Author's response to reviews

Title: Personalized medicine in psychiatry: problems and promises

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Author's response to reviews: see over
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Comments: Commissioned content.
Introduction to the Revised Manuscript

We would like to thank the review committee for their insightful and valuable comments on the previous manuscript submission. We were quite pleased that they found that this proposal is “topical” and is “is impressive in its attempted scope.” Furthermore, “the authors have gone beyond risk factors related to the consequences of treatment (which could be a narrow definition of personalized medicine) to address risk factors and biomarkers associated with disease and diagnosis.” This revised manuscript has built on these strengths and addressed the concerns that were raised. This document outlines our response to the reviewers’ critiques. As such, we have extensively restructured and enhanced the manuscript. Substantial changes to the manuscript are indicated by a margin sidebar (as shown, left), and track changes are embedded in the text.

Critique 1:

1. “…is difficult to read and overloaded with details.”
   - The manuscript has been substantially streamlined by focusing on the affective disorders and schizophrenia. When appropriate, we reference the accompanying tables for additional information when extensive discussion of particular examples is superfluous. This has improved the readability of the manuscript as well.

2. “…A guideline on the impact on personalized medicine is missing…”
   - This review provides highlights of some of the work that has been done toward attaining the goal of personalized medicine. We conclude that many of the goals of personalized medicine have yet to be realized, as personalized medicine in psychiatry is still in an information gathering stage. Thus, to specify an impact (beyond information attainment) of a particular component or example on personalized medicine would seem premature.
   - We have added a contents section for an overview and general guide.

3. “…the conclusion that to date we have no causative treatment strategies for severe psychiatric disorders and therefore personalized medicine is in its infancy is missing.”
   - This point has been added to the conclusion.

4. “The manuscript should be re-written considerably, splitted and focus only on psychosis (schizophrenia and affective disorders). PTSD and alcohol dependence should be described in separate manuscripts, the switching between these disorders is confusing.”
   - Please refer to comments in #1.
5. “...some genes from association studies are described in detail while other - and more important- findings from GWAS studies are ignored. Therefore the review seems to be unbalanced and not up to date.”
   - Please note, that we have included additional GWAS and large linkage study results in the Genetics section (including ZNF804A and NRG1).

6. “The neuroimaging part is rudimental and should be improved including results from structural, functional imaging and spectroscopy.”
   - This section is not meant to be comprehensive of all imaging in neuropsychiatric disorders, but it puts forth some of the results that have achieved a considerable consensus in the field. Given the length of the manuscript, we have opted to summarize imaging findings mainly in tabular form and we have included this reference to the table as well.

7. “In the environment chapter, drug abuse (e.g. cannabis) is missing.”
   - The primary foci for the revised manuscript are affective disorders and schizophrenia.

Critique 2:

- “…the depth of coverage of each topic is very variable, with some aspects only dealt with superficially...however the reader needs some explicit guidance where a comprehensive assessment of the literature has not been attempted and the information provided is solely an example of the various findings…”
  - So as not to overwhelm the reader with too many studies, we have attempted to direct the reader to understand that the examples given are illustrative of some of the work done on the path to personalized medicine in psychiatry. Both the positive achievements and the setbacks in the field have helped to inform our current knowledge; however, comprehensive coverage of this type would ultimately detract from the broader message.

1. “p2 the authors refer to "rare genetic variants" although many are in fact common but still account for a small percentage of heritability.”
   - This text has been adjusted to reflect the impact that common variants have in disease susceptibility/heritability.

2. “p3 In addressing TPH they authors could make clear the different functionality, distribution and hence relevance of the two subtypes.”
   - The text now includes the distinction between the homologs.

3. “p12 The authors make useful reference to SZgene but miss a w from the web address. However, they mention few of the risk genes, and arguably not the most important. Neuregulin1, RGS4, ZNF804A and others deserve some mention here, or at least there should be an indication that the genes discussed are two examples of several more.”
• The web address has been corrected.
• Though the text references SZGene as a repository of the “more than 1000 candidate genes”, it has been more explicitly stated in the text, that NRG1, ZNF804A, COMT and DISC1 and DISC2 are examples within a larger collection of proposed risk genes or susceptibility loci.
• This section has now incorporated findings from neuregulin 1 and ZNF804A. The evidence for RGS4 is less compelling than NRG1 or ZNF804A, thus we have not included it as an example here.

4. “p15 There is far more work on gene methylation in depression than the authors indicate. Essentially only one example is given here, although given the later discussion of BDNF, why do they not discuss this gene and its methylation, at least in relation to early life stress as a further risk factor, and in PTSD. Other genes - HTT, MTHFR - may also be relevant here.”
• We have included one illustrative example of methylation in MDD (and other examples for BDD and SZ). As mentioned in our previous response, the review is not meant to be comprehensive, and this has been clarified in the text. This review no longer addresses PTSD.

5. “The same goes for BD and schizophrenia - discussion of recent HTR1A and HTR2A methylation results would be valuable.”
• We have added a discussion of HTR2A in SZ. HTR1A findings are preliminary, with less support than HTR2A.
• There is mixed evidence for association with more studies demonstrating lack of a genetic association between HTR2A and BD (positive studies listed first):
  i. (+) Manchia et al. (2010): HTR2A SNPs were genotyped in 230 BD patients and inserted as covariates in a mixture regression model of age at onset (AAO). HTR2A interaction term associated with early onset under dominant, recessive and additive model.
  ii. (+) McAuley et al. (2009): Significant assn of rs2224721 (P = 0.02) and borderline significance of rs1923886 (P = 0.05) were observed. The former remained significant after multiple testing corrections using the rough False Discovery Rate method, but did not exceed the more conservative Bonferroni’s correction threshold. Haplotype association analysis suggests that the haplotype CCGCA (at SNPs rs3125, rs6314, rs1923886, rs2224721 and rs2770296) is protective against bipolar disorder (P = 0.021, odds ratio 0.63) and the rarer haplotype CCACG confers risk to the disorder (P = 0.0065, odds ratio 3.08).
  iii. (+) Brezo et al. (2010): longitudinal cohort of 1255 followed for 22 years. HTR2A variation influenced both suicide attempts (rs6561333, rs7997012 and rs1885884) through interactions with histories of sexual and physical abuse and mood disorders (rs9316235), through one main effect.
  iv. (+) Ranade et al. (2003): conducted TDT and case-control comparisons involving nine HTR2A SNPs (four SNPs of HTR2A exons and five flanking SNPs). Comparison of BD1 cases (n = 93) with a group of population based controls (n = 92) revealed associations with SNPs on
exons 2 and 3 (516C/T and 1354C/T, respectively), consistent with haplotype-based differences. Analysis of the cases and their available parents using the TDT suggested linkage and associations with 1354C/T, as well as haplotypes bearing this SNP. In view of the relatively small sample, replicate studies using large samples are needed.

v. (+) Chee et al. (2001): assn study of 142 Korean BD cases and 148 controls of HTR2A. Found an assn with G-1438A \( p=0.007 \).

vi. (+) Bonnier et al (2002): assn study of 127 CAU BD cases and 142 controls of HTR2A. Association with A-1438G \( p=0.015 \).

vii. (-) Arranz et al. (1997): assn study of 176 British BD cases and 183 controls of HTR2A SNPs. Trend twd assn w/ C516T \( p=0.06 \).

viii. (-) Mahieu et al. (1997): assn study of 83 CAU BD cases and 129 controls of HTR2A T102C SNP: no evidence of assn \( p=1.0 \).

ix. (-) Gutiérrez et al. (1997): studied four HTR2A polymorphisms in BD in 88 BD patients and 113 healthy controls and reports no significant assn between any of the polymorphisms and BD (whether tested individually or as haplotypes).

x. (-) Vincent et al. (1999): assn study of HTR2A in 103 BD patients and 103 controls. Identified a significant assn w/ the C102 SNP and BD \( p=0.07 \); OR:1.79; \( \chi^2 \) (2)= 9.58 but unable to replicate the assn in an independent sample of 109 BD and 109 controls \( p=0.47; \chi^2 \) (2)= 1.51.

xi. Tut et al. (2000): assn study of 72 Chinese BD cases and 74 controls: no assn between HTR2A SNP T102C and BD \( p=0.93 \).

xii. (-) Massat et al. (2000): case-control: 309 BD and 309 matched controls. No significant assn w/ BD and HTR2A SNP T102C.

xiii. (-) Blairy et al. (2000): investigated genetic contribution of T102C to BD in 40 BD and 89 normal subjects using genotyping. Concluded that the results exclude a major effect of the SNP on BD, though given limited sample size can’t exclude minor effect.

xiv. (-) Levinson (2005): meta-analysis of genetic linkage and association of pooled OR of HTR2A T102C SNP supported an assn to SZ but not to BD.

xv. (-) De Luca et al. (2007): family trio study of HTR2A T102C in BD and SZ: no assn with either. Report their data “essentially exclude[s] imprinting at this locus as a potential explanation for the complex inheritance observed in major psychosis.”

xvi. (-) Shaikh et al. (2007): case-control and family based assn study of 203 patients to 203 matched controls and analyzed 448 families for an association between HTR2A and HTR1B SNPs and childhood-onset mood disorder. Both case-control and family based assessments showed no association between the childhood-onset mood disorders and the SNPs.


xviii. (-) Abdolmaleky et al (2011): studied HTR2A gene expression and methylation patterns in postmortem brains of 35 SZ, 35 BD and 35 controls. They found no significant difference in allelic frequencies for the T102C polymorphism between groups.
Epigenetic dysregulation of HTR2A has been suggested by one group (Abdolmaleky et al. (2011)) in postmortem brain; however, this has yet to be independently replicated. This group also published a study using HTR2A methylation status as a peripheral biomarker; however, they do not indicate whether the SZ and BZ patients were taking antipsychotics. This group (and others) have demonstrated that antipsychotics have an effect on the methylation status of HTR2A, thus this omission is particularly salient.

i. Abdolmaleky et al. (2011) postmortem findings: qPCR detected differences in HTR2A gene expression between the T102C genotypes with CC and TC having significantly greater HTR2A gene expression (regardless of disease status). When the TC heterozygotes were divided into SZ, BD and control groups, SZ and BD both had lower HTR2A gene expression than controls, though only the SZ group (and antipsychotic-free BD subgroup) was (were) significantly lower than the control group.

ii. Ghadirivasfi et al. (2011) (last author: Abdolmaleky) peripheral biomarker findings: “bisulfite sequencing was used to screen DNA methylation status of the HTR2A promoter CpGs and qMSP was used to quantify the degree of cytosine methylation at differentially methylated sites. Most of the cytosines of the HTR2A promoter were unmethylated. However, CpGs of the -1438A/G polymorphism site, -1420 and -1223 were >95% methylated. The CpG at T102C polymorphic site and neighboring CpGs were ≥70% methylated both in the patients and controls. qMSP analysis revealed that the cytosine of the T102C polymorphic site was significantly hypo-methylated in SCZ, BD, and first-degree relatives compared to controls.”

Similarly, the evidence for epigenetic dysregulation HTR1A is preliminary.

i. Carrard et al. (2011): 58 BD, 40 SZ and 67 control subjects were compared with regard to methylation status of the promoter region of HTR1A in blood. Found increased methylation in BD and SZ relative to controls.

ii. López-Figueroa et al. (2004): postmortem brain study of MDD, BD and SZ (15 subjects per group). HTR1A mRNA expression differed significantly according to diagnosis. In DLPC: BD and MDD had significant decrease in HTR1A mRNA relative to controls. In the hippocampus: BD and SZ had significantly increased HTR1B mRNA and decreased HTR2A mRNA.

iii. Burnett et al. (1996): postmortem brain study in SZ: DLPFC: no significant change in HTR1A expression relative to controls. PFC: HTR2A mRNA levels were significantly lower than controls.

6. “In discussion of biomarkers, there is some imbalance and selectivity in the topic discussed - measurement of CSF metabolites is not currently a realistic acceptable approach for clinical biomarker determination.”

We appreciate this feedback and acknowledge that there is bound to be selectivity in a review that is not comprehensive. However, we have decided that to continue to feature the biomarker examples as written.
7. “p22 BDNF discussion of serum vs plasma is not clear - the authors continue to mention "blood" levels.”
   • This has been clarified in the text.

8. “p24 there is surprising focus on how the amount of alcohol intake is a correlate of alcoholism. It is hard to take a valuable message from this point.”
   • This section dealing with substance dependence has been removed.

9. “The authors discuss with clarity and depth the issue of environmental factors.”
   • We have not changed the environmental section in this revision.

10. “Much of the discussion around genetic correlates of treatment - which comes closer to what many think of as personalised medicine - is variable in its depth of coverage. There is a lot on MDD response, but little (perhaps rightly) on antidepressant side effects. In contrast in schizophrenia, the authors start by addressing genetics of TD, not a major problem for patients starting on current therapies, and then mention weight gain pharmacogenetics. This has been reviewed extensively in several recent reviews, and involves far more than 5-HT2C and leptin gene polymorphisms which have been frequently replicated, not withstanding some failures.”
    • Please refer to comments above re: examples vs. comprehensive coverage of all of personalized medicine.
    • “The authors are incorrect in stating that "the T-allele previously reported to be protective against weight gain was demonstrated to be associated with weight gain ... was subsequently supported by a meta-analysis".”
      i. This statement has been removed from the text.
    • “They also incorrectly imply weight gain to be a class effect of second generation drugs - early drugs such as chlorpromazine show weight gain.”
      i. The text has now reflects that weight gain is a side effect of several of the second-generation antipsychotics. Chlorpromazine has also been listed as potentially contributing to weight gain.

11. “Other than the results from the CATIE study, the authors only address dopamine D2 and D3 polymorphisms in schizophrenia symptom response, yet many other (and some replicated) candidate genes have been shown to have associations with response.”
    • We have included discussion of current findings in KCNH2 and atypical antipsychotic treatment response.

12. “In the "emerging applications" section it would be valuable to acknowledge that there are already some gene-environment interaction studies yielding results in treatment response.”
    • We have added some specific examples of gene-environment interaction studies that are yielding results in treatment response.
“...the authors have lost both depth (in many sections) and balance. This might be acceptable if they were to indicate what is being used solely as an example, acknowledging their lack of comprehensive coverage. Better, however, would be to focus on a component of this work - fewer disorders, perhaps, or the HPA axis

o We have adjusted the manuscript to strike a better balance between breadth and depth. Additionally, there are now several references to the work as an illustration/sample/selection of a larger body of work.

o We appreciate the suggestion to focus on a component of this work and believe a current and focused manuscript on the HPA axis would be a valuable addition to dialogue in the field. However, the subject matter for this invited review (“generalised review on personalized medicine”) is broader in its intended scope.