Meta-Analysis of Genome-Wide Linkage Scans of Attention Deficit Hyperactivity Disorder

Kaixin Zhou,1 Astrid Dempfle,2 Mauricio Arcos-Burgos,3,4 Steven C. Bakker,5 Tobias Banaschewski,6 Joseph Biederman,7 Jan Buitelaar,8 F.Xavier Castellanos,9 Alysa Doyle,7 Richard P. Ebstein,10 Jenny Ekholm,11 Paola Forabosco,1,12 Barbara Franke,8,11 Christine Freitag,10 Susann Friedel,16 Michael Gill,17 Johannes Hebebrand,16 Anke Hinney,16 Christian Jacob,17 Klaus Peter Lesch,18 Sandra K. Loo,19 Francisco Lopera,17 James T. McCracken,17 James J. McGough,7 Ana Miranda,22 Maximilian Muenke,4 Fernando Mulas,23 Stanley F. Nelson,11 T. Trang Nguyen,7 Robert D. Oades,24 Matthew N. Ogdie,25 Juan David Palacio,20 David Pineda,20 Andreas Reif,18 Tobias J. Renner,26 Herbert Roeyers,27 Marcel Romanos,26 Aribert Rothenberger,28 Helmut Schäfer,2 Joseph Sergeant,29 Richard J. Sinke,5 Susan L. Smalley,19,30 Edmund Sonuga-Barke,1,9,31 Hans-Christoph Steinhausen,32 Emma van der Meulen,33 Susanne Walitza,34 Andreas Warnke,26 Cathryn M Lewis,1,12 Stephen V. Faraone,7,34 and Philip Asherson1*

1Social, Genetic, and Developmental Psychiatry Centre, Institute of Psychiatry, King’s College London, London, UK
2Institute of Medical Biometry and Epidemiology, Philipps-University Marburg, Marburg, Germany
3Department of Psychiatry and Behavioral Sciences, Leonard M. Miller School of Medicine, University of Miami, Miami, Florida
4Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland
5Department of Medical Genetics, University Medical Center Utrecht, Utrecht, The Netherlands
6Department of Child and Adolescent Psychiatry and Psychotherapy, Central Institute of Mental Health, University of Heidelberg, Mannheim, Germany
7Department of Psychiatry, Harvard Medical School, Massachusetts General Hospital, Boston, Massachusetts
8Department of Psychiatry, Radboud University Nijmegen, Donders Centre for Neuroscience, Medical Centre, Nijmegen, The Netherlands
9Child Study Center, New York University, New York, New York
10Geha MHC, Petach-Tikva, Israel
11Department of Human Genetics, UCLA, Los Angeles, California
12Department of Medical and Molecular Genetics, King’s College London, London, UK
13Istituto di Genetica delle Popolazioni—CNR, Alghero, Italy
14Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands
15Department of Child and Adolescent Psychiatry, Saarland University Hospital, Homburg, Germany
16Department of Child and Adolescent Psychiatry, University of Duisburg-Essen, Essen, Germany
17Department of Psychiatry, Trinity Centre for Health Sciences, St. James’s Hospital, Dublin, Ireland
18ADHD Clinical Research Program, Department of Psychiatry and Psychotherapy, University of Wuerzburg, Wuerzburg, Germany
19Department of Psychiatry and Biobehavioral Sciences, Semel Institute for Neuroscience & Human Behavior, UCLA, Los Angeles, California
20Neurosciences Group, University of Antioquia, Medellin, Colombia
21Department of Neurobehavioral Genetics, University of Trier, Trier, Germany
22Department of Developmental and Educational Psychology, University of Valencia, Valencia, Spain
23Department of Neuropaediatrics, La Fe University Hospital, Faculty of Medicine, Valencia, Spain
24University Clinic for Child and Adolescent Psychiatry, Essen, Germany
25The Broad Institute, MIT, Cambridge, Massachusetts
26ADHD Clinical Research Program, Department of Child and Adolescent Psychiatry and Psychotherapy, University of Wuerzburg, Wuerzburg, Germany
27Ghent University, Dunantlaan, Ghent, Belgium
28Child and Adolescent Psychiatry, University of Gottingen, Gottingen, Germany
29Vrije Universiteit, De Boelelaan, Amsterdam, The Netherlands
30Center for Neurobehavioral Genetics, Semel Institute for Neuroscience & Human Behavior, UCLA, Los Angeles, California
31School of Psychology, Institute for Disorder on Impulse and Attention, University of Southampton, Highfield, Southampton, UK
32Department of Child and Adolescent Psychiatry, University of Zurich, Zurich, Switzerland
33Department of Child and Adolescent Psychiatry, University Medical Center Utrecht, Utrecht, The Netherlands
34Department of Psychiatry, SUNY Upstate Medical University, Syracuse, New York

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*Correspondence to: Dr. Philip Asherson, MRC Social, Genetic, and Developmental Psychiatry Centre, Institute of Psychiatry, King’s College London SE5 8AF, UK. E-mail: p.asherson@iop.kcl.ac.uk

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Attention deficit hyperactivity disorder (ADHD) is one of the most common childhood behavioral disorders characterized by early onset of age-inappropriate hyperactivity, impulsivity, and inattentiveness [Asherson, 2004]. Family and twin studies have consistently shown that genetic factors play an important role in ADHD etiology with heritability estimated around 76% [Faraone et al., 2005]. Meta-analysis of candidate gene studies has confirmed small but significant association with variants within or close to genes such as dopamine D4 (DRD4) and D5 (DRD5) receptor genes [Faraone et al., 2005]. Novel genes are still to be discovered through hypothesis free genome-wide linkage and association studies.

To date, seven genome-wide ADHD linkage scans have been published and some chromosome regions such as 5p13, 14q12, and 17p11 have been indicated in multiple studies [Fisher et al., 2002; Bakker et al., 2003; Arcos-Burgos et al., 2004; Hebebrand et al., 2006; Ogdie et al., 2006; Faraone et al., 2007; Asherson et al., 2008; Romanos et al., 2008]. However, no chromosome region has been consistently identified across the scans and the majority of the findings were unique to each study. This is not unexpected because the power of individual scans is expected to provide more power to detect true linkage signals.
used method in linkage meta analysis [Levinson et al., 2003; Lewis et al., 2003]. For a more detailed list of GSMA case studies, please go to the homepage (http://www.kcl.ac.uk/deptsa/memoge/gsma/).

Heterogeneity among studies can be assessed by the Q statistic, which is defined as the sum of the squared deviations of each study’s bin rank from the mean bin rank within the GSMA framework [Zintzaras and Ioannidis, 2005a]. The significance of Q statistics can be determined by permutations and it can be adjusted for differing sample sizes as well. Low between-study heterogeneity indicates consistency of study results in the same bin. Moreover, since the Q statistic is associated with the mean rank, an adjusted statistics Q_adjusted can also be computed by permuting only the ranks within the GSMA framework [Zintzaras and Ioannidis, 2005a].

In the current study we used the GSMA program to get the summed rank SR, P_SR, and P_OR statistics through 10,000 permutations [Pardi et al., 2005]. The 22 autosomes were divided into 120 bins according to the original GSMA protocol and the genome-wide significant threshold is 0.05/120 = 0.000417, and the threshold for suggestive evidence of linkage is 0.0083 [Wise et al., 1999; Levinson et al., 2005]. The program HEGESMA was used to get the Q_adjusted and its P-values through 10,000 simulations [Zintzaras and Ioannidis, 2005b]. Both weighted and un-weighted GSMA analysis was performed. The un-weighted analysis assumes each study has the same statistical power. To address the power difference across studies, the weight given to each study in the weighted analysis was computed as the squared root of the number of cases in each study as shown in Table I. This weight is not idea across studies, the other criteria in the sample ascertainment process but used different data capture instruments. In some studies, cases with sub-threshold diagnosis or comorbid autism were also included in the analysis. For the current meta-analysis, only linkage statistics based on the stringent diagnostic criteria were included. The four studies published earlier were genotyped on microsatellite panels mapped on the Marshfield genetic map while the three recent scans were genotyped with SNP microarrays from the Decode genetic map [Kong et al., 2002]. For the genetic map positions presented below, all the original Marshfield map positions were transformed into Decode map positions. The linkage statistics varied across studies due to the differences in their original study design and analysis methods.

RESULTS

The un-weighted and weighted P_SR statistics for each of the 120 bins are plotted in Figure 1. Significant thresholds for nominal (P < 0.05) suggestive (P < 0.0085) and genome-wide significant (P < 0.000417) linkage are marked. Table II shows the full details of both weighted and un-weighted GSMA results, including P_SR, P_OR and the adjusted heterogeneity test p-values P_un in the 10 bins with at least nominal linkage signals (P < 0.05) from the un-weighted analysis.

Linkage signals from both the weighted (SR = 718, P_SR = 0.00038, P_OR = 0.041) and un-weighted (SR = 714, P_SR = 0.00034, P_OR = 0.04) analyses in bin 16.4 (16q23.1-qter) were genome-wide significant according to Lander and Kruglyak’s criteria after a Bonferroni correction for the number of bins) [Lander and Kruglyak, 1995]. The P_OR of around 0.04 from both the weighted and un-weighted analyses enhances the evidence that this bin is linked to ADHD. Nine additional bins on chromosomes 5, 6, 7, 8, 9, 15, 16, 17 showed nominal linkage signals (P_SR < 0.05) from the un-weighted analysis. For each of the 10 bins with linkage signals, the P_SR statistics did not differ dramatically between the weighted and un-weighted analyses with the highest weighted P_SR < 0.08 as shown in Table II. Furthermore, no significant rank heterogeneity among the studies was observed for any of the 10 bins. This heterogeneity test result was expected because the total number of seven studies provides limited statistical power to detect heterogeneity when the gene effect is relatively small or moderate [Lewis and Levinson, 2006].

DISCUSSION

In the current study, our primary un-weighted GSMA analysis identified a total number of 10 chromosomal regions with nominal linkage signals (P_SR < 0.05). Under the null hypothesis of no linkage in any of the 120 bins, only 6 such bins are expected by chance and the probability of observing 10 or more is 0.077 [Wise et al., 1999]. These results suggest that some of the bins in our primary GSMA analysis, as nominated by individual linkage scans collectively, are likely to harbor ADHD genes.

The most significant finding in this GSMA analysis was identified in bin 16.4 which covers the chromosome region from 16q23.1 to the q terminal. Details of the linkage statistics within this bin are plotted in Figure 2. This bin had the maximum rank (rank = 120) in two scans with multipoint nonparametric LOD > 3.1 in the Asherson et al. [2008] study and MODglobal > 3.2 in the Romanos et al. [2008] study. Nominal linkage signals were also observed in two scans with Multipoint Nonparametric MLS of 1.05 (rank = 109) and 1.08 (rank = 105) in the Ogdie et al. [2003] study and the Bakker et al. [2003] study respectively. Even in the other three scans with no linkage signal, the ranks for this bin are also higher than average with ranks of 80, 73 and 112 in the Faraone et al. [2007] study, the Hebebrand et al. [2006] study and the Arcos-Burgos et al. [2004] study respectively. Although none of these scans reached genome-wide significance on their own, these moderate findings had collectively contributed to a genome-wide significant linkage signal as identified by GSMA.

Interestingly bin 16.3, which is next to bin 16.4 also showed nominal linkage signal (P = 0.017) from the un-weighted GSMA analysis and suggestive linkage (P = 0.0072) in the weighted GSMA analysis. This observation of clustered significant linkage bins could be explained by the fact that one multipoint linkage signal could extend 30–50 cM and affect the ranks of adjacent bins [Wise et al., 1999]. To explore this possibility, we repeated the GSMA analysis by shifting the bin boundaries 15 cM forward [Levinson et al., 2003]. The new bin covering chromosome 16q21–16q24 remained genome-wide significant and the adjacent bins showed no linkage signals. These results suggest that one strong linked locus within the new bin (64–83 Mb on the NCBI genome build 35) may account for both 16.3 and 16.4 signals in our primary GSMA analysis. It is also supported by the details of the linkage statistics as shown in Figure 2 that most of the linkage peaks in bin 16.4 extended to bin 16.3.
<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Caucasian + African American</th>
<th>Caucasian</th>
<th>Paisa decent</th>
<th>Caucasian</th>
<th>Caucasian + African American</th>
<th>Caucasian</th>
<th>Caucasian</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
<td>USA</td>
<td>Dutch</td>
<td>Columbia</td>
<td>German</td>
<td>USA</td>
<td>8 European countries</td>
<td>German</td>
</tr>
<tr>
<td>Design</td>
<td>Affected sib pair</td>
<td>Affected sib pair</td>
<td>Multigenerational pedigree</td>
<td>Affected sib pair</td>
<td>Affected sib pair</td>
<td>Affected sib pair</td>
<td>Multigenerational pedigree</td>
</tr>
<tr>
<td>Sample size</td>
<td>266 families, 308 pairs, 519 Cases</td>
<td>106 families, 132 pairs, 238 cases</td>
<td>18 pedigrees, 126 cases</td>
<td>102 families, 127 pairs, 229 cases</td>
<td>217 families, 384 pairs, 601 cases</td>
<td>134 families, 142 pairs, 276 cases</td>
<td>8 pedigrees, 95 cases</td>
</tr>
<tr>
<td>GSMA weight</td>
<td>1.38</td>
<td>0.94</td>
<td>0.68</td>
<td>0.92</td>
<td>1.49</td>
<td>1.01</td>
<td>0.59</td>
</tr>
<tr>
<td>Markers</td>
<td>423 LMS STRs</td>
<td>Marshfield 402 STRs</td>
<td>~400 STRs (CIDR)</td>
<td>475 STRs</td>
<td>IL-IV 5800 SNPs</td>
<td>IL-IV 5800 SNPs</td>
<td>10 k SNP from Affymatrix 50 k Array</td>
</tr>
<tr>
<td>Type of analysis</td>
<td>Multipoint nonparametric MLS</td>
<td>Multipoint nonparametric MLS</td>
<td>Combined parametric multipoint linkage LOD</td>
<td>Multipoint nonparametric LOD</td>
<td>Multipoint nonparametric LOD</td>
<td>Multipoint nonparametric LOD</td>
<td>MOD global parametric LOD</td>
</tr>
<tr>
<td>Analysis program</td>
<td>Mapmaker/sibs</td>
<td>Mapmaker/sibs</td>
<td>FASTLINK</td>
<td>Merlin</td>
<td>Merlin</td>
<td>Merlin</td>
<td>Genehunter</td>
</tr>
<tr>
<td>Categorical diagnosis</td>
<td>Any DSM-IV diagnosis through “best-estimate procedure” 95% definitive, 5% probable</td>
<td>DSM-IV diagnosis through “best-estimate procedure” Narrow and Broad with autism disorder</td>
<td>DSM-IV diagnosis through “best-estimate procedure”</td>
<td>DSM-IV criteria applied in K-SADS-PL</td>
<td>DSM-IV criteria</td>
<td>DSM-IV criteria applied in PACS and Connor’s teachers long rating scale</td>
<td>DSM-IV criteria with 23 subclinicals</td>
</tr>
<tr>
<td>Exclusion criteria</td>
<td>IQ ≤ 70; schizophrenia; autism</td>
<td>IQ ≤ 80; confounding psychiatric disorder</td>
<td>Not available</td>
<td>IQ ≤ 75; confounding psychiatric disorder; neurological disorder; physical brain damage; perinatal and postnatal environment factors</td>
<td>IQ ≤ 70; confounding psychiatric disorder; neurological disorder; physical brain damage</td>
<td>IQ ≤ 80 confounding psychiatric disorder</td>
<td>Not available</td>
</tr>
</tbody>
</table>
There are more than 200 annotated genes within bin 16.4, none of which have been previously examined in ADHD candidate gene association studies due to their lack of known functional relevance to the disorder. However, a recent genome-wide association scan found that the CDH13 (a cell adhesion molecule), which is located on chromosome 16q24, is associated with methamphetamine dependence [Uhl et al., 2008]. Another genome-wide QTL association scan using the IMAGE sample also found markers within CDH13 to be strongly associated with total ADHD symptom scores within children diagnosed with ADHD [Jessica Su et al., in this issue]. Whether genetic variations of CDH13 explain the linkage signals in this region is beyond the scope of the current study. Further fine mapping studies or combined linkage and association analysis are expected to address this issue.

Bin 5.3, which covers chromosome 5q11.2–q14.3, is another region with a nominal linkage signal in our GSMA analysis. It is worth noting that this bin is 40 cM away from the chromosome 5p13 region that was indicated as a potential locus for ADHD by two previous linkage scans [Hebebrand et al., 2006; Ogdie et al., 2006]. It is unlikely that the GSMA signal observed in bin 5.3 is contributed by linkage to 5p13 as the other five studies showed no linkage at this locus. However, it does not mean we should not pursue the 5p13 linkage region either, because GSMA only identifies promising regions and is not used for exclusion mapping. Indeed, further fine mapping of the 5p13 region has identified genetic variation of SLC6A3 (dopamine transporter gene) as a potential explanation for the linkage signal [Ogdie et al., 2004; Friedel et al., 2007].

### TABLE II. Bins With Linkage Signals From the Un-Weighted GSMA

<table>
<thead>
<tr>
<th>Bin</th>
<th>Boundary</th>
<th>Un-weighted</th>
<th>Weighted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Genetic(^a) (cM)</td>
<td>Physical(^b) (Mb)</td>
<td>SR</td>
</tr>
<tr>
<td>5.3</td>
<td>71–103</td>
<td>56–88</td>
<td>582</td>
</tr>
<tr>
<td>6.3</td>
<td>65–98</td>
<td>43–91</td>
<td>583</td>
</tr>
<tr>
<td>6.4</td>
<td>98–131</td>
<td>91–132</td>
<td>577</td>
</tr>
<tr>
<td>7.3</td>
<td>60–91</td>
<td>39–78</td>
<td>631</td>
</tr>
<tr>
<td>8.1</td>
<td>0–25</td>
<td>0–13</td>
<td>595</td>
</tr>
<tr>
<td>9.4</td>
<td>81–107</td>
<td>85–106</td>
<td>594</td>
</tr>
<tr>
<td>15.1</td>
<td>0–29</td>
<td>0–31</td>
<td>578</td>
</tr>
<tr>
<td>16.3</td>
<td>65–99</td>
<td>51–78</td>
<td>617</td>
</tr>
<tr>
<td>16.4</td>
<td>99–130</td>
<td>78–88</td>
<td>714</td>
</tr>
<tr>
<td>17.1</td>
<td>0–32</td>
<td>0–11</td>
<td>602</td>
</tr>
</tbody>
</table>

\(^{a}\)Genetic map positions are according to Decode genetic map.

\(^{b}\)Physical map positions are according to NCBI Genome Build 35.
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REFERENCES


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Although the majority of the subjects included in this study have white European ancestry, the potential influence of genetic heterogeneity (namely population specific loci) on the GSMA analysis should not be ignored [Zintzaras and Ioannidis, 2005a; Lewis and Levinson, 2006]. We conclude that chromosome regions such as 16q22, which was confirmed by further fine mapping [Arcos-Burgos et al., 2004].

In summary, this GSMA analysis of all seven published ADHD linkage scans suggests that some chromosome regions identified in the original studies might harbor ADHD genes. As shown by the recent identification of CNTNAP2 as an autism susceptibility gene, linkage evidence can play an important role in gene discovery [Alarcon et al., 2008; Arking et al., 2008; Stephan, 2008]. We conclude that chromosome regions such as 16q22–16q24 which show genome-wide significant linkage are worthy of attention even in the era of genome-wide association studies.
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