**In Silico Whole Genome Association Scan for Murine Prepulse Inhibition**

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**Abstract**

**Background:** The complex trait of prepulse inhibition (PPI) is a sensory gating measure related to schizophrenia and can be measured in mice. Large-scale public repositories of inbred mouse strain genotypes and phenotypes such as PPI can be used to detect Quantitative Trait Loci (QTLs) in silico. However, the method has been criticized for issues including insufficient number of strains, not controlling for false discoveries, the complex haplotype structure of inbred mice, and failing to account for genotypic and phenotypic subgroups.

**Methodology/Principal Findings:** We have implemented a method that addresses these issues by incorporating phylogenetic analyses, multilevel regression with mixed effects, and false discovery rate (FDR) control. A genome-wide scan for PPI was conducted using over 17,000 single nucleotide polymorphisms (SNPs) in 37 strains phenotyped. Eighty-nine SNPs were significant at a false discovery rate (FDR) of 5%. After accounting for long-range linkage disequilibrium, we found 3 independent QTLs located on murine chromosomes 1 and 13. One of the PPI positives corresponds to a region of human chromosome 6p which includes DTNBP1, a gene implicated in schizophrenia. Another region includes the gene Tsn which alters PPI when knocked out. These genes also appear to have correlated expression with PPI.

**Conclusions/Significance:** These results support the usefulness of using an improved in silico mapping method to identify QTLs for complex traits such as PPI which can be then be used for to help identify loci influencing schizophrenia in humans.

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**Introduction**

Traditional approaches for mapping quantitative trait loci (QTLs) in mice usually involve crossing two strains that differ in a trait of interest, followed by phenotyping and genotyping a large number of the resulting progeny. The chromosomal regions identified with this approach are large, typically 20–40 cM [1], and further work is therefore needed to pinpoint the specific gene(s) and causal mutation(s) responsible for the QTL effect. The whole process is expensive and may require many years of study. However, databases have recently been created that contain data from large scale genotyping projects involving many common inbred mouse strains. Combining this data with phenotypic information on the same strains creates the opportunity to map QTLs “in silico” [2]. Since all mice from an inbred strain are genetically identical and homozygous, genotyping need only occur once and their haplotypes can be derived unambiguously from their genotypes. Once the phenotype is known one can 1) group the mice from strains with similar genotypes and then 2) test for the phenotypic differences between the mice with the different genotypes. By repeating this for a genome-wide panel of markers, a whole-genome scan can be performed in silico for detecting haplotypes that harbor variants influencing the trait.

There seems little doubt that in silico scans are useful to detect highly penetrant mutations [3–5] and a number of successful examples can be found in the literature [6–8]. However, the utility of this method for finding QTLs for complex traits is more controversial. Some criticisms involve the specific execution of the method, such as the use of a very small number of inbred strains, or insufficient control of false discoveries due to multiple testing problems [9,10]. In principle, these criticisms can be easily addressed, by increasing the number of strains in the analysis or using new and more powerful methods to control false discoveries [11]. Other criticisms may be more fundamental, such as difficulties arising from the complex haplotype structure of inbred mice or the risk of false discoveries due to the presence of genotypic and phenotypic subgroups of mouse strains [12]. Other fast and cost-effective in silico methods exist, such as using a panel of recombinant inbred lines derived from only 2 parental inbred lines [13–15] that are much less affected by these phenomena.
However, it may be premature to discard in silico mapping of QTLs for complex traits using common inbred strains. The method is new and at least some of the criticisms may be addressed by further developing our analytical strategies. For example, the lack of randomness in breeding histories of inbred strains in combination with the fact that subgroups may also differ phenotypically can create spurious associations. That is, all genetic differences between the subgroups will tend to be associated with the phenotype and there would be no possibility to distinguish true and spurious associations. In human studies, an analogous issue is population stratification, which is a great concern. In samples containing subjects with multiple ancestries, this must be addressed using appropriate statistical controls [16]. Otherwise, tens of thousands or markers will appear significant in the genome-wide association studies using up to one million genetic markers. Approaches to control for stratification include using of self-report of ancestry or genetically derived principle components in the analysis. For studies using inbred mouse lines, a cladogram which is a hierarchical grouping based on phylogenetic analysis of strain relatedness can be created to subdivide inbred strains into more genetically homogenous subgroups. By testing whether or not haplotypes are associated with the phenotypes within these cladistic subgroups which are akin to branches in the tree, we reduce the risk of false positives. This is because genetic variation is now related to deviations from the subgroup mean, so that phenotypic differences between strains are no longer necessarily associated with genetic differences between strains.

If we assume that the methodological problems can be addressed, in silico scans do have a number of potential advantages. First, since the average ancestral segment length among classical inbred strains has been estimated to be 1.0–1.5 megabases (Mb) in size, the resolution is relatively good in comparison to traditional QTL mapping methods. Second, the costs for many common inbred mouse lines are relatively low in comparison to recombinant inbred lines. Third, the amount of phenotypic and genotypic information on common inbred strains is increasing rapidly. Examples of freely available repositories such as the Mouse Phenome Database (MPD - [17]) and WebQTL continue to grow. These resources include not only strain phenotypes but also genotypes from large scale projects that have recently been completed (http://www.well.ox.ac.uk/mouse/INBREDS/) and others in progress. The availability of all this information has the potential to produce novel results with the only cost being analysis time. Finally, the presence of multiple founder lines as well as wild derived inbred strains can be advantageous. First, there is more genetic and phenotypic diversity when many strains are used. Therefore the potential to detect more causal variants is increased. In mapping using F2 crosses of only two strains, much of the variation that is present in the population from which the two lines were drawn is excluded and not detectable. Although the use of multiple founder lines introduces more alleles and decreases relative effect size, the process is much more analogous to human association mapping. Therefore, the results may also be more generalizable across lines and perhaps species. This is important because the generally accepted eventual goal of using model organisms is to generalize the knowledge to humans. Results such as these can be used in cross species data integration[18] which can lead to the identification of novel associations in humans[19]. Although clearly of potential utility, in silico scans alone will probably not be able to identify the actual causal variants. Instead they may better be viewed as part of a fast and inexpensive method to identify and prioritize complex-trait candidate genes without requiring the construction of sub(congenic mouse strains [8]. The likely outcome of an in silico scan is a number of small chromosomal regions that contain causal variants. Existing databases can then be used to identify the candidate genes in the regions and look for corroborating evidence. Furthermore, other “omic” platforms (e.g. expression arrays) could be use to further reduce the list of candidate genes and refine the region [20].

In this study we performed an in silico scan using phenotypic data generated by Willott and colleagues [21] for the complex trait prepulse inhibition (PPI), a sensory gating measure thought to be related to schizophrenia. A recent review cited 13 different studies that found PPI deficits in schizophrenic patients [22]. PPI is also variable and heritable in humans with and without psychiatric diagnoses [23,24] and in model organisms. Deficits in PPI can be induced pharmacologically and reversed with antipsychotics [25]. PPI has also been the subject of phenotypic characterization[26,27] and QTL mapping efforts in rodents [28–35]. QTLs identified in silico were compared against evidence from a variety of sources including previous mouse PPI QTLs, meta-analysis of human schizophrenia genome scans, and microarray experiments in an attempt to find convergent or consistent patterns of evidence.

Results

In silico scan

Our base model was a 2-level model where mice were nested in strains with sex and clade membership included as covariates. SNPs were added to this base model and tests performed to examine whether this significantly improved model fit. Figure 1 plots the p-values for all SNPs across the mouse genome. The conservative “lowest slope” method (Hsueh et al., 2003) estimated the proportion of true null hypotheses to be 0.991017. Using this estimate, we found 89 significant SNPs when the FDR was controlled at the 0.05 level. Because of the large number of tests, this means that the estimated proportion of false discoveries among the 89 significant tests was 5%. The number of significant SNPs dropped noticeably from 89 to 20 when the FDR was controlled at the 0.045 rather than 0.05 level, which corresponded with a threshold p-value of 3.0e-5. We focused these SNPs in order to have tractable number of results to interpret. The full list of results satisfying a FDR of 5% are contained in Table S1.

The top 20 significant SNPs map to 8 regions with 5 isolated SNPs, 2 clusters of 3 to 4 SNPs, and one cluster of 8 SNPs. Details are contained in Table 1. Examination of linkage disequilibrium (LD) using $r^2$ between the top 20 markers revealed meaningful LD (<1% for genome-wide marker-marker $r^2$) between many of the markers pairs including those on different chromosomes. Table 2 contains these results and shows that within the set of 20 there are three sets of mirror markers containing 7, 3, and 2 markers. However, they all contain at least one marker from the cluster of positive markers on chromosome 1 between 113.9–118.9 Mb. SNP and gene positions are based on the May 2004 assembly (Build33) of the mouse genome at the UCSC Genome Browser [36]. After examining the $r^2$ for each of the top 20 markers with every other marker in the genome and the 2 marker association results within each mirror (data not shown), we believe that the most parsimonious explanation of the pattern of results is that the cluster on chromosome 1 is origin of the mirrors. After accounting for mirrors that reflect the cluster of significant markers on chromosome 1, only 2 additional independent signals remain and are rs3698264 (chromosome 1, 79.9 Mb, index 575) and rs3724682 (chromosome 13, 46.8 Mb, index 12594).

Support for results

The identified QTLs were compared with a variety of sources including previous mouse PPI QTLs, meta-analysis of human...
schizophrenia genome scans, and microarray experiments. The mouse/human chain track within the genome browser was used to compare regions homologous between mouse and human genomes [37,38].

Replication of previous mouse QTLs

There are previous studies attempting to map QTLs for PPI in mice. Joober and colleagues provisionally mapped PPI QTLs using recombinant congenic strains based on inbred lines C57BL/6J and A/J [29]. For auditory PPI, they initially reported 7 QTLs common across all acoustic intensities studied and an additional 25 loci linked to at least one acoustic intensity for a total of 32 provisional loci. However, the analytical methodology was criticized [39] and a more appropriate analysis showed a more modest list of significant loci which included chromosomes 2, 3, 5, 7, 11, and 16 [40]. The results from chromosome 16 have been investigated further by Petryshen [31] who performed QTL mapping by intercrossing chromosome substitution strains (CSS). The parental CSSs carried an A/J chromosome 16 on a C57BL/6J background. The 2 initial QTL intervals described by Joober and colleagues on 16 were confirmed and the interval narrowed.

We do not believe our results robustly replicate any reported QTL on 16. Joober et al. have since expanded upon their auditory work using tactile PPI which didn’t replicate their auditory PPI results[35].

PPI QTL mapping has also been performed using an F2 cross of C57BL/6 and C3H/He lines and identified a PPI locus at the Fabp7 gene [33] on chromosome 10. We did not detect any significant markers in the region of Fabp7. Watanabe et al. also reported provisional QTLs on chromosomes 1, 3, 7, 11, and 13. However, the sizes of the linked regions were not reported and therefore any overlap with loci on chromosomes 1, 11, and 13 in the current study could not be compared directly.

Finally, Hitzemann and colleagues have attempted to map PPI QTLs using selectively bred lines from a heterogeneous stock derived from four inbred lines including C57BL/6J, DBA/2J, BALB/cJ and LP/J[34]. This effort is the most analogous to the current study due to the use of multiple founder lines. However, the study was directed at previously implicated chromosomes 3, 11, and 16. The signal we detected at rs6299418 on chromosome 11 is consistent with the interval reported by Hitzemann et al [34].

Loci for human schizophrenia

We examined if the three independent in silico mouse PPI QTLs results mapped to the regions implicated by the [41] meta-analysis of human schizophrenia genome scans. The meta-analysis is a large study using 20 linkage scans with a total of 1,208 pedigrees and 2,945 affecteds. In the study, the genome was divided into 120 separate 30-cM bins. The top ten bins represented different regions comprising 300 cM or ~8% of the human genome. Four of the eight homologous mouse regions contained at least 1 significant SNP in our scan, when the FDR was controlled at the 0.05 level, including the signals on chromosomes 1 and 13.

On chromosome 1 in the area surrounding marker rs3674655 (p-value 8.14×10^-7, index814), which is homologous to human 2q14, the LD pattern shown in Figure 2 is irregular. There are no obvious places to define a boundary, even when attempting to use an arbitrary standard such as r^2 above a whole genome cut-off of one percentile. By examining individual haplotypes (data not shown), we estimate the core of the association signal extends at least 3 megabases (Mb) from rs13476069 (index 802, 115.9 Mb) to rs13476078 (index825, 118.9 Mb) but may extend as much as

Figure 1. Plot of p-values from PPI scan across mouse genome with corresponding FDR thresholds.
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5.7 Mb from mCV23695506 (index 792, 114.4 Mb) to rs3696498 (index 833, 120.1 Mb).

The 5.7 Mb interval includes genes Tsn, Inhbb, Railh, Epib4.115, Ptpn4, Sctr, Dbi, and Steap3, several of which are good functional candidates for PPI and schizophrenia. Ptpn4 (the protein tyrosine phosphatase, non-receptor type 4) interacts with glutamate receptors, Grid2, Pln, and Grin2a, and TRAX/DISC1 fusion protein [50]. Therefore, Tsn, Inhbb, Railh, Epib4.115, Ptpn4, Sctr, Dbi, and Steap3 are in LD with the SNPs showing association at either end. Also mapping to this interval is Cap2. The human homolog of this gene has been reported to show altered expression in human schizophrenic brain [61]. This interval was tentatively implicated by Joober et al. in their study of mouse PPI [29]. However, it was not one of the six chromosomes that remained after reanalysis [40].

The results for the third in silico QTL at rs3698264 (chromosome 1, 79.9 Mb, index 575, p-value 0.000034) appeared to be an isolated signal when only single marker analysis was considered. However, tests of sliding 2 marker windows showed additional evidence independent of rs3698264. In the 1 megabase interval surrounding but not including rs3698264, the p-values ranged from 0.045 to 0.000083. The most significant 2 marker result which includes rs3698264 is for rs3698264-rs8253473 (p-value 5.8×10⁻⁷) and defines an 89.5 kilobases (kb) interval containing part of the secretogranin II (Scg2) gene. Indeed, Scg2 also known as chromogranin C contains rs8253473. This marker defines the end of the most associated two marker haplotype in the genome and not just the region nearby. Scg2 is a plausible candidate for influencing PPI. Phencyclidine (PCP) modulates Scg2 expression in rats [62,63]. Genes responding to PCP are good candidates for schizophrenia since PCP produces effects similar to schizophrenia in humans. In model organisms, PCP creates PPI deficits that can be ameliorated with administration of atypical antipsychotics [25]. There are also positive human schizophrenia association studies with chromogranin B which is a closely related gene [64,65].

### Table 1. Chromosomal band, megabase (Mbp) location, and p-value of SNPs that are significant when the FDR is controlled at 0.045 level.

| Cluster | Marker       | Index | chr | Mbp   | Cytogenetic location | p-value |
|---------|--------------|-------|-----|-------|----------------------|---------|
| 1       | rs6404446    | 140   | 1   | 20.994482 | 1qA4 | 4.39E-06 |
| 1       | rs3716569    | 141   | 1   | 21.012714 | 1qA4 | 2.21E-06 |
| 1       | rs4222181    | 142   | 1   | 21.024567 | 1qA4 | 3.33E-06 |
| 2       | rs3698264    | 575   | 1   | 79.865390 | 1qC4 | 3.39E-05 |
| 3       | rs2686443    | 657   | 1   | 93.319361 | 1qD  | 4.86E-06 |
| 4       | rs3022830    | 802   | 1   | 115.928102 | 1qE2 | 1.78E-05 |
| 4       | rs3694226    | 811   | 1   | 117.143094 | 1qE2 | 4.39E-06 |
| 4       | rs3662732    | 813   | 1   | 117.337845 | 1qE2 | 1.20E-06 |
| 4       | rs3674655    | 814   | 1   | 117.379659 | 1qE2 | 8.14E-07 |
| 4       | CEL-1_117526378 | 816 | 1   | 117.526378 | 1qE2 | 8.14E-07 |
| 5       | rs6216134    | 820   | 1   | 118.236415 | 1qE2 | 1.29E-05 |
| 5       | rs3719973    | 824   | 1   | 118.834067 | 1qE2 | 1.29E-05 |
| 5       | rs13476078   | 825   | 1   | 119.302133 | 1qE2 | 2.67E-05 |
| 5       | rs6215373    | 5262  | 5   | 42.914749 | 5qB3 | 3.27E-05 |
| 5       | mCV22331571  | 5265  | 5   | 43.271643 | 5qB3 | 5.00E-05 |
| 5       | rs3669254    | 5266  | 5   | 43.347335 | 5qB3 | 5.59E-06 |
| 5       | rs3663092    | 5270  | 5   | 43.668813 | 5qB3 | 5.15E-06 |
| 6       | rs3691954    | 8102  | 8   | 21.206986 | 8qA2 | 4.09E-05 |
| 7       | rs6299418    | 11098 | 11  | 66.784499 | 11qB3 | 4.39E-06 |
| 8       | rs3724682    | 12594 | 13  | 46.810500 | 13qA5 | 4.75E-05 |

Index is the marker order across the genome and is used in subsequent tables and figures instead of marker name.

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### Table 2. LD pattern of 20 SNPs FDR <= 0.045.

| Marker             | Index# | 140 | 141 | 142 | 575 | 657 | 802 | 811 | 813 | 814 | 816 | 820 | 824 | 825 | 5262 | 5265 | 5266 | 5270 | 8102 | 11098 | 12594 |
|--------------------|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|------|------|------|-------|-------|
| rs6404446          | 140    | 1   | 1   | 1   | 0.05| 0.51| 0.62| 1   | 0.77| 0.77| 0.77| 0.51| 0.51| 0.77| 0.62| 0.51| 1    | 1    | 0.51  | 1     | 0.24  |
| rs3716569          | 141    | 1   | 1   | 1   | 0.06| 0.51| 0.62| 1   | 0.77| 0.77| 0.77| 0.51| 0.51| 0.77| 0.62| 0.51| 1    | 1    | 0.51  | 1     | 0.24  |
| rs4222181          | 142    | 1   | 1   | 1   | 0.05| 0.51| 0.62| 1   | 0.77| 0.77| 0.77| 0.51| 0.51| 0.77| 0.62| 0.51| 1    | 1    | 0.51  | 1     | 0.38  | 1     | 0.23  |
| rs3698264          | 575    | 0.33| 0.42| 0.38| 1   | 0.09| 0.1 | 0.05| 0.08| 0.06| 0.06| 0.09| 0.09| 0.08| 0.08| 0.09| 0.05| 0.06| 0.01 | 0.05 | 0.1   |
| rs6268443          | 657    | 1   | 1   | 1   | 0.33| 1   | 0.82| 0.51| 0.66| 0.66| 0.66| 0.67| 0.67| 0.37| 0.51| 0.67| 0.62| 0.51| 0.51 | 0.51 | 0.47  |
| rs302830           | 802    | 1   | 1   | 1   | 0.5 | 1   | 1   | 0.62| 0.81| 0.8  | 0.8  | 0.82| 0.82| 0.47| 0.63| 0.51| 0.62| 0.62| 0.47 | 0.62 | 0.39  |
| rs3694226          | 811    | 1   | 1   | 1   | 0.33| 1   | 1   | 1   | 0.77| 0.77| 0.77| 0.51| 0.51| 0.77| 0.62| 0.51| 1    | 1    | 0.51  | 1     | 0.24  |
| rs3662732          | 813    | 1   | 1   | 1   | 0.5 | 1   | 1   | 1   | 1   | 1   | 1   | 0.66| 0.66| 0.47| 0.8  | 0.37| 0.77| 0.63| 0.64 | 0.77 | 0.31  |
| rs3674655          | 814    | 1   | 1   | 1   | 0.33| 1   | 1   | 1   | 1   | 1   | 1   | 0.66| 0.66| 0.58| 0.8  | 0.37| 0.77| 0.77| 0.77 | 0.77 | 0.31  |
| CEL-1_117526378    | 816    | 1   | 1   | 1   | 0.33| 1   | 1   | 1   | 1   | 1   | 1   | 0.66| 0.66| 0.58| 0.8  | 0.37| 0.77| 0.77| 0.77 | 0.77 | 0.31  |
| rs6216134          | 820    | 1   | 1   | 1   | 0.33| 0.82| 1   | 1   | 1   | 1   | 1   | 0.66| 0.51| 0.4  | 0.51| 0.51| 0.51 | 0.51 | 0.51 | 0.51  |
| rs3719973          | 824    | 1   | 1   | 1   | 0.33| 0.82| 1   | 1   | 1   | 1   | 1   | 0.66| 0.51| 0.4  | 0.51| 0.51| 0.51 | 0.51 | 0.51 | 0.51  |
| rs13476078         | 825    | 1   | 1   | 1   | 0.5 | 0.74| 0.76| 1   | 0.76| 0.76| 0.76| 1   | 1   | 0.46| 0.37| 0.77| 0.78 | 0.22 | 0.77 | 0.15  |
| rs6215373          | 5262   | 1   | 1   | 1   | 0.33| 0.79| 0.79| 1   | 1   | 1   | 1   | 0.79| 0.79| 0.75| 1   | 0.27| 0.62| 0.62| 0.62 | 0.62 | 0.38  |
| mCV22331571        | 5265   | 1   | 1   | 1   | 0.33| 0.82| 0.79| 1   | 0.74| 0.74| 0.74| 0.63| 0.63| 0.74| 0.57| 1   | 0.62| 0.51| 0.24 | 0.51 | 0.47  |
| rs3669254          | 5266   | 1   | 1   | 1   | 0.35| 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 0.51 | 1    | 0.27  |
| rs3663092          | 5270   | 1   | 1   | 1   | 0.5 | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 0.51 | 1    | 0.31  | 1     | 0.24  |
| rs3691954          | 8102   | 0.71| 0.72| 0.71| 0.2 | 1   | 0.76| 0.71| 0.8 | 1   | 1   | 1   | 0.52| 1   | 0.68| 0.71| 0.7  | 1    | 0.51  | 0.24  |
| rs6299418          | 11098  | 1   | 1   | 1   | 0.33| 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 0.71 | 1    | 0.24  |
| rs3724682          | 12594  | 1   | 1   | 1   | 0.4 | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1    | 1    | 1     |      |

$r^2$ is above diagonal. D' is below diagonal.
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Consortium on M430 arrays using hippocampus tissue and
analyzed using the RMA method. Data was available for 12 of
the 40 lines with PPI data. We tested for correlation between PPI
and hippocampus expression of candidate genes selected from the
top 3 regions. Due to the small number of lines (n = 12) with both
PPI and expression information, we chose only to test microarray
probesets in a limited number of genes that we had prior evidence
for a relationship to schizophrenia or PPI. These genes were Scg2,
Dtnbp1, Cap2, and Tsn. Details of the results are shown in Table 3.
PPI was significantly correlated with gene expression for one of
two probesets in Cap2 (r = 0.6, p-value = 0.039) and approached
significance using at least one probeset in Dtnbp1 (p-value 0.1) and
Tsn (p-value 0.085). We performed exploratory analysis looking for
statistical interactions between gene expression levels and PPI
using linear regression and mixed models. We observed that
different probesets within the same gene such as with Tsn gave
different results. Although this may seem inconsistent, further
examination of the alignment of probes to gene revealed that
different probesets for Tsn aligned to different populations of
alternatively polyadenylated transcripts. Further analysis using
univariate mixed models revealed the same pattern results across
the probesets but with increased significance. A highly significant
interaction was detected between Dtnbp1 and Scg2 (p-value = 0.00024).
Although Tsn (mixed model p-value 0.052) and Cap2 (mixed model p-value 0.02) are significant when considered individually and together (mixed model p-value 0.009, based on
2df), they do not contribute significantly in the presence of the
Dtnbp1-Scg2 interaction.

Discussion

We found 89 SNPs that were likely to have real effects on PPI
(FDR<0.05). When we conservatively considered only the top 20
based on FDR and the distinct LD pattern of the inbred mouse
genome, these 20 collapsed into 3 probable distinct independent
regions. These 3 independently associated loci are likely to affect
PPI (FDR 0.045) including a 3 to 6 megabase interval on
chromosome 13 and two separate loci on chromosome 1. Next,
we showed that 2 of these regions correspond to regions implicated
in human linkage studies of schizophrenia. The region on
chromosome 13 that is implicated by both our in silico PPI analyses
and human linkage studies was also implicated in a provisional QTL
mapping study of PPI in mice by Joober et al [29] using
recombinant congenic strains. However, chromosome 13 did not
remain significant when a more appropriate analysis was conducted[40]. The gene Dtnbp1 is in the middle of this region. This is an
encouraging finding as several association and expression studies
suggest that the human homolog of Dtnbp1 is one of the strongest
candidates for schizophrenia [51–60]. In addition, the human
homolog of another gene in this region, Cap2, is reported to show
altered expression in schizophrenic brain [61].

The region on chromosome 1 that is implicated by both the in
silico PPI analyses and human linkage studies contains the genes
Tsn and Scg2. Tsn is directly implicated in mouse PPI as it is known
to alter PPI when knocked out [46] and Scg2 is PPI candidate due
to multiple lines of evidence [25,62–65]. Finally, we found that
hippocampus expression is at least suggestively significantly related
to PPI for all four genes Dtnbp1, Cap2, Tsn, Scg. Analyses of these
expression data also showed a highly significant relationship
between PPI and a statistical interaction between PPI and a statistical interaction between Dtnbp1 and Scg2.

In sum, results suggest that the in silico mapping of QTLs can be
improved and successfully adapted to help map loci for complex
traits. That is, the obtained results were supported by converging
evidence from a variety of sources including previous mouse PPI
QTLs, meta-analysis of human schizophrenia genome scans, and
microarray experiments. In silico scans have several attractive
properties such as the low costs of the mice, relatively more genetic
variation due to multiple ancestral strains, and public availability

Discussion

We found 89 SNPs that were likely to have real effects on PPI
(FDR<0.05). When we conservatively considered only the top 20
based on FDR and the distinct LD pattern of the inbred mouse
of genotype/phenotype information. This suggests that these scans can be a valuable addition to our method arsenal for mapping genetic variation affecting complex traits. Although the resolution is relatively good in comparison to traditional QTL mapping methods, the QTLs detected by the in silico methods still spanned 2–4 MB. However, we also demonstrated how public resources can be used to add weight to findings and identify specific candidates. As the amount and quality of information in public databases increases, we would expect this ability to refine the location of relevant genetic variation to improve in parallel. Finally, as the focus in the present study was to demonstrate the usefulness of the method, we focused on genes and loci for which there is already a considerable amount of evidence in the literature. However, this does not mean that the method cannot generate novel candidates and even in our case we expect that other previously less studied genes could affect schizophrenia and are performing association studies to follow up these leads.

**Materials and Methods**

**Sample and measurements**

To perform an in silico scan, we first matched PPI data for 37 different strains to 17,757 SNPs contained in the MPD. The 37 phenotyped strains with genotype information were from a study 40 strains and represented 805 individual inbred mice with approximately 10 animals of each sex per strain [21]. The majority (~13 k) of the SNP data came from the Wellcome-CTC Mouse Strain SNP Genotype Set (www.well.ox.ac.uk/rmott/MOUSE/INBREDS) and the remainder from a variety of other sources including dbSNP, the Jackson Lab [66], and The Scripps Research Institute [5]. The PPI variable analyzed was PPI total, which is a summary measure across three different acoustic startle frequencies (70 dB at 4, 12, and 20 kHz). Although other PPI

| Gene      | Probe | r     | adj r² | p-val |
|-----------|-------|-------|--------|-------|
| Cap2      | 1450910 | -0.14 | -0.08  | 0.67  |
| Cap2      | 1423222 | -0.60 | 0.30   | 0.039 |
| Dtnbp1    | 1431619 | -0.50 | 0.18   | 0.10  |
| Sgc2      | 1450708 | -0.47 | 0.14   | 0.12  |
| Tsn       | 1448516 | 0.52  | 0.19   | 0.085 |
| Tsn       | 1448515 | 0.40  | 0.08   | 0.19  |
| Tsn       | 1416908 | 0.20  | -0.05  | 0.53  |
| Tsn       | 1416907 | 0.20  | -0.06  | 0.53  |

| Gene expression microarray analysis method is RMA.
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variables were generated by Willott and colleagues, they argue that PPI total is the best measure for sensory gating. Ambiguous genotypes and heterozygotes were removed. One hundred seventy-three markers were found to be named duplicates and removed. A further 725 markers were removed from analysis because they were not polymorphic between our 37 selected strains. This left a total panel of 16,859 SNPs.

Phylogenetic analyses

The lack of randomness in the breeding history of inbred strains in combination with the fact that strain subgroups may also differ phenotypically can create spurious associations. Our approach to minimize such spurious findings mimics “within family” based analyses often applied in human association studies to avoid similar problems due to population stratification. By testing whether a locus within a family is associated with the outcome, spurious associations are avoided because all family members come from the same subpopulation. To define “families” of inbred strains of mice we estimated the phylogenetic relationship for 480 inbred strains of mice using the APE (Analyses of Phylogenetics and Evolution) extension to the R language (results not shown). Because an extensive search is impossible with as many as 15 k SNPs we used the maximum parsimony algorithm that minimizes the number of steps, or tree length, needed to account for the differences between strains. The main groupings we observed replicated those found by Petkov et al. who constructed a “family tree” for 102 strains using 1,638 SNPs [66]. Each of the 37 strains used in the current study were assigned to one of 7 possible phylogenetic subgroups or clades from our cladistic analysis. Family trees and cladograms are not synonymous since cladistics has its own set of rules for defining family trees. Therefore, not all hierarchical arrangements of strains are cladograms since cladograms reflect similarity and not descent. Details of the strains used and clade assignment are contained in Table S2.

Mixed/multilevel models

The tests between phenotype and genotype were performed using multilevel or mixed modeling [67,68]. Multilevel models are particularly suitable for analyzing samples with a hierarchical or clustered structure. Clustered data are present here because multiple animals from the same strain are assessed. The inclusion of SNPs as well as subgroup membership in multilevel models is straightforward [69]. Specifically for the current study, let \( \alpha \) be an overall constant, \( \sigma \) the effect of phylogenetic subgroup \( k \), \( g \) the effect of SNP \( j \), and \( r_{ik} \) a residual score of mouse \( i \) with genotype \( j \) from subgroup \( k \) consisting of the effects of other unlinked loci and environmental factors. The trait score \( x_{ijk} \) of mouse \( i \) with SNP \( j \) from subgroup \( k \) can then be written as: \( x_{ijk} = \alpha + \sigma g_k + r_{ijk} \). The statistical test will involve effect \( g_k \). Because \( g_k \) is the deviation from the subgroup mean it will only be significant if within the subgroup SNP \( j \) has an effect. Scripts were written and analyses performed in the R statistical environment. Specifically, the nlme package was used to perform mixed/multilevel model analysis. The maximum likelihood (ML) method was used instead of the default restricted maximum likelihood (REML) to be able to perform tests for fixed plus random effects in the model. Two times the difference between log-likelihoods of the model with and without genetic effect \( g_k \) is asymptotically chi-square distributed, with the difference in estimated parameters of the nested models as the degrees of freedom. After single marker analyses were completed, haplotype analyses with multiple markers were performed to determine risk haplotype and estimate the size of the associated region.

Control of false discoveries

In our in silico scan, the vast majority of the SNPs will not be associated with the dependent variables and this creates a considerable risk of false discoveries. In this article, we control the so-called false discovery rate [70,71]. Because of the large number of tests that are performed in this study, we can interpret the FDR as the proportion of false discoveries to the total discoveries we would on average introduce into the literature through this study. Alternatively, FDR can be interpreted as the probability that a randomly selected discovery from this study is false [71–73].

An important advantage of the FDR in this context is that it provides a better balance between finding true effects and controlling false discoveries compared to more traditional “family-wise” methods that control the probability of finding one or more false discoveries in the whole study (e.g. the single step Bonferroni correction). The problem is that family-wise error methods control exclusively to the risk of even a single false discovery. Because this risk is high in genome-wide scans, these studies will be heavily penalized via very small threshold p-values. As a result power will be low to detect genetic effects.

In addition to its pleasant interpretation, the FDR appears fairly robust against the effects of correlated tests in general [11,70,71,74–77] and the correlational structure of linkage disequilibrium (LD) studies in particular [78,79]. An intuitive explanation is that these methods use estimates of the ratio of false to total discoveries in a study. Correlated tests mainly increase the variance of these estimates. However, the FDR statistics themselves that are the means of these estimates tend to remain similar. To avoid that the FDR is controlled too conservatively we need to estimate the proportion of tests for which the null-hypothesis is true. For this purpose we used the “lowest slope” method, known to be conservatively biased toward one [80].

Defining the QTL interval

Inbred laboratory mouse strains originated from a mixed but limited founder population [81]. Although recombination breaks up chromosomes when they are passed on to the next generation, the number of generations that occurred before inbreeding was limited. As a result current inbred mouse strains share extensive haplotypes from their founder strains, causing LD or associations among markers that are close to each other on the mouse genome. Indeed, by typing a large set of SNPs in nine inbred strains, [3] found that for most of the chromosomal regions few (e.g. two) different founder haplotypes were observed. Thus, for each of the significant markers, the QTL interval needs to be defined. LD can extend over several Mb and we therefore included wild derived inbred strains that may not share the same ancestral haplotypes in order to achieve the greatest mapping resolution.

To determine how far out meaningful LD extended from highly significant SNPs, we first calculated the \( r^2 \) between the top SNPs and every other marker in the genome. The \( r^2 \)s were then ranked and the meaningful LD threshold was defined as being ranked in the one percentile. The ranking and threshold calculation was done separately for each of the top markers and showed that each marker has a different distribution of \( r^2 \) across the genome. Therefore the threshold \( r^2 \) for each marker was also different. Other rank thresholds were examined but the 0.01 level seemed to be the most useful in relation to the expected decay of LD as a function of physical distance. Physically nearby (<5 megabases) markers above the 0.01 rank threshold were considered to be in real LD and not imperfect mirrors (see below). Finally, in regions surrounding multiple associated SNPs, all marker to marker \( r^2 \)s were calculated.
In addition to LD caused by the presence of common haplotypes, there is the phenomenon of markers sharing the same or highly similar pattern of genotypes across strains but that may be on different chromosomes. These ‘mirrors’ can occur by chance and the phenomenon is aggravated by the non-random mating history of the common inbred strains. These mirrors are characterized by \( r^2 \) values close or equal to 1. The problem is that mirrors will give very similar association results making it difficult to identify the exact location of the QTL. Distinguishing between meaningful LD between physically related markers caused by shared haplotypes versus mirror effect is challenging.

However, we used the following procedure based on the parsimony principle to address this issue. After completing the in silico scan and controlling the FDR at the 0.045 level, all pairwise marker-to-marker \( r^2 \)’s for the significant SNPs were calculated. To determine the origin or the source of the true signal of a set of mirrors, the mirrors were first physically mapped and then the various solutions with different origins were plotted. The plot with the fewest number of origins was determined to be the most parsimonious.

References
1. Van Ooijen JW (1999) LOD significance thresholds for QTL analysis in experimental populations of diploid species. Heredity 83 (3 Pt 5): 611–624.
2. Grupe A, Germer S, Usuka J, Aud D, Belknap JK, et al. (2004) In silico mapping of complex disease-related traits in mice. Science 292: 1913–1918.
3. Wade CM, Kalbokan EJ III, Kirby AW, Zody MC, Mullikin JC, et al. (2002) The mosaic structure of variation in the laboratory mouse genome. Nature 420: 574–578.
4. Liao G, Wang J, Guo J, Allard J, Cheng J, et al. (2004) In silico genetics: identification of a functional element regulating H2-Kd allele gene expression. Science 306: 608–614.
5. Pletcher MT, McClurg P, Batalov S, Su AI, Barnes SW, et al. (2004) Use of a dense single nucleotide polymorphism map for in silico mapping in the mouse. PLoS Biol 2: e303. doi:10.1371/journal.pbio.0020303.
6. Wang N, Akey JM, Zhang K, Chakrabrety R, Jan L (2002) Distribution of recombination crossovers and the origin of haplotype blocks: the interplay of population history, recombination, and mutation. Am J Hum Genet 71: 1227–1234.
7. Smith JD, James D, Danky HM, Witkowski KM, Moore KJ, et al. (2003) In silico quantitative trait locus map for atherosclerotic susceptibility in apolipoprotein E-deficient mice. Arterioscler Thromb Vasc Biol 23: 117–122.
8. Park YG, Clifford R, Buettow KH, Hunter KW (2003) Multiple cross and inbred strain haplotype mapping of complex-trait candidate genes. Genome Res 13: 118–121.
9. Chester EJ, Rodriguez-Zas SL, Mosgl JS (2001) In silico mapping of mouse quantitative trait loci. Science 294: 2423.
10. Darvasi A (2001) In silico mapping of mouse quantitative trait loci. Science 294: 2423.
11. van den Oord EJ, Sullivan PF (2003) False discoveries and models for gene discovery. Trends Genet 19: 537–542.
12. Mhiyer TR, Chester EJ, Tranchselvam M, Lunga C, Cory-Slechta DA, et al. (2002) Heritability, correlations and in silico mapping of locomotor behavior and neurochemistry in inbred strains of mice. Genes Brain Behav 4: 209–228.
13. Peirce JL, La L, Gu J, Silver LM, Williams RW (2004) A new set of BXD recombinant inbred lines from advanced intercross populations in mice. BMC Genet 5.
14. Williams RW, Gu J, Qi S, La L (2001) The genetic structure of recombinant inbred mice: high-resolution consensus maps for complex trait analysis. Genome Biol 2: RESEARCH0046.
15. Williams RW, Bennett R, La L, Gu J, DeFries JC, et al. (2004) Genetic structure of the LXS panel of recombinant inbred mouse strains: a powerful resource for complex trait analysis. Mamm Genome 15: 637–647.
16. Tian C, Gregersen PK, Seldin MF (2000) Accounting for ancestry: population substructure and genome-wide association studies. Hum Mol Genet 17: R143–R150.
17. Grubb SC, Churchill GA, Bogus MA (2004) A collaborative database of inbred mouse strain characteristics. Bioinformatics 20: 2057–2058.
18. Guo AV, Webb BT, Miles MF, Zimmerman MP, Kendler KS, et al. (2009) ERGR: An ethanol-related gene resource. Nucleic Acids Res 37: D180–D184.
19. Webb BT, Sullivan PF, Skelly T, van den Oord EJ (2008) Model-based gene selection shows engrailed 1 is associated with antipsychotic response. Physiogenomix Genomics 10: 751–759.
20. Chester EJ, Lu L, Shou S, Yu G, Gu J, et al. (2005) Complex trait analysis of gene expression uncovers polygenic and pleiotropic networks that modulate nervous system function. Nat Genet 37: 233–242.
21. Willott JF, Tanner L, O’Steen J, Johnson KR, Bogue MA, et al. (2003) Acoustic startle and prepulse inhibition in 40 inbred strains of mice. Behav Neurosci 117: 716–727.
22. Braff DL, Geyer MA, Swerdlov NR (2001) Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. Psychopharmacology (Berl) 156: 234–250.
23. Anokhin AP, Heath AC, Myers E, Ralano A, Wood S (2003) Genetic influences on prepulse inhibition of startle reflex in humans. Neurosci Lett 355: 45–48.
24. Cadenhead KS, Swerdlov NR, Shafer KM, Diaz M, Braff DL (2000) Measurement of the startle reaction in normal subjects: implications for schizophrenia in patients and in subjects with schizotypal personality disorder: evidence of inhibitory deficits. Am J Psychiatry 157: 1660–1668.
25. Geyer MA, Krebs-Thomson K, Braff DL, Swerdlov NR (2001) Pharmacological studies of prepulse inhibition modulation of sensorimotor gating deficits in schizophrenia: a decade in review. Psychopharmacology (Berl) 156: 117–154.
26. McCaughran J Jr, Bell J, Hitzemann R (1999) On the relationships of high-frequency hearing loss and cochlear pathology to the acoustic startle response (ASR) and prepulse inhibition of the ASR in the BXD recombinant inbred series. Behav Genet 29: 21–30.
27. Kline I, Decena E, Hitzemann R, McCaughran J Jr (1998) Acoustic startle, prepulse inhibition, locomotion, and latent inhibition in the neuroleptic-responsive (NR) and neuroleptic-nonresponsive (NNR) lines of mice. Psychopharmacology (Berl) 139: 322–331.
28. McCaughran J Jr, Mahjubi E, Decena E, Hitzemann R (1997) Genetics, haloperidol-induced catalepsy and haloperidol-induced changes in acoustic startle and prepulse inhibition. Psychopharmacology (Berl) 134: 131–139.
29. Joober R, Zarate JM, Rosdolc GA, Skamene E, Boks P (2002) Provisional mapping of quantitative trait loci modulating the acoustic startle response and prepulse inhibition of acoustic startle. Neuropsychopharmacology 27: 765–781.
30. Palmer AS, Breen LL, Fiseum P, Combi LH, Spence MA, et al. (2003) Identification of quantitative trait loci for prepulse inhibition in rats. Psychopharmacology (Berl) 165: 270–279.
31. Petryshen TL, Kirby A, Hammer Jr RP, Purcell S, Singer JB, et al. (2005) Two QTLs for prepulse inhibition of startle identified on mouse chromosome 16 using chromosome substitution strains. Genetics 171: 1955–1964.
32. Vendruscolo LF, Terenina-Rigalde E, Raba P, Ramos A, Takahashi RN, et al. (2006) A QTL on rat chromosome 7 modulates prepulse inhibition, a neuro-behavioral trait of ADHD, in a Lewa × SHR intercross. Behav Brain Funct 2(1): 21.
33. Watanabe A, Toyota T, Owada Y, Hayashi T, Iwayama Y, et al. (2007) Fabp7 maps to a quantitative trait locus for a schizophrenia endpoint phenotype. PLoS Biol 5: e287. doi:10.1371/journal.pbio.0050287.
34. Hitzemann R, Malmanger B, Belknap J, Darakjian P, McWeeny S (2008) Short-term selective breeding for high and low prepulse inhibition of the acoustic startle response; pharmacological characterization and QTL mapping in the selected lines. Pharmacol Biochem Behav 90: 525–533.
35. Tsukamachi A, Roka P, Joober R (2008) Prepulse inhibition (PPN) of tactile startle response in recombinant congenic strains of mice: QTL mapping and comparison with acoustic PPI. J Genet Genomics 35: 139–151.
36. Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, et al. (2002) The human genome browser at UCSC. Genome Res 12: 996–1006.
37. Kent WJ, Baertsch R, Hinrichs A, Miller W, Haussler D (2003) Evolution’s cauldron: duplication, deletion, and rearrangement in the mouse and human genomes. Proc Natl Acad Sci U S A 100: 11484–11489.
40. Schwartz S, Kent WJ, Smit A, Zhang Z, Baertsch R, et al. (2003) Human-mouse alignments with BLASTZ. Genome Res 13: 105–107.

41. Palmer AA, Airey DC (2003) Inappropriate choice of the experimental unit leads to a dramatic overestimation of the significance of quantitative trait loci for prepulse inhibition and startle response in recombinant congeneric mice. Neuruppsychoarmacology 28: 818.

42. Jeoer R, Zaratie JM, Rouleau GA, Skamene E, Boka P (2003) Reply: Inappropriate Choice of the Experimental Unit Leads to a Dramatic Overestimation of the Significance of Quantitative Trait Loci for Prepulse Inhibition and Startle Response in Recombinant Congenic Mice. Neuruppsychoarmacology 28: 819.

43. Lewis CM, Levinson DF, Wise LH, Delisi LE, Straub RE, et al. (2003) Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: Schizophrenia. American Journal of Human Genetics 73: 34–48.

44. Myers KM, Goulet M, Rusche J, Boisnenu R, Davis M (2003) Partial reversal of phencyclidine-induced impairment of prepulse inhibition by secretin. Biol Psychiatry 54: 67–75.

45. Stein JM, Bergman W, Fang Y, Davison L, Brensinger C, et al. (2000) Behavioral and neurochemical alterations in mice lacking the RNA-binding protein translin. J Neurosci 26: 2184–2196.

46. Cho YS, Chennathukuzhi VM, Handel MA, Eppig J, Hecht NB (2004) The relative levels of translin-associated factor X (TRAX) and testis brain RNA-binding protein determine their nucleocytoplasmic distribution in male germ cells. J Biol Chem 279: 31514–31523.

47. Myerson KM, Goulet M, Rusche J, Boisnenu R, Davis M (2005) Partial reversal of phencyclidine-induced impairment of prepulse inhibition by secretin. Biol Psychiatry 54: 67–75.

48. Shen J, Chen X, Xu X, Wu R, Zhao J, et al. (2006) Significant linkage and association between a functional (GT)n polymorphism in the GRIN2A promoter with Japanese schizophrenia. Neurosci Lett 378: 102–105.

49. Tsai CA, Hsueh HM, Chen JJ (2003) Estimation of false discovery rates in genome scan meta-analysis of schizophrenia and bipolar disorder, part II: Schizophrenia. American Journal of Human Genetics 73: 34–48.

50. van den Oord EJCG (2005) Controlling false discoveries: application to high-dimensional genomic data. Journal of Biopharmaceutical Statistics 15: 544–555.

51. Williams NM, Preece A, Morris DW, Sparkes G, Bray NJ, et al. (2004) Identification in 2 independent samples of a novel schizophrenia risk haplotype of the dystrohexin binding protein gene (DTNBP1). Arch Gen Psychiatry 61: 544–555.

52. van den Oord EJ (2003) Estimating effects of latent and measured genotypes in multilocus models. Stat Methods Med Res 10: 393–407.

53. van den Oord EJ (2003) Estimating effects of latent and measured genotypes in multilocus models. Stat Methods Med Res 10: 393–407.

54. Weickert CS, Straub RE, McClintock BW, Matsumoto M, Hashimoto R, et al. (2004) Differential regulation of chromatin on the dystrohexin binding protein gene (DTNBP1) in rat brain by phencyclidine treatment. Neuroscience 104: 325–333.

55. van den Oord EJ (2003) Estimating effects of latent and measured genotypes in multilocus models. Stat Methods Med Res 10: 393–407.

56. van den Oord EJ (2003) Estimating effects of latent and measured genotypes in multilocus models. Stat Methods Med Res 10: 393–407.

57. van den Oord EJ (2003) Estimating effects of latent and measured genotypes in multilocus models. Stat Methods Med Res 10: 393–407.

58. van den Oord EJ (2003) Estimating effects of latent and measured genotypes in multilocus models. Stat Methods Med Res 10: 393–407.

59. van den Oord EJ (2003) Estimating effects of latent and measured genotypes in multilocus models. Stat Methods Med Res 10: 393–407.

60. van den Oord EJ (2003) Estimating effects of latent and measured genotypes in multilocus models. Stat Methods Med Res 10: 393–407.

61. van den Oord EJ (2003) Estimating effects of latent and measured genotypes in multilocus models. Stat Methods Med Res 10: 393–407.

62. van den Oord EJ (2003) Estimating effects of latent and measured genotypes in multilocus models. Stat Methods Med Res 10: 393–407.

63. van den Oord EJ (2003) Estimating effects of latent and measured genotypes in multilocus models. Stat Methods Med Res 10: 393–407.

64. van den Oord EJ (2003) Estimating effects of latent and measured genotypes in multilocus models. Stat Methods Med Res 10: 393–407.

65. van den Oord EJ (2003) Estimating effects of latent and measured genotypes in multilocus models. Stat Methods Med Res 10: 393–407.

66. van den Oord EJ (2003) Estimating effects of latent and measured genotypes in multilocus models. Stat Methods Med Res 10: 393–407.

67. van den Oord EJ (2003) Estimating effects of latent and measured genotypes in multilocus models. Stat Methods Med Res 10: 393–407.

68. van den Oord EJ (2003) Estimating effects of latent and measured genotypes in multilocus models. Stat Methods Med Res 10: 393–407.

69. van den Oord EJ (2003) Estimating effects of latent and measured genotypes in multilocus models. Stat Methods Med Res 10: 393–407.

70. van den Oord EJ (2003) Estimating effects of latent and measured genotypes in multilocus models. Stat Methods Med Res 10: 393–407.

71. van den Oord EJ (2003) Estimating effects of latent and measured genotypes in multilocus models. Stat Methods Med Res 10: 393–407.

72. van den Oord EJ (2003) Estimating effects of latent and measured genotypes in multilocus models. Stat Methods Med Res 10: 393–407.

73. van den Oord EJ (2003) Estimating effects of latent and measured genotypes in multilocus models. Stat Methods Med Res 10: 393–407.

74. van den Oord EJ (2003) Estimating effects of latent and measured genotypes in multilocus models. Stat Methods Med Res 10: 393–407.

75. van den Oord EJ (2003) Estimating effects of latent and measured genotypes in multilocus models. Stat Methods Med Res 10: 393–407.

76. van den Oord EJ (2003) Estimating effects of latent and measured genotypes in multilocus models. Stat Methods Med Res 10: 393–407.

77. van den Oord EJ (2003) Estimating effects of latent and measured genotypes in multilocus models. Stat Methods Med Res 10: 393–407.

78. van den Oord EJ (2003) Estimating effects of latent and measured genotypes in multilocus models. Stat Methods Med Res 10: 393–407.

79. van den Oord EJ (2003) Estimating effects of latent and measured genotypes in multilocus models. Stat Methods Med Res 10: 393–407.

80. van den Oord EJ (2003) Estimating effects of latent and measured genotypes in multilocus models. Stat Methods Med Res 10: 393–407.