eddahibi et al., 2003, Circulation 108: 1839-1844.). More recent studies challenged these data and concluded that "variation of the serotonin transporter gene appears unlikely to confer significant susceptibility to pulmonary arterial hypertension" (Machado et al., 2006, Am J Respir Crit Care Med 173: 793-797). This later study emphasizes the need for adequately powered cohorts for association analyses to identify not only genetic determinants of disease susceptibility but also inherited modifiers for disease development, but is not mentioned.

- The authors claim that "Attenuation of IPAH in experimental mice can occur pharmacologically through the administration of a 5-HTT inhibitor", although they published paradoxical reports showing that some 5-HTT inhibitors have putative beneficial effects in pulmonary hypertension (Marcos et al., 2003, Am J Respir Crit Care Med 168: 487-493.), while injection of serotonin potentiates the development of pulmonary hypertension in rats exposed to chronic hypoxia (Eddahibi et al., 1997, Am J Physiol 272: H1173-1181). In addition the putative protective effect of SSRIs has been challenged by the recent warning on the association between the maternal use of SSRIs in late pregnancy and persistent pulmonary hypertension of the newborn in the offspring (Chambers et al., 2006, N Engl J Med 354: 579-587).

- The authors called dexfenfluramine as "a 5-HTT inhibitor", which is misleading when it is in fact a "5-HT releaser": Anorectic agents that increase the risk of developing primary pulmonary hypertension share the common property of being 5-HT transporter substrates and potent 5-HT releasers (Rothman et al., 2002, Pharmacol Biochem Behav 71: 825-836.). These properties are clearly distinct from SSRI which are 5-HTT blockers (simple inhibitors of 5-HT reuptake). Furthermore, fenfluramine exposure has been established as "a potent trigger for pulmonary arterial hypertension" not a protecting agent in humans, see a recent review by (Weir et al., 2008, Eur Respir J 31: 232-235).

- The statement "only 5-HT internalized by 5-HTT exert mitogenic effects on PASMC" is not supported by any reference.
- The statement "5HTT-/- mice have substantially lowered circulating 5-HT (Eddahibi et al., 2000, J Clin Invest 105: 1555-1562), which should result in decreased 5-HT-derived tone and thus protection against primary pulmonary hypertension", is incomplete, this work having assessed total blood 5-HT content which corresponds mainly to platelet stored 5-HT. Platelet stored 5-HT is not free and cannot reach lung serotonin transporter or receptors, while an increase in plasma (free) serotonin is a well established clinical criteria of primary pulmonary hypertension (Hervé et al., 1995, Am J Med 99: 249-254).

The discussion is very weak. The statement "The classes of genes discovered (%) closely mirror the classes of genes dysregulated in mice expressing a mutant BMPR2" is again misleading since very few (about four) are common and they are not modified in a coherent way with this study.

Minor

- Fold change and absolute change are not defined in Table 2-3
- The selected primers are not all generating products spanning introns; How the authors assessed they are not amplifying contaminating genomic DNA?
- How the mice were genotyped?
- The Western blot quality is low: the authors should show how they determined the specific band.
- The differences shown in qPCR of fig2 do not seems statistically different.

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I have no competing interests in relation to this paper.