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Genome Wide Linkage Analysis of Multiple Measures of Neuroticism of two large cohorts from Australia and the Netherlands

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Abstract

Context—People meeting diagnostic criteria for anxiety or depressive disorders tend to score high on the personality scale of neuroticism. Studying this personality dimension can give insights into the aetiology of these important psychiatric disorders.

Objective—To undertake a comprehensive genome-wide linkage study of neuroticism, using large study samples that have been measured multiple times. To compare the results between countries for replication and across time within countries for consistency.

Design—Genome wide linkage scan.

Setting—Twin individuals and their family members from Australia (AU) and the Netherlands (NL).

Participants—19,635 sibling pairs completed self-report questionnaires for neuroticism up to five times over a period of up to 22 years. 5,069 sibling pairs were genotyped with microsatellite markers.

Methods—Non-parametric linkage analyses were conducted in Merlin-Regress for the mean neuroticism scores averaged across time. Additional analyses were conducted for the time specific measures of neuroticism from each country to investigate consistency of linkage results.

Results—Three chromosomal regions exceeded empirically-derived thresholds for suggestive linkage using mean neuroticism scores: 10p 5 cM (NL), 14q 103 cM (NL) and 18q 117 cM (AU & NL combined), but only 14q retains significance after correction for multiple testing. These regions all showed evidence for linkage in individual time-specific measures of neuroticism and one (18q) showed some evidence for replication between countries. Linkage intervals for these regions all overlap with regions identified in other studies of neuroticism or related traits and/or in studies of anxiety in mice.

Conclusions—Our results demonstrate the value of the availability of multiple measures over time and add to the optimism reported in recent reviews for replication of linkage regions for neuroticism. These regions are likely to harbour causal variants for neuroticism and its related

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psychiatric disorders and can inform prioritisation of results from genome-wide association studies.

Introduction

The personality trait of "neuroticism" is defined as a tendency to experience psychological distress. Individuals with high neuroticism scores are characterized by emotional instability, low self-esteem, and feelings of anxiety, depression, and guilt[1]. Neuroticism scores are found to be high in those suffering from psychiatric disorders such as major depression and anxiety disorders[2] and this association appears to be reciprocal. Prospective studies demonstrate that neuroticism or neuroticism-like traits predict future major depression[3–7] and not merely because of overlap with prodromal symptoms of major depression. Self-report questionnaires can be used to score neuroticism as a quantitative trait measurable on large population cohorts[8, 9]. Therefore, study of neuroticism in large populations is relatively easy and can give insights into the aetiology of important psychiatric disorders.

Neuroticism scores have been found to be robust measures with test-retest correlations of 0.79[10] to > 0.90[9, 11] for scores measured up to two years apart, and approximately 0.60 for scores measured up to six years[12] or 19 years[11] apart. It is well established that neuroticism is partially under genetic control[13, 14], with heritability estimates of 30%–54%[8, 12, 15, 16]. Twin studies have consistently shown no evidence for a shared common environmental component[12, 15, 17]. Genetic correlations between neuroticism scores taken over a six year period were above 0.88 for all age groups[12]. On average, women score higher for neuroticism than men, but heritability estimates are mostly consistent across sexes[14–16]. However, opposite sex sibling correlations[16, 17] and mother-son correlations[15] have been reported as lower, suggesting that different genes may be of importance in men and women. Estimates of the genetic correlation between neuroticism and depression or anxiety range from 0.4 to 0.8[17–20].

Four previous linkage studies of neuroticism have been published[10, 16, 21, 22]; three of these studies used a single measure of neuroticism and one[10] used an average of two measures taken six months apart. For two of the studies, the linkage analyses for neuroticism were secondary to the analyses of the ascertainment criteria of their study cohorts, namely alcohol[22] or nicotine[21] dependence. Recent reviews[14, 23] summarised the linkage analysis results from the three earliest published of these studies and from an additional 14 studies of psychiatric disorders considered to be genetically related to neuroticism and concluded that some consistency is starting to emerge across studies.

Examples of genetic linkage analysis of longitudinal data on any trait in adults are rare[24] despite recognition that use of multiple measures can increase power by reducing between sib residual non-shared variance[25]. Consistency in linkage regions across repeated measures cannot be considered as a replication, as this requires identification of the same linkage region in independent data sets. Nonetheless, inconsistency in linkage regions identified from repeated measures might indicate type I error and biological implausibility of the putative region.

In this study, we report a linkage analysis of neuroticism from two large study samples of twin families from Australia and the Netherlands. Individuals in the Australian study have been measured up to four times over a 22 year period and on different scales. Individuals in the Dutch study sample have been measured up to five times over an 11 year period using the same scale. These data sets are independent between countries and therefore provide an opportunity to investigate replication of linkage results. Within countries, there are partly overlapping samples of participants at each measurement occasion, providing an opportunity to investigate consistency of linkage results.

Methods

Australian study sample: Participants and measures of neuroticism

All participants were adult twins and their families recruited through the Australian Twin Registry and were of North European ancestry. All provided written informed consent under study protocols approved by the Queensland Institute of Medical Research Human Research Ethics Committee. Participants completed one or more personality questionnaires: the Eysenck Personality Questionnaire revised 23-item (EPQ-R)[26], the shortened 12-item subset (EPQ-R-S) or the NEO five factor inventory personality questionnaire[27] which includes 12 items in the neuroticism domain and compared to the EPQ-R probes angry hostility, self-consciousness, impulsiveness and vulnerability as well as anxiety and depression. Each individual could have up to four measures of neuroticism measured at four different times, these (or their transformations, discussed below) are referred to as AU_{80} (EPQ-R), AU_{89} (EPQ-R-S), AU_{99} (EPQ-R) and AU_{02} (NEO) with these trait codes reflecting the approximate year in which the scores were collected. The participants contributing AU_{80}, AU_{89} and AU_{99} measures are described in detail elsewhere[8]. Briefly, participants contributing AU_{80} or AU_{89} scores were ascertained solely on the status of being a twin registered through the Australian Twin Registry or, in the case of AU_{89}, being a family member of a registered twin. The participants contributing AU_{99} measures were ascertained as siblings pairs selected for discordance or concordance with respect to extreme neuroticism or anxiety or depression scores: one sibling in the top or bottom decile, the other sibling in the top or bottom quintile, excluding monozygotic (MZ) twin pairs and allowing for selection of multiple siblings per family, in an Extreme Discordant and Concordant (EDAC) design[28] (for full details see Kirk et al[8]). The EDAC design identifies the sibling pairs that are most informative for genetic studies[29]. The participants in the 1999 study had the opportunity to complete the EPQ-R by telephone interview and/or by mail; ~80% completed both within six months with a test-retest correlation of 0.9[8, 11]. The two scores were averaged for analysis in this study. The long-term stability of the AU_{80}, AU_{89} and AU_{99} measures are reported in Birley et al[11] (in which the 1980, 1989 and 1999 studies are named Canberra, Alcohol cohorts (where “Alcohol” does not refer to any ascertainment criteria) and Anxiety studies). The participants contributing AU_{02} measures were ascertained as being extended twin families with a high incidence of smokers as part of an ongoing Nicotine Addition Genetics study[30]. Where possible, blood (or buccal) samples were obtained from the study participants and their parents.

Dutch study sample: Participants and measures of neuroticism

Families with adolescent and adult twins have been assessed roughly every two years since 1991 as part of an ongoing longitudinal survey study of the Netherlands Twin Register (NTR). Participants are of Dutch ancestry[31] and were recruited under informed consent. Each survey, with the exception of the 1995 wave, collected information on personality and psychopathology[31, 32] and was conducted under protocols approved by ethics committee of the Free University Hospital, Amsterdam. Consequently, each individual could have up to five measures of neuroticism measured at five different times, these (or their transformations, discussed below) are referred to as $NL_{91,}$ NL_{93}, NL_{97}, NL_{99} and NL_{02} with subscript codes reflecting the approximate year in which the scores were collected (corresponding to Waves 1,2 and 4–6 of data collection[32]). Neuroticism was measured using the Amsterdamse Biografische Vragenlijst (ABV)[33], a self-report questionnaire similar in content to the EPQ-R[34]. The neuroticism scale comprises 30 questions with a 3-item response scale (no, don’t know, yes). The neuroticism score is a weighted sum of the item responses.

Neuroticism scores

Neuroticism scores are sum scores and such data typically deviate from normality by having heavy tails. The averaged angular transformation[35] was used to normalise the distribution, as in other studies[8, 11, 16, 36]. The neuroticism scores used in the analysis were residuals from regression of the transformed neuroticism scores on age, sex and age*sex (and age^2 and age^2*sex for AU_{89}) which were standardised separately for each sex. The mean AU_{89} score of those selected for measurement in the AU_{99} study sample was not significantly different from that of the full study group, but the variance was higher. Therefore, the AU_{99} measures were standardised using the variance of the AU_{89} cohort so that the higher variance of AU_{99} measures was maintained. Finally, an average neuroticism score was calculated for each person within each country, denoted by AU and NL. The number of measures contributing to each average was recorded and used as a weight in the repeated measures linkage analysis. Descriptions of the phenotype (all those measured) and genome scan (only those used in the linkage analysis) data sets are given in Tables 1 and 2.

Genotyping

The genotypic data available for the Australian study resulted from submission of DNA samples to one or more of six genotyping centres, namely Gemini P/L (G), Sequana Therapeutics Inc (S), Leiden University Medical Centre(L), the Mammalian Genotyping Service, the Center for Mammalian Genetics at the Marshfield Clinic Research Foundation (M), the Australian Genotyping Research Facility, AGRF (A) and the Finnish Genome Center, University of Helsinki (H). A description of the G,L,M,S genotyping and the subsequent merging and cleaning of the marker data sets is described in detail elsewhere[37, 38]. Since then, additional M, A and H genotypes have been merged using the same protocol. Family members were submitted to the same genotyping facility. Participants with measure AU_{99} were submitted preferentially for genotyping ((Figure 1b), but this was not the sole criterion used to select families for genotyping and so the impact of the EDAC design was less marked for the AU_{89} measure which was available on the largest subset of samples (Figure 1a). Data cleaning based on Mendelian errors, unlikely genotypes and consistency of pedigree and marker relationships was undertaken as described by Cornes et al[37].

Dutch samples were genotyped by the M or L laboratories. The genotype data from these screens were combined. Allele calling and binning were equalized between markers that were present in multiple scans, using ~ 30 control samples. Data cleaning based on Mendelian errors, unlikely genotypes and consistency of pedigree and marker relationships was undertaken as described by Middeldorp et al[39]. The distributions of the neuroticism measures for those with and without genome scan data were similar.

Map positions of all genotyped markers were estimated in Kosambi cM by locally weighted linear regression (http://www.qimr.edu.au/davidD) from the NCBI Build 35.1 physical map positions and published Decode and Marshfield genetic map positions[40]. Identical markers genotyped at different genotyping facilities were all included, separated by 0.001cM on the genetic map. Using markers genotyped in common, the F_{st} between the Australian and Dutch samples was estimated to be 0.30%, implying that these samples can be combined for joint genetic analysis[41]. Individuals were required to have genotypes on more than 280 markers resulting in an average distance of 8.2 cM (AU) and 11.0 cM (NL) between genotyped markers of sib pairs. 38% (AU) and 51% (NL) of parents were genotyped.

Preliminary Analyses

Phenotypic (test-retest) correlations between the EPQ measures AU_{80}, AU_{89} and AU_{99} correlations range between 0.59–0.62[11, 36]. Test-retest correlations of these measures with

AU_{02} are lower 0.46–0.54[36] reflecting the different emphasis of some of the items included in the NEO personality inventory neuroticism domain. The average phenotypic correlation between the Dutch measures is 0.65, range 0.56–0.77, with higher correlations between consecutive measures. The highest sib-pair correlations (estimated in Sib-Pair[42], Table 1) were for the youngest cohorts NL_{91} and NL_{93}. The high sib-correlation for the AU_{99} is a reflection of the EDAC selection. The lowest sib-correlation was for AU_{02} scored on the NEO scale. Analyses of subsets of the Australian[11, 15] and Dutch[32] data have consistently shown no evidence for influence of common environmental effects. Genetic correlations were estimated in ASReml[43] and ranged between 0.91–0.95 between the EPQ measures (AU_{80}, AU_{89} and AU_{99}) and ranged between 0.80 and 0.95 between these measures and the AU_{02} NEO measure. Formal testing showed that the measures can be considered as repeated measures of the same trait[36]. The genetic correlations between the five Dutch traits range from 0.84 to 0.95. Across all neuroticism measures, averaged estimates of heritability, phenotypic and genetic correlations were 0.32, 0.61 and 0.90 respectively. Preliminary linkage analyses conducted using a full multivariate model (not presented) suggested that there was little to be gained compared to the repeated measures model with genetic correlations of this magnitude.

Linkage analyses

Genetic linkage analysis of the autosomes was conducted in *Merlin-Regress*[44] which regresses estimated identity-by-descent between relative pairs on the squared sums and squared differences of trait values of the pairs. Investigation of the properties of the method by simulation[44] showed it to be powerful and efficient even for selected samples (EDAC designs). It requires phenotypic measures to be standardised in the unselected population sample and uses the population parameters (mean, variance, heritability) derived from the full population sample rather than the selected or genotyped sample. The method is also appropriate for general pedigrees including multiple sibs per family. However, simulation studies[44] showed that, although large sibships can increase power, the distribution of the test statistic can become distorted if the contributions from families become highly skewed. For this reason sibships were limited to a maximum of 5, selecting sibs that maximised either the discordance or concordance of each family. Mean neuroticism scores were analysed in Merlin-Regress options *–mean 0 –var 1*, with heritabilities entered as twice the sib correlations (Table 1) and using the *–testretest* option with correlation of 0.61. Analyses were repeated using mean measures from only males and only females because other studies have reported sex-specific linkage regions (summarised in[16]). Analyses using scores of males or females only are denoted with subscripts m or f respectively. Linkage analysis for the X-chromosome was conducted in *Merlin MINX*. In all analyses, multipoint LOD scores for presence of a quantitative QTL were estimated every 5cM (a 1 cM grid was used to determine linkage region confidence intervals, as the region bounded by 1 LOD less than the maximum observed). Using the 5cM grid allows the linkage statistic to be collected over all families even when families were genotyped for different markers. Option *–singlepoint* was used to identify the individual marker contributing most to regions showing evidence of linkage. Linkage analyses were repeated using individual measures of neuroticism to allow examination of consistency in linkage signal between time-specific measures for each country.

Autosomal genome-wide empirical significance thresholds were derived from 1000 gene drop simulations as implemented in *Merlin –simulate* which utilises the allele frequencies, marker positions and missing genotype patterns of the real data set and simulates under a model which assumes random linkage between genotype and phenotypes. All phenotypes were analysed using the same simulated data sets, which maintains the correlation structure between phenotypic measures. The maximum LOD scores from each chromosome of each

simulation replicate were retained and were used to derive the empirical LOD thresholds for Lander-Kruglyak[45] suggestive linkage (1 LOD exceeding the threshold per genome scan) and significant linkage (1 LOD exceeding the threshold per 20 genome scans) for each neuroticism measure analysed and for the nine mean measures of neuroticism simultaneously to derive thresholds that account for multiple testing.

Within *Merlin-Regress* option *–rankFamilies* gives an ELOD20 score for each phenotypic measure. ELOD20 is the LOD expected given the data of a quantitative trait locus (QTL) that accounts for 20% of the phenotypic variance, assuming fully informative markers. Observed marker informativeness (I) was estimated as the average information content of the 5cM estimates across the autosomes. ELOD20 scores corrected for observed marker informativeness were calculated as $ELOD20_{(I)} = ELOD20*I$. Both $ELOD20_{(I)}$ and $ELOD10_{(I)}$ scores were used to calculate the power of our study samples given the phenotypic and genotypic information to detect QTL that account for 20% and 10% of the total variance at the empirical significant [45] or suggestive[45] thresholds for linkage using the 'Probability Function Calculator' of the 'Genetic Power Calculator'[46], where $ELOD10_{(I)} = ELOD20_{(I)}/4$.

Results

Empirically derived suggestive and significant LOD thresholds for samples with each level of neuroticism are listed in Table 1. The lowest thresholds are for the samples comprised predominantly of a single sib pair per family: AU_{80}, NL_{91} and NL_{93}. The empirical threshold for suggestive and significant linkage accounting for the multiple testing of the 9 mean measures of neuroticism are 2.5 and 4.1, respectively.

The mean ± standard deviation of the information content across the autosomes as calculated every 5cM in Merlin-Regress was 0.73 ± 0.08 (AU) and 0.51 ± 0.10 (NL), the difference reflecting the average distance of genotyped markers between sib-pairs. The ELOD20 scores are listed in Table 1. By accounting for the observed informativeness of the genotyped markers, we estimate that the study samples AU&NL, AU and NL have 100%, 99% and 86% power to detect a QTL that accounts for 20% of the total variance at the significant threshold of linkage. These samples have power of 60%, 27% and 9% to detect a QTL that accounts for 10% of the total variance at the significant thresholds, and of 89%, 65%, 37% at the suggestive thresholds. The power of sex specific analyses is much lower, as expected from the number of same sex sib-pairs contributing to the analysis. The sex specific AU&NL, AU and NL measures have, for females, 99%, 86% and 64% and, for males, 69%, 40% and 24% power to detect a QTL that accounts for 20% of the total variance at the suggestive threshold of linkage.

The genome-wide linkage plot for AU, NL and the joint analysis of AU&NL (Figure 2) show three regions that exceed the empirical threshold for suggestive linkage for their respective measures: 18q 116 cM for AU&NL, 14q 104cM for NL and 10p 5 cM for NL. An additional two regions just fail to reach the this threshold: 8q 132 cM for AU and 6cen 75 cM for NL. The chromosomal position with the maximum LOD score based on a 1 cM grid scan, linkage intervals, corresponding cytogenetic band and the marker with the maximum LOD score within the region based on a singlepoint analysis are listed in Table 3. To investigate these results, we looked for consistency in linkage signal in time-specific measures of neuroticism within the linkage intervals and found that for all five regions at least two individual measures achieved LOD >1 (Table 3). In contrast, an additional 10 chromosomal regions (NL_{97}: Chr 14 22 cM; AU_{80}: Chr16 56 cM, AU_{02}: Chr 3 14 cM, AU_{80}: Chr 6 178 cM, NL_{02}: Chr 9 149 cM, NL_{97}: Chr 16 124 cM, AU_{99}: Chr 17 14 cM, NL_{93}: Chr 18 60 cM, AU_{80}: Chr19 104 cM, AU_{89} chr 21 21 cM) achieved LOD scores that

exceeded the empirical suggestive threshold for significance for an individual measure of neuroticism and only the first two listed achieved a LOD >1 for any other individual measure within the 1 LOD drop confidence interval. Within country, some datasets were longitudinal (e.g., > 90% of those included in the AU_{80} or AU_{99} analysis were also included in the AU_{89} analysis, Table 2), whilst some data sets were largely independent (e.g., < 10% of participants with AU_{80} or AU_{99} scores were also scored for the AU_{02}, Table 2). The most extreme example of inconsistency was for AU_{89} which achieved a LOD of 2.7 for 21p 21 cM, yet no evidence for linkage was found with the AU_{99} measure (maximum LOD within the region of 0.1), a difference which persisted when the analysis was limited to include only phenotypes of individuals who were measured in both studies. Examination of the sib-pair phenotypic scores and IBD sharing from families that contribute most to these linkage signals, showed nothing that could not reasonably be attributed to stochastic variation.

Five regions which exceed empirical suggestive thresholds of linkage are reported for analyses of single sex average neuroticism scores (Table 3). For the linkage interval of these regions, analyses were conducted for the relevant sex for the nine time-specific data sets. Of the five regions, three (2p, 5q and 15cen) were supported by more than one sex specific individual measure with LOD > 1.0 (Table 3). Of the 8 regions listed in Table 3 that exceed the empirical suggestive threshold for significance only the 14q region exceeds the threshold that accounts for multiple testing of the 9 mean measures.

Discussion

We have performed a linkage analysis for neuroticism using two large independent study samples of North European descent. In total, 5,069 sibling pairs contributed to the linkage analysis which used mean neuroticism scores from both Australia and the Netherlands to maximise sample size and power. Linkage analyses of mean neuroticism score for each country separately allowed us to look for replication between independent data sets. The mean neuroticism measure of each participant could comprise between 1 and 5 individual measures and we used individual neuroticism scores to look for consistency of linkage results. Although subjects with more than one measure of neuroticism age over time, the high genetic correlations between measures would not lead us to expect different genetic variants to be identified in the linkage analysis of different measures. Using mean neuroticism score, we identified five regions where LOD > 1.5, for three of these the LOD score exceeded the empirical threshold for significance. All five regions showed some consistency in linkage scores for individual time-specific measures within country and two regions (8q 134 cM and 18q 117 cM) showed some evidence for replication between countries. Other studies that have reported linkage to these regions are listed in Table 3; we include studies reviewed by Fullerton[14] plus a small number of additional, mostly subsequent, publications. Region 18q 117 cM overlaps the linkage intervals reported by three other studies: recurrent early onset and major depression[47] 73 cM, neuroticism in females[22], 91 cM and 115 cM; harm avoidance[22] 109 cM. Region 14q 103 cM has previously been identified in a linkage analysis of the Dutch study samples for a broad anxiety phenotype[39] but also in an independent study of extended families with a high occurrence of anxiety disorders[48]. Region 10p 5cM was estimated from the linkage graph for EPQ neuroticism presented by Fullerton et al[16] to have exceeded the level of suggestive linkage. Only the confidence interval of the 18q region overlaps with a region considered to have “reasonable support for linkage” by Fullerton[14] (10 regions were identified comprising ~9% of the genome). Also listed in Table 3 are human chromosomal regions homologous to linkage regions from studies of anxiety in mouse as summarised by Smoller et al[49]; 11 homologous human chromosomal regions were identified which totalled ~17% of the human genome. Linkage studies in mice are relevant because similar brain processes are likely to exist for anxiety in mice and neuroticism in humans[13] and the powerful design of studies

that are possible in mice can lead to highly significant linkage regions bounded by tight confidence intervals. Of the five regions we have identified (Table 3) 4 overlap with regions identified by Smoller, an overlap that exceeds chance expectations (Binomial p= 0.003)

Five sex specific linkage regions exceeded thresholds of suggestive linkage (Table 3) of which 2cen 112 cM (males) shows evidence for replication between countries and 5q 191 cM (males) shows evidence for consistency between the Dutch time-specific measures. Of these, region 8p has previously been identified in other linkage studies including two male specific reports (Table 3) and linkage with suicide and recurrent early major depression and been reported for 2p[50]. Two of the five sex specific regions overlap with homologous regions identified by Smoller[49] from mouse linkage studies. Analysis of male and female mean scores separately had much reduced power compared to the joint sex analyses, particularly the male specific analyses and so we place less emphasis on the sex specific results.

For a study of its kind our sample size is large (Table 4), yet the number of linkage regions that we identified for AU&NL, AU and NL were 2, 0 and 3 respectively, not very different from the one per linkage scan expected by chance. Of the other linkage studies for neuroticism (Table 4), only the study of Fullerton et al[16] has more power to detect a QTL. Based on observed phenotypic and marker information we had 100% (or 89%) power in the combined Australian and Dutch sample to detect a QTL that accounts for 20% (or 10%) of the total variance at the suggestive[45] threshold for linkage. For a trait with a heritability of 30% these are perhaps optimistic power calculations, none-the-less the next largest neuroticism linkage study[10] to date, assuming fully informative marker information, reports only 72% power for a QTL that accounts for 20% of the variance. We note that studies likely to have much less power to detect QTL have identified more suggestive and significant linkage regions (Table 5). Theoretically, sample sizes of more than 50 sib pairs should not result in a biased number of linkage statistics exceeding suggestive or significant linkage thresholds under the null hypothesis[45]. Although under the alternate hypothesis (when a QTL does exist) an inverse correlation between sample size and LOD score is expected[51]. One conclusion is that there simply are no variants that explain 10% or more of the genetic variance. When do our suggestive linkage peaks represent false positives and when does their low significance reflect variants of smaller effect size? It is not possible to answer this question but by considering multiple measures of neuroticism, we reduce the impact of the environmental noise surrounding chance extreme concordance or discordance of measures and therefore reduce one cause of the occurrence of false positive linkage signals. The examination of linkage analyses from the individual measures of neuroticism provides some evidence for the robustness of our results using mean score.

Limitations of our study include different measures of neuroticism, both between countries and within the Australian sample. The Dutch participants came from a younger cohort than the Australian participants. A recent study has suggested that subtle differences in the EPQ-R and NEO neuroticism instruments may be important for genetic studies[52]. However, the high genetic correlations between different measures suggest the different measurement instruments are probing the same underlying trait, at least within country. As in other studies, we have undertaken some multiple testing (sex dependent analyses, both between and within countries) which has not been accounted for in the empirical thresholds derived for each mean measure. The empirical threshold (LOD 2.5) derived to account for the multiple testing of the 9 mean neuroticism measures (including sex specific means) is exceeded only for 14q 103 cM. None-the-less, the robustness of our results as measured by consistency in linkage score between time-specific measures combined with the high rate of overlap with regions reported in other studies add to the optimism reported in recent reviews[14, 47] for replication of linkage regions, even though the true effect sizes of

underlying variants are unlikely to be large. A recent genome-wide association study of neuroticism using DNA pooling[53] failed to identify any loci that explained more than 1% of the variance. It is unlikely that the consensus in linkage signals across studies is driven by single variants of such a small magnitude, but more likely implies allelic heterogeneity of causal variants within functionally important genes. Consistently identified regions from linkage analyses will be important in prioritisation of results from genome-wide association studies (GWAS). Time will tell if GWAS result in the identification of causal variants which account for the majority of the observed genetic variance. International collaborations compiling large family-based study samples for linkage analysis may well be necessary for identification of genes that contain multiple but rare causal variants.

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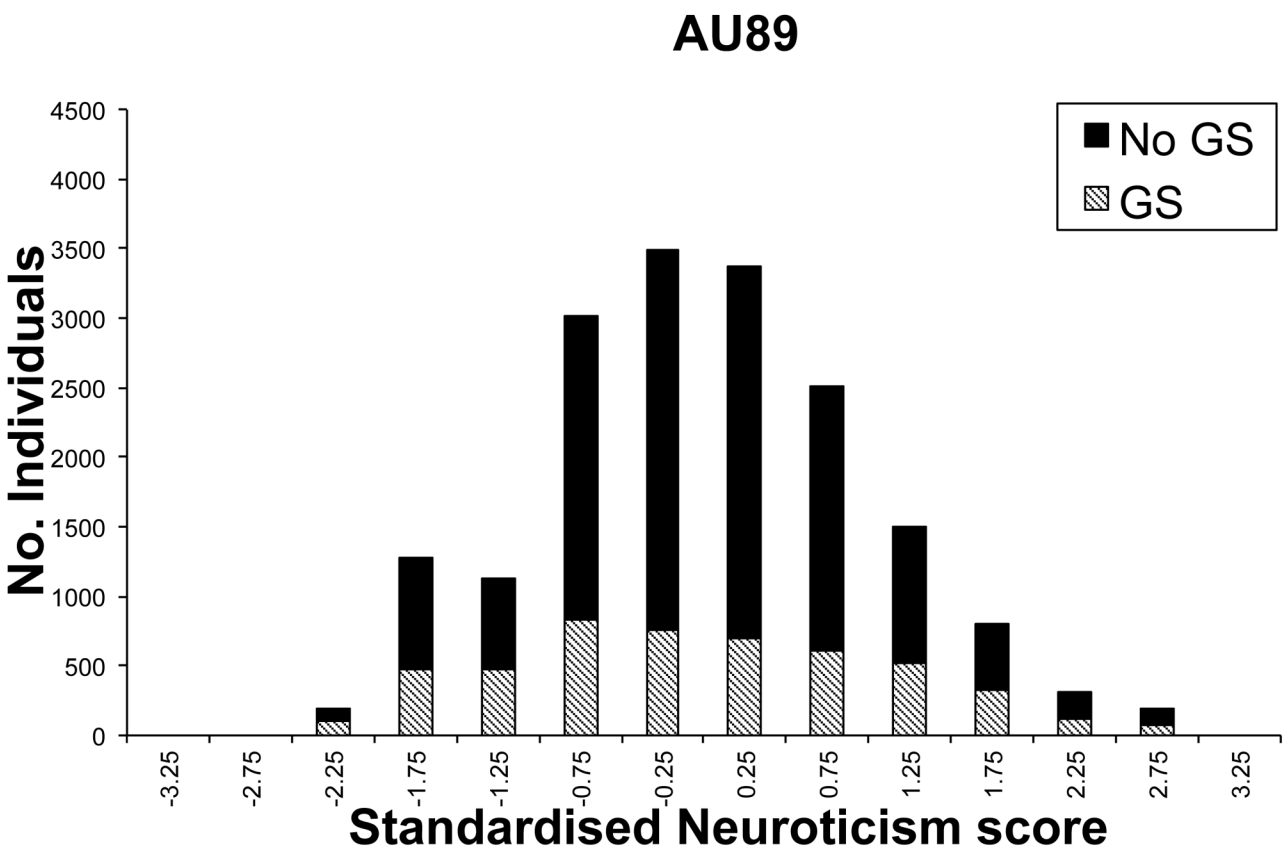

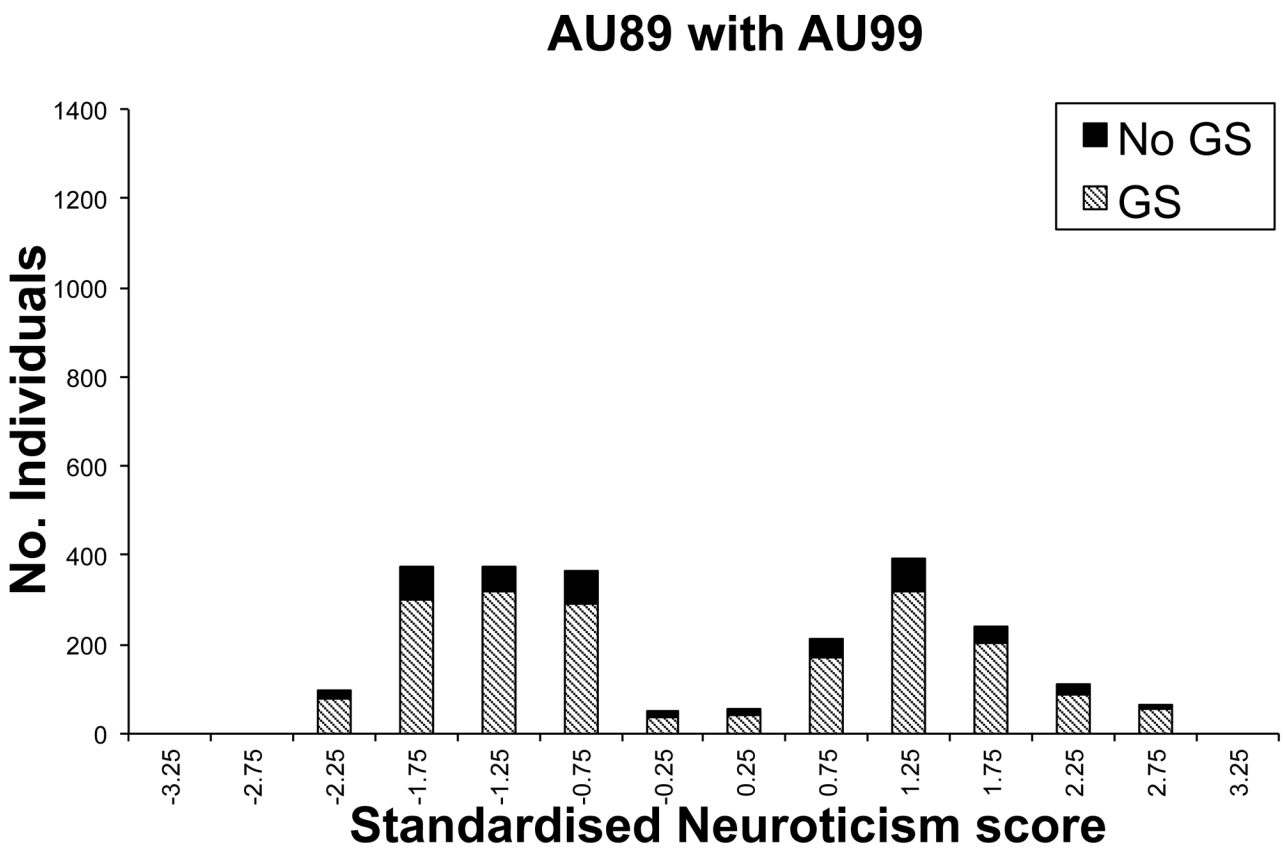

Figure 1.
Distribution of a)standardised AU_{89} Neuroticism scores for those in the genome scan (GS) and those not in the genome scan (No GS) and b) the same but only for those selected to have AU_{99} scores.

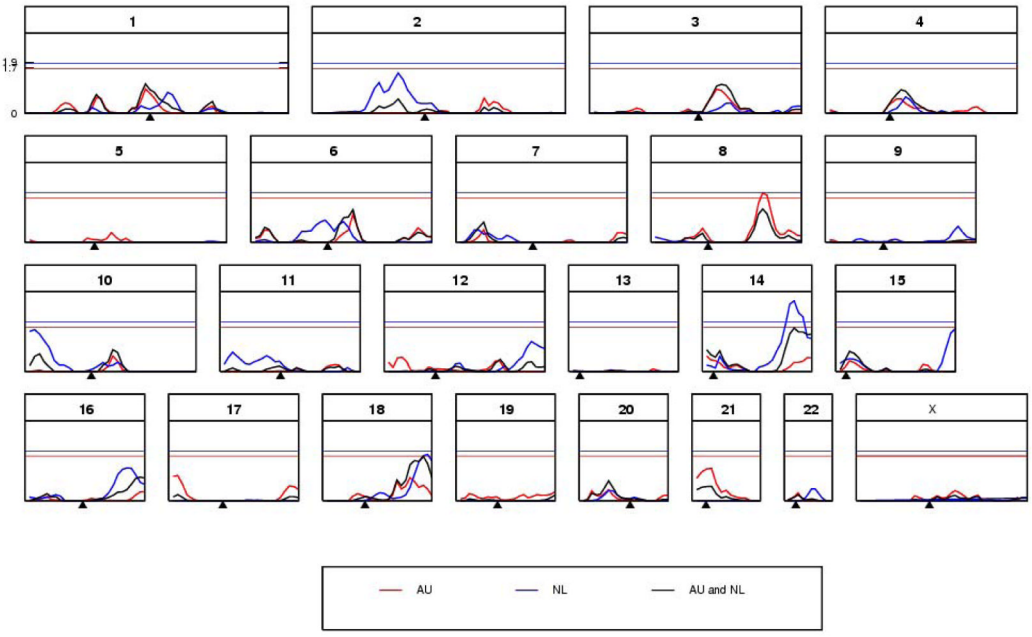

Figure 2.

Merlin-Regress linkage results of LOD score (y-axis) for each chromosome (1–22, X) based on a 5 cM grid (x-axis) for mean neuroticism score of the Australian (AU), Dutch (NL) and combined (AU&NL) data sets. Empirical thresholds for suggestive linkage were 1.7 (red horizontal) for AU and AU&NL and 1.9 (blue horizontal) for NL.

Table 1

Description of data sets. For inclusion in a) the phenotype data set, families needed to have at least two individuals with neuroticism scores and known age, only one of an MZ pair included and b) the linkage analysis data set, families size limited to 2–5 siblings with neuroticism scores, known age and genotype data on > 280 markers.

	Australia					Netherlands						
	AU_{80}	**AU_{89}**	**AU_{99}**	**AU_{02}**	**AU**	**NL_{91}**	**NL_{93}**	**NL_{97}**	**NL_{99}**	**NL_{02}**	**NL**	**AU&NL**
a) Phenotype												
# families	2017	4253	938	576	4999	1661	2014	1283	1332	2069	3808	8807
# sib-pairs	2033	10513	1709	1589	12772	997	1148	3220	2691	2553	6863	19635
Mean age	33.4	35.1	43.1	40.2		17.7	19.2	26.6	30.9	32.8		
Sib-correlations	0.18	0.14	0.27[4]	0.10	0.14	0.25	0.28	0.16	0.20	0.17	0.20	0.16
Female-female	0.25	0.16	0.31[4]	0.13	0.14	0.25	0.34	0.20	0.19	0.25	0.23	0.17
Male-male	0.18	0.13	0.30[4]	0.15	0.18	0.27	0.34	0.07	0.18	0.13	0.17	0.18
Female-Male	0.12	0.12	0.24[4]	0.07	0.11	0.23	0.21	0.18	0.21	0.12	0.19	0.14
b) Linkage analysis												
# families	1035	1634	802	306	1945	133	224	410	367	359	564	2509
# individuals with G[1]	3209	5523	2862	1378	6522	558	834	1533	1405	1376	2030	8552
# full sib-pairs[2]	1046	2988	1350	912	3870	133	226	825	678	613	1199	5069
#female-female	427	11191	512	325	1501	47	86	319	268	261	461	3542
#male-male	168	431	202	154	586	30	48	161	124	106	219	805
Proportion of families												
2 sibs	1.00	0.75	0.75	0.52	0.73	1.00	1.00	0.65	0.69	0.72	0.62	
3 sibs	0.00	0.17	0.19	0.24	0.17	0.00	0.00	0.23	0.22	0.21	0.23	
4+ sibs	0.00	0.08	0.06	0.24	0.10	0.00	0.00	0.12	0.09	0.08	0.12	
LOD suggestive[3]	1.6	1.7	1.7	1.7	1.7	1.5	1.5	1.7	1.7	1.7	1.6	1.7
LOD significant[3]	2.9	3.0	3.0	3.5	3.1	2.7	2.8	3.1	3.4	3.3	3.1	3.1
ELOD20[4]	1.4	8.0	2.3	1.3	11.9	0.4	0.9	2.0	1.7	1.6	10.0	20.9[6]

[1] G =genome scan data;

[2] MZ pairs excluded; a small number of families only contributed half-sib pairs;

[3] empirical suggestive and significant LOD score thresholds from 1000 gene drop simulation genome scans.

[4] Expected LOD of a QTL that accounts for 20% of the phenotypic variance.

[5] the high full-sib correlation reflects the ascertainment for inclusion in the Australian 1999 study.

[6] ELOD20 for AU&NL ≠ ELOD20 for AU + ELOD20 for NL, because of different heritabilities used.

Table 2

Overlap in data sets used in linkage analysis: the proportion of full-sib pairs who both have the row measure of neuroticism that also both have the column measure of neuroticism.

	AU_{80}	**AU_{89}**	**AU_{99}**	**AU_{02}**		**NL_{91}**	**NL_{93}**	**NL_{97}**	**NL_{99}**	**NL_{02}**
AU_{80}		0.82	0.17	0.06	NL_{91}		0.81	0.62	0.53	0.50
AU_{89}	0.28		0.43	0.12	NL_{93}	0.48		0.65	0.56	0.52
AU_{99}	0.13	0.97		0.04	NL_{97}	0.09	0.15		0.59	0.51
AU_{02}	0.06	0.33	0.06		NL_{99}	0.09	0.16	0.74		0.64
AU	0.26	0.76	0.34	0.26	NL_{02}	0.10	0.17	0.71	0.71	
					NL	0.10	0.17	0.71	0.57	0.51

Table 3

Chromosomal regions where the LOD >1.5 for either AU&NL, AU or NL. Evidence for consistency of signal for individual measures within country and evidence for support from other studies.

Measure[a]	Chr	Position cM[b]	LOD[b]	Linkage interval[b] cM	Linkage interval cytogenetic band	Singlepoint Marker[c], LOD, position	LOD > 1.5[d]	1.0 < LOD < 1.5[e]	Fullerton[14] identified region[f]	Human region homologous mouse linkage region[g]	Primary sourcs for human linkage studies[h]
NL	6	75	1.5	59–111	6p21.2-6q21	D6S2410, 2.4, 75 cM	NL_{99}* NL_{02}	AU&NL		99-190 cM	
AU	8	134	1.6	125–145	8q24.12-8q24.21	D8S592, 1.7, 124 cM	AU_{89} $AU_{99,}$	NL_{91}		104-154 cM	
NL	10	5	2.0*	0–29	10p15.3-10p14	D10S1412, 1.8, 25 cM	NL_{99}* NL_{97}* NL_f* NL_m				*Neuroticism [16]
NL	14	103	2.6*	94–118	14q32.12-14q32.31	D14S1434, 1.7, 104 cM	NL_{99}* AU&NL*	NL_{02}		76-134 cM	* Anxiety 105 cM[39] R Anxiety 105 cM[48]
AU&NL	18	117	1.9*	95–125	18q21.33-18qter	D18S61, 1.4, 99 cM	NL*	NL_{99} NL_{02} AU_{89}	R 80 cM	85-109 cM	** Neuroticism Female 91 cM[22] * RE-MDD or anxiety 73 cM [47] Neuroticism Female 115 cM[22] R Harm avoidance 109 cM [54] Panic Disorder 104 cM[55]
Sex specific											
AU_m &NL_m	2	112	1.6*	94–118	2p13.2-2q11.2	D2S1790, 1.6, 111 cM	NL_{02}*	AU_m NL_m AU_{89}		102-151 cM	** Suicide & RE-MDD 100 cM
NL_m	5	191	2.2*	185–199	5q35.1-5q35.2	D5S211, 1.4, 191 cM	NL_{99m}* NL_{97m}	NL_{02m}			
AU_m	8	45	1.6*	34–53	8p22-8p21.1	D8S1771, 1.6, 49 cM		AU_{89m}	M 50cM		** Harm avoidance 60 cM [56] Suicide & RE-MDD 37 cM [50] * RE-MDD 60 cM Anxiety 21 cM [48] RE-MDD 25 cM Male pairs[57] Neuroticism both sexes and male pairs[14]
NL_m	10	175	1.7*	164–175	10q26.3	D10S212, 1.1, 173 cM	NL_{99m}			70-171 cM	
NL_f	15	17	1.8 *	0–35	15p11.2-15q14	GTTTT001, 1.3, 24 cM	NL_{99f}*	NL_{02f}			

[a] Mean neuroticism measure with highest LOD score in region based on 5cM grid search.

[b] Maximum LOD score from 5cM grid search. Position and linkage interval (1 LOD less than the maximum LOD) based on 1 cM grid search of identified regions; those marked with * are significant at the suggestive threshold for linkage

[c] Marker within interval which shows largest singlepoint LOD score.

[d] other measures within linkage interval boundaries with LOD > 1.5, those marked with * are significant at the suggestive threshold for linkage.

[e] other *individual* measures with 1< LOD <1.5 scores within linkage interval boundaries.

[f] Regions identified by Fullerton[14] as having support from multiple (M) studies or having reasonable (R) support for linkage from analyses of neuroticism, major depression, anxiety and panic disorder as listed in the primary sources for results where linkage intervals overlap (or are likely to overlap if not presented).

[g] Regions identified by Smoller et al[49] (S) as being homologous to linkage regions identified in studies of anxiety in mice.

[h] Linkage studies used in the review by Fullerton[45], plus additional studies[22, 39, 48, 50, 55, 58]. RE-MDD recurrent early onset major depression. ** significant, * suggestive (these include estimated suggestive regions from the linkage study of Fullerton et al[16] who discussed in detail only significant linkage results; from their Figure 2 we have estimated which additional peaks may have exceeded a suggestive threshold for linkage, −logP >2.5), R reported (approx LOD > 1.5)

Table 4

Summary of linkage studies of neuroticism ordered by number of sibpairs measured for neuroticism

a. Country b. Study Name (if any) c. Reference d. Measure of neuroticism. e. No. of measures if >1	Ascertainment of base population	# sibpairs measured	Selection criterion for inclusion in linkage analysis	# sibpairs in linkage analysis. Inter-marker distance.	Analysis	No. linkage peaks identified[1]			
						SIG	SIGsex	SUG	SUGsex
a UK **c** Fullerton et al[16] **d** EPQ-R	Community-based sample	34,580	Used neuroticism score to select 2.5% most discordant and 2.5% most concordant, 78%[2] response rate	629 10cM[¶]	Visscher-Hopper[59] regression	2	3	8[3]	3[3]
a Australia & Netherlands **c** This paper See next 2 rows.	See next 2 rows	19,635	See next 2 rows	5,424	Merlin-Regress[44]	0	0	2	1
a Australia **c** This paper **d** EPR-R/EPQ-R-S/NEO **e** Mean score of up to 4 measures over 22 years	**i.** Twin individuals and their families. **ii.** Large families with high incidence of smokers	**i.** 11,665 **ii.** 1,107 independent	**i.** Although based on an EDAC design in which neuroticism score to select one sib in top/ bottom 10%, other sib in top bottom 20%, 91%[2] response rate[8], the selectively genotyping overall was weak Figure 1a **ii.** None	**i.** 3,364 **ii.** 702 8cM[§]	Merlin-Regress	0	0	0	1
a. Netherlands **b.** NETSAD **c.** This paper **d.** ABV	Twin individuals and their families	6,863	None. No difference in distribution of neuroticism scores for those with and without genotypes.	1,358 11cM[§]	Merlin-Regress	0	0	3	3

a. Country b. Study Name (if any) c. Reference d. Measure of neuroticism. e. No. of measures if >1	Ascertainment of base population	# sibpairs measured	Selection criterion for inclusion in linkage analysis	# sibpairs in linkage analysis. Inter-marker distance.	Analysis	No. linkage peaks identified[1]			
						SIG	SIGsex	SUG	SUGsex
e. Mean score of up to 5 measures over 11 years									
a. UK **b.** GENESiS **c.** Nash et al[10] **d.** EPQ-R-S **e.** Means score of up to 2 (78% with 2 measures) 6 months apart.	Community-based sample	4,824	Most informative 10%, Response rate 65%[2]	297 9 cM	Merlin-Regress	0	1	0[4]	2
a Ireland & Northern Ireland **c** Kuo et al[22] **d** EPQ-R-S	Sibships concordant for alcohol dependence	714	None	714 4cM	Merlin-Regress	1	4	2[5]	4[5]
a New Zealand **c** Neale et al[21] **d** EPQ-R-S	Sibships concordant for nicotine dependence	201	None	201 10cM[¶]	Merlin-Regress	0	N/A	5	N/A

[1] Exceed Lander and Kruglyak[12] empirical thresholds for significant (SIG) or suggestive (SUG) linkage: One linkage test statistic of observed magnitude or greater expected by chance every twenty genome scans (SIG) or each genome scan (SUG). Sex specific linkage peaks, SIGsex, SUGsex.

[2] Response rates are for individuals and reflect the success of the selection criterion for entry into the linkage study; response rate for sibpairs are expected to be the square of this number.

[3] Estimated from their Figure 2 assuming threshold of −logP = 2.5.

[4] One suggestive region identified but listed as significant sex specific region.

[5] Estimated as those with LOD >1 (as empirical threshold for SIG was 1.29).

[§] Calculated as the average distance between markers genotyped in both members of sib pairs, which is likely to be higher than the average distance between markers reported for the other studies.

¶estimated from number of markers