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BRIEF COMMUNICATION

Neuroticism and polymorphisms in the serotonin transporter gene

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ABSTRACT

Background. There is evidence for an association between two different polymorphisms of the human serotonin transporter gene (5-HTT) and the personality trait of neuroticism and affective disorder.

Methods. We studied the association between neuroticism and polymorphisms in the 5HTT-linked promoter region and in a variable number tandem repeat region (VNTR) of the 5-HTT gene in 204 people aged over 60 derived from a random sample of men and women in the general population. Approximately half of the subjects were in the top 20% of neuroticism scorers and half in the bottom 20%.

Results. There were no significant differences in allelic or genotypic frequencies between the high and low neuroticism scorers. There was highly significant linkage disequilibrium between the two 5-HTT gene polymorphisms, and haplotype analysis showed no association between neuroticism level and haplotype.

Conclusions. Reports of an association between two 5-HTT gene polymorphisms and the personality trait of neuroticism are not supported by these results.

INTRODUCTION

The converging consensus about the number of principal human personality traits ranges from between three (Eysenck, 1991) and five (Costa & McCrae, 1992; Goldberg, 1993) to seven (Almagor *et al.* 1995; Cloninger *et al.* 1993). Among these models there is much agreement, especially with regard to two traits (Matthews & Deary, 1998). First, extraversion is an important, reliable, stable and valid source of individual differences with respect to sociability. Secondly, the broad tendency to experience negative emotions is described in the dimension of neuroticism.

Neuroticism has appeared as a clear dimension within self- and peer-reports of personality since the beginnings of scientific research on personality (Deary, 1996). Neuroticism appears in most systems of personality traits and also in disparate cultures (Barrett & Eysenck, 1984). Individual differences in neuroticism are highly stable across many years in adulthood (Conley, 1985). High neuroticism predisposes people to clinical depression and affects the time to relapse in people who are already depressed (Surtees & Wainwright, 1996). Neuroticism has a large effect on self-reports of physical health and the experience of functional (medically unexplained) symptoms (Kirmayer *et al.* 1994).

What are the biological bases of neuroticism? One suggestion was that people high in neuroticism have greater reactivity of the autonomic nervous system (Eysenck, 1967). This suggestion has proved difficult to confirm or refute (Zuckerman, 1991). The main evidence for a biological basis for neuroticism comes from

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heritability studies. Family, twin and adoption studies indicate that about 27–31% of the variance in neuroticism is accounted for by additive genetic variance, and a further 14–17% by non-additive genetic variance (Loehlin, 1992). Neuroticism appears to be a genetic risk factor for anxiety and depressive disorders (Jardine *et al.* 1984).

The link between neuroticism and affective disorder suggests that the serotonin (or 5-hydroxytryptamine or 5-HT) neurotransmitter system might make a contribution to neuroticism differences. Functioning of this system is altered in depression, and antidepressant drugs act upon it (Ogilvie & Harmar, 1997). Newer antidepressant drugs are serotonin specific reuptake inhibitors. Thus, the system of presynaptic serotonin reuptake and the serotonin transporter that is responsible for 5-HT reuptake might be implicated in neuroticism differences. The human serotonin transporter is encoded by a single gene on chromosome 17q11.1–17q2 (Ramamoorthy *et al.* 1993; Ogilvie & Harmar, 1997). It spans approximately 31 kb and comprises 14 exons.

Information about biological mechanisms underlying personality differenes can come from molecular genetic methods, especially the technique of quantitative trait loci (QTL; Plomin *et al.* 1994). Phenotypic characteristics often have multiple genetic determinants and the percentage of variance in the phenotype accounted for by the action of any one site of genetic variability might be small. In QTL as applied to personality research, candidate genes should have two features. First, the candidate gene should have some *prima facie* relevance to the personality dimension in question. Secondly, the gene in question should show polymorphism; that is, it should show variability in the population. These characteristics are met by two recently-described polymorphisms in the serotonin transporter gene (5-HTT; Ogilvie & Harmar, 1997).

The first polymorphism is in the 5-HTT-linked promoter region (5-HTTLPR; Heils *et al.* 1996). It involves a 44 base-pair insertion/deletion. Decreased serotonin transporter expression is associated with the short form of the allele. Having single or double copies of the short form accounted for about 4% of the variance in neuroticism (Lesch *et al.* 1996). However, subsequent reports failed to replicate the association (Ball *et al.* 1997; Ebstein *et al.* 1997). The short form has been associated with affective disorder (Collier *et al.* 1996) but this was not replicated in a subsequent report (Rees *et al.* 1997).

The second relevant polymorphism of the 5-HTT gene is a variable number tandem repeat region (VNTR) within the second intron (Ogilvie & Harmar, 1997), containing variable numbers of copies of a 16–17 base-pair element. The three alleles contain 9, 10 and 12 repeats. People with a history of unipolar depression are about four times more likely to carry the allele with nine copies of the repeated element (Battersby *et al.* 1996; Harmar *et al.* 1996; Ogilvie *et al.* 1996). One study (Stober *et al.* 1996) failed to find an association between the uncommon nine repeat allele and affective disorder, whereas a second suggested a weak association with unipolar disorder (Rees *et al.* 1997). The 12 repeat allele might be more common in people with manic depressive disorder (Collier *et al.* 1996, Kunugi *et al.* 1997; Rees *et al.* 1997). A study of German twins found no association between the 12 repeat allele and self- and peer-reported neuroticism (Ball *et al.* 1997).

In the present study we report the association between the above two polymorphisms in the serotonin transporter gene – 5-HTTLPR and VNTR – and self-report neuroticism levels in a large, random sample of the older adult population.

METHOD

Subjects

Participants were members of the Edinburgh Artery Study, a randomly selected sample of 809 men and 783 women in the general population in Edinburgh, aged 55–74 years at recruitment in 1988 (Fowkes *et al.* 1991). Participants were sampled from age-sex registers of general practices. A follow-up examination took place between November 1992 and March 1994, when blood was taken for genetic analyses.

NEO-Five Factor Inventory (NEO-FFI; Costa & McCrae, 1992)

Subjects completed the NEO-FFI, a self-report personality instrument that measures personality factors of neuroticism, extraversion, openness, agreeableness and conscientiousness. Only neur-

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oticism was studied with respect to the two variations in the serotonin transporter gene. The NEO-FFI was sent to all surviving members of the cohort who were still participating and contactable between March 1995 and November 1995. There had been 269 (17%) deaths, 27 (1·6%) were no longer participating in the study, and 20 (1·3%) participants were not traceable. There were, therefore, 1196 eligible participants, and 1028 personality questionnaires were received (86% response rate). Of these, 901 had valid neuroticism scores (75%). A small proportion (16%, 189 subjects) filled in their questionnaires at a university clinic, the remainder at home.

For the genetic analyses the top and bottom 150 scorers on neuroticism were chosen. An additional 60 from each extreme were identified to be used as substitutes. Blood samples were sent to the MRC Brain Metabolism Unit, Edinburgh, for genotyping. Not all participants with valid neuroticism scores had viable blood samples, and the final groups for analysis contained 100 high and 104 low neuroticism scorers. The range of the neuroticism scores in the top group was 23–47, and in the bottom group 0–12. There were 259 (92 men, 167 women) who fell into the top range of scores, and the final sample was, therefore, 39% (38 men, 62 women) of them. In the low scoring group there were 171 (109 men, 62 women) people, and the sample represented 61% (66 men and 38 women) of them.

Genotyping

During the 1992–1994 clinic visit, 30 ml of venous blood was withdrawn. A tourniquet was used but released as quickly as possible. A 10 ml genetic sample, placed in an EDTA tube and inverted 5 or 6 times, was stored in a 4 °C refrigerator and then posted to the Duncan Guthrie Institute of Medical Genetics, Glasgow. DNA was extracted from lymphocytes using standard techniques (Kunkel *et al.* 1977).

Amplification of the VNTR region in intron 2 of the HSERT gene was carried out as described previously (Battersby *et al.* 1996). For the 5-HTTLPR polymorphism PCR amplification was carried out using forward primer 5′-CACCTA-ACCCCTAATGTCCCTACT and reverse primer 5′-GGACTGAGCTGGACAACCAC in a reaction volume of 50 μl. The mixture contained 1 × Pfu buffer (Stratagene), 100 ng of each primer, 1 μl of a 1 in 10 dilution of DNA, 200 μM each of dATP, dCTP and dTTP, together with 100 μM- dGTP and 100 μM 7-deaza-GTP. The reaction was heated to 98 °C for 5 min and 2·5 U Pfu exo-minus polymerase (Stratagene) was added in 5 μl of 1 × Pfu buffer. Amplification consisted of 40 cycles of 98 °C for 45 s, 65 °C for 45 s, and 72 °C for 90 s, with a final extension for 5 min at 72 °C. Products were resolved on 3% agarose gels and visualized by ethidium bromide staining under UV transillumination.

RESULTS

The 5-HTTLPR serotonin transporter marker's allelic ($\chi^2 = 0{\cdot}00$, df = 1, NS) and genotypic ($\chi^2 = 2{\cdot}44$, df = 2, NS) frequencies were similar in the low and high neuroticism groups (Table 1*a*). In addition, the 5-HTT VNTR serotonin transporter marker's allelic ($\chi^2 = 0{\cdot}86$, df = 2, NS) and genotypic ($\chi^2 = 2{\cdot}22$, df = 5, NS) frequencies were similar in the high and low neuroticism groups (Table 1*b*).

Maximum likelihood estimates of haplotype distributions in the total population and in the high and low neuroticism groups were made and compared with the Arlequin software package (Schneider *et al.* 1996). There was highly significant linkage disequilibrium between 5-HTTLPR and VNTR polymorphisms ($P < 0{\cdot}0001$ for the total sample and the high neuroticism sample, and $P < 0{\cdot}02$ in the low neuroticism sample), with allele 9 of the VNTR associated exclusively with the long form of the 5-HTTLPR and a progressive increase in frequency of haplotypes containing the short form of the 5-HTTLPR with increasing number of repeats in the VNTR. However, there was no evidence for any association between haplotype and neuroticism.

DISCUSSION

The present study did not find support for an association between self-reported neuroticism score and two polymorphisms on the human serotonin transporter gene. Compared with other published studies on this topic the present study is large and involved a normal sample of the older population. This negative result is unlikely to be due to sample size; we had a power of 80% to detect an effect at a significance

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Table 1. *Allelic and genotypic frequencies for the (a) 5-HTTLPR and (b) 5-HTT VNTR serotonin transporter marker in groups with high and low NEO-FFI neuroticism scores*

(*a*)	Allelic frequency		Genotypic frequency		
	S	L	SS	SL	LL
High neuroticism	0·456	0·544	0·235	0·441	0·324
Low neuroticism	0·455	0·545	0·180	0·550	0·270

(*b*)	Allelic frequency			Genotypic frequency					
	9	10	12	9–9	9–10	9–12	10–10	10–12	12–12
High neuroticism	0·035	0·353	0·611	0·010	0·030	0·020	0·131	0·414	0·394
Low neuroticism	0·024	0·329	0·648	0·000	0·019	0·029	0·095	0·448	0·410

level of 0·05. The closest comparable study is that of Ball *et al*. (1997). Their study of individuals from a German twin sample examined the top and bottom 5% of neuroticism scorers on the NEO-FFI. They suggested that a wider range of the population distribution should be tested in case the 5-HTT genes were associated with the normal range of neuroticism but not the extremes. No evidence of this may be found in the present study. The other negative study of personality traits and a 5-HTT related gene was that by Ebstein *et al*. (1997) in which TPQ harm avoidance showed no relationship with 5-HTTLPR allelic and genotypic variation in over 120 normal subjects.

There remain some substantial positive findings. The association of the short form of the 5-HTTLPR gene and neuroticism and anxiety traits in a large sample (Lesch *et al*. 1996), and the possibility that affective disorder is related to the 9 and/or the 12 repeat alleles of the VNTR gene (Collier *et al*. 1996; Harmar *et al*. 1996) remain to be replicated or refuted. The importance of further studies lies in the wide publicity that the original, positive results have received (Craddock, 1996; Goldman, 1996; Eley & Plomin, 1997; Ogilvie & Harmar, 1997).

Further attempts at replications of recent findings with respect to genes and personality traits and mood disorders will clarify the current contradictory account. In addition, it will be interesting to investigate new candidate genes as they appear. In addition to research on humans, leads may be expected from some animal models of human personality traits and mood disorders, such as the research that has shown that individual differences in 'emotionality' in mice are influenced by loci on murine chromosomes 1, 12 and 15 (Flint *et al*. 1995).

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