The role of genetic variability in the GABRA6, 5-HTT and BDNF genes in anxiety-related traits.

Bárbara Arias*[1], Mari Aguilera*[1], Jorge Moya[2], Pilar A Sáiz[3], Helena Villa[2], Manuel I. Ibáñez[2], Maria P García-Portillo[3], Julio Bobes[3], Generós Ortet[2], Lourdes Fañanás[1].

[1] Department of Animal Biology, Anthropology Section, Faculty of Biology, University of Barcelona and Biomedicine Institute of the University of Barcelona (IBUB), and Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), Instituto de Salud Carlos III, Spain.
[2] Department of Basic Psychology, Clinical and Psychobiology, Faculty of Human and Social Sciences, Jaume I University
[3] Department of Psychiatry, School of Medicine, University of Oviedo, and Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), Instituto de Salud Carlos III, Spain.

*Authors contributed equally to the manuscript

Abstract

Objective: The aims of this study were to test the individual association of the 5-HTT, BDNF, and GABRA6 genes with anxiety-related traits and to explore putative GxG interactions in a healthy sample. **Method**: A sample of 937 individuals from the general population completed the TCI questionnaire; a subsample of 553 individuals also filled in a brief version of the NEO inventory. The whole sample was genotyped for the 5-HTTLPR polymorphism (5-HTT gene), the Val66Met polymorphism (BDNF gene) and the T1521C polymorphism (GABRA6 gene). **Results**: Individuals carrying the TTgenotype of the T1512C polymorphism presented slightly higher scores for Harm Avoidance (HA) than C allele carriers ($F=2.96$, $p=0.051$). In addition, there was a significant GxG interaction on HA between the 5-HTTLPR and Val66Met polymorphisms ($F=3.4$, $p=0.009$). **Conclusion**: GABRA6 emerges as a putative gene may be involved in the variability of HA. The effect of a significant GxG interaction between the 5-HTT and BDNF genes on HA could explain part of the genetic basis underlying anxiety-related traits.

Key Words: harm avoidance, 5-HTT gene, BDNF gene, GABRA6 gene.

Significant outcomes

• A complex gene–gene interaction between the 5-HTT and BDNF genes explained some of the variability in the harm avoidance dimension.

• Genetic variability related to the gabaergic system is associated with the harm avoidance dimension in a sample of the Spanish general population.

• The harm avoidance dimension of the TCI questionnaire is more strongly supported by genetic components than the neuroticism dimension of the NEO inventory.

Limitations

• The two Spanish populations analyzed presented differences with respect to the genotype distribution of the Val66Met polymorphism (BDNF gene).

• Neuroticism data from the NEO inventory were only available for the sample from Castelló.

Introduction

Anxiety-related traits that are continuously distributed in the normal human personality (1) have been described as individual differences in emotional reactivity, proneness to worry and susceptibility to negative affect. They are mainly captured by the presence of neuroticism (2, 3) or harm avoidance (4). Neuroticism (N) includes traits such as anxiety, anger, hostility, depression, self-consciousness, impulsiveness and vulnerability, while harm avoidance (HA) characterizes individuals with high scores for being cautious, tense, apprehensive, fearful, inhibited, shy, easily fatigued and worried. Both N and HA have been considered as markers for vulnerability to depressive disorders (5-8).

Twin studies have suggested that the estimated heritability for personality traits ranges between 30 and 50% (9). Specifically, these studies on the genetics of personality have demonstrated that the genetic component of anxiety-related traits accounts for 40 to 60% of the observed variance (10, 11).

There is growing interest in the idea that neurotransmitter functions relate to normal variations in personality traits (12, 13). In this sense, serotonin neurotransmission (5-HT) has a fundamental role in the modulation of emotional behaviour and in brain development. Genetic variability associated with 5-HT function is likely to influence behavioural predispositions such as anxiety-related traits (14, 15).The serotonin transporter (5-HTT) has a key role in 5-HT neurotransmission, since it is the main reuptake mechanism and the main biological target for antidepressant drugs. 5-HTT is encoded by the SLC6A4 gene, which contains an insertion/deletion in the 5' promoter region of 44bp (5-HTTLPR), with reduced transcription of the 5-HTTLPR short (S) allele in comparison with the long L allele (16, 17).

The seminal study carried out by Lesch and collaborators (1996) showed an association between anxiety-related traits and the 5-HTT gene (17). Although inconsistencies have emerged as shown in a recent meta-analysis (see review (18, 19)), one recent meta-analyses have shown the definite effect of variability at 5-HTT gene on depression and stress sensitivity (20).

Besides the 5-HTT gene, the brain-derived neurotrophic factor (BDNF) from the neurotrophin family could be of special interest because of its critical role in normal adaptive responses to stress as well as in the response to antidepressant treatment (21, 22). The BDNF gene (chromosome 11p14) presents an SNP (196 G/A -Val 66 Met) located in the 5' pro-BDNF precursor peptide sequence that may affect intracellular processing and secretion of the mature protein (23). Sen et al. (2003) reported that the Met allele was associated with lower levels of neuroticism (24), although later studies failed to replicate such findings (25-27). Recently, the Met allele of the Val66Met polymorphism at the BDNF gene has been shown to play a putative role in anxiety disorders. Met carriers showed impaired learning of cues that signal safety versus threat that rely on extinction mechanisms (28) .

Another neurotransmitter that might be involved in the propensity for a fearful or anxious temperament is γ-aminobutyric acid (GABA), the main inhibitory neurotransmitter in the CNS (29, 30). The inhibitory effect of GABA is mediated by GABAA receptors, which are ionotropic GABA-gated chloride channel receptors.

GABA acts as an agonist by inducing conformational changes in the GABAA receptor, increasing the permeability of the central pore to chloride ions. The chloride influx hyperpolarizes the neuron, reducing its excitability and having a general inhibitory effect on neuronal activity. In addition, benzodiazepines, one of the pharmacological treatments for anxiety disorders, increase the efficiency of inhibitory GABAergic neurotransmission by means of agonists binding to the receptor $GABA_A$.

It has been suggested that individual differences in the frontal cortical $GABA_A$ receptor complex and GABA systems could modulate patterns of brain activity associated with individual differences in threat-related responses (29). Uhart and collaborators (2004) analysed a single-nucleotide polymorphism (SNP) consisting of a T-to-C substitution (position 1521) in the 3' non-coding region of the $GABA_A\alpha_6$ receptor subunit gene GABRA6 (cr. 5q34) in a healthy population (31). The results revealed that homozygotes for the C allele showed an attenuated hormonal and physiological response to acute psychological stress. Therefore, this polymorphism could be involved in the individual differences underlying part of the biological basis of anxiety-related traits.

Materials and Methods

Sample

Our sample consisted of 937 subjects from the general population (47.8% males; total mean age=30.5, SD=12.2) who were recruited from the campus of the University Jaume I (Castelló, Spain) and from primary care settings from Oviedo (Asturias, Spain).

In terms of education 12.2% of individuals had completed elementary school, 56.8% had completed high school, and 25% had received a university education. Sociodemographic data divided by the geographic origin of the samples (Castelló/Asturias) are displayed in Table 1.

Table 1. Sociodemographic data according to geographic origin

Geographic origin	Gender distribution *	Mean age (SD; range)**	Education ***
Castelló (Comunitat Valenciana) (n=533)	242 males 291 females	22.9 (5.4; 18-55)	Elementary 14 (3%) High School 424 (84%) University 67 (13%)
Oviedo (Asturias) (n=404)	202 males 202 females	40.5 (11.3; 20-60)	Elementary 104 (26%) High School 125 (31%) University 175 (43%)

*$\chi2$= 1.95 p=0.163; **F=996.8 p<0.0001; ***$\chi2$=271.8 p<0.0001

Exclusion criteria were the presence of any past or present major psychiatric disorder and/or a history of any severe mental disorder in first-degree relatives. These aspects were screened by means of a short interview designed ad hoc for this study on the basis of selected items of structured scales such as SCID-I (32) and FIGS (33). All participants were of Spanish (Caucasian) ancestry to reduce the possibility of

confounding genetic differences by population stratification (34).

Ethical approval was obtained from local Spanish research ethic committees in each Institution. All participants provided written informed consent before inclusion in the study.

Measurements

Personality assessment

All participants filled out the self-reported Temperament and Character Inventory (TCI;(35)) composed of 240 items. Given the aims of this study, the analyses focused on the dimension of Harm Avoidance (HA), which has four subscales: Anticipatory worry (HA1), Fear of uncertainty (HA2), Shyness with strangers (HA3) and Fatigability (HA4). Good psychometric properties have been described for TCI (35).

In addition, 533 individuals completed the Brief Big Five Questionnaire (BFQ; (36)). This test has high test-retest reliability and longitudinal stability (37). The analyses focused on the dimension of Neuroticism (N). Subscales corresponding to the N dimension were not available.

Laboratory methods

Genomic DNA from the Castelló subsample was extracted from saliva samples using the Collection Kit BuccalAmp DNA extraction kit (Epicentre® Biotechnologies, Madison, WI) and that from the Oviedo subsample was extracted from blood samples using the salting-out technique (38). The 5-HTTLPR polymorphism of the serotonin transporter gene was analyzed using the protocol previously described by Lesch and collaborators (1996) (17). The SNP rs6265 (Val66Met) of the BDNF gene and the SNP rs3219151 (T1521C) of the GABRA6 gene were genotyped using Applied Biosystems (AB) TaqMan technology. An AB assay-on-demand service supplied the probes.

Those individuals who were not genotyped were not included in the final statistical analyses (see Table 2 for final sample).

Random individuals were re-genotyped in order to confirm the reproducibility of the pattern.

Statistical analyses

Analyses were performed using STATA 9.1 (39) and EpiInfo (40).

The main effects of polymorphisms on harm avoidance and neuroticism were analyzed separately for each of the following polymorphisms: the 5-HTTLPR genotype, BDNF genotype, and GABRA6 genotype using linear regression analysis. Regressions were performed for the harm avoidance dimension and separately for each individual subscale of harm avoidance. The Wald test was performed to test the overall main effect of each polymorphism on each dimension and subscale.

The xi3 command was then used to test interactive effects with categorical predictors

(41). The interactive effects between SLC6A4 5-HTTLPR and BDNF Val66Met, between SLC6A4 5-HTTLPR and GABRA6, and between BDNF Val66Met and GABRA6 T1512C were fitted in models of harm avoidance and neuroticism. The Wald test was used to assess the interaction effect. When a two-way interaction was significant, further simple effects were assessed, that is, the effect of VI1 at each level of VI2.

All regression analyses were controlled for age, gender and demographic origin.

Results

The genotype, allele distribution and Hardy-Weinberg equilibrium of each population (Castelló and Oviedo) as well as those of the total sample are shown in Table 2.

Table 2. Genotype distribution of the three polymorphisms analysed according to geographic origin

	5-HTTLPR polymorphism (SLC6A4 gene)				
	Genotype distribution			***Allele distribution***	
	L/L	L/S	S/S	L allele	S allele
Castelló (n=475)	119 (25.1%)	230 (48.4%)	126 (26.5%)	468 (49.2%)	482 (50.8%)
Oviedo (n=404)	122 (30.2%)	192 (47.5%)	90 (22.3%)	436 (53.9%)	372 (46.1%)
Total sample (n= 879)	241 (27.4%)	422 (48%)	216 (24.6%)	904 (51.4%)	854 (48.6%)
**	χ^2=3.75, df=2, p=0.15			χ^2=3.86, df=1, p=0.05	
	Val66Met polymorphism (BDNF gene)				
	Genotype distribution			***Allele distribution***	
	Val/Val	Val/Met	Met/Met	Val allele	Met allele
Castelló (n=470)	282 (60%)	159 (33.8%)	29 (6.2%)	723 (76.9%)	217 (23.1%)
Oviedo (n=385)	269 (69.9%)	106 (27.5%)	10 (2.6%)	644 (83.6%)	126 (16.4%)
Total sample (n=855)	551 (64.4%)	265 (31%)	39 (4.6%)	1367 (79.9%)	343 (20.1%)
**	χ^2=11.83, df=2, p<0.01			χ^2=11.93, df=1, p<0.01	
	T1512C polymorphism (GABRA6 gene)				
	Genotype distribution			***Allele distribution***	
	T/T	T/C	C/C	T allele	C allele
Castelló (n=481)	161 (33.5%)	236 (49.1%)	84 (17.5%)	558 (58.1%)	404 (41.9%)
Oviedo (n=370)	120 (32.4%)	193 (52.2%)	57 (15.4%)	433 (58.5%)	307 (41.5%)
Total sample (n=851)	281 (33%)	429 (50.4%)	141 (16.6%)	991 (58.2%)	711 (41.8%)
**	X^2=1, df=2, p=0.61			χ^2=0.04, df=1, p=0.83	

* Frequencies were in Hardy-Weinberg equilibrium for the Castelló subsample (5-HTTLPR polymorphism χ^2_2=0.24, p=0.88; Val66Met polymorphism χ^2_2=0.52, p=0.77; T1512C polymorphism χ^2_2=0.02, p=0.99); the Oviedo subsample (5-HTTLPR polymorphism χ^2_2=0.36,p=0.83; Val66Met polymorphism χ^2_2=0.00, p=0.99; T1512C polymorphism χ^2_2=1.06, p=0.59); and for the total sample (5-HTTLPR polymorphism χ^2_2=0.70, p=0.70; Val66Met polymorphism χ^2_2=0.51, p=0.76; T1512C polymorphism χ^2_2=0.60, p=0.74).
** Statistical results derived from the comparison between the Castelló and Oviedo subsample distributions.

The genotypic frequencies that we observed in these Spanish populations were similar to those frequencies described for European Caucasians (26, 31, 42, 43).

The two populations presented similar genotype and allele distributions for the 5-HTTLPR polymorphism (5-HTT gene) and for the T1512C polymorphism (GABRA6 gene). However, the genotype and allele distribution differed between Castelló and Oviedo for the Val66Met polymorphism (BDNF gene) (genotype frequencies: χ_2=11.83, df=2, p=0.003; allele frequencies: χ_2=11.93, df=1, p<0.001). These differences were taken into account when we merged the samples for subsequent statistical analyses.

We found no significant associations between the 5-HTTLPR (5-HTT gene) or Val66Met (BDNF gene) polymorphisms and HA or its subscales.

The effect of GABRA6 T1512C polymorphisms on HA approached statistical significance, that is, the TT group had higher scores for HA than TC or CC subjects (F=2.96, p=0.051). In addition, a significant main effect of the GABRA6 T1512C polymorphism was found on HA1 (Anticipatory worry) (F=4.07; p=0.017). Specifically, the TT group had higher scores compared to TC or CC. No significant effect was found on the other subdimensions (see Table 3).

Table 3. Mean scores (SD) and results (Wald test) per polymorphism analysed in the whole sample for Harm Avoidance and its subscales (n=937).

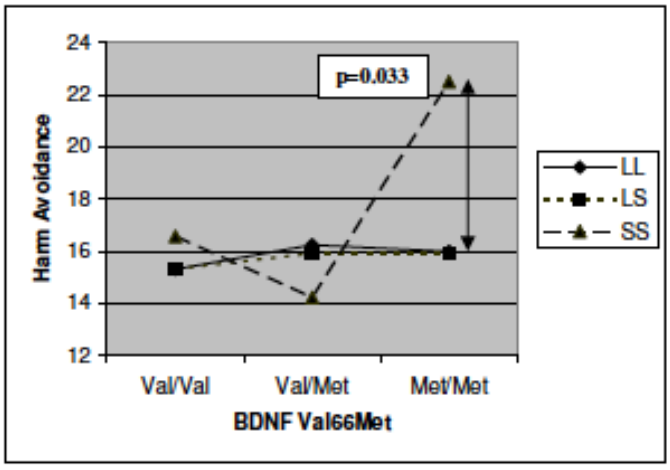
	5-HTTLPR polymorphism				BDNF Val66Met polymorphism				GABRA6 T1512C polymorphism			
	LL (n = 241)	LS (n = 422)	SS (n = 216)	F	Val/Val (n = 551)	Val/Met (n = 265)	Met/Met (n = 39)	F	TT (n = 281)	TC (n = 429)	CC (n = 141)	F
Harm Avoidance	15.6(6.25)	15.5(6.06)	15.9(6.51)	0.52	15.57(6.09)	15.47(6.44)	17.07(7.33)	0.13	16.29(6.60)	15.31(6.34)	15.21(6.33)	**2.96***
HA1	4.24(2.49)	4.19(5.59)	4.16(2.69)	0.00	4.17(2.57)	4.14(2.67)	4.76(2.95)	1.38	4.58(2.75)	4.05(2.59)	4.07(2.66)	**4.07****
HA2	4.22(1.91)	4.16(1.89)	4.25(1.89)	0.38	4.22(1.89)	4.17(1.91)	4.32(1.96)	0.34	4.34(1.99)	4.18(1.93)	4.08(1.87)	2.10
HA3	3.58(2.14)	3.55(2.06)	3.82(2.00)	1.71	3.59(2.08)	3.63(2.10)	4.12(2.17)	2.01	3.69(2.05)	3.56(2.23)	3.60(2.08)	0.39
HA4	3.52(1.92)	3.61(1.89)	3.69(1.89)	0.14	3.59(1.89)	3.54(1.86)	3.88(1.79)	0.60	3.69(1.84)	3.52(1.96)	3.46(1.96)	1.23

*p=0.051; **p=0.017 (All analyses controlled for age, gender and geographic origin) **Abbreviations: HA1** (Anticipatory worry); **HA2** (Fear of uncertainty); **HA3** (Shyness with strangers); **HA4** (Fatigability)

Gene and anxiety-related trait interactions

We found a significant two-way interaction in relation to HA. An interaction between the 5-HTTLPR and BDNF Val66Met polymorphisms modulated the HA scores (F=3.4, p=0.009). Analysis of simple effects showed that subjects with Met/Met and S/S genotypes had higher scores on HA compared to Met/Met-LS or Met/Met-LL (F=3.43, p=0.033) (see Figure 1). No other two-way interactions were significant with regards to HA.

Figure 1. The effect of the BDNF Val66Met polymorphism on HA is moderated by the 5-HTTLPR polymorphism. Met-carriers with the S/S genotype (n=6) showed higher scores for HA compared to LS (n=23) or LL (n=16).



We found no association between either of the polymorphisms analyzed and N, or any gene–gene interaction effect on N.

Discussion

With regards to the main effect of the 5-HTTLPR polymorphism (5-HTT gene) on anxiety-related traits, we did not succeed in replicating the previously reported association between the S allele and high anxiety-related traits reported by Lesch et al. (1996) (17). In contrast, our results agree with most of the studies that have reported negative results in Caucasian populations (44-51).

It has been suggested that important sources of heterogeneity between studies could be due to the use of slightly different personality constructs, the inclusion of psychiatric populations, or the use of small sample sizes (52). In the present study, both neuroticism and harm avoidance were explored; in addition, the whole sample was screened for any current or lifetime psychiatry disorder and, finally, the sample size was larger than in previous studies (44-51).

The Val66Met polymorphism (BDNF gene) has previously been associated with anxiety-related traits by Sen et al. (2003) (24). The study revealed that Val allele carriers presented higher levels of neuroticism compared to Met allele carriers. However, later studies, in agreement with our findings, did not detect this independent effect in relation to those personality traits (25-27).

Interestingly, a gene–gene interaction was detected between the 5-HTTLPR polymorphism (5-HTT gene) and Val66Met polymorphism (BDNF gene): among individuals with the Met/Met genotype, carriers of the SS genotype exhibited significantly higher scores for harm avoidance than L allele carriers. This gene–gene interaction may underlie the inconsistencies found in studies exploring only the main effects of one of these genes and anxiety-related traits.

The results of our study provide evidence for a role of the GABRA6 T1512C polymorphism in harm avoidance variability in a large sample from the general population. Specifically, the TT genotype of this polymorphism seemed to be strongly associated with high scores on the subscale “anticipatory worry” (HA1). These results together with the results reported by Uhart et al. (2004)(31) suggest that the GABRA6 T1512C polymorphism, in accordance with our hypothesis, is strongly associated with individual differences in anxiety-related traits, specifically in those traits that are closely related to the anxiety spectrum, such as anticipatory worry. However, we know that this polymorphism located at 3’ region do not seem to display functional relevance. As α6 subunit is included into a cluster in chromosome 5q with α1, β2 and α2 subunit genes (53), the analyzed polymorphism may be in linkage disequilibrium with another site within the GABRA6 gene or these genes located in close proximity. Moreover, $GABA_A$ receptors are classified as ionotropic receptors which are involved in fast events in the neuron, as opposite to other type of receptors (such 5-HTT transporter) that mediate more long-term effects modifying the responsiveness and plasticity of the neuron (54). We could hypothesized that subtle genetic changes in this fast-response receptors such as this SNP, may alter the signal transmission in the neuron impacting, then, in the final anxiety related-phenotype.

Finally, our results seem to identify genetic components involved in harm avoidance, derived from Cloninger's tridimensional theory of personality. However, negative results were found for the neuroticism dimension derived from the Big Five model developed by Costa and McCrae. These results are consistent with a recent metaanalysis reporting the existence of a small, but significant genetic background for the harm avoidance dimension but not for the neuroticism dimension (19). Taken together, these findings suggest that the HA construct seems to be more strongly associated with genetically related traits than the neuroticism construct. Nevertheless, further research is needed to elucidate the complex biological and genetic architecture of anxiety-related traits.

Our study has some limitations. Firstly, we found differences between the Castelló and Oviedo populations with respect to the genotype distribution of the Val66Met polymorphism (BDNF gene); these differences were basically related to an increase in the frequency of the Val/Val genotype in the subsample from Oviedo. However, when we merged the two populations, the genotype frequencies were similar to those expected in a Caucasian population (26). Thus, it is unlikely that the significant results obtained were due to a bias in the genotype distribution. In fact, the effect of interaction between 5-HTT and BDNF on HA was found with the Met allele and not with the Val allele, as might be expected due to the overrepresentation of this allele in the Oviedo sample.

With a sample of almost a thousand individuals, our study was larger tan previous studies (44-51), and therefore the likelihood of Type I or II errors was reduced. However, some of our findings might not have been statistically significant if multiple testing corrections had been carried out. These corrections are likely to be excessively exclusive in the context of the present study since the selection of the genetic polymorphisms, the sample size and the analyses performed had a directional hypothesis based on previous findings (55).

In conclusion, the GABRA6 T1512C polymorphism has emerged as a putative genetic factor involved in human anxiety-related trait variability, specifically HA. Moreover, the effect of a significant GxG interaction between the 5-HTT gene and BDNF gene on HA could partly explain some of the inconsistencies found in previous studies and should be taken into account in the understanding of individual differences in complex constructs such as anxiety-related traits.

Disclosure/Conflicts of interest

The author(s) declare that, except for income received from their primary employer, no financial support or compensation has been received from any individual or corporate entity over the past three years for research or professional services and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest

Acknowledgements

Mari Aguilera thanks the Departament d'Universitats, Recerca i Societat de la Informació (DURSI) de la Generalitat de Catalunya for a predoctoral grant (2004 FI 00673). This study was supported by the Ministerio de Educación y Ciencia (SAF 2005-

07852- C02-01), the Ministerio de Ciencia e Innovación (SAF2008-05674-C03-01), the Ministerio de Sanidad y Consumo (05-317), the Fondos FEDER and the Ministerio de Ciencia e Innovación (PSI2008-05988). Support was also received from the Spanish Ministry of Health, CIBERSAM and the Institut de Biomedicina de la Universitat de Barcelona (IBUB). Thanks to Dr. Marieke Wichers for her statistical advice.

References

1. LESCH KP, BENNINGHOFF J, SCHMITT A. The psychopharmacogeneticneurodevelopmental interface in serotonergic gene pathways. In: Lerer B, ed. Pharmacogenetics of psychotropic drugs. Cambridge, United Kingdom: Cambridge University Press; 2002. p. 95-126.
2. COSTA PT, ., MCCRAE RR. The NEO Personality Inventory Manual. Odessa, FL: Psychological Assessment Resources; 1985.
3. COSTA PT, MCCRAE RR. NEO PI-R. The revised NEO Personality Inventory. Odessa, FL: Psychological Assessment Resources; 1992.
4. CLONINGER CR, SVRAKIC DM, PRZYBECK TR. A psychobiological model of temperament and character. Arch Gen Psychiatry. 1993;50:975-90.
5. DUGGAN C, SHAM P, LEE A, MINNE C, MURRAY R. Neuroticism: a vulnerability marker for depression evidence from a family study. J Affect Disord. 1995;35:139-43.
6. FANOUS AH, NEALE MC, AGGEN SH, KENDLER KS. A longitudinal study of personality and major depression in a population-based sample of male twins. Psychol Med. 2007;37:1163-72.
7. KENDLER KS, KESSLER RC, NEALE MC, HEATH AC, EAVES LJ. The prediction of major depression in women: toward an integrated etiologic model. Am J Psychiatry. 1993;150:1139-48.
8. KENDLER KS, MYERS J. The genetic and environmental relationship between major depression and the five-factor model of personality. Psychol Med. 2010;40:801-6.
9. PLOMIN R, DE FRIES JC, MCCLEARN GC, RUTTER M. Behavioral genetics. 3rd Edition ed. New York; 1997.
10. BOUCHARD TJ, JR., LOEHLIN JC. Genes, evolution, and personality. Behav Genet. 2001;31:243-73.
11. HEIMAN N, STALLINGS MC, HOFER SM, HEWITT JK. Investigating age differences in the genetic and environmental structure of the tridimensional personality questionnaire in later adulthood. Behav Genet. 2003;33:171-80.
12. DEPUE RA, COLLINS PF. Neurobiology of the structure of personality: dopamine, facilitation of incentive motivation, and extraversion. Behav Brain Sci. 1999;22:491-517; discussion 8-69.
13. ZUCKERMAN M. Psychobiology of personality. New York: 2nd Ed. Cambride University Press.; 2005.
14. CARVER CS, MILLER CJ. Relations of serotonin function to personality: current views and a key methodological issue. Psychiatry Res. 2006;144:1-15.
15. HANDLEY SL. 5-Hydroxytryptamine pathways in anxiety and its treatment. Pharmacol Ther. 1995;66:103-48.
16. HEILS A, MOSSNER R, LESCH KP. The human serotonin transporter gene polymorphism--basic research and clinical implications. J Neural Transm. 1997;104:1005-14.
17. LESCH KP, BENGEL D, HEILS A, et al. Association of anxiety-related traits with

a polymorphism in the serotonin transporter gene regulatory region. Science. 1996;274:1527-31.
18. EBSTEIN RP. The molecular genetic architecture of human personality: beyond self-report questionnaires. Mol Psychiatry. 2006;11:427-45.
19. MUNAFO MR, CLARK T, FLINT J. Does measurement instrument moderate the association between the serotonin transporter gene and anxiety-related personality traits? A meta-analysis. Mol Psychiatry. 2005;10:415-9.
20. KARG K, BURMEISTER M, SHEDDEN K, SEN S. The Serotonin Transporter Promoter Variant (5-HTTLPR), Stress, and Depression Meta-analysis Revisited: Evidence of Genetic Moderation. Arch Gen Psychiatry. 2011;(online first).
21. DUMAN RS. Synaptic plasticity and mood disorders. Mol Psychiatry. 2002;7 Suppl 1:S29-34.
22. DUMAN RS, MONTEGGIA LM. A neurotrophic model for stress-related mood disorders. Biol Psychiatry. 2006;59:1116-27.
23. EGAN MF, KOJIMA M, CALLICOTT JH, et al. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell. 2003;112:257-69.
24. SEN S, NESSE RM, STOLTENBERG SF, et al. A BDNF coding variant is associated with the NEO personality inventory domain neuroticism, a risk factor for depression. Neuropsychopharmacology. 2003;28:397-401.
25. WILLIS-OWEN SA, FULLERTON J, SURTEES PG, WAINWRIGHT NW, MILLER S, FLINT J. The Val66Met coding variant of the brain-derived neurotrophic factor (BDNF) gene does not contribute toward variation in the personality trait neuroticism. Biol Psychiatry. 2005;58:738-42.
26. LANG UE, HELLWEG R, KALUS P, et al. Association of a functional BDNF polymorphism and anxiety-related personality traits. Psychopharmacology (Berl). 2005;180:95-9.
27. TSAI SJ, HONG CJ, YU YW, CHEN TJ. Association study of a brain-derived neurotrophic factor (BDNF) Val66Met polymorphism and personality trait and intelligence in healthy young females. Neuropsychobiology. 2004;49:13-6.
28. SOLIMAN F, GLATT CE, BATH KG, et al. A genetic variant BDNF polymorphism alters extinction learning in both mouse and human. Science. 2010;327:863-6.
29. KALIN NH. Nonhuman primate studies of fear, anxiety, and temperament and the role of benzodiazepine receptors and GABA systems. J Clin Psychiatry. 2003;64 Suppl 3:41-4.
30. SEN S, VILLAFUERTE S, NESSE R, et al. Serotonin transporter and GABAA alpha 6 receptor variants are associated with neuroticism. Biol Psychiatry. 2004;55:244-9.
31. UHART M, MCCAUL ME, OSWALD LM, CHOI L, WAND GS. GABRA6 gene polymorphism and an attenuated stress response. Mol Psychiatry. 2004;9:998- 1006.
32. FIRST MB, SPITZER RL, GIBBON M, WILLIAMS JBW. Structured Clinical Interview for DSM-IV Axis I Disorders-Cliniciam Version (SCID-CV). Washington, DC: American Psychiatric Press; 1997.
33. MAXWELL ME. A Manual for FIGS. Bethesda, Maryland: Clinical Neurogenetics Branch, Intramural Research, National Institute of Mental Health; 1992.
34. FREEDMAN ML, REICH D, PENNEY KL, et al. Assessing the impact of population stratification on genetic association studies. Nat Genet. 2004;36:388-93.
35. CLONINGER CR, PRZYBECK TR, SVRAKIC DM, WETZEL RD. The Temperament and Character Inventory (TCI): a guide to its development and use.: St

Louis: Center for Psychobiology of Personality; 1994.
36. BENET-MARTINEZ V, JOHN OP. Los Cinco Grandes across cultures and ethnic groups: multitrait multimethod analyses of the Big Five in Spanish and English. J Pers Soc Psychol. 1998;75:729-50.
37. COSTA PT, JR., MCCRAE RR. Stability and change in personality assessment: the revised NEO Personality Inventory in the year 2000. J Pers Assess. 1997;68:86-94.
38. MILLER SA, DYKES DD, POLESKY HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988;16:1215.
39. STATACORP. STATA statistical software: Release 9.1. 9.1 ed. College Station, Texas: Statacorp; 2005.
40. CDC EI. Epi Info version 6. 6 ed. Atlanta, Georgia USA; 1996.
41. CHEN X, ENDER P, MITCHELL M, WELLS C. Regression with Stata. http://www.ats.ucla.edu/stat/stata/webbooks/reg/default.htm; 2003.
42. ARIAS B, CATALAN R, GASTO C, GUTIERREZ B, FANANAS L. 5-HTTLPR polymorphism of the serotonin transporter gene predicts non-remission in major depression patients treated with citalopram in a 12-weeks follow up study. J Clin Psychopharmacol. 2003;23:563-7.
43. ROSA A, CUESTA MJ, FATJO-VILAS M, PERALTA V, ZARZUELA A, FANANAS L. The Val66Met polymorphism of the brain-derived neurotrophic factor gene is associated with risk for psychosis: evidence from a family-based association study. Am J Med Genet B Neuropsychiatr Genet. 2006;141:135-8.
44. BENJAMIN J, OSHER Y, KOTLER M, et al. Association between tridimensional personality questionnaire (TPQ) traits and three functional polymorphisms: dopamine receptor D4 (DRD4), serotonin transporter promoter región (5-HTTLPR) and catechol O-methyltransferase (COMT). Mol Psychiatry. 2000;5:96-100.
45. COMINGS DE, GADE-ANDAVOLU R, GONZALEZ N, et al. A multivariate analysis of 59 candidate genes in personality traits: the temperament and carácter inventory. Clin Genet. 2000;58:375-85.
46. EBSTEIN RP, GRITSENKO I, NEMANOV L, FRISCH A, OSHER Y, BELMAKER RH. No association between the serotonin transporter gene regulatory region polymorphism and the Tridimensional Personality Questionnaire (TPQ) temperament of harm avoidance. Mol Psychiatry. 1997;2:224-6.
47. FLORY JD, MANUCK SB, FERRELL RE, DENT KM, PETERS DG, MULDOON MF. Neuroticism is not associated with the serotonin transporter (5- HTTLPR) polymorphism. Mol Psychiatry. 1999;4:93-6.
48. HERBST JH, ZONDERMAN AB, MCCRAE RR, COSTA PT, JR. Do the dimensions of the temperament and character inventory map a simple genetic architecture? Evidence from molecular genetics and factor analysis. Am J Psychiatry. 2000;157:1285-90.
49. HUNNERKOPF R, STROBEL A, GUTKNECHT L, BROCKE B, LESCH KP. Interaction between BDNF Val66Met and dopamine transporter gene variation influences anxiety-related traits. Neuropsychopharmacology. 2007;32:2552-60.
50. LANG UE, BAJBOUJ M, WERNICKE C, ROMMELSPACHER H, DANKERHOPFE H, GALLINAT J. No association of a functional polymorphism in the serotonin transporter gene promoter and anxiety-related personality traits. Neuropsychobiology. 2004;49:182-4.
51. MAZZANTI CM, LAPPALAINEN J, LONG JC, et al. Role of the serotonin transporter promoter polymorphism in anxiety-related traits. Arch Gen Psychiatry. 1998;55:936-40.

52. SAVITZ JB, RAMESAR RS. Genetic variants implicated in personality: a review of the more promising candidates. Am J Med Genet B Neuropsychiatr Genet. 2004;131B:20-32.
53. FARRANT M. Amino acids: inhibitory. In: R W, ed. Neurotransmitters, Drugs and Brain Function. New York: John Wiley & sons; 2001. p. 225-50.
54. WEBSTER RA. Neurotransmitter systems and function: overview. In: Webster RA, ed. Neurotransmitters, Drugs and Brain Function. New York: John Wiley & sons; 2001. p. 3-32.
55. CARDON LR, BELL JI. Association study designs for complex diseases. Nat Rev Genet. 2001;2:91-9.