Selection in the dopamine receptor 2 gene: a candidate SNP study

Tobias Göllner and Martin Fieder

Department of Anthropology, University of Vienna, Vienna, Austria

ABSTRACT

Dopamine is a major neurotransmitter in the human brain and is associated with various diseases. Schizophrenia, for example, is treated by blocking the dopamine receptors type 2. *Shaner, Miller & Mintz (2004)* stated that schizophrenia was the low fitness variant of a highly variable mental trait. We therefore explore whether the dopamine receptor 2 gene (*DRD2*) underwent any selection processes. We acquired genotype data of the 1,000 Genomes project (phase I), which contains 1,093 individuals from 14 populations. We included single nucleotide polymorphisms (SNPs) with two minor allele frequencies (MAFs) in the analysis: MAF over 0.05 and over 0.01. This is equivalent to 151 SNPs (MAF > 0.05) and 246 SNPs (MAF > 0.01) for *DRD2*. We used two different approaches (an outlier approach and a Bayesian approach) to detect loci under selection. The combined results of both approaches yielded nine (MAF > 0.05) and two candidate SNPs (MAF > 0.01), under balancing selection. We also found weak signs for directional selection on *DRD2*, but in our opinion these were too weak to draw any final conclusions on directional selection in *DRD2*. All candidates for balancing selection are in the intronic region of the gene and only one (rs12574471) has been mentioned in the literature. Two of our candidate SNPs are located in specific regions of the gene: rs80215768 lies within a promoter flanking region and rs74751335 lies within a transcription factor binding site. We strongly encourage research on our candidate SNPs and their possible effects.

INTRODUCTION

The catecholamine dopamine is a neurotransmitter in the human brain. Dopaminergic neurons can be divided into four major pathways: nigrostriatal, mesolimbic, mesocortical and tuberoinfundibular (*Andén et al., 1964*; *Dahlström & Fuxe, 1964*). These neurons play an important role in voluntary movement, feeding, reward and learning, as well as certain other functions. Outside the brain, dopamine takes on a physiological role in cardiovascular functions, hormonal regulation, renal and other functions (*Snyder et al., 1970*; *Missale et al., 1998*; *Sibley, 1999*; *Carlsson, 2001*; *Iversen & Iversen, 2007*). Due to this involvement in many different processes and systems, dopamine is also related to a variety of diseases. Parkinson's disease, caused by a loss of dopaminergic innervations in the striatum, is a prominent example (*Ehringer & Hornykiewicz, 1960*). Additionally, the expected associations between the dopaminergic system and schizophrenia stem from the fact that various dopamine receptor 2 blockers are used as antipsychotics in treating that
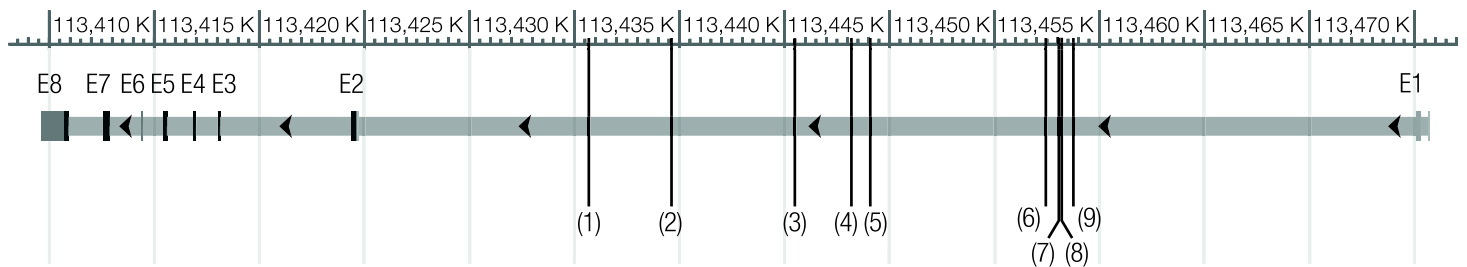
Figure 1 Location of candidate SNPs under balancing selection in *DRD2*. E1-8 are exons 1 to 8. (1) rs60599314, (2) rs79549222, (3) rs12574471, (4) rs80215768, (5) rs76581995, (6) rs80014933, (7) rs74751335, (8) rs77264605, (9) rs76499333.

condition (*Snyder et al., 1970*; *Creese, Burt & Snyder, 1976*; *Seeman et al., 1976*; *Carlsson et al., 2001*). Further relationships with dopamine dysregulation are expected in Tourette's syndrome and attention deficit hyperactivity disorder (ADHD) (*Mink, 2006*; *Swanson et al., 2007*; *Gizer, Ficks & Waldman, 2009*). The strong involvement of dopamine in the reward system suggests an association with drug abuse and addiction (*Hyman, Malenka & Nestler, 2006*; *Di Chiara & Bassareo, 2007*; *Koob & Volkow, 2010*). Many more diseases and conditions are expected to involve dopamine dysfunctions. (As reviewed by *Beaulieu & Gainetdinov (2011)*).

In humans, five different dopamine receptors exist. They are classified into two categories based on their structure and their pharmacological and biochemical properties. The D1-class includes the dopamine receptors 1 and 5, while the D2-class consists of the dopamine receptors 2, 3 and 4 (*Andersen et al., 1990*; *Niznik & Van Tol, 1992*; *Sibley & Monsma, 1992*; *Sokoloff et al., 1992*; *Civelli, Bunzow & Grandy, 1993*; *Vallone, Picetti & Borrelli, 2000*). The focus of our study is on the dopamine receptor 2 and its gene *DRD2*. The dopamine receptor 2 gene lies on the long arm of chromosome 11 (11q23.1). It spans from 113,280,317 to 113,346,413 for a total of 66,096 base pairs (bp) (information accessed on NCBI in the GnRH37 assembly). For the gene card, see Fig. 1. *DRD2* has six introns (*Gingrich & Caron, 1993*). Alternative splicing between intron 4 and 5 of an 87 bp exon generates two variants of the dopamine receptor 2. The difference between D2S (short) and D2L (long) is a 29-amino-acids-long chain in the third intercellular loop of the protein (*Giros et al., 1989*; *Monsma et al., 1989*). While the short form (D2S) is mainly expressed at the presynapse, the long form (D2L) is expressed postsynaptically (*Usiello et al., 2000*; *De Mei et al., 2009*). The D2S are mainly autoreceptors, i.e., they reduce the expression of dopamine when activated. This leads to an important negative feedback mechanism (*Wolf & Roth, 1990*; *Missale et al., 1998*; *Sibley, 1999*). (Again, as reviewed by *Beaulieu & Gainetdinov, 2011*).

Among the many single nucleotide polymorphisms (SNPs) of *DRD2*, one prominent example is rs6277, also known as C957T. It has been associated with schizophrenia in Han Chinese in Taiwan (*Glatt et al., 2009*), in Russians (*Monakhov et al., 2008*) and in Bulgarians (*Betcheva et al., 2009*). Together with the -141C allele, the 957T allele is associated with the diagnosis of anorexia nervosa (*Bergen et al., 2005*). A meta-analysis showed that the Ser311Cys polymorphism (rs1801028) in *DRD2* is a risk factor for schizophrenia.

The heterozygotes (Ser/Cys) and the homozygotes for Cys were both at elevated risk for schizophrenia when compared to the Ser/Ser genotypes (*Glatt & Jönsson, 2006*). In a study with alcoholic patients and controls, the A allele of rs1076560 was more frequent in alcoholic patients (*Sasabe et al., 2007*). In 2012, Mileva-Seitz et al. conducted a study with Caucasian mothers and their infants. They taped mother-infant behaviour and genotyped various SNPs of *DRD2* and also *DRD1*. rs1799732 and the previously mentioned rs6277 were both associated with direct vocalization of the mother towards the infant.

The body of literature on SNPs and their possible effects is growing rapidly. Considering the influences those SNPs could have on human behaviour, and bearing in mind the different ecological habitats of *Homo sapiens*, we explore if *DRD2* underwent any selection processes. In *2004*, an interesting proposal by *Shaner, Miller & Mintz* stated that schizophrenia was the low fitness variant of a highly variable mental trait. Based on the connection between dopamine receptor 2 and schizophrenia, as stated above, we focused our analysis on *DRD2*.

To reduce false-positives, we used two selection detection algorithms to explore *DRD2*. This exploratory ("hypothesis-free") approach is designed to find candidate SNPs that were under selection. The data basis of our analysis is the 1,000 Genomes Project samples.

MATERIAL AND METHODS

We acquired data from the 1,000 Genomes Project (phase I) through SPSmart engine v5.1.1 (http://spsmart.cesga.es/engines.php; *Amigo et al., 2008*), using the search term "DRD2." We included all single nucleotide polymorphisms (SNPs) with a minor allele frequency (MAF) greater than 0.05 ($N = 151$ SNPs) to include only the more frequently occurring SNPs. To verify our results also on the basis of a higher number of SNPs (which occur less frequently), we conducted the same analysis also based on a MAF > 0.01 sample ($N = 246$ SNPs; data presented in Supplemental Information). The structure of the *DRD2* gene (113,280,317–113,346,413 in the GnRH37.p13 primary assembly) is shown in Fig. 1. The populations used for our analysis are shown in Table 1.

The data were converted by hand into the CONVERT format. All further format conversions were performed by PGD Spider 2.0.5.2 (*Lischer & Excoffier, 2012*).

Two different programs were used to detect selection; both use F_{ST} approaches to detect outliers. The program LOSITAN calculates FDIST, which uses F_{ST} and the expected heterozygosity. It assumes an island model of migration with neutral markers. An expected distribution of Wright's inbreeding coefficient is calculated and then outliers are identified. A neutral mean F_{ST} was computed by the program before the 50,000 simulations were performed. The infinite alleles model was used. To avoid false positive detection, we set the significance level to $p < 0.01$ (P(Simulation F_{ST} < sample F_{ST})) (*Antao et al., 2008*).

BayeScan is a Bayesian statistics program. Basically, it calculates two simulations for every locus: one in which it assumes the locus is under selection and the other one in which this assumption is dropped. It splits the F_{ST} coefficient into two parts. The alpha value is a locus-specific component shared by all populations. The beta value is a population-specific component shared by all loci. This is achieved via logistic regression and provides insight

Table 1 Populations of the 1,000 genomes project.

Superpopulation (code)	Population code	Population	Number of individuals
Africa (AFR)	ASW	African ancestry in Southwest USA	61
	LWK	Luhya in Webuye, Kenya	97
	YRI	Yoruba in Ibadan, Nigeria	88
Europe (EUR)	CEU	Utah residents with Northern and Western European ancestry	87
	FIN	Finnish from Finland	93
	GBR	British from England and Scotland	88
	IBS	Iberian populations in Spain	14
	TSI	Toscani in Italy	98
East Asia (ASN)	CHB	Han Chinese in Bejing, China	97
	CHS	Han Chinese South	100
	JPT	Japanese in Tokyo, Japan	89
America (AMR)	CLM	Colombians from Medellin, Colombia	66
	MXL	Mexican ancestry from Los Angeles USA	60
	PUR	Puerto Ricans from Puerto Rico	55
		All populations	1,093

into selection. The alpha value serves as an indicator for selection. Significant positive values of alpha indicate directional selection, whereas significant negative values indicate balancing selection. The posterior probabilities are estimated using a reversible-jump Markov Chain Monte Carlo (MCMC) approach. The posterior probabilities are gained by counting how many times alpha is included in the model. Before computing the Markov chains, we calculate 20 pilot runs with 5,000 iterations each. The initial burn-in is set to 50,000 steps and the chains are run with 5,000 iterations and a thinning interval of 10. The program output consists of a posterior probability, the logarithm (base 10) of the posterior odds and a q value. These three values are all for the model with selection. Furthermore, the alpha value is reported along with an F_{ST} coefficient average of all population per locus. In BayeScan the threshold of a posterior P of >0.99 and a log10(PO) of 2 or higher is used. This threshold is labelled as "Decisive" by BayeScan (see the program manual at http://cmpg.unibe.ch/software/BayeScan/files/BayeScan2.1_manual.pdf) (*Foll & Gaggiotti, 2008*).

To compute linkage disequilibrium (LD) of the SNPs, we used the R "genetics package" (http://cran.r-project.org/web/packages/genetics/genetics.pdf; *Warnes et al., 2013*). *Mueller (2004)* states that D' is particularly useful to assess the probability for historical recombination in a given population and r^2 is useful in the context of association studies. We therefore primarily calculate D', but we also calculated r^2 , which is presented in Supplemental Information.

In most populations one or more SNPs had to be excluded to successfully run the computation. The population IBS was excluded entirely from this computation. IBS is a very small population ($n = 14$), and 30 SNPs caused the computation to fail. For a detailed view on all excluded SNPs, see Table S1.

We accessed information on the gene via NCBI (http://www.ncbi.nlm.nih.gov/) and on the specific SNPs via Ensembl (http://www.ensembl.org/).
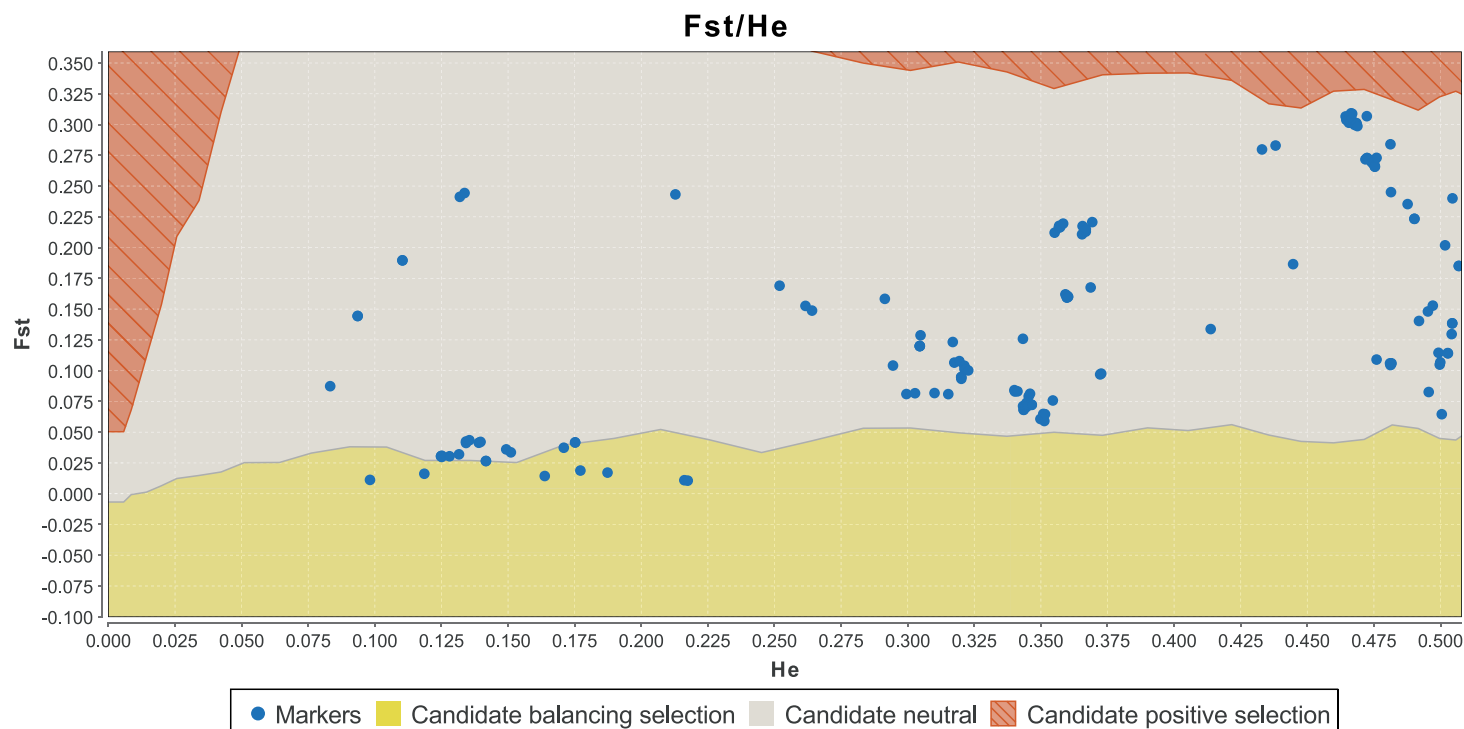
Fst/He

Figure 2 Detection of outlier SNPs of the *DRD2* gene using FDIST (LOSITAN). *X*-axis: estimated heterozygosity values. *Y*-axis: F_{ST}-values. The upper area indicates positive directional selection, the middle area neutrality, and the lower area balancing selection. Confidence intervals represent borders between "selection areas". See Table S2 for the exact results on the SNPs.

RESULTS

The combined results of LOSITAN and BayeScan yielded nine candidate SNPs under balancing selection (MAF > 0.05); see Table 2. Figure 2 shows the graphical output of LOSITAN and Fig. 3 the output of BayeScan.

For a detailed view on the results of LOSITAN and BayeScan for all SNPs, see Table S2 in the supplementary material. Figure 1 provides a gene view of *DRD2* with labels for the candidate SNPs.

The same calculations based on the MAF > 0.01 sample revealed only 2 SNPs (rs60599314, rs79549222) under balancing selection by both LOSITAN and Bayescan (Fig. S1, Table S3A and Table S3B).

Three SNPs (rs6277, rs12800853, rs11608109) that do not reach significance in the MAF > 0.05 sample (Table S3A) do reach significance in the MAF > 0.01 sample (Table S3B), for directional selection. They barely reach significance ($P < 0.01$) based on the MAF > 0.01 sample in LOSITAN (Fig. S1 and Table S3B). However if we applied more stringent detection prerequisites ("force mean F_{ST}" and "neutral mean F_{ST}"; increasing computational load, but also increasing convergence and lowering the bias in F_{ST} estimation) in LOSITAN, none of these three SNPs reaches significance (Fig. S2). The results for balancing selection in BayeScan remained nearly unchanged in the MAF > 0.01 sample, with the exception of rs12574471, which did not reach significance (Table S3B).
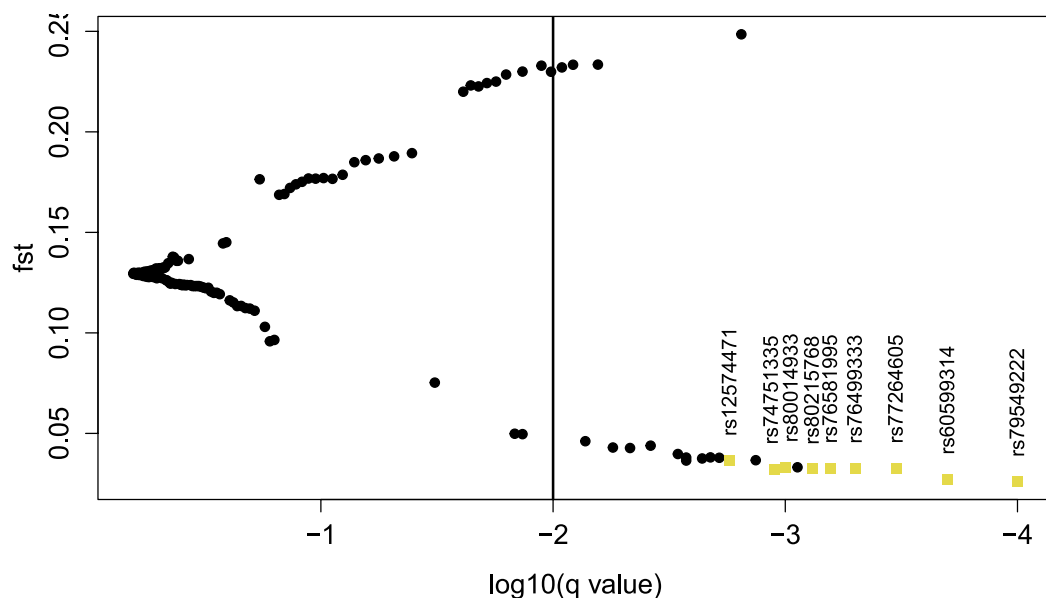
Figure 3 Graphical output of BayeScan. *X*-axis: $\log10(q$ values) for all SNPs, the threshold is -2. *Y*-axis: F_{ST}-values, where high values indicate directional selection, low values balancing selection. For exact results see Table S2. Squares: the nine SNPs in the MAF > 0.05 sample that BayeScan and LOSITAN find.

Table 2 The dopamine receptor 2 gene's nine candidate SNPs for balancing selection (MAF > 0.05).

Locus (#)	Major allele (Frequency)	Minor allele (Frequency)	F_{ST} (Lositan)	F_{ST} (BayeScan)	Location
rs60599314 (1)	C (0.871)	T (0.129)	0.0110	0.0272	113,306,431 (Intronic region)
rs79549222 (2)	T (0.87)	G (0.13)	0.0106	0.0260	113,310,340 (Intronic region)
rs12574471 (3)	C (0.891)	T (0.109)	0.0172	0.0364	113,316,236 (Intronic region)
rs80215768 (4)	G (0.925)	A (0.075)	0.0304	0.0328	113,318,880 (Intronic region)
rs76581995 (5)	C (0.925)	A (0.075)	0.0304	0.0328	113,319,835 (Intronic region)
rs80014933 (6)	T (0.923)	C (0.077)	0.0304	0.0332	113,328,135 (Intronic region)
rs74751335 (7)	G (0.915)	C (0.085)	0.0266	0.0322	113,328,810 (Intronic region)
rs77264605 (8)	A (0.915)	G (0.085)	0.0266	0.0327	113,328,913 (Intronic region)
rs76499333 (9)	G (0.925)	A (0.075)	0.0299	0.0327	113,329,449 (Intronic region)

All nine SNPs detected based on MAF > 0.05 are intron variants (Fig. 1). Only rs12574471 (3) is mentioned in the literature because it is near a supposed recombination hotspot (*Glatt et al., 2009*). rs80215768 (4) lies within a promoter flanking region; rs74751335 (7) lies within a transcription factor binding site. Nonetheless, we found no known associations for those two SNPs.

The F_{ST} values of these nine loci indicate an overall low genetic differentiation, as well as a low differentiation between populations (Table 2). This is in accordance with balancing selection acting on the gene. The differences in F_{ST} values stem from different algorithms used by the programs.

The Linkage Disequilibrium measurements D' and r^2 were used. The heat maps for all nine populations are shown in the supplementary material (Figs. S3–S15 for D' and Figs. S16–S28 for r^2). The relative position of the marked SNPs change because different populations had different SNPs excluded (see Table S1 for the list).

DISCUSSION

We found nine SNPs to be candidates for balancing selection based on the sample MAF > 0.05; of those, two had been also detected under balancing selection based on the MAF > 0.01 sample. We found no SNPs based on the MAF > 0.05 sample and the MAF > 0.01 sample, under directional selection, that are detected by both algorithms (on $P < 0.001$ in LOSITAN) and if more stringent detection criteria were applied in LOSITAN. We therefore conclude that, if directional selection has been acting on $DRD2$, then the signs are rather weak, i.e., too weak to make definitive conclusions.

Checking all nine SNPs under balancing selection based on the MAF > 0.05 sample with Ensembl reveals that they are all intronic region variants. For rs60599314 (1) and rs79549222 (2) that are found by LOSITAN and BayeScan on both the MAF >0.05 sample and the MAF >0.01 sample, no particular additional information is known. We therefore suggest that these two SNPs may provide interesting candidates for future functional studies.

rs80215768 (4) lies within a promoter flanking region and rs74751335 (7) lies within a transcription factor binding site (TFBS) (both SNPs detected based on the MAF >0.05 sample). Many studies are available on the possible effects of mutations in such regions (*Hayashi, Watanabe & Kawajiri, 1991*; *In et al., 1997*; or for a more general review on the topic, *Jaenisch & Bird, 2003*). Nonetheless, the SNPs show low F_{ST} values, which is congruent with the finding of balancing selection. Sewall Wright's guidelines for interpreting F_{ST} values suggest little genetic differentiation in our populations (as cited by *Jobling et al., 2013*; Chapter 5, Box 5.2). As silent mutations in $DRD2$ are known to alter the mRNA stability and even the synthesis of the receptor itself (*Duan et al., 2003*), we call for exploring the possible effects of these SNPs.

Additionally, the levels of the linkage disequilibrium measurement D' are typical for the respective populations: African populations show a dispersed pattern and no clear LD blocks (Figs. S3, S11, and S15). While the LD blocks are visible in American populations (Figs. S7, S12 and S13), they are not as clear as in Asian (Figs. S5, S6 and S10) or European populations (Figs. S4, S8, S9 and S14). Our candidate SNPs are part of tight LD blocks ($D' > 0.8$), which prevents us from making any further interpretations. We also examined the measurement r^2 for all populations (again, excluded SNPs are listed in Table S1), which revealed no new insight.

The finding of balancing selection suggests that in our sample the minor alleles bear some fitness disadvantage. Note that some individuals are homozygous for the minor allele (0.8–2.3% per SNP, over all populations). Fitness is altered if survival or reproduction of an organism is affected. This raises the possibility of a connection between our candidate SNPs and diseases or malfunctions of dopamine receptor 2. In the list of diseases associated with dopamine (see "Introduction") the most striking example is schizophrenia because dopamine receptor 2 blockers can successfully treat patients.

Albeit we aim to avoid overhasty conclusions regarding directional selection on *DRD2*, the three SNPs detected by BayeScan under positive selection—and that are found by LOSITAN just below significance—could be of interest. This is particularly the case for rs6277, with its known phenotypic associations: rs6277 has been associated with schizophrenia in Han Chinese in Taiwan (*Glatt et al., 2009*), in Russians (*Monakhov et al., 2008*) and in Bulgarians (*Betcheva et al., 2009*).

Nonetheless, rs6277 was not identified among the 108 schizophrenia-associated loci that have recently been published based on 36,989 cases and 113,075 controls by the *Schizophrenia Working Group of the Psychiatry Genomics Consortium* (*Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014*). Nevertheless, *DRD2* is one of the genes that was confirmed as being associated with schizophrenia by the *Working Group*. Thus, even weak signs of directional selection on *DRD2* might be important to (i) identify potential new disease-related phenotypical associations and (ii) to speculate on what the "selective force" could have been bringing mutations on *DRD2* towards fixations and (iii) what potential condition-related consequences selection on *DRD2* could have. The question is whether these conditions affect fitness. Accordingly, *Bassett et al. (1996)* showed that reproductive fitness is reduced in groups of familial schizophrenia, which suggests a selection process. Puzzlingly enough, they also found some evidence for an increased fitness of a small subsample of sisters. *Shaner, Miller & Mintz (2004)* proposed that schizophrenia is the low-fitness trait of a highly variable mental trait. They argue that the persistence of the illness at about 1% globally is too high for new mutations. Thus, mainly balancing selection would fit this hypothesis very well, and our candidate SNPs under balancing selection could be viable indicators for this.

DRD2 is clearly associated with schizophrenia. Schizophrenia, however, is a "polygenic condition" including genetic loci over the whole human genome (*Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014*). Accordingly, the importance of *DRD2* should not be over-estimated. Moreover, the method of selection detection does not allow direct inferences about a phenotype (e.g., schizophrenia). Our overall results can serve as a valuable precursor to future studies on the subject.

To untangle the possible effects of our SNPs, we propose a study in which our candidate SNPs are investigated in schizophrenic and non-schizophrenic persons. A simple comparison of the SNPs and the different haplotypes between the two groups should efficiently help assess our findings. If this proposed study finds differences in those two groups, then the mechanisms of those SNPs and their possible haplotypes must be investigated.

CONCLUSION

We found nine candidates for balancing selection on *DRD2* but only a weak signs for directional selection. Interestingly, rs6277, a SNP known to be associated with schizophrenia, is among those SNPs for which we found weak evidence for directional selection. Some of the SNPs under balancing selection are potentially associated with various diseases. These SNPs could be important as biomarkers due to their very low F_{ST} values: the genetic differentiation of one population compared with the whole sample is very small. While all candidate SNPs may be worth exploring, we definitely recommend using rs60599314 and rs79549222 (as these were detected under balancing selection based on a MAF >0.05 and a MAF >0.01 sample). We also recommend rs80215768 and rs74751335, found under balancing selection, for further studies on *DRD2* because the former within a promoter flanking region and the latter lies in a transcription factor binding site.

ACKNOWLEDGEMENTS

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

Grant Disclosures

Competing Interests

The authors declare that there are competing interests.

Author Contributions

- Tobias Göllner conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures and/or tables.
- Martin Fieder conceived and designed the experiments, contributed reagents/materials/analysis tools, reviewed drafts of the paper.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.1149#supplemental-information.

REFERENCES

Amigo J, Salas A, Phillips C, Carracedo. 2008. SPSmart: adapting population based SNP genotype databases for fast and comprehensive web access. *BMC Bioinformatics* **9(1)**:428 DOI 10.1186/1471-2105-9-428.

Andén NE, Carlsson A, Dahlström A, Fuxe K, Hillarp NÅ, Larsson K. 1964. Demonstration and mapping out of nigro-neostriatal dopamine neurons. *Life Sciences* **3(6)**:523–530 DOI 10.1016/0024-3205(64)90161-4.

Andersen PH, Gingrich JA, Bates MD, Dearry A, Falardeau P, Senogles SE, Caron MG. 1990. Dopamine receptor subtypes: beyond the D 1/D 2 classification. *Trends in Pharmacological Sciences* **11(6)**:231–236 DOI 10.1016/0165-6147(90)90249-8.

Antao T, Lopes A, Lopes RJ, Beja-Pereira A, Luikart G. 2008. LOSITAN: a workbench to detect molecular adaptation based on a Fst-outlier method. *BMC Bioinformatics* **9(1)**:323 DOI 10.1186/1471-2105-9-323.

Bassett AS, Bury A, Hodgkinson KA, Honer WG. 1996. Reproductive fitness in familial schizophrenia. *Schizophrenia Research* **21(3)**:151–160 DOI 10.1016/0920-9964(96)00018-7.

Beaulieu JM, Gainetdinov RR. 2011. The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacological Reviews* **63(1)**:182–217 DOI 10.1124/pr.110.002642.

Bergen AW, Yeager M, Welch RA, Haque K, Ganjei JK, Van den Bree MB, Kaye WH. 2005. Association of multiple *DRD2* polymorphisms with anorexia nervosa. *Neuropsychopharmacology* **30(9)**:1703–1710 DOI 10.1038/sj.npp.1300719.

Betcheva ET, Mushiroda T, Takahashi A, Kubo M, Karachanak SN, Zaharieva IT, Vazharova RV, Dimova II, Milanova VK, Tolev T, Kirov G, Owen MJ, O'Donovan MC, Kamatani N, Nakamura Y, Toncheva DI. 2009. Case—control association study of 59 candidate genes reveals the DRD2 SNP rs6277 (C957T) as the only susceptibility factor for schizophrenia in the Bulgarian population. *Journal of Human Genetics* **54(2)**:98–107 DOI 10.1038/jhg.2008.14.

Carlsson A. 2001. A paradigm shift in brain research. *Science* **294(5544)**:1021–1024 DOI 10.1126/science.1066969.

Carlsson A, Waters N, Holm-Waters S, Tedroff J, Nilsson M, Carlsson ML. 2001. Interactions between monoamines, glutamate, and GABA in schizophrenia: new evidence. *Annual Review of Pharmacology and Toxicology* **41(1)**:237–260 DOI 10.1146/annurev.pharmtox.41.1.237.

Civelli O, Bunzow JR, Grandy DK. 1993. Molecular diversity of the dopamine receptors. *Annual Review of Pharmacology and Toxicology* **33(1)**:281–307 DOI 10.1146/annurev.pa.33.040193.001433.

Creese I, Burt DR, Snyder SH. 1976. Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. *Science* **192(4238)**:481–483 DOI 10.1126/science.3854.

Dahlström A, Fuxe K. 1964. Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta Physiologica Scandinavica* **SUPPL 232**:1–55.

De Mei C, Ramos M, Iitaka C, Borrelli E. 2009. Getting specialized: presynaptic and postsynaptic dopamine D2 receptors. *Current Opinion in Pharmacology* **9(1)**:53–58 DOI 10.1016/j.coph.2008.12.002.

Di Chiara G, Bassareo V. 2007. Reward system and addiction: what dopamine does and doesn't do. *Current Opinion in Pharmacology* **7(1)**:69–76 DOI 10.1016/j.coph.2006.11.003.

Duan J, Wainwright MS, Comeron JM, Saitou N, Sanders AR, Gelernter J, Gejman PV. 2003. Synonymous mutations in the human dopamine receptor D2 (*DRD2*) affect mRNA stability and synthesis of the receptor. *Human Molecular Genetics* **12(3)**:205–216 DOI 10.1093/hmg/ddg055.

Ehringer H, Hornykiewicz O. 1960. Distribution of noradrenaline and dopamine (3-hydroxytyramine) in the human brain and their behavior in diseases of the extrapyramidal system. *Klinische Wochenschrift* **38**:1236–1239. DOI 10.1007/BF01485901.

Foll M, Gaggiotti O. 2008. A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. *Genetics* **180(2)**:977–993 DOI 10.1534/genetics.108.092221.

Gingrich JA, Caron MG. 1993. Recent advances in the molecular biology of dopamine receptors. *Annual Review of Neuroscience* **16(1)**:299–321 DOI 10.1146/annurev.ne.16.030193.001503.

Giros B, Sokoloff P, Martres MP, Riou JF, Emorine LJ, Schwartz JC. 1989. Alternative splicing directs the expression of two D2 dopamine receptor isoforms. *Nature* **342**:923–926 DOI 10.1038/342923a0.

Gizer IR, Ficks C, Waldman ID. 2009. Candidate gene studies of ADHD: a meta-analytic review. *Human Genetics* **126(1)**:51–90 DOI 10.1007/s00439-009-0694-x.

Glatt SJ, Faraone SV, Lasky-Su JA, Kanazawa T, Hwu HG, Tsuang MT. 2009. Family-based association testing strongly implicates DRD2 as a risk gene for schizophrenia in Han Chinese from Taiwan. *Molecular Psychiatry* **14(9)**:885–893 DOI 10.1038/mp.2008.30.

Glatt SJ, Jönsson EG. 2006. The Cys allele of the DRD2 Ser311Cys polymorphism has a dominant effect on risk for schizophrenia: evidence from fixed-and random-effects meta-analyses. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics* **141(2)**:149–154 DOI 10.1002/ajmg.b.30273.

Hayashi SI, Watanabe J, Kawajiri K. 1991. Genetic polymorphisms in the 5′-flanking region change transcriptional regulation of the human cytochrome P450IIE1 gene. *Journal of Biochemistry* **110(4)**:559–565.

Hyman SE, Malenka RC, Nestler EJ. 2006. Neural mechanisms of addiction: the role of reward-related learning and memory. *Annual Review of Neuroscience* **29**:565–598 DOI 10.1146/annurev.neuro.29.051605.113009.

In KH, Asano K, Beier D, Grobholz J, Finn PW, Silverman EK, Silverman ES, Collins T, Fischer AR, Keith TP, Serino K, Kim SW, De Sanctis GT, Yandava C, Pillari A, Rubin P, Kemp J, Israel E, Busse W, Ledford D, Murray JJ, Segal A, Tinkleman D, Drazen JM. 1997. Naturally occurring mutations in the human 5-lipoxygenase gene promoter that modify transcription factor binding and reporter gene transcription. *Journal of Clinical Investigation* **99(5)**:1130–1137 DOI 10.1172/JCI119241.

Iversen SD, Iversen LL. 2007. Dopamine: 50 years in perspective. *Trends in Neurosciences* **30(5)**:188–193 DOI 10.1016/j.tins.2007.03.002.

Jaenisch R, Bird A. 2003. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nature Genetics* **33**:245–254 DOI 10.1038/ng1089.

Jobling M, Hollox E, Hurles M, Kivisild T, Tyler-Smith C. 2013. *Human evolutionary genetics*. New York and London: Garland Science.

Koob GF, Volkow ND. 2010. Neurocircuitry of addiction. *Neuropsychopharmacology* **35(1)**:217–238 DOI 10.1038/npp.2009.110.

Lischer HEL, Excoffier L. 2012. PGDSpider: an automated data conversion tool for connecting population genetics and genomics programs. *Bioinformatics* **28(2)**:298–299 DOI 10.1093/bioinformatics/btr642.

Mink JW. 2006. Neurobiology of basal ganglia and Tourette syndrome: basal ganglia circuits and thalamocortical outputs. In: *Advances in neurology*. vol. 99. New York: Raven Press, 89.

Missale C, Nash SR, Robinson SW, Jaber M, Caron MG. 1998. Dopamine receptors: from structure to function. *Physiological Reviews* **78(1)**:189–225.

Monakhov M, Golimbet V, Abramova L, Kaleda V, Karpov V. 2008. Association study of three polymorphisms in the dopamine D2 receptor gene and schizophrenia in the Russian population. *Schizophrenia Research* **100(1)**:302–307 DOI 10.1016/j.schres.2008.01.007.

Monsma FJ, McVittie LD, Gerfen CR, Mahan LC, Sibley DR. 1989. Multiple D2 dopamine receptors produced by alternative RNA splicing. *Nature* **342**:926–929 DOI 10.1038/342926a0.

Mueller JC. 2004. Linkage disequilibrium for different scales and applications. *Briefings in Bioinformatics* **5(4)**:355–364 DOI 10.1093/bib/5.4.355.

Niznik HB, Van Tol HH. 1992. Dopamine receptor genes: new tools for molecular psychiatry. *Journal of Psychiatry and Neuroscience* **17(4)**:158–180.

Sasabe T, Furukawa A, Matsusita S, Higuchi S, Ishiura S. 2007. Association analysis of the dopamine receptor D2 (DRD2) SNP rs1076560 in alcoholic patients. *Neuroscience Letters* **412(2)**:139–142 DOI 10.1016/j.neulet.2006.10.064.

Schizophrenia Working Group of the Psychiatric Genomics Consortium. 2014. Biological insights from 108 schizophrenia-associated genetic loci. *Nature* **511(7510)**:421–427 DOI 10.1038/nature13595.

Seeman P, Lee T, Chau-Wong M, Wong K. 1976. Antipsychotic drug doses and neuroleptic/dopamine receptors. *Nature* **261(5562)**:717–719 DOI 10.1038/261717a0.

Shaner A, Miller G, Mintz J. 2004. Schizophrenia as one extreme of a sexually selected fitness indicator. *Schizophrenia Research* **70(1)**:101–109 DOI 10.1016/j.schres.2003.09.014.

Sibley DR. 1999. New insights into dopaminergic receptor function using antisense and genetically altered animals 1. *Annual Review of Pharmacology and Toxicology* **39(1)**:313–341 DOI 10.1146/annurev.pharmtox.39.1.313.

Sibley DR, Monsma FJ. 1992. Molecular biology of dopamine receptors. *Trends in Pharmacological Sciences* **13**:61–69 DOI 10.1016/0165-6147(92)90025-2.

Snyder SH, Taylor KM, Coyle JT, Meyerhoff JL. 1970. The role of brain dopamine in behavioral regulation and the actions of psychotropic drugs. *American Journal of Psychiatry* **127(2)**:199–207 DOI 10.1176/ajp.127.2.199.

Sokoloff P, Andrieux M, Besançon R, Pilon C, Martres MP, Giros B, Schwartz JC. 1992. Pharmacology of human dopamine D 3 receptor expressed in a mammalian cell line: comparison with D 2 receptor. *European Journal of Pharmacology: Molecular Pharmacology* **225(4)**:331–337 DOI 10.1016/0922-4106(92)90107-7.

Swanson JM, Kinsbourne M, Nigg J, Lanphear B, Stefanatos GA, Volkow N, Wadhwa PD. 2007. Etiologic subtypes of attention-deficit/hyperactivity disorder: brain imaging, molecular genetic and environmental factors and the dopamine hypothesis. *Neuropsychology Review* **17(1)**:39–59 DOI 10.1007/s11065-007-9019-9.

Usiello A, Baik JH, Rougé-Pont F, Picetti R, Dierich A, LeMeur M, Borrelli E. 2000. Distinct functions of the two isoforms of dopamine D2 receptors. *Nature* **408**(**6809**):199–203 DOI 10.1038/35041572.

Vallone D, Picetti R, Borrelli E. 2000. Structure and function of dopamine receptors. *Neuroscience & Biobehavioral Reviews* **24**(**1**):125–132 DOI 10.1016/S0149-7634(99)00063-9.

Warnes G, Leisch F, Man M, Warnes MG. 2013. *Package 'genetics'. Available at* https://cran.r-project.org/web/packages/genetics/index.html.

Wolf ME, Roth RH. 1990. Autoreceptor regulation of dopamine synthesis. *Annals of the New York Academy of Sciences* **604**(**1**):323–343 DOI 10.1111/j.1749-6632.1990.tb32003.x.