Author’s response to reviews

Title: Family-based clusters of cognitive test performance in familial schizophrenia

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Version: 3 Date: 11 Jun 2004

PDF covering letter
Dear Editor,

Below is the point-by-point description of the changes we made to our manuscript in response to the comments of the referees.

Sincerely yours,

Response to the referees comments

Irving I. Gottesman

COMPULSORY

1. Given the focus on methodological innovations using sophisticated clustering techniques and multidimensional scaling, a more appropriate journal with a more appreciative audience may be Psychometrika or Biometrika.

We agree that journals like those Prof. Gottesman suggests might have been appropriate for the present paper. However, we chose to submit the paper to the BMC Psychiatry, because the results of the study should be easily accessible for the readers of psychiatric genetics and general psychiatry. We find that in addition to methodological innovations, our paper adds to psychiatric literature by presenting the cluster solution, which clearly distinguishes subgroups of schizophrenia families in their cognitive functioning.

2. Absent a normal control group, there seems to be no way to talk about the specificity of your findings for schizophrenia. That is, your results may be true of normal families with respect to these same neuropsychological variables. A discussion of this point could show the importance of your findings for all families. Discuss the implications of NOT having a normal control group.

The following text has now been added to the Discussion, on page 12:

In the absence of a control sample, the present study could not test the possibility that the same clustering solution would emerge in normal families from the population. However, to our knowledge, such family clustering studies have not been conducted. In a study by Horan and Goldstein (ref 33), a cluster analysis was conducted both in a patient and in a non-psychotic patient control group. The clustering solutions in these groups did not resemble each other, suggesting a specific pattern in the schizophrenic population. It is known that family members of schizophrenia patients tend to perform worse than subjects from control populations (refs 1,2,3), and particularly those in multiply affected families (ref 32). Indeed, the aim of the present study was to explore
the clustering of families in multiply affected families with schizophrenia. Thus the generalizability of the results may be limited to such samples representing one fifth of all schizophrenia cases (ref 34).

Another place where the lack of control group is now taken into account is the text on page 11, in the part of the Discussion that refers to Palmer et al. on the possibility of a group of schizophrenia patients with no observed global impairment in cognition.

3. Among the suggested criteria for an endophenotype (Gottesman & Gould, 2003) are their demonstrable heritability via twin and family studies and the power of them to distinguish between schizophrenics and normals. Heinrichs (2001, Oxford U. Press) in his book "In Search of Madness--Schizophrenia and Neuroscience" provides important meta-analyses of relevant variables showing that most have low power (small effect sizes) to distinguish between patients and controls. Please discuss your selected variables in this light.

We are aware that the issue of cognitive endophenotypes is a complex one, and too straightforward arguments for them may not be warranted. Our starting point in the present study was to include traits that have been found to be heritable and distinguish patients from their unaffected relatives, who in turn differ from normal controls. We have previously demonstrated in a family study that many of the cognitive traits we use in the clustering analysis do show moderately significant heritabilities (Tuulio-Henriksson et al. 2002). Furthermore, a twin study (Cannon et al. 2000) showed that there is a strong genetic component again in some of the functions derived from the same neuropsychological tests we apply, and also that the unaffected co-twins showed impairments compared with controls. Although we can not argue that all the traits we use are valid endophenotypes for schizophrenia, many of them are (please see also answer 1 to Prof. Leboyer).

We have added discussion on these issues on page 11.

We tested the differences between the affected and unaffected individuals, and in all clusters, the individuals without any psychiatric diagnosis performed better than the affected subjects. This was true both in the whole sample of 54 families, and in the clusters. However, in the intermediate cluster, the differences were not always significant, although the patients’ level of performance was always below that of the unaffected individuals (data not shown).

4. Please comment on your inclusion of cases with a diagnosis of schizophreniform disorder. They are likely to add noise to the data, although given your cases being familial may be an exception. Is the diagnosis given after 6 months of follow without any change of diagnosis being considered?

The DSM-IV diagnoses of all patients were assigned based on 1) case note records from the whole illness history, 2) OPCRIT, and 3) SCID-interview. If any of these three sources showed that a diagnosis of schizophrenia could have been considered (or assigned) at any point of time, then the final research diagnosis was schizophrenia. Thus the patients with schizophreniform disorder can be considered true cases with this disorder. We agree with Prof. Gottesman that the 10 cases with schizophreniform disorder may add noise. On the other hand, each family contained at least one patient
with pure schizophrenia, which implies that on family level, the sample represents this disorder.

This has now been more emphasized in the Methods/subjects subsection, on pages 3-4.

MINOR

5. OPCRIT is the usual designation for what you call OCCPI.

It seems that many researchers nowadays use "OCCPI" instead of "OPCRIT", which is, in fact the designation used in the article that we refer to. We have now returned the original designation OPCRIT.

DISCRETIONARY

6. Your text gives the impression that the term and use of endophenotype is a recent development. It traces back to 1972 when it was introduced into psychopathology by Gottesman & Shields in their book "Schizophrenia and Genetics". Further, the term is not restricted to psychological test data, but can be used with imaging and biochemistry etc.

We have now added the 1972 reference in the Background section.

7. Your findings are supported by a number of other investigators should you choose to add them: e.g. Braff, Greene, Erlenmeyer-Kimling, Leonard & Freedman, etc

We are aware of the most invaluable work done by the authors Prof. Gottesman mentions. However, to restrict the reference list, we have added only one paper by Freedman, Adler and Leonard (Biol Psych 1999), which is closest to the subject matter of the present study (ref 6).

8. You may wish to point out that a very large proportion of cases of schizophrenia DO NOT have a positive family history, some 81% do not have an affected parent or sibling.

We have added a sentence on this issue in the Discussion, on page 12, where we discuss the generalizability of our results.

Marion Leboyer

COMPULSORY

1. Could the authors explain why they consider the affected and unaffected individuals as an homogeneous population. This strategy would eventually be fruitful
if the tests used here had previously been demonstrated as being endophenotypes which is not the case for any of the neuropsychological tests used here.

We do not, a priori, consider that the affected and unaffected individuals represent a homogeneous population. In fact, the aim of our study was to explore whether the clustering process could identify such homogeneity among the sample of patients and their first-degree relatives, and whether this homogeneity would be present in definable groups of families. The clustering solution could have been quite different, for example five small clusters, just one cluster, or no clusters.

We agree with Prof. Leboyer that some of the tests we used are not enophenotypic traits in schizophrenia (please see also answer 3 to Prof. Gottesman). This is the case particularly with the variables derived from the WAIS. However, all other tests (or rather the functions they measure), well represent such methods. We have measured attention, working memory, verbal memory, visual memory, and executive function, all of which have been considered important endophenotypic functions in previous literature. Based on this, we had a reasonable rationale to expect some proximity in the test scores between the patients and their healthy first-degree relatives. We included the WAIS ability functions to partly represent such relevant other variables that may have a direct effect on performance in the endophenotypic traits, and which correlate with e.g. education.

We have now added discussion on this issue on page 11.

2. Could the authors explain what were the reasons to choose these tests. They only specify the test-retest stability which has been assessed in general population, but not in a psychiatric population. Also, on page 8, it is specified that more neuropsychological test variables have been added: what are these variables and why they have been discarded.

Administering the neuropsychological test battery lasted approximately 2½-3 hours for each individual. As we only had a single session with the subjects, including both the interview and testing, it was unavoidable that some subjects could not complete the whole battery, which resulted in missing values in some test methods. These were, e.g. TRAILS and Stroop, which were not included in the present study because of too many missing cases (about 30%). In addition, the CVLT produces a large number of variables, but only three of them were included here, as they were considered to best represent the function of verbal learning (total recall in five subsequent trials, using a learning strategy=semantic clustering, and recognition memory).

We have omitted the sentence "planned to be extensive" from the original page 8. It gave a slightly wrong impression of the test battery.

3. The cluster analysis used here is new for psychiatry genetics and probably unknown to most psychiatrists readers. Thus, this method should be more clearly described. Concerning the method, it is not explained, why gender and age are variables included in the cluster analysis and not other potentially relevant variables (clinical characteristics, treatment, education etc.). It is also awkward to note that to normalize data, mean and SD have been used although it is widely demonstrated that schizophrenic subjects and normals have very different results to neuropsychological
data. It is also difficult to understand how the variable sex has been normalized since its is a binary variable.

We have clarified the explanation of the clustering procedure by adding a figure (new Figure 1) together with associated text that describe the idea in a concrete fashion. The visualization part of the procedure has also been improved, e.g. by employing a dendrogram to illustrate the step-wise formation of the clusters. Please see also our response to Prof. Horan in this regard.

The basic idea was to perform clustering using the neuropsychological test variables and not let the clinical and treatment characteristics affect the results. While the number of education years per se were not used in the clustering process they do affect the clustering solution through the WAIS ability functions included among the test variables (see also the response to item 1). As the basic units in clustering are families which usually include members of both sex and of various age, it was not surprising that no significant differences were found between the clusters in this respect. Also, as mentioned in the results section, removing the age and sex variables from the clustering procedure had only minor effects on the clustering solution.

A variable was normalized by computing its mean and SD over all subjects, normal or schizophrenic. In a distance based clustering process it is important that the variables have approximately similar magnitudes. Otherwise the solution could be dominated simply by those variables with the largest unnormalized (absolute) values rather than by the variables with the highest clustering potential. For the same reason it is also important to rescale any binary variables. This motivation for normalization is now mentioned in the text.

4. It would have been interesting to describe which neuropsychological tests differentiate best the different clusters as for further genetic analysis, it would save time to use a restricted battery.

Tables 3 and 4 give the overall differences between the tests. In addition, we calculated the effect sizes to examine which tests best differentiate the clusters in the present study. Between all clusters, tests measuring verbal learning and memory (CVLT), long-term verbal memory (WMS-R Story), visual working memory (WMS-R Visual Span), and verbal fluency were the best discriminators. Between the good and impaired cluster, effect sizes for these tests were over 1.0, between the good and the intermediate 0.7, and between the poor and intermediate 0.5-0.7, thus reflecting rather good effects. Although the functions that these methods assess are relevant for genetic analyses, better test methods may be available than those we have used, e.g. for assessing visual working memory.

We did not include the effect size analyses in the paper. The present knowledge does not suggest specificity to e.g. discriminate schizophrenia from bipolar disorder with any neuropsychological test method. Besides, other endophenotypic traits, such as eye movements, or certain neurophysiological measures (e.g. P300), may eventually be more valid than the complex cognitive variables.

William Horan
COMPULSORY

Topic 1. Identification of meaningful subgroups of families with schizophrenia

1. The authors provide a reasonable rationale for why the identification of more homogeneous subgroups of families would benefit genetic studies of schizophrenia. However, a compelling case that three valid, meaningful subgroups that are not merely arbitrary demarcations on a continuum of severity of cognitive impairment is not provided. The fact that the cluster algorithm identified three clusters only partly supports the validity of these putative subgroups. The clusters demonstrate hardly any differences on the clinical variables examined. The authors do not discuss whether the clusters demonstrate any meaningful differences in their patterns of cognitive impairment, aside from overall severity of cognitive impairment. If I understand the results correctly, the 3 clusters account for only 27% of the total variation in the neuropsychological performance.

One interesting difference in clinical variables between the clusters was that, unlike the well-performing and the intermediate cluster, the poor cluster included no patients with bipolar or other affective psychoses. This was not sufficiently emphasized, and we have now given more information on this in the section describing the demographic and clinical variables (page 9). We also discuss this on page 10.

The emerging of the three separate clusters was the product of a complex clustering algorithm applied to set of families. It is not clear how the families could be placed on a continuum with respect to their cognitive impairment without such a mathematical procedure based on distances between the family members. Given the multidimensionality of the situation, it was by no means clear that a relatively simple overall cognitive ordering for the clusters would emerge.

The 27% is not the percentage of variance explained by the clusters. It is simply the percentage of variance accounted for the two principal component directions used in projecting the 19-dimensional space onto the two dimensional display of Figure 4.

2. The approach of using families, rather than individuals, in a cluster analysis is novel in schizophrenia research and seems potentially informative. However, might there be potential downsides to lumping affected and unaffected relatives into a single cluster analysis? For example, might the inclusion of unaffected siblings, who presumably have minimal cognitive deficits, merely add "noise" in the identifications of patient subgroups that differ in patterns as well as level of cognitive impairment? If the three identified subgroups are indeed meaningful, would one also expect a similar structure to emerge in cluster analyses conducted separately for affected and unaffected subjects?

Our specific and explicit interest was to explore whether it is possible to detect separate and definable clusters from a sample comprising both schizophrenia patients and their family members. We have added discussion on this on page 12, and added a reference by Horan and Goldstein, 2003 (ref 33).
3. The description of the affected and unaffected cases on p. 4 is quite cursory. What were the overall exclusion criteria (e.g. neurological conditions, substance use)? What was the patients’ symptom status at the time of testing? It is stated that the final sample includes 165 subjects with a psychiatric diagnosis - I don’t get the same total - 82 schizophrenia + 13 schizoaffective + 12 bipolar + 48 non-psychotic. Were there more family members with psychiatric diagnoses? P. 10 refers to subjects with "affective psychotic disorders" - does this refer to bipolar subjects?

We regret having used both letters and numbers when describing the material: the 10 subjects suffering from schizophreniform disorder were referred to as "ten" and thus it was difficult to detect them from the text. Affective psychotic disorders refers to both psychotic bipolar and psychotic unipolar disorders.

We have now better described the material and given the reasons for excluding cases on pages 3-4.

4. Were the unaffected siblings screened for schizophrenia spectrum disorders (e.g. paranoid, schizotypal, schizoid)? There seems to be a fairly high prevalence of other psychiatric disorders in unaffected relatives. Might any cognitive deficits in these "unaffected" relatives reflect diagnosable spectrum disorders or other Axis 1 disorders rather than latent vulnerability to schizophrenia?

All subjects without a consensus diagnosis of a psychotic disorder (please see answer 4 to Prof. Gottesman) were screened with the SCID-II for personality disorders. SCID-II was missing in the original manuscript and has now been added on the Methods/Subjects section, on page 3. Only those individuals to whom no present or life-time psychiatric diagnosis was assigned were considered unaffected.

5. The characteristics of this particular sample (i.e. multiply affected siblings) are unique. While the authors mention this as a potential limitation, the generalizability of these findings seems highly questionable.

We agree that the generalizability of our results is limited, and have emphasized it more on pages 11 and 12. Please see also answer 2 to Prof. Gottesman.

6. In the Discussion section (p. 10, paragraph 1), the authors suggest that the severely impaired subgroup would be the most informative in genetic analyses. Further explanation of the logic here would be helpful. If the clusters each identify meaningful schizophrenia subgroups, why would one particular cluster be more appropriate or promising? The final sentence of this paragraph ("In our analysis...") is difficult to follow.

We agree with Prof. Horan that this statement is not well rationalized. We have now omitted this claim, and also formulated better the idea in the unclear sentence (page 10).

Topic 2. The introduction of a new clustering algorithm

7. My main comment on this section is that the description of the procedure that determines the degree of similarity/dissimilarity within each family is far too brief (p.
6) - pairwise test performance differences between the family members are only briefly mentioned on p. 7. This within-family index of similarity seems to be the main contribution of the new procedure - significantly more detailed explanation would increase the potential usefulness of this procedure for other researchers.

In fact, the within-family distance is irrelevant for the clustering process. It only affects the ordering of the family members in the data image of the clusters (old Figure 1 A). To avoid misunderstanding, we do not any more pay attention to the within-family order so this part of the clustering algorithm description has also been removed.

8. Much of the description of the visualization procedure appears in the figure captions. I would suggest providing a more comprehensive, sequential description of the cluster and visualization procedures within the methods section that guides the reader through the process. I wonder whether a psychiatric journal is the most appropriate medium for a full description of the cluster analysis and visualization procedure.

The visualization part of the clustering procedure has been improved. The dendrogram, a standard tool in cluster analysis, is now used to visualize the stepwise formation of the clusters. The detailed description of the algorithm has also been moved from the figure captions to the main text. See also the response to the item 3 of Prof. Leboyer.

As new visually enhanced data mining and clustering techniques are increasingly being used also in the analysis of biomedical data, we believe that it is useful to introduce them to the psychiatric research community as well.

9. A third figure (scatter diagram) is included but is not discussed in the text.

This figure was in fact discussed in the text. In the revised manuscript the discussion is on pages 8 and 9.