ADHD Candidate Gene Study in a Population-Based Birth Cohort: Association with DBH and DRD2

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ABSTRACT

Objective: Attention-deficit/hyperactivity disorder (ADHD) is a common childhood-onset disorder with a significant impact on public health. Although a genetic contribution to risk is evident, predisposing genetic determinants remain largely unknown despite extensive research. So far, the most promising candidate genes have been those involved in dopamine and serotonin pathways. This study tests a series of allelic variants within such candidate genes to determine their potential influence on ADHD susceptibility.

Method: We used a population sample ascertained from a birth cohort of a subpopulation of Finland, characterized by founder effect and isolation, thus minimizing genetic heterogeneity. The subjects were systematically ascertained using DSM-IV diagnostic criteria for ADHD from the Northern Finland Birth Cohort 1986 of more than 9,000 individuals, resulting in the study sample of 188 ADHD cases and 166 controls. We genotyped markers in 13 candidate genes, including critical components of dopamine and serotonin pathways.

Results: We report evidence for association of ADHD with allelic variants of the dopamine $\beta$-hydroxylase (DBH) and dopamine receptor D2 (DRD2) genes.

Conclusions: Our study supports the involvement of the dopamine pathway in the etiology of ADHD; specifically the genes DBH and DRD2 deserve more attention in further studies. J. Am. Acad. Child Adolesc. Psychiatry, 2007;46(12):1614–1621. Key Words: attention-deficit/hyperactivity disorder, birth cohort, DRD2, DBH.
allele detection (Service et al., 2006; Varilo and Peltonen, 2004).

In an initial exploration of the genetic basis of ADHD in the Northern Finland Birth Cohort 1986 (NFBC), we investigated genes within the dopaminergic, norepinephrinergic, and serotonergic neurotransmitter systems, which have previously been suggested to be involved in the etiology of ADHD (Olfson, 2004; Quist and Kennedy, 2001).

**METHOD**

**Subjects**

The ADHD study sample was ascertained as described in detail by Smalley et al. (2007), using DSM-IV diagnostic criteria for ADHD cases and controls from the Northern Finland Birth Cohort 1986 (N = 9,432 live-born individuals), a 1-year population-based birth cohort from the two northernmost provinces of Finland. This regional subpopulation of Finland represents an isolated population characterized by founder effect and more genetic homogeneity. Prospective longitudinal collection of an extensive amount of phenotype data has been carried out on the cohort members from the prenatal period onward (Järvelin et al., 1993). At age 15 the subjects’ parents were requested to complete the Strengths and Weaknesses of ADHD-Symptoms and Normal-Behaviors (SWAN) rating scale (Swanson et al., 2001a, 2001b). Based on SWAN screening, subjects were invited for further evaluation to determine their participation as either cases or controls. Clinical assessment included the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version, a semistructured interview for the assessment of ADHD and other psychiatric disorders (Kaufman et al., 1997). Final ADHD diagnoses were based on all of the information gathered on subjects using a best estimate procedure and DSM-IV criteria. Cases included in this study met a definite or probable lifetime diagnosis of ADHD. Probable cases fell one symptom short of diagnosis but met age of onset and impairment criteria. Controls were included if they met best estimate criteria as unaffected and were classified as SWAN controls. For a more detailed description of the sample and assessments, see Smalley et al. (2007). In the total study sample, 188 cases and 166 controls were identified (228 males and 126 females). Of these, at least 159 cases and 151 controls were genotyped for each of the attempted markers. All of the genotyping occurred without knowledge of case-control status. All of the study subjects provided informed consent under the University of Oulu and University of California, Los Angeles institutional review boards.

**Genotyping Methods**

Candidate genes were selected based on relevance in ADHD literature and include all of the known dopamine receptor genes, **DRD1-DRD5**, as well as **DAT1, DBH, TH, 5HTT, COMT, GRIN2A, MAOA**, and **SNAP25** (Table 1). We selected genotyped polymorphisms from the literature and additional single nucleotide

<table>
<thead>
<tr>
<th>Gene</th>
<th>Marker</th>
<th>Gene</th>
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</tr>
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</tr>
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<td>rs265973</td>
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<td>rs362599</td>
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</table>

Note: Marker assay quality control information is available from the authors. VNTR = variable number of tandem repeats.
polymorphisms (SNPs) from SNPBrowser version 3.1 or dbSNP and Celera databases, with minor allele frequencies >0.05 in white samples, according to data provided by dbSNP, the International HapMap Project, or Applied Biosystems. The markers included were relatively evenly spaced, covering the genomic region of the genes and flanking regions.

DNA was extracted from study participants’ blood according to standard protocols (Vandenplas et al., 1984). SNPs in DRD1–DRD5 were genotyped using Sequenom’s MassARRAY technology (Sequenom, San Diego, CA). Polymerase chain reaction (PCR) and extension primers were designed using SpectroDESIGNER 2.0 software (sequences available on request), and SNPs were genotyped in 3- to 6-plex reactions using the standard protocols. The average success rate for the Sequenom SNP data was 97%. SNPs in DAT1, DBH, 5HTT, COMT, GRIN2A, MAOA, and SNAP25 were genotyped using TaqMan SNP Genotyping Assays and the 7900HT Fast Real-Time PCR System for fluorescent read detection and allelic discrimination using the manufacturers’ specifications. The average success rate for the TaqMan SNP data was 99%. SNPs not failing the Hardy-Weinberg equilibrium (p < .05) were included in the analysis.

The 48-base pair variable number of tandem repeats (VNTR) polymorphism in DRD4 exon 3 was amplified using primers 5'-GCGACTACGTGTTCTACCTG-3' and 5'-AGGACCCCTCATGGCCTTG-3' in a modification of a previously described method (Lichter et al., 1993). The length of the fragments was determined using an ABI 3730 DNA analyzer (Applied Biosystems), called with GeneMapper version 3.0 software and verified manually. The DBH microsatellite (GT repeat) was genotyped according to previously described specifications (Wei et al., 1997) using primers 5'-TATGGAGAAAGAGAAGCAGG-3' and 5'-CTCTGGGCTCATGCTACATATA-3'. Genotypes were determined using an ABI 3700 DNA analyzer and called using Genotyper version 2.1 software. The 40-base pair (bp) VNTR in the 5'-UTR was genotyped according to a method described previously (Cook et al., 1995) using primers 5'-CTTCTCTGGAGGCTACGGCTAGG-3' and 5'-TGTGTTGTAGGGAAACGGCCCTGAG-3'. The length of the fragments was determined by 3% agarose gel electrophoresis. Genotypes were inspected visually and called twice independently. The 30-bp MAOA VNTR was genotyped according to a previously described procedure (Sabol et al., 1998) using primers 5'-ACACGCTGACGTTGGAGAAGG-3' and 5'-GAAACGACCTCATTGGCAAGG-3'. Products were separated on an ABI 3700 DNA analyzer and genotypes were called using Genotyper version 2.1 software. The 40-base pair (bp) VNTR in the DAT1 3'-untranslated region (UTR) was genotyped according to a method described previously (Cook et al., 1995) using primers 5'-CTTCTCTGGAGGCTACGGCTAGG-3' and 5'-TGTGTTGTAGGGAAACGGCCCTGAG-3'. The length of the fragments was determined by 3% agarose gel electrophoresis. Genotypes were inspected visually and called twice independently. The 30-bp MAOA VNTR was genotyped according to a previously described procedure (Sabol et al., 1998) using primers 5'-ACAGCCTGACGTTGGAGAAGG-3' and 5'-GAAACGACCTCATTGGCAAGG-3'. Products were separated on an ABI 3700 DNA analyzer and genotypes were called using Genotyper version 2.1 software. The 5HTTLPR (5HTT gene-linked polymorphic region), a 44-bp insertion/deletion, was genotyped using a method described previously (Manor et al., 2001) with the primers 5'-GGGCTTGGCCGCTCTGAATGC-3' and 5'-GGGCTTGGCCGCTCTGAATGC-3'.

VNTR, 5HTTLPR, and microsatellite polymorphisms were tested for Hardy-Weinberg equilibrium using the GENEPOP or PEDSTATS software program, and markers not failing the Hardy-Weinberg equilibrium (p < .05) were included in the study. The average success rate was 97%.

Statistical Analyses

The DRD4 VNTR was analyzed both as a multiallelic marker and as a biallelic marker based on carrier status of the long alleles (≥6 repeats). The DAT1 VNTR was analyzed as a biallelic marker after removing two individuals with a rare allele. Haploblocks were determined using HAPLOVIEW, and haplotypes were constructed using PHASE2.1.1 software (Adkins, 2004; Stephens and Donnelly, 2003; Stephens et al., 2001). Rare (frequency <0.05) haplotypes were clustered together into composite haplotypes. Analyses of biallelic marker data were performed using the SAS software package version 9.1.3. Logistic regression was used for testing association between genotypes or haplotypes and ADHD. Modeling was done using sex as a covariate and for males and females separately. Comparisons were made between different genotypes and carriers of different alleles for single markers, and between presence versus absence or different copy counts for haplotypes. Analyses of multiallelic marker data were done using LRTCC, an in-house program performing genotype- and allele-based likelihood ratio and randomization tests for calculating p values. Power calculations were performed using the Genetic Power Calculator program (Purcell et al., 2003). Results were not corrected for type I errors due to limitations of sample size and magnitude of gene effects investigated. Instead, reliability of the findings is to be evaluated based on independent replications or refutations.

RESULTS

Logistic regression analyses provide evidence of association with ADHD for markers in four genes, DRD2, DBH, DAT1, and 5HTT (Table 2).

We detected association for an SNP (rs2073837) in the DBH intron 12. The minor homozygote was risk conferring compared with the major homozygote adjusted for sex (OR 2.7, p = .0055) and an even stronger effect was seen in males (OR 5.0, p = .0027).

We also observed association in males only for three intronic DRD2 SNPs (rs1079727, rs1079595, rs1124491) and an SNP in the 3'-UTR (rs1800497), the DRD2 Taq1A RFLP. For all of the SNPs the minor allele was risk conferring, giving odds ratios between 1.9 and 2.1 (p_{min} = .017, p_{max} = .039). No evidence of association was seen in the complete sample or in females.

For DAT1 we found some evidence of association for an SNP (rs12516948) in the 3'-UTR. However, the result is only marginally significant, and no evidence of association is seen for the other DAT1 markers.

Although four SNPs in 5HTT are nominally associated, including an SNP in the 3' flanking region (rs1979572) and three intronic SNPs (rs4325622, rs140701, rs2066713), we consider the results inconclusive because the effect was seen only for heterozygotes, and no risk (or protective) allele could be identified.

We constructed allelic haplotypes using associated DRD2 SNPs (Fig. 1). These SNPs belong to the same haplblock (in DRD2, D' > 0.8 for SNPs between rs1079727 and rs1800497, and for SNPs rs1799978 and rs4245149). We discarded one SNP (rs1079595) because it was in full LD (D' = 1, r^2 = 1) with another (rs1124491). The DRD2 minor allelic haplotype gave
evidence of association for males, being risk-conferring. The odds ratio of the haplotype carriers compared to the rest was 2.1 \((p = .017)\). No evidence of association was seen in the complete sample or in females.

**DISCUSSION**

ADHD is a common childhood-onset disorder that often persists in some form into adulthood and has a marked effect on public health. Despite extensive research on the genetic basis of ADHD, results to date remain inconclusive. From the available genetic studies, genetic heterogeneity is evident. We attempted to address the genetic heterogeneity by studying the genetics of ADHD within a case-control study sample, ascertained using DSM-IV diagnostic criteria from the NFBC, representing an isolated population.

Our study sample provided evidence of association with ADHD for a single SNP in DBH (rs2073837), and for four SNPs in DRD2 (rs1079727, rs1079595,

### TABLE 2
Logistic Regression Results for Single Markers

<table>
<thead>
<tr>
<th>Gene</th>
<th>Marker</th>
<th>Alleles</th>
<th>N Cases</th>
<th>N Controls</th>
<th>OR [95% CI]</th>
<th>(p)</th>
<th>Males</th>
<th>N Cases</th>
<th>N Controls</th>
<th>OR [95% CI]</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBH</td>
<td>rs2073837</td>
<td>22 vs. 11</td>
<td>35 vs. 50</td>
<td>18 vs. 60</td>
<td>2.69 [1.34–5.43]</td>
<td>0.0055</td>
<td></td>
<td>24 vs. 39</td>
<td>5 vs. 41</td>
<td>5.05* [1.75–14.54]</td>
<td>0.0027</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11/12 vs. 22</td>
<td>124 vs. 35</td>
<td>133 vs. 18</td>
<td>0.44 [0.23–0.82]</td>
<td>0.0099</td>
<td></td>
<td>45 vs. 77</td>
<td>22 vs. 71</td>
<td>1.89 [1.03–3.45]</td>
<td>0.0393</td>
</tr>
<tr>
<td>DRD2</td>
<td>rs1079727</td>
<td>22/12 vs. 11</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>43 vs. 72</td>
<td>21 vs. 70</td>
<td>1.99 [1.07–3.69]</td>
<td>0.0287</td>
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<td></td>
<td>rs1079595</td>
<td>22/12 vs. 11</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>47 vs. 73</td>
<td>22 vs. 71</td>
<td>2.08 [1.14–3.80]</td>
<td>0.0174</td>
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<td></td>
<td>rs1124491</td>
<td>22/12 vs. 11</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>45 vs. 73</td>
<td>22 vs. 69</td>
<td>1.93 [1.05–3.55]</td>
<td>0.0333</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>rs1800497</td>
<td>22/12 vs. 11</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>44 vs. 29</td>
<td>50 vs. 14</td>
<td>0.43 [0.20–0.91]</td>
<td>0.0264</td>
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<tr>
<td>DAT1</td>
<td>rs12516948</td>
<td>11 vs. 22</td>
<td>85 vs. 12</td>
<td>69 vs. 22</td>
<td>2.20 [1.01–4.80]</td>
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<td>22/12 vs. 11</td>
<td>101 vs. 53</td>
<td>114 vs. 33</td>
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<td>0.51 [0.27–0.97]</td>
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<td>67 vs. 37</td>
<td>81 vs. 24</td>
<td>0.54 [0.29–0.99]</td>
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<td>45 vs. 73</td>
<td>22 vs. 69</td>
<td>1.93 [1.05–3.55]</td>
<td>0.0064</td>
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*Note: Uncorrected results with \(p < .05\) are shown (full data set is available from the authors). Allele coding: 1 = major, 2 = minor.

* Five minor allele homozygotes in male controls.

Fig. 1 Logistic regression results for DRD2 haplotypes in males.
rs1124491, rs1800497) for males. For the DBH SNP (rs2073837) minor allele, an odds ratio of 5.0 ($p = .0027$) for males is exceptionally high, but it needs to be interpreted with caution because there were only five minor allele homozygotes in male controls. However, we also detected similar evidence of association in the complete study sample (both sexes) adjusted for sex (OR 2.7, $p = .0055$). It is interesting to note, based on across species comparisons, the risk-conferring minor allele represents the more distant ancestral allele, raising the possibility that negative selection may have decreased its frequency if the carriage ship would have reduced our ancestors’ fitness.

Previous studies on DBH have concentrated mostly on the intron 5 Taq1 polymorphism (Comings et al., 1996b; Daly et al., 1999; Roman et al., 2002; Smith et al., 2003; Wigg et al., 2002), and a small association effect was detected in a recent meta-analysis of all of the studies to date (Faraone et al., 2005), but not in our study. Although association results for other adjacent polymorphisms have been negative (Hawi et al., 2003; Payton et al., 2001; Smith et al., 2003; Wigg et al., 2002), the GT microsatellite studied here has been associated with serum dopamine β-hydroxylase levels (Cubells et al., 1998). Our findings introduce a novel intronic DBH SNP showing putative association with ADHD. This SNP appears to define an allele that is probably unrelated to previous positive findings because it resides outside the haploblock defined by the other DBH SNPs investigated (in DBH, $D' > 0.8$ for SNPs rs1108581 and rs1611125, and for SNPs rs1611125 and rs2519152). DBH is located within a suggestive linkage peak evident in a Dutch genome-wide scan for ADHD (Bakker et al., 2003), but not in the United States (Ogdie et al., 2004), German (Hebebrand et al., 2006), or Colombian populations (Arcos-Burgos et al., 2004). Because the protein product of DBH is an enzyme catalyzing the conversion of dopamine to norepinephrine, DBH can be considered a functionally relevant candidate gene.

Evidence of association in males for four DRD2 SNPs (rs1079727, rs1079595, rs1124491, rs1800497, $p_{\min} = .017, p_{\max} = .039$) and for a three-SNP haplotype ($p = .017$) was observed, with odds ratios of approximately 2. For SNP rs1079727, the major allele is ancestral. As in the case of the DBH SNP discussed above, the risk-conferring minor allele of the Taq1A RFLP (rs1800497) is ancestral. Previous studies on DRD2 have concentrated mainly on the Taq1A RFLP in the DRD2 3′-UTR. It has been associated with a reduction in dopamine receptor D2 density in the brain (Jonsson et al., 1999; Pohjalainen et al., 1998), although there are also opposing results (Laruelle et al., 1998). There have been both positive (Comings et al., 1991; Comings et al., 1996a) and negative (Huang et al., 2003; Kustanovich et al., 2004; Rowe et al., 1999) ADHD association findings, with the positive ones coming from case-control studies and the negative ones from family studies. The discordance has been suggested to be related to differences in study populations because subjects in the positive studies had comorbid Tourette syndrome. Our study provides evidence for the association of DRD2 with ADHD in males without reference to comorbidity. Because the NFBC 1986 has substantial comorbidities (Smalley et al., 2007), further exploration of gene-phenotype associations will be examined taking into account other measures of clinical variability. Our study further supports the significance of the Taq1A RFLP and introduces three novel SNPs showing putative association with ADHD in males and sharing a haploblock with the Taq1A RFLP. It is not surprising that the DRD2 association was detected in males only because there is evidence of estrogen-dependent sex differences in dopaminergic function (Sawada and Shimohama, 2000). Sex differences in dopamine receptor density have also been observed, and age-dependent changes in dopamine receptor D2 density coincide with changes in ADHD occurrence in males (Andersen and Teicher, 2000).

The DRD2 Taq1A RFLP was recently found to be located in a novel kinase gene ankyrin repeat and kinase domain containing 1 (ANKKI), coded by the antisense strand (Neville et al., 2004). The ANKK1 protein product is a member of a family of proteins involved in signal transduction pathways. In ANKK1 the Taq1A SNP causes a Glu713Lys substitution, which may affect the protein’s substrate-binding specificity. Our finding of a putative association of the SNP also raises the possibility of involvement of ANKK1 in ADHD.

In regard to 5HTTLPR, for which a recent pooled analysis (Faraone et al., 2005) of previous studies (Beitchman et al., 2003; Cadoret et al., 2003; Kent et al., 2002; Manor et al., 2001; Retz et al., 2002; Seeger et al., 2001; Zoroglu et al., 2002) showed evidence of association, we observed a trend toward association.
The short allele homozygote showed a trend toward being risk conferring compared with the other homozygote, with an odds ratio of 1.87 ($p = .083$) adjusted for sex.

The findings of the present study support involvement in ADHD of two dopamine pathway genes suggested by previous studies (Bobb et al., 2005; Faraone et al., 2005): DRD2 and DBH. Despite support for DRD4, DRD5, DAT1, 5HTT, and SNAP25 reported in other study populations (Bobb et al., 2005; Faraone et al., 2005), we did not see considerable support for association of these genes in the NFBC 1986. Our study included the specific polymorphisms that have been associated (Faraone et al., 2005), except for DRD5, in which only a single, novel SNP passed all of the genotyping quality controls. The lack of association with this SNP does not rule out association with other allelic variants of DRD5. Considering sample size, limitations of power may preclude us from detecting modest effects. For example, assuming full LD ($D' = 1$) between the predisposing variant and detected marker and frequencies of 0.10 for both, a study sample of our size provides 80% power to detect genotypic relative risks of $>2$ at $p < .05$, under a dominant model. However, with a relative risk of 1.5, more typical of the variants identified in complex traits, our study has only 35% power to detect the effect. Alternatively, because our study sample is ascertained from a genetically homogeneous isolated population, some of the genetic risk loci present in other populations may not exist within the NFBC 1986. Conversely, the same population will allow us to identify genetic risk loci that may be missed in the screening of other more heterogeneous populations.

Our recent data indicate that the DRD2 haplotype associated with ADHD in this study is also associated with the persistence personality trait in a similar but earlier collected population-based birth cohort of 12,058 live births from northern Finland, the Northern Finland Birth Cohort 1966 (NFBC 1966, unpublished data). The minor allelic haplotype, which is implicated as conferring risk to ADHD in males in the NFBC 1986, was found to be associated with low persistence in NFBC 1966 especially in females ($p = .0023$), but also adjusted for sex ($p = .033$) and in males and females together ($p = .031; n = 1434$). Low persistence and ADHD share some phenotypic similarity; the inattentiveness and impulsiveness that is characteristic of ADHD subjects can be viewed as low persistence to maintain a task. Therefore, low persistence could be envisioned as an endophenotype for ADHD. Extreme variation in persistence in the low side, combined with other features characteristic of ADHD, may comprise the clinical entity of ADHD. There is some prior association evidence of low persistence with ADHD (Rettew et al., 2004; Yoo et al., 2006).

Limitations

The results of our study add further support for the involvement of DBH and DRD2 in ADHD. However, these findings require replication in independent populations of various ethnic origins. Interpretation difficulties in all complex trait research arise from shortcomings including limited sample size, variation of causative genetic variants across and within populations, environmental effects, and ambiguity of the relevant phenotype. An important limitation in our study is the restricted sample size. Phenotypic ambiguity may be reduced by determining endophenotypes that are relevant to ADHD (Doyle et al., 2005a, 2005b). For instance, temperament may prove to be an important endophenotype for ADHD as indicated from associations of temperament with ADHD (Lynn et al., 2005; Rettew et al., 2004; Yoo et al., 2006).

Clinical Implications

Advances in uncovering ADHD susceptibility genes are steps toward a future in which information on the genetic architecture of ADHD may be used to improve clinical practice. Genetic data may lead to new directions in research on novel pharmacological interventions or aid in the development of new diagnostic regimes. Improved treatment options combined with easier and more reliable diagnostics may facilitate intervention at an early age, possibly affecting the subject’s well-being also in later life. Our study further advances the likely role in ADHD of two genes involved in dopamine regulation, DBH and DRD2.

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REFERENCES

Adkins RM (2004), Comparison of the accuracy of methods of computational haplotype inference using a large empirical dataset. *BMC Genet* 5:2-8

Andersen SL, Teicher MH (2000), Sex differences in dopamine receptors and their relevance to ADHD. *Neuropsychobiology* 42:137–141


Cadoret RJ, Langbehn D, Caspers K et al. (2003), Associations of the serotonin transporter promoter polymorphism with aggressivity, attention deficit, and conduct disorder in an adoptee population. *Compr Psychiatry* 44:88–101

Comings DE, Comings BG, Muhleman D et al. (1991), The dopamine D2 receptor locus as a modifying gene in neuropyschiatric disorders. *JAMA* 266:1793–1800


Comings DE, Muhleman D, Gyorg R (1996a), Dopamine D2 receptor (DRD2) gene and susceptibility to posttraumatic stress disorder: a study and replication. * Biol Psychiatry* 40:368–372


Faraone SV, Perlis RH, Doyle AE et al. (2005), Molecular genetics of attention-deficit/hyperactivity disorder. * Biol Psychiatry* 57:1313–1323

Hawi Z, Lowe N, Kiley A et al. (2003), Linkage disequilibrium mapping at DAT1, DRD5 and DBH narrows the search for ADHD susceptibility alleles at these loci. * Mol Psychiatry* 8:299–308


Huang YS, Lin SK, Wu YY, Chao CC, Chen CK (2003), A family-based association study of attention-deficit hyperactivity disorder and dopamine D2 receptor Taq1 A alleles. *Chang Gung Med J* 26:897–903


Kent L, Doerry U, Hardy E et al. (2002), Evidence that variation at the serotonin transporter gene influences susceptibility to attention deficit hyperactivity disorder (ADHD): analysis and pooled analysis. *Mol Psychiatry* 7:908–912


Ogdie MN, Fisher SE, Yang M et al. (2004), Attention deficit hyperactivity disorder: fine mapping supports linkage to 5p13, 6q12, 10p13, and 17p11. *Am J Hum Genet* 75:661–668


Pohjalainen T, Rinne JO, Nagren K et al. (1998), The A1 allele of the dopamine D2 receptor Taq1 A alleles. *J Neural Transm* 105:912–917


Rettew DC, Copeland W, Stanger C, Hudziak JJ (2004), Associations

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The purpose of this work was to describe levels of maternal anxiety, depressive symptoms, and perceptions of infant vulnerability associated with newborn genetic screening for susceptibility to type 1 diabetes. Patients and Methods: Mothers of infants tested at birth for genetic susceptibility to type 1 diabetes as part of a prospective study investigating potential environmental triggers of autoimmunity were recruited to this study. Three mother-infant cohorts were studied: 58 infants at increased genetic risk, 73 at low risk, and 76 who had not undergone testing. The Vulnerable Baby Scale, Edinburgh Postnatal Depression Scale, and state subscale of the State Trait Anxiety Inventory were administered at the 9-week, 4-month, and 1-year postnatal ages. Genetic-risk notification occurred at the 10-week postnatal age. Mothers whose infants had undergone genetic testing were also asked to subjectively rate how much they thought and worried about their child’s genetic test result. Statistical analyses were conducted to test for differences in questionnaire scores among the 3 groups. Results: No difference among the groups was detected in Vulnerable Baby Scale or Edinburgh Postnatal Depression Scale scores using linear mixed-effects model analysis. Maternal anxiety was paradoxically slightly lower in the increased-risk group shortly after notification of results, but there were no significant differences among the groups by 1 year. Mothers of infants in the high-risk group reported thinking and worrying about their child’s test result significantly more than mothers of low-risk infants at both time points after notification of results. Conclusions: Newborn genetic screening to identify infants at risk for type 1 diabetes is not associated with elevated levels of maternal anxiety, depressive symptoms, or heightened perceptions of infant vulnerability. However, responses to subjective assessment questions suggest that it is possible that more subtle effects on mothers do occur, and this requires further investigation. Pediatrics 2007;120:e324–e335.