Linkage to Chromosome 1p36 for Attention Deficit Hyperactivity Disorder Traits in School and Home Settings

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ABSTRACT

Background: Limited success has been achieved through previous ADHD linkage scans which were all designed to map genes underlying the dichotomous phenotype. The International Multi-centre ADHD Genetics (IMAGE) project performed a whole genome linkage scan specifically designed to map ADHD quantitative trait loci (QTL).

Methods: A set of 1,094 single selected Caucasian ADHD nuclear families was genotyped on a highly accurate and informative single nucleotide polymorphism (SNP) panel. Two quantitative traits measuring the children’s symptoms in home and school settings were collected and standardized according to a population sample of 8000 children to reflect the developmental nature and gender prevalence difference of ADHD. Univariate linkage test was performed on both traits and their mean score.

Results: A significant common linkage locus was found at chromosome 1p36 with a locus-specific heritability of 5.1% and a genome-wide empirical p<0.04. Setting-specific suggestive linkage signals were also found: LOD=2.2 at 9p23 for home trait and LOD=2.6 at 11q21 for school trait.

Conclusions: These results indicate that given large samples with proper phenotypic measures, searching for ADHD genes with a QTL strategy is an important alternative to using the clinical diagnosis. The fact that our linkage region 1p36 overlaps with the dyslexia QTL DYX8 further suggests it is potentially a pleiotropic locus for ADHD and dyslexia.

INTRODUCTION

Attention deficit hyperactivity disorder (ADHD [MIM143465]) is a common childhood developmental behavioral disorder which has a prevalence of 4-10% in the general population, depending on the precise way the diagnostic criteria are implemented (1). It occurs 4 to 8 times more frequently in boys than in girls (2). Converging evidence from family, twin and adoption studies suggests a significant genetic contribution to ADHD, with an estimated sib relative risk (λs) of 4 to 8 and average heritability of 0.76 (3, 4). Both categorical diagnosis and dimensional ratings of ADHD symptoms have been used in molecular genetic studies designed to identify the genes that confer risk to the disorder. Convincing evidence of candidate gene association has been obtained for genetic variants within or close to the dopamine D4 and D5 receptor genes from the combined analysis of numerous independent datasets in different populations (4, 5). In addition, associations are suggested in several other genes following the meta-analysis of association findings reported in three or more studies, although further independent replications are still required (4). However, the genes identified so far contribute only a small elevated risk to ADHD and a large amount of the population variance is still to be accounted for (6, 7).

The main method applied during the last decade to localize novel genes that increase the risk for ADHD has been the use of genome-wide linkage tests to identify chromosomal regions containing putative risk loci. To date, six independent genome-wide ADHD linkage scans have been conducted, all of which were designed to adopt the categorical diagnosis as the phenotype (8-14). These scans nominated several novel genomic regions that might harbor ADHD susceptibility genes; with the most prominent findings on chromosomes 5p and 17p being detected in several studies. However, none of these loci were consistently detected and no genes that can explain these linkage peaks have been clearly identified, indicating the complexity of the genetic risk factors underlying ADHD.
Although not originally designed to map quantitative trait loci (QTL) for ADHD symptoms, three of these linkage studies also used QTL linkage methods by using the total DSM-IV symptom count (DSM-TOT) as a quantitative measure of ADHD severity (11-13). No clear QTL linkage evidence was discovered in these studies and there was hardly any overlap between the dichotomous and quantitative trait linkage test results. There are a few study design issues that could explain these unimpressive results apart from the complexity of the genetic influences on ADHD. First, the power to detect QTLs of small to moderate effect was limited in these studies, which consisted of relatively small samples of 100 to 300 sib pairs. Second, the DSM-TOT measures were not adjusted for age and gender in these studies to reflect the developmental course of ADHD symptoms and the gender prevalence difference. Third, the variance of DSM-TOT in the clinical samples is less than that from the standardized scores derived from standard ratings scales of ADHD symptoms that in most cases adopt 4-point scales of severity for each symptom.

Another concern for QTL mapping of ADHD is that only parental DSM-TOT but no teacher data were applied in previous linkage scans. Three twin studies have examined this question and while all show that parent and teacher rated ADHD reflecting behavior in the home and school settings are highly heritable, there are substantial unique genetic effects (setting-specific loci) in addition to shared genetic effects between the two measures (common loci) (15-18). Here we report results from the first QTL linkage scan designed specifically to use age and gender standardized quantitative scores of ADHD symptoms from both home and school settings, using a large single-proband ascertained sample.

**MATERIALS & METHODS:**

For this study, the International Multi-centre ADHD Genetics (IMAGE) project recruited 1,094 European Caucasian nuclear families ascertained through DSM-IV combined-subtype ADHD probands. The study design was originally established with the specific aim of completing genome-wide linkage using QTL approaches. Detailed recruitment procedures have been published previously (19). Families were identified at 12 specialist clinical centers across 8 European countries: Belgium (Ghent), Germany (Essen and Göttingen), Ireland (Dublin), Israel (Tel-Aviv and Jerusalem), Netherlands (Nijmegen and Amsterdam), Spain (Valencia), Switzerland (Zurich) and the United Kingdom (London and Southampton). The probands and their siblings were aged between 5 and 17 at the time of assessment and at least one and in most cases two of their biological parents were also available for DNA collection. Clinical research assessments were administered by qualified child psychiatrists or trained interviewers, after which blood or buccal samples were collected for DNA extraction either directly or from cell lines that were established at Rutgers University, New Jersey. The following exclusion criteria were applied for the probands and their sibs: (i) IQ < 70; (ii) a diagnosis of schizophrenia or autism or neurological disorders such as epilepsy and brain injury; (iii) any genetic or medical disorder associated with externalizing behaviors that might mimic ADHD, (iv) ethnicity other than white European origin, (v) not living at home with at least one biological parent.

The final sample of 4,529 genotyped subjects reported in this linkage study included 2,545 children and their parents. Ethical approval for the study was obtained from National Institute of Health.
registered ethical review boards for each centre. All the families gave informed consent for the collection of clinical data, DNA and cell line storage at Rutgers, and sharing of anonymous clinical and genotype data with the scientific community.

Two quantitative traits measuring the children’s ADHD symptoms at home and at school were obtained from their parents and teachers respectively by using the revised Long Versions of the Conners’ Rating Scales (20). The DSM-IV symptom subscales (N-subscases), consisting of four-point scales for each of the 18 DSM-IV ADHD symptom items, were used to rate the children’s behavior. The raw scores from both informants were adjusted for age and gender according to a normative profile which has a distribution of Mean=50 and STD=10 derived from a population sample of 8,000 children (20). After the transformation, the standardized scores from parents (PNscore) and teachers (TNscore) were used as the quantitative trait measures in the QTL linkage analysis.

Table 1
Phenotypic features of the 2545 children from 1094 nuclear families

<table>
<thead>
<tr>
<th></th>
<th>ADHD(^1)</th>
<th>Non-ADHD(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n=1141)</td>
<td>(n=1404)</td>
<td></td>
</tr>
<tr>
<td>Male : Female</td>
<td>975 : 166</td>
<td>714 : 690</td>
</tr>
<tr>
<td>Age</td>
<td>10.7 ±2.8</td>
<td>11 ±3.3</td>
</tr>
<tr>
<td>PNscore</td>
<td>77.3 ±9.4</td>
<td>54.8 ±13</td>
</tr>
<tr>
<td>TNscore</td>
<td>73.8 ±11.6</td>
<td>55.6 ±13.3</td>
</tr>
<tr>
<td>Mean Score</td>
<td>75.5 ±8.2</td>
<td>55.1 ±11.2</td>
</tr>
<tr>
<td>Correlation(^2)</td>
<td>0.21</td>
<td>0.44</td>
</tr>
</tbody>
</table>

ADHD attention-deficit/hyperactivity disorder; PNscore, standardized scores from parents; TNscore, standardized scores from teachers.

1. A total number of 1141 children (967 probands and 174 sibs) made the research diagnosis criteria of DSM-IV Combined type ADHD. 2. Pearson’s correlation coefficient between PNscore and TNscore.

**Figure 1.** Genome wide QTL linkage test results for the three ADHD measures. Multipoint LOD scores were calculated with Merlin using a regression based method for (A) the PNscore, (B) the TNscore, and (C) the mean score of the PNscore and the TNscore. ADHD, attention-deficit/hyperactivity disorder; LOD, logarithm of odds; PNscore, standardized scores from parents; QTL, quantitative trait loci; TNscore, standardized scores from teachers.
Descriptive statistics for the two quantitative traits are listed in Table 1. The distribution of both traits is shifted towards the more severe end in the ADHD children and their unaffected sibs compared to the normal population mean of 50. As expected from the bivariate distribution of the PNscore and TNscore in the population, both proband and sibling scores are distributed closer to the extreme and the correlation in children with ADHD is lower than that in the unaffected group. In order to detect QTLs that could account for the overlap between parent and teacher scores, we tested for QTL linkage using both the PNscore and TNscore independently, in addition to the mean of the two scores. The use of the mean score for the parent and teacher rated ADHD symptoms is expected to reduce the variance specific to each informant and improve the power to detect common genetic factors.

Genotyping was completed by the Center for Inherited Disease Research (CIDR) using Illumina’s BeadArrayTM technology (http://www.illumina.com) on a BeadLab system. A total number of 5,545 autosomal SNPs from the Illumina Linkage IVB SNP panel were successfully assayed with a call rate of 99.6% and reproduction rate of 99.994%. The markers were ordered and placed on the physical map according to Genome Build 35 (National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/). Interpolated genetic distances from the deCODE genetic map (Sturlugotu 8, IS-101, Reykjavik, Iceland) were used in the following linkage analysis (21).

We identified and corrected 40 pedigree errors by testing pair-wise subject relationships throughout the sample with RELPAIR (http://csg.sph.umich.edu/boehnke/relpair.php) (22). One hundred and five SNPs were dropped due to significant departure (p<0.01) from Hardy-Weinberg Equilibrium reported by Pedstats (http://www.sph.umich.edu/csg/abecasis/PedStats/) (23). Another 33 SNPs were dropped due to excessive Mendelian inconsistencies found by PEDCHECK (http://watson.hgen.pitt.edu/register/docs/pedcheck.html) (24). The remaining sporadic Mendelian inconsistencies were removed by dropping all the family members’ genotypes on the erroneous SNP. Genotypes leading to unlikely recombinants were removed by Merlin (http://www.sph.umich.edu/csg/abecasis/Merlin/) (25). After the above data cleaning process, a total number of 5,407 autosomal SNPs entered into our linkage analysis with an average resolution of 1.66 SNPs/cM. The average entropy information content was 95.2% across the genome and never dropped below 81% (25).

Univariate QTL linkage was examined for the PNscore, the TNscore and their mean score by Merlin, which implements a regression-based procedure using trait-squared sums and differences to predict IBD sharing between any non-inbred relative pairs (25, 26). With the population distribution parameters of mean, variance and heritability specified, this method can be applied to selected samples with similar statistical power to the variance component linkage tests. Since linkage disequilibrium between adjacent SNPs can lead to inflated LOD scores, we applied the criteria of $r^2 < 0.05$ to cluster correlated SNPs into combined markers (23).

**RESULTS:**

Figure 1 and Table 2 describe the univariate QTL linkage test results. Setting-specific suggestive linkage signals (LOD ≥ 2.2) were detected according to the criteria proposed by Lander and Kruglyak (27). A LOD score of 2.2 for PNscore was found on 9p23 and a LOD score of 2.6 for TNscore was identified on 11q21. The
most prominent results for the setting specific results were LOD scores of 2.7 and 2.4 on 1p36 for PNscore and TNscore respectively, indicating a possible common locus in this region influencing both parent and teacher ratings in the home and school settings. In the mean score test that has better power to detect common loci, the linkage signal at 1p36 increased to 3.2 with a locus-specific heritability of 5.1%, whereas the other two setting-specific linkage signals at 9p23 and 11q21 became undetectable.

Table 2
Suggestive Linkage results

<table>
<thead>
<tr>
<th>Region</th>
<th>Location</th>
<th>PNscore</th>
<th>TNscore</th>
<th>Mean Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1p36</td>
<td>45</td>
<td>2.7</td>
<td>2.4</td>
<td>3.2</td>
</tr>
<tr>
<td>9p23</td>
<td>27</td>
<td>2.2</td>
<td>0.2</td>
<td>0.8</td>
</tr>
<tr>
<td>11q21</td>
<td>100</td>
<td>0.05</td>
<td>2.6</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Multipoint LOD>2.2: cM, centimorgan; LOD, logarithm of odds; PNscore, standardized scores from parents; TNscore, standardized scores from teachers.

To provide an empirical evaluation of the common linkage finding at the chromosome 1p36 locus, we performed simulations to establish the genome-wide level of significance. Merlin was used to generate 1,000 replicates of genome scan genotypes under the null hypothesis of no linkage, while preserving the original phenotypes, family structures, allele frequencies, LD structure and missing data pattern (25). The same univariate linkage tests for the PNscore and the TNscore were repeated. In each simulated data set, we defined a common linkage using two criteria: (i) peak LOD scores higher than 2.2 for both traits; (ii) overlapped LOD-1 support intervals. These criteria were relaxed compared to our experimental observations where the peak LOD scores of 2.4 and 2.7 were found at exactly the same location. Only 39 such common linkage signals were found in 1,000 simulated genome scans. According to Lander and Kruglyak, a statistically significant linkage may be expected to occur 50 times in 1,000 random genome scans (27). Our common linkage finding at 1p36 has therefore surpassed this threshold with an empirical genome-wide significance of p<0.04.

DISUSSION:

In the current study, we recruited 1,094 single ascertained nuclear families and performed a QTL linkage scan with 90% statistical power to detect a 10% QTL and around 50% power for a 5% QTL (28). We identified a common locus influencing both parent and teacher ratings of ADHD symptoms that was significant at the genome-wide level following simulation to determine the empirical level of significance. Additionally, we identified two setting-specific loci that were suggestive of linkage but did not pass genome-wide levels of significance.

The use of QTL approaches to map genes for ADHD has been discussed in the literature with no firm conclusions drawn on the added value of this approach over the analysis of diagnosed cases alone. We have previously discussed the potential value of this approach that is supported by available family and twin data and for the following reasons consider that QTL strategies provide an important complementary strategy (29). First the approach is supported by our quantitative genetic analyses of the IMAGE sample family data, that shows a familial association between ADHD and quantitative trait measures of ADHD symptom scores in siblings; with estimated proband-sibling correlations in the region of 0.3 (29). Furthermore, the sibling distributions show no evidence of bimodality that might suggest some discontinuity of the genetic influences between the probands and siblings. Furthermore, since the diagnosis can only be made when the number of symptoms
exceeds a threshold, the dichotomous trait is by its very nature dimensional (2).

Second, nearly all twin studies that demonstrate high heritability for ADHD symptoms have used quantitative measurements in general population twin samples. Group heritability approaches that estimated the heritability of ADHD using various thresholds for the extreme group also provide no evidence of discontinuity of the genetic influences (29, 30). In contrast however, the analysis of twin data using latent class approaches has suggested that the genetic influences on extreme groups may not be the same as those that influence levels of ADHD symptoms throughout the general population (31).

QTL studies of ADHD therefore add to the literature in two important ways. First if the QTL model holds true, that states that different frequencies of the same risk alleles will influence the level of ADHD symptoms throughout the population, then we can expect a greatly increased power for the detection of risk loci from the adoption of QTL strategies (26). On the other hand, the alternative finding that some genetic risks may be specific to the extreme group would also be important with different predictions for the severity, course and outcome of ADHD symptoms related at least in part to presence of specific genetic risk factors. The relative unpopularity of QTL studies for molecular genetic studies of ADHD has arisen mainly from concerns over the lack of direct clinical relevance and uncertainty that the QTL model holds true (4). Furthermore, in the linkage studies to date, little additional information has been obtained from the small number of QTL studies which were mostly designed to replicate the findings from dichotomous trait studies (3).

Our study was specifically designed to increase the power for QTL approaches through the selection of extreme cases (ADHD combined type was recently estimated to occur in 2.34% of male children (32). In the original design we intended to select only the most informative subset of extreme high and low scoring siblings for QTL linkage (33), however the study design was modified to include probands plus all of their siblings; since the selection process would have excluded many suitable families for association studies and made the ascertainment process considerably more complex. Furthermore our power calculations indicated that we would need to screen many more proband-sibling pairs to achieve greater power using the selected sibling design. The most significant common linkage locus detected in this study on chromosome 1p36 explains 5.1% of the total variance. Since the overall phenotypic variance from additive genetic factors is around 76%, this suggests that many more QTLs exist of far smaller effect that were not detectable using the study design and sample size implemented in this study. In fact this is expected since linkage has far less power to detect small to moderate genetic effects than association; and recent studies using whole genome association approaches suggest that for most common complex disorders only small genetic effects will exist (34). The genes identified as being associated with ADHD so far confer odds ratios of around 2.2 – 1.4 which is equivalent to QTL effects of < 1% assuming a simple additive genetic model (6). This partially explains why the three previous ADHD linkage scans that used between 100 to 300 sib pairs failed to detect any of the QTLs reported in this study. It also suggests that only very large samples will have the required power to replicate a QTL finding that contributes 5% to the total phenotypic variance using linkage approaches. However this would not be the case if the linkage finding were explained by a single common variant. For
example, to replicate a 5% QTL in a threshold-selected case-control study, assuming a liability threshold model with cases selected 2 standard deviations above the mean and risk-increasing allele frequency of 50%, only 740 cases and controls would be needed to obtain 80% statistical power at $\alpha = 10^{-7}$ (35). However, if the 5% QTL detected in our linkage scan is a cumulated effect of 5 variants each accounting for only 1% of the phenotypic variance, then the sample size needed to detect each of them would be 3,400 cases and controls (35).

A major question that we had to address in this study was the best way to select suitable quantitative traits for QTL studies of ADHD. Although a large number of instruments are available, assessing ADHD as a quantitative trait for molecular genetic studies remains challenging due to its developmental nature, sex differences and rater specificity. The D4-TOT score that has been used in previous ADHD QTL analyses is not an ideal measurement despite being the most comparable quantitative trait to the DSM-IV categorical diagnosis. The symptom count is generally less informative than rating scales that show increased variance in the general population and the proper adjustment for age and gender is not clearly established. In contrast, the standardized scores of the Connors’ rating scales were developed from a large population survey and are well adjusted for the covariates of age and gender to have uniform population distributions. These rating scales are therefore suitable for QTL studies since they can be informative when applied to either clinical or population samples or both.

Another question we needed to address was the number of different quantitative phenotypes to include in this analysis. There is evidence of both unique and common genetic effects on parent and teacher scores, but also on hyperactivity versus inattention scores. Since our main objective was to determine whether the QTL approach could identify loci related to the combined type of ADHD we decided to focus on the overlap between the measures; both in terms of symptom subtype and situational pervasiveness. Furthermore, we did not wish to reduce our power further by inclusion of multiple different phenotypic measures, so decided to analyze inattention and hyperactivity as a single combined measure since estimates of the genetic correlation of around 0.6, suggest that the majority of genetic effects are shared between the two domains (36).

In contrast, when considering the combined inattention-hyperactivity/impulsivity scores from parent and teacher ratings, both the phenotypic and genetic correlations between the two situation-specific measures were relatively low; suggesting that the there may be substantial differences in the genes underlying genetic risk for the two situations (16). For this reason we included the two scales as separate dimensions in addition to taking the mean. By testing PNScore and TNScore individually we identified suggestive evidence of setting specific QTLs for both traits. The greatest level of evidence came from the mean score test, which has a statistical power advantage for the detection of common loci, however because this failed to detect the setting-specific loci, this suggests that the analysis of each trait separately may be required to detect setting-specific loci.

Our finding that the common locus on 1p36 was detected with suggestive evidence of linkage for the single trait tests, while the mean score test detected a stronger linkage signal (LOD=3.2), further suggests that the combined index can outperform the single trait in mapping
the common loci. Although the mean score linkage test did not reach genome-wide significance at the 1p36 locus according to Lander and Kruglyak’s criteria, our simulations based on the two single trait linkage tests showed that this common linkage was statistically significant. We concluded from these results that there might be better ways to combine the parent and teacher ratings rather than the simple use of mean score. A genetic factor score derived from twin modeling, as described in a previous study is theoretically very informative for mapping common QTL (15). However, since a comparable twin sample with similar demographic features and phenotypic measures was not available to derive the factor loadings we were not able to perform this analysis in the current study.

Another theoretically desirable approach to map common QTL is through multivariate linkage analysis (37). This approach has been successfully adopted in the study of complex traits such as dyslexia (38). However, its applicability to the current study is hindered by a number of factors. First, the regression-based linkage test used in this study is powerful and robust to sample selection but has not been extended to the multivariate case (26). Second, the modified Haseman-Elston method is available for multivariate linkage test and robust to selection, but possesses less statistical power (39, 40). Thirdly, the multivariate variance component based method is powerful, but requires accurate ascertainment adjustment when applied to our selected sample (41, 42). Moreover, the variance component based method does not always provide increased power compared to the individual univariate analysis (28, 43, 44).

Since one of the main aims of the study was to provide a contrasting and hopefully more informative approach to the detection of QTLs related to the combined subtype of ADHD, it is pertinent to compare the results of this study with those of our previous analysis of the subset of 142 combined subtype affected sibling pairs (ASP) from 134 of the families used in this study (9). Using the ASP approach we identified suggestive linkage on chromosomes 9 and 16 (9). Furthermore, there appears to be no overlap between the two sets of findings, which could be explained in two main ways. First, both these studies might be underpowered to detect true linkage signals, especially for the dichotomous trait analysis. Second, genes that influence the quantitative trait may not be the same as those that influence the clinical disorder. Some of our previous work investigating the QTL associations of candidate genes in a recent scan of 51 genes supports the possibility of this type of heterogeneity since in our recent analyses we have found that most of the positive genetic findings with the diagnostic category (e.g. DRD4 and DAT1) could not be replicated using QTL association approaches (19). The possibility that QTLs for ADHD traits in the population are different from genetic effects on the core disorder has potentially important implications for the way that ADHD is conceptualized and requires further investigation.

Finally, it is worth noting that the chromosome 1p36 region overlaps with the linkage region DYX8, which was reported as a QTL significantly linked with dyslexia (45, 46). This is particularly interesting given that multivariate behavior genetic studies had consistently found some shared genetic components between reading difficulty and the inattention domain of ADHD (47). Since there is some previous evidence that dyslexia may be particularly associated with inattentive symptoms rather than hyperactive-impulsive symptoms, we
repeated the QTL linkage test at this locus with the combined parent and teacher rated DSM-IV subscales for the inattention and hyperactivity-impulsivity scales separately. As shown in Figure 2, the QTL linkage signal detected at chromosome 1p36 in our study is mainly from the inattentive subscale. These results suggest that further investigation is warranted to clarify whether this region harbors a pleiotropic QTL affecting both inattention and dyslexia.

Figure 2:
QTL linkage for different ADHD domains. Chromosome 1 multipoint LOD scores for the three combined teacher and parent DSM-IV ADHD ratings in different domains as rated by Conners’ Rating Scales questionnaires: red = hyperactivity-impulsivity domain; blue = DSM-IV inattention domain; black = combined index of inattention domain and hyperactivity-impulsivity domain. ADHD, attention-deficit/hyperactivity disorder; LOD, logarithm of odds; QTL, quantitative trait loci.

REFERENCES


