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# A survey of putative anxiety-associated genes in panic disorder patients with and without bladder symptoms

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## Abstract

**Background—**We have previously described a subtype of panic disorder (PD) that we termed 'bladder syndrome', characterized by urological and bladder symptoms (and possibly interstitial cystitis) in the patients and/or their family members and confirmed the validity of this subset in family linkage and association analysis. In this study, we determine (a) whether 20 single-nucleotide polymorphisms (SNPs) reported in the literature can be replicated in a new PD dataset and (b) whether dividing the sample into those with and without the 'bladder syndrome' can help to resolve the genetic heterogeneity within this new sample.

**Methods—**We selected 20 putative associated SNPs from the literature, taken from studies published since 2004. We tested these SNPs for association in a sample of 351 PD patients and 552 controls, and then divided them into subgroups of 92 patients from bladder families and 259 from nonbladder families.

**Results—**(a) When analyzed in all PD patients, none of the 20 SNPs appeared to be replicated (except for *SLC6A4* from our previous study, but in a sample that overlaps substantially with that in our previous report). (b) However, some intriguing findings emerged when we separated bladder from nonbladder families: *SLC6A4*, reported by us previously, yielded stronger evidence than before ($P = 0.0018$) when examined only in nonbladder families, and in contrast, is not statistically

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**Conflicts of interest**

There are no conflicts of interest.

significant in bladder families. Two other markers yielded nominally significant results in bladder families – rs5751876 in *ADORA2A* (*P* = 0.046) and rs12579350 in *TMEM16B* (*P* = 0.035) – but were not significant in nonbladder families. (c) Two markers had noticeably lower *P*-values when we differentiated the women and analyzed them separately – rs12579350 in *TMEM16B* (*P*-value decreased from 0.035, as above, to 0.00055) and a different SNP in *ADORA2A*, rs4822492 (*P*-value decreases from 0.07 to 0.028).

**Significance—**Our results indicate that most of the 20 reported associations do not hold up when PD is analyzed as one group. However, our findings provide further evidence that PD with bladder symptoms may be genetically different from PD without bladder. We suggest that it is worth pursuing *SLC6A4* in nonbladder PD, and *ADORA2A* and *TMEM16B* in bladder PD. Also, the possibility of a male–female difference in PD is worth pursuing. We also briefly discuss issues of replication and multiple tests.

### Keywords

*ADORA2A*; *ANO2*; genetics; interstitial cystitis; panic disorder; *SLC6A4*

## Introduction

### Panic disorder

Panic disorder (PD) is a debilitating yet common psychiatric condition, with a lifetime prevalence of 3.4–4.7% in the USA (Kessler *et al.*, 2006; Schumacher *et al.*, 2011). Although it is clear that PD shows at least moderate heritability (Hettema *et al.*, 2001; Schumacher *et al.*, 2011), the genes responsible for this effect have remained elusive (Hamilton, 2009; Maron *et al.*, 2010). Linkage studies have failed to identify specific PD genes (with one exception; see below), and there have been only a handful of reports of genetic association, with even fewer being replicated (Maron *et al.*, 2010).

Failure to replicate can result from spurious initial associations that arise from small sample sizes. However, other explanations are also possible, including undetected population stratification, the so-called ‘replication fallacy’ (Gorroochurn *et al.*, 2007 and see the Discussion section), sample heterogeneity between studies, etc.

One method of minimizing potential heterogeneity in PD studies has been to focus on populations that are presumably more genetically homogenous (Thorgeirsson *et al.*, 2003). Another important safeguard against heterogeneity comes from paying very careful attention to phenotype (e.g. Greenberg, 1992; Ioannidis *et al.*, 2009), and stratifying affected individuals by subphenotypes (Hamilton *et al.*, 2003).

### The bladder syndrome

In a previous work, we identified a syndrome characterized by specific bladder, thyroid, and cardiovascular problems, as well as severe migraines. Several of these symptoms had already been reported in PD (Gorman *et al.*, 1988; Lydiard *et al.*, 1994; Stewart *et al.*, 1994; Zaubler and Katon, 1996; Placidi *et al.*, 1998). In our genetic studies of 128 multiplex

families with PD (Fyer and Weissman, 1999; Weissman *et al.*, 2000; Hamilton *et al.*, 2003), we observed that these symptoms aggregated nonrandomly in some families, but not others.

Whereas some of the symptoms, such as migraines, are relatively common in the general population, the bladder manifestations are relatively rare, making it all the more intriguing that the bladder phenotype led to the strongest findings in our genetic studies (discussed below). Thus, we refer to the collection of bladder, thyroid, and cardiovascular symptoms as the bladder syndrome.

In subsequent studies, several lines of evidence converged to support the hypothesis that this syndrome represents a separate genetic entity within PD. Linkage analysis found significant evidence for linkage to chromosome 13q (LOD = 3.52) when we analyzed the syndrome families separately (Weissman *et al.*, 2000). As mentioned above, the most striking symptoms were bladder problems, and examination of case records by an urologist indicated that these symptoms represented interstitial cystitis (IC), a chronic and debilitating urological disorder (Hanno, 1994) that is relatively rare [lifetime prevalence around 0.5% (Jones and Nyberg, 1997)]. In an independent sample of patients with IC, we observed a four-fold higher lifetime prevalence of PD than in urological controls without IC; also, the IC patients were significantly more likely to have a first-degree relative with PD than were probands without PD (Weissman *et al.*, 2004). Further evidence for familiality emerged from another study where we included an independent PD case–control sample (Talati *et al.*, 2008). We found an increase in IC, as defined by the consensus criteria developed by NIDDK, in probands with PD, as opposed to controls; also, first-degree relatives of PD probands were at an increased risk for IC, whether or not the proband had IC.

Whether this connection between PD and IC is because of pleiotropy or autonomic dysregulation in both conditions or other factors altogether is not yet known. For further discussion, see Weissman *et al.* (2004), Talati *et al.* (2008).

In this study, we do two things. First, we attempt to replicate the findings of 20 positive association reports from the literature. We do this in our PD sample, using the standard approach of including all cases in the association analysis. Second, on the basis of the above evidence for genetic differences between families with and without the bladder syndrome, we analyze the two types of cases separately. We believe that this second approach may help resolve the genetic heterogeneity within PD and potentially provide additional evidence for two distinct forms of PD. In addition, given the complex nature of PD associations, at the end of the paper, we also briefly discuss our negative and positive results, with an emphasis on replication and multiple tests.

## Methods and materials

We draw data from two of our previous studies, referred to as the panic linkage study (Hamilton *et al.*, 2003) and the anxiety study (Gyawali *et al.*, 2010; Strug *et al.*, 2010), respectively.

## Sample description

**Cases—**All cases for this study had a definite or a probable *Diagnostic and Statistical Manual of Mental Disorders, 4th ed.*, diagnosis of PD with onset by the age of 31 years and were of non-Hispanic White ancestry. The PD cases were drawn from two previously described populations, one a case–control study of anxiety disorders including panic (Gyawali *et al.*, 2010; Strug *et al.*, 2010) and the other a linkage study of panic (Hamilton *et al.*, 2003). Both datasets had data on bladder symptoms that allowed us to generate PD +bladder and PD −bladder groups. Of the 259 nonbladder cases, 248 were drawn from the anxiety disorder study and 11 from the panic linkage study. In addition, we had 92 ‘PD with bladder’ (PDB) cases, 59 from the anxiety study and 33 from the linkage sample. To qualify as PDB, an individual had to have PD and also had to have at least one first-degree relative with PD, and the individual and/or the first-degree relative also had to have bladder symptoms consistent with an NIDDK definition of IC. Sex distribution was heavily skewed toward women: 207 women and 52 men among the nonbladder cases, and 80 women and 12 men among the PDB cases. The 307 cases from the anxiety study and the 44 from the linkage study represented all cases that fulfilled our criteria and for whom we had DNA.

**Controls—**The first set of controls ($n = 80$), all ethnically White, were recruited in the same manner as the independently recruited cases above (Talati *et al.*, 2008). They were interviewed on the Schedule for Affective Disorders and Schizophrenia Lifetime Version modified for Anxiety Disorders (SADS-LA-IV) and were required to have (a) no anxiety disorder, (b) no family history of anxiety disorder, and (b) to be at least 30 years of age, so that they had passed through the age of risk requirement for the onset of PD in the cases. To increase the number of controls, we screened the 4035 individuals who make up the National Institute of Mental Health Human Genetics Initiative psychiatric control sample recruited from across the USA. There were 534 individuals from the National Institute of Mental Health Human Genetics Initiative psychiatric controls older than 30 years of age who screened negative for all psychiatric categories assessed on the Composite International Diagnostic Interview Short Form (CIDI-SF). Of these, we were able to genotype 472 Whites, bringing our total anxiety-free control group to a total of 552 (278 women and 274 men). Table 1 summarizes the characteristics of the cases and controls.

## Single-nucleotide polymorphism selection

At the start of this study, we carried out a PubMed search on ‘panic disorder genetic association’ and identified 25 single-nucleotide polymorphisms (SNPs) with statistical significance for association with PD reported since 2004. [For one SNP, rs4822492 in *ADORA2A* (#5 in our list; see Table 2), the statistical significance was for anxiety disorder, not panic, but we included this SNP as it is also in tight linkage disequilibrium with the other *ADORA2A* SNP, rs5751876, #4 in our list.] In all cases, we used the same SNPs reported in each study, as our goal was to replicate these associations. (That is, we did not use SNPs in reported linkage disequilibrium with the reported SNPs as proxies or rely on a gene-based approach with other markers within the gene of the reported SNP.) Of these 25 SNPs, five were excluded because of multiplex assay incompatibility as scored by the Sequenom ‘Assay design algorithm’ (Sequenom Inc., San Diego, California, USA) or failure

to pass quality control (see below), leaving 20 SNPs. Table 2 summarizes information about these 20 SNPs.

### Genotyping and quality control

All SNPs were genotyped using Sequenom iPLEX MassARRAY through the Roswell Park Cancer Institute Genomic Shared Resources (Buffalo, New York, USA). All DNA was prepared as recommended by Roswell Park Cancer Institute Genomic Shared Resources. For quality control, 20 individuals were genotyped in duplicate for all SNPs and four SNPs were genotyped in duplicate for all individuals. SNPs and individuals who yielded nonconcordant duplicates were excluded. In addition, we excluded individuals who yielded genotypes for fewer than 90% of all SNPs and SNPs that yielded calls for fewer than 90% of individuals. When tested for Hardy–Weinberg equilibrium, the 20 SNPs had *P*-values ranging from 0.01 to 1.00, with a median of 0.59. The two SNPs with *P*-values below 0.05 (rs17466684 of *CLU*, #16 in our tables; and rs9302001of *BC045767*, #18 in the tables) did not yield any results of interest. We had rs3813034 (#11 in Tables 2 and 3), genotypes for 307 individuals from a previous study (Gyawali *et al.*, 2010) and regenotyped this SNP for the same individuals in this study. On comparing the overlap between studies, we observed complete concordance, indicating genotyping fidelity across platforms.

### Statistical analysis

Allelic association tests were carried out with PLINK (Purcell *et al.*, 2007) to generate $\chi^2$ *P*-values, as well as odds ratios and 95% confidence intervals. Strict Bonferroni correction would require a *P*-value below 0.000513 to achieve an overall significance level of 0.05 (see the Discussion section). However, given the high priors accompanying the SNPs examined, along with the nonindependent nature of some of these SNPs, we report all uncorrected *P*-values below 0.05 as being nominally significant (also see the remarks on issues of multiple testing in the Discussion section). With a significance of 0.05, power calculations (http://pngu.mgh.harvard.edu/~purcell/gpc/cc2.html) suggest between 72% power for our smallest stratified sample and 99% power for the total case–control sample to detect true associations, assuming a relative risk of 2.0 and complete LD between the disease allele and the marker allele.

## Results

### Results when all panic disorder cases are analyzed together

When we analyzed all PD cases as one group, 19 of the 20 SNPs tested failed to achieve even nominal significance, and thus did not replicate the association with PD in our sample of 351 cases and 552 controls. The one exception, rs3813034 in *SLC6A4*, will be discussed separately below. The only other SNP that even approached nominal statistical significance ($P = 0.06$) was rs3816995 in the *SDK2* gene on chromosome 17 (#19 in the tables), which encodes a protein of unknown function homologous to the *Drosophila melanogaster* sidekick gene product and that was previously found to be associated with PD by GWAS (genome-wide association studies) (Otowa *et al.*, 2009). The ‘all PD’ columns in Table 3 show the results.

### Results when panic disorder patients are differentiated into bladder versus nonbladder

The rs3813034 SNP (#11 in the tables), which is functional in the *SLC6A4* gene (see the Discussion section), yielded interesting results. Note that the sample in this paper overlaps substantially with the sample in the paper where we initially reported an association with PD (Gyawali *et al.*, 2010; Strug *et al.*, 2010). Thus, the positive result with this SNP does not represent an independent replication. However, what is new is that the significant result when the SNP is analyzed in all PD patients ($P = 0.01$) becomes even more striking when we consider only the nonbladder cases (present in 52.1% of alleles in cases vs. in 43.8% in controls, $P = 0.0018$), but yields no evidence at all of association in the bladder families ($P = 0.93$). A direct comparison of nonbladder vs. bladder cases for this SNP is significant as well (52.1% in nonbladder cases vs. 43.5% in bladder cases, $P = 0.044$). Note also that the frequency of the risk allele in the bladder PD cases is essentially the same as the frequency in the controls.

Two other SNPs show differences between bladder and nonbladder families – in the opposite direction from the *SLC6A4* SNP. The rs5751876 SNP in the *ADORA2A* gene (#4 in the tables) is not significant when analyzed in all PD patients ($P = 0.29$) or only in the nonbladder cases ($P = 0.81$), but it is nominally significant when analyzed in the bladder cases alone [84 of 182 (46.2%) vs. 363 of 940 (33.1%), $P = 0.046$]. The rs12579350 SNP in the *TMEM16B* gene (#17 in the tables) shows a similar pattern, with nominal significance in bladder cases [64 of 692 (9.2%) vs. 87 of 1100 (7.9%), $P = 0.035$], but no significance when all cases are analyzed together or when the nonbladder cases are analyzed separately. Table 3 shows these results (‘no bladder’ and ‘bladder’ columns).

### Results when only female bladder and nonbladder patients are analyzed

Both PD and its associated bladder symptoms occur significantly more frequently in women (Crowe *et al.*, 1983; Weissman *et al.*, 1997). The female-to-male ratio for PD is over 4 to 1 in our sample and it is over 6 to 1 for the bladder cases considered separately, whereas the 552 controls are almost identically matched for sex. Therefore, in both the nonbladder and the bladder groups, we reanalyzed the data using only the women. These analyses reduced the number of controls from 552 to 278, with the nonbladder cases reduced from 259 to 207 and the bladder cases from 92 to 80. For the rs3813034 SNP in *SLC6A4* (#11), the results among women remained nominally significant in the nonbladder cases [217 of 412 alleles in cases (52.7%) vs. 241 of 556 alleles in controls (43.3%), $P = 0.0041$] and with no evidence of association in the bladder group ($P = 0.92$). For the two SNPs that had shown nominal significance in bladder cases and no significance in nonbladder cases, this pattern remained in the women-only analysis. The *P*-values improved slightly for #4, rs5751878 [74 of 158 (46.8%) vs. 204 of 556 (36.7%), $P = 0.021$], and improved considerably for #17, rs12579350 [23 of 158 (14.6%) vs. 34 of 556 (6.1%), $P = 5.5 \times 10^{-4}$). The odds ratio for this SNP was 2.62, with a 95% confidence interval [1.49, 4.59]. This may have been because of the fact that all 12 PD-bladder men were homozygous for the nonrisk allele. These results indicate a possible sex-specific role for the *TMEM16B* in the PD-bladder syndrome. The ‘no bladder (fm)’ and ‘bladder (fm)’ columns of Table 3 summarize these results.

## Discussion

### Summary of findings

Using our own PD sample, we examined a panel of 20 SNPs that had been reported in the literature to be associated with PD. In the most pessimistic interpretation of our results, one would look at the *P*-values in our 'all PD' analysis (first column of results in Table 3) and conclude that none appeared to be replicated (but also see the points discussed below, under the Issues of replication section), except for the rs3813034 SNP in *SLC6A4* on chromosome 17 (#11 in the tables); moreover, this would not count as a replication, as the sample overlaps considerably with the sample in our original report of that association (Gyawali *et al.*, 2010; Strug *et al.*, 2010). [The current sample contains the 307 cases from the anxiety study (Gyawali *et al.*, 2010; Strug *et al.*, 2010), plus 44 additional cases from (Hamilton *et al.*, 2003); see Table 1].

However, a more nuanced look at the results indicates intriguing differences. Our current findings suggest that the previously identified finding of an association at *SLCL6A4* (#11 in the tables) may be driven by the PD cases without bladder symptoms, whereas two other loci (*ADORA2A* and *TMEM16B*, #4 and #17) provide potential evidence of an association only in the PD cases with bladder symptoms. However, it should be noted that the latter two loci are on chromosomes 22 and 12, respectively, not on 13, where we had found our peak linkage evidence. Yet, as we already have several lines of evidence pointing toward the bladder symptoms representing a separate genetic syndrome (see the Introduction section), these findings buttress the hypothesis that the 'bladder syndrome' represents a distinct genetic subtype of PD.

Differentiation of the female PD cases has, perhaps, less prior justification than differentiation of the bladder and nonbladder cases. However, other genes such as the PD candidate gene *BDKRB2* have been shown to have sex-specific effects in a range of phenotypes (Madeddu *et al.*, 1996; Asselbergs *et al.*, 2006; Bourdet *et al.*, 2010). For one of our tested SNPs, rs12579350 (#17), the change when we differentiate by sex is striking. Therefore, for future investigations into PD, it may be worthwhile to analyze female cases separately.

### The *SLC6A4*, *ADORA2A*, and *TMEM16B* genes

Understanding the network of polymorphisms associated with PD is an important step in modeling risk and outcome for patients. More importantly, it provides an insight into the biology of disease expression, an essential component of developing more effective treatments. The genes we find to be most important in our sample by association analysis are *SCL6A4*, *ADORA2A*, and *THEM16B*. The *SCL6A4* gene encodes the mammalian serotonin transporter and has been studied in many psychiatric disorders (Goldberg *et al.*, 2009; Caspi *et al.*, 2010). The pathways and signaling surrounding the neurotransmitter serotonin (5-hydroxytryptamine) have been recognized for their important roles in behavior and mood for decades. However, the role of specific genetic variants in the risk for PD has been unclear. Our studies suggest that the functional SNP rs3813034, which alters polyadenylation and the abundance of the *SCL6A4* message (Gyawali *et al.*, 2010), is

involved in a subset of PD not necessarily accompanied by bladder symptoms. The *ADORA2A* gene has also been the focus of studies examining the genetics of mood disorders. We had previously reported linkage of *ADORA2A* to PD in a study of 153 families (including the families of 44 cases from this study) (Hamilton *et al.*, 2004). Subsequently, *ADORA2A* was found to be associated with PD phenotypes (Deckert *et al.*, 1998; Childs *et al.*, 2008; Hohoff *et al.*, 2010) and anxiety (Deckert *et al.*, 1998; Childs *et al.*, 2008; Hohoff *et al.*, 2010). Deckert *et al.* (1998) found an association with rs5751876 in a group of 89 PD patients. This was replicated by Hohoff *et al.* (2010) when the sample was expanded to 457 cases. In our study, its association seems to depend on the bladder cases. Perhaps the most unexpected association in our study is that found in *TMEM16B*. The *TMEM16B* gene, also known as *ANO2*, encodes the $Ca^{2+}$ -activated chloride channel family member anoctamin 2 (Hartzell *et al.*, 2009; Stephan *et al.*, 2009; Stohr *et al.*, 2009). The rs12579350 SNP of this gene had yielded a genome-wide *P*-value of $3.7 \times 10^{-9}$ in a Japanese GWAS of 200 PD cases and 200 non-PD controls (Otowa *et al.*, 2009), although it was not replicated in a follow-up study that increased the sample size (Otowa *et al.*, 2010). In this study, rs12579350 appears to be associated with PD bladder, particularly in women. The biological connection of *TMEM16B* to PD is unclear, but provides an impetus for further functional studies. These three PD-associated genes, each associated with a slightly different phenotype, may help us understand the biological networks contributing to different kinds of PD syndromes.

## Issues of replication

For all complex diseases, especially psychiatric disorders, finding genetic associations that hold up under further scrutiny has proven challenging. It is widely accepted that if an association is not replicated, then the initial finding was likely a false positive (Greene *et al.*, 2009). However, this is not necessarily the case. Possible alternative explanations include the following.

One alternative explanation is the ‘replication fallacy,’ already known in some areas of applied statistics, and elucidated with particular application to genetic association studies by Gorroochurn *et al.* (2007). Depending on how low the *P*-value in an initial study was, a second study may have a probability as low as 50% of replicating the study in a sample of the same size, even when the initial study’s result was in fact a true positive. If the second study has a smaller sample than the first study, the replication probability can be even lower than 50%.

A second possibility is that the initial association may be real for the sample collected, but not for a subsequent independently sampled population (Vieland, 2001). With complex diseases, it is very difficult to ensure that the sample collected for the follow-up study is truly comparable with the sample used in the initial study.

Third, there may be unrecognized etiological heterogeneity within any one sample. This is where very careful attention to phenotype can play a critical role. For example, a study of the COMT V158M (rs4680) polymorphism (Domschke *et al.*, 2008) stratified PD cases by neuronal response to fearful faces and in this way was able to replicate a previous reported association. In the current study, the differentiation of bladder from nonbladder cases yields

a quite different picture than analysis of all PD cases together. This separation is not *ad hoc* but is based on our published studies – both linkage and association – indicating genetic differences between the two types of cases.

Finally, there is the question of what significance level may be required in a follow-up study. As pointed out by Vieland (2001), if a significance level of $\alpha_1$ is required in an initial study and a significance level of $\alpha_2$ in a follow-up study, a significance level of $\alpha_1 \times \alpha_2$ overall is actually required. Thus, if both $\alpha_1$ and $\alpha_2$ are set at 0.05, a significance level of 0.0025 may actually be required. At first glance, it may seem harmless to set such a strict criterion, but unfortunately if one does so, one pays the price in greatly reduced statistical power.

Thus, although replication studies are certainly important, they do not necessarily provide definitive answers to our questions, even when they are negative.

## Issues of multiple testing

When attempting to 'correct' for multiple analyses on data, logical difficulties and contradictions may develop; in fact, for this reason, some investigators recommend following a completely different paradigm for statistical testing (Royall, 1997, 2000; Strug and Hodge, 2006a, 2006b). However, in the current study, we followed the standard statistical paradigm, and thus we need to address the issue of multiple testing.

The underlying concern in multiple testing arises from the fact that if a number of statistical tests are carried out at, say, the 5% level, the probability that at least one of these tests will yield a 'significant' result, if there is no association with any of the markers, will be greater than 5%. We can designate 5% as the 'nominal' or the 'desired' type I error level, whereas the true probability, the increased one, is the 'corrected' level. Turning the question around, if $N$ tests are carried out and a $P$-value of, say, 0.001 is obtained, this should be considered the nominal $P$-value; to determine the corrected $P$-value, the Bonferroni correction formula $P_{corr} = 1 - (1 - P_{nom})^N$, or, in the usual approximation, $P_{corr} \approx N \times P_{nom}$, should be used.

However, applying this Bonferroni correction leads to two questions. (i) It assumes the $N$ analyses are all statistically independent; if they are correlated, then the approach is overconservative and the Bonferroni approach loses power. (ii) It assumes that none of the $N$ analyses has prior support; thus, this approach is appropriate for a GWAS, for example. However, this logic does not allow for the possibility that some or all of the analyses are potentially replicating earlier studies or that there may be biological arguments supporting any of the markers.

Returning to our study – on the face of it, Table 3 shows 100 analyses (five each of 20 markers). Of the eight $P$-values below 0.05, only one – rs12579350 (#17) – is small enough to 'almost' survive the standard Bonferroni correction with $N = 100$: inserting $P_{nom} = 0.0005$ and $N = 100$ into the Bonferroni formula above yields $P_{corr} = 1 - (0.99945)^{100} \cong 0.0535$. However, both the issues raised above have relevance for our study: the five tests carried out on each marker are not independent, and therefore, the 100 analyses are not independent [cf. issue (i)]; all 20 loci had been published previously, with some of them having already been replicated [issue (ii)]; and, as discussed in the Introduction section, we

have strong previous evidence that the bladder syndrome is a separate genetic entity within PD [also issue (ii)].

Because of these complications, we cannot state exactly what the 'corrected' *P*-value(s) should be for this study, but we believe that it is reasonable to argue that our results – those for rs5751876 and rs4822492 in *ADORA2A*, rs3813034 in *SLC6A4*, and rs12579350 in *TMEM16B*, as well as those supporting the separation of bladder from nonbladder families, and the possible differentiation of women from men with PD – should not be dismissed automatically because of multiple tests. They deserve serious consideration, and we hope that we or other investigators will be able to study them further, toward the goal of either replicating or rejecting them.

## Conclusion

Achieving definitive and unassailable results in studying the genetics of complex diseases is difficult. Findings that do not meet the general standards for statistical significance need to be replicated in an independent sample before strong conclusions can be drawn. We do not claim that our findings are definitive. Nevertheless, they suggest the potential value of differentiating families with bladder symptoms in future genetic studies of PD.

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**Table 1**

Description of the sample

| Panic disorder cases | Female | Male | Total |
|---|---|---|---|
| No bladder syndrome | 207 | 52 | 259 (248[a] + 11[b]) |
| Bladder syndrome | 80 | 12 | 92 (59[a] + 33[b]) |
| Total cases | 287 | 64 | 351 (307[a] + 44[b]) |
| Controls | 278 | 274 | 552 |

[a]From the anxiety study (Gyawali *et al.*, 2010; Strug *et al.*, 2010).

[b]From the panic linkage study (Hamilton *et al.*, 2003).

**Table 2**

Descriptions and sources of the 20 single-nucleotide polymorphisms analyzed in this study

| # | SNP | Gene | Chromosome | Study | Cases | OR or RR | *P*-value | Replicated? |
|---|---|---|---|---|---|---|---|---|
| 1 | rs2296972 | *HTR2A* | 13 | Unschuld *et al.* (2007) | 154 | NR | 0.0006 | – |
| 2 | rs6313 | *HTR2A* | 13 | Unschuld *et al.* (2007) | 154 | NR | 0.009 * | – |
| 3 | rs6311 | *HTR2A* | 13 | Unschuld *et al.* (2007) | 154 | NR | 0.009 * | – |
| 4 | rs5751876 | *ADORA2A* | 22 | Deckert *et al.* (1998) | 457 | 1.51 | 0.01 | Hohoff *et al.* (2010) |
| 5 | rs4822492 | *ADORA2A* | 22 | Childs *et al.* (2008) | 62 | NR | 0.007 | – |
| 6 | rs4680 | *COMT* | 22 | Domschke *et al.* (2008) | 115 | NR | 0.01 | Rothe *et al.* (2006)) |
| 7 | rs8016905 | *BDKRB2* | 14 | Gratacos *et al.* (2009) | 173 | 3.06/2.79 | $10^{-7}/10^{-6}$ | – |
| 8 | rs10414815 | *ZNF574* | 19 | Gratacos *et al.* (2009) | 173 | 2.03 | 0.001 * | – |
| 9 | rs6136667 | *PDYN* | 20 | Gratacos *et al.* (2009) | 173 | NR | 0.018 * | – |
| 10 | rs945032 | *BDKRB2* | 14 | Gratacos *et al.* (2009) | 173 | 3.06 | $1.0 \times 10^{-7}$ | – |
| 11 | rs3813034 | *SLC6A4* | 17 | Gyawali *et al.* (2010) | 307 | 1.56–1.81 | 0.007 | – |
| 12 | rs5326 | *DRD1* | 5 | Maron *et al.* (2005) | 127 | 1.25 | 0.02 | – |
| 13 | rs1386494 | *TPH2* | 12 | Maron *et al.* (2007) | 213 | 1.88 | 0.01 | Maron *et al.* (2008) |
| 14 | rs860554 | *PKP1* | 1 | Otowa *et al.* (2009) | 200 | NR | $4.6 \times 10^{-8}$ | – |
| 15 | rs9372078 | *PLEKHG1* | 6 | Otowa *et al.* (2009) | 200 | NR | $4.9 \times 10^{-7}$ | – |
| 16 | rs17466684 | *CLU* | 8 | Otowa *et al.* (2009) | 200 | NR | $6.9 \times 10^{-7}$ | – |
| 17 | rs12579350 | *TMEM16B* | 12 | Otowa *et al.* (2009) | 200 | NR | $3.7 \times 10^{-9}$ | – |
| 18 | rs9302001 | *BC045767* | 13 | Otowa *et al.* (2009) | 200 | NR | $3.0 \times 10^{-7}$ | – |
| 19 | rs3816995 | *SDK2* | 17 | Otowa *et al.* (2009) | 200 | NR | $2.1 \times 10^{-7}$ | – |
| 20 | rs8192506 | *DBI* | 2 | Thoeringer *et al.* (2007) | 126 | 0.43 | 0.0298 | – |

NR, not reported; OR, odds ratio; RR, relative risk; SNP, single-nucleotide polymorphism.

[a]Denotes failure to pass multiple test correction for panic disorder.

**Table 3**

*P*-values and odds ratios for 20 single-nucleotide polymorphisms, under different analyses

| | | All PD Cases 351 Controls 552 | | No bladder Cases 259 Controls 552 | | No bladder (fm) Cases 207 Controls 278 | | Bladder Cases 92 Controls 552 | | Bladder (fm) Cases 80 Controls 278 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| # | SNPs | *P*-value | OR | *P*-value | OR | *P*-value | OR | *P*-value | OR | *P*-value | OR |
| 1 | rs2296972 | 0.77 | 1.03 | 0.52 | 1.08 | 0.43 | 1.12 | 0.58 | 0.91 | 0.82 | 0.96 |
| 2 | rs6313 | 0.51 | 1.06 | 0.43 | 1.09 | 0.80 | 1.03 | 0.98 | 1.01 | 0.77 | 0.95 |
| 3 | rs6311 | 0.35 | 1.10 | 0.27 | 1.13 | 0.69 | 1.05 | 0.92 | 1.02 | 0.77 | 0.95 |
| 4 | rs5751876 | 0.29 | 1.11 | 0.81 | 1.03 | 0.31 | 1.15 | 0.046 | 1.38 | 0.021 | 1.52 |
| 5 | rs4822492 | 0.24 | 1.12 | 0.62 | 1.06 | 0.10 | 1.25 | 0.07 | 1.33 | 0.028 | 1.49 |
| 6 | rs4680 | 0.69 | 0.96 | 0.92 | 1.01 | 0.95 | 0.99 | 0.27 | 0.84 | 0.58 | 0.90 |
| 7 | rs8016905 | 0.17 | 1.16 | 0.27 | 1.14 | 0.78 | 1.04 | 0.26 | 1.21 | 0.53 | 1.13 |
| 8 | rs10414815 | 0.63 | 0.93 | 0.26 | 0.83 | 0.85 | 0.96 | 0.33 | 1.24 | 0.16 | 1.42 |
| 9 | rs6136667 | 0.40 | 0.89 | 0.22 | 0.83 | 0.83 | 0.96 | 0.74 | 1.08 | 0.56 | 1.15 |
| 10 | rs945032 | 0.20 | 0.85 | 0.46 | 0.90 | 0.27 | 1.20 | 0.11 | 0.70 | 0.64 | 0.89 |
| 11 | rs3813034 | 0.0125 | 1.27 | 0.0018 | 1.40 | 0.0041 | 1.46 | 0.93 | 0.99 | 0.93 | 1.02 |
| 12 | rs5326 | 0.78 | 1.04 | 0.70 | 1.06 | 0.92 | 1.02 | 0.94 | 0.98 | 0.66 | 0.89 |
| 13 | rs1386494 | 0.65 | 1.06 | 0.49 | 1.11 | 0.85 | 1.04 | 0.81 | 0.94 | 0.44 | 0.81 |
| 14 | rs860554 | 0.31 | 0.89 | 0.30 | 0.88 | 0.38 | 0.88 | 0.70 | 0.93 | 0.66 | 0.91 |
| 15 | rs9372078 | 0.89 | 1.01 | 0.72 | 1.04 | 0.42 | 1.12 | 0.72 | 0.94 | 0.94 | 0.99 |
| 16 | rs17466684 | 0.91 | 0.99 | 0.96 | 0.99 | 0.93 | 1.02 | 0.87 | 0.96 | 0.92 | 1.02 |
| 17 | rs12579350 | 0.32 | 1.19 | 0.93 | 1.02 | 0.11 | 1.49 | 0.035 | 1.68 | 0.00055 | 2.62 |
| 18 | rs9302001 | 0.79 | 1.03 | 0.82 | 1.03 | 0.43 | 1.11 | 0.86 | 1.03 | 0.38 | 1.17 |
| 19 | rs3816995 | 0.059 | 1.20 | 0.15 | 1.17 | 0.35 | 1.13 | 0.09 | 1.31 | 0.12 | 1.32 |
| 20 | rs8192506 | 0.97 | 1.01 | 0.79 | 0.93 | 0.87 | 0.95 | 0.57 | 1.24 | 0.89 | 1.06 |

Shaded fields indicate nominally significant results ($P \leq 0.05$).

Numbers (#) correspond to those in Table 2.

PD, panic disorder; SNP, single-nucleotide polymorphism.