Statistical methods for analysing complex genetic traits

El Galta, Rachid

CHAPTER 7

Phenotypic Subtypes in Attention Deficit Hyperactivity Disorder in an Isolated Population

E.A. Croes, R. el Galta, J.J. Houwing-Duistermaat, R.F. Ferdinand, S. Lopez Leon, T.A. Rademaker, M.C. Dekker, B.A. Oostra, F. Verhulst and C.M. Van Duijn

Abstract

Background: We address the use of two informants in genetic studies and whether familial aggregation is similar for the three phenotypic subtypes of ADHD. Methods: Lifetime ADHD was diagnosed in a Dutch isolated population using parents and teachers as informants, creating two subgroups (one or two informants), then further divided into three phenotypic categories (inattentive, hyperactive/impulsive, combined). Genealogy was collected for all patients. Mean kinship coefficients for the subgroups were calculated. Results: Fifteen of twenty-six children were linked to a common ancestor within ten generations. The mean kinship coefficient of patients confirmed by two informants was significantly higher than in patients only scored positive by one informant (p=0.03). All patients of the inattentive subtype were connected to a common ancestor, which was significantly higher (0.028) than expected. 81% of these patients derive of consanguineous marriages, also higher than expected. This means that recessive mutations may be involved in the inattentive subtype. These patients were more closely related than those with the other phenotypes ($p < 0.01$). Conclusion: Our data suggests that using two informants in diagnosing ADHD helps identify a phenotype with a strong genetic component. The inattentive phenotype showed strong familial clustering and evidence of a recessive origin.

Published in *Eur J Epidemiol*. 2005; **20**:789-94

7.1 Introduction

Attention Deficit Hyperactivity Disorder (ADHD) is one of the most common psychiatric disorders in children (Verhulst et al., 1997). It is characterised by inattention, distractibility, over-activity and poor impulse control (Barkley, 2003). Males are more frequently affected than females (Gaub and Carlson, 1997). It has been suggested that ADHD is a risk for academic problems, antisocial behaviour and substance abuse in adolescence and adulthood (Barkley, 1996; Cantwell, 1996; Hill and Schoener, 1996). There is strong familial aggregation of ADHD in families (Faraone et al., 2001). The heritability of ADHD has been estimated to be 0.50-0.98 (Faraone et al., 2000; Levy et al., 1997; Thapar et al., 1995). The mode of inheritance is thought to involve genes with dominant effects (Lopera et al., 1999), but others have argued that the inheritance is more complex because many different genes are involved. A major problem hampering genetic research of ADHD, and psychiatric genetic research in general, is the difficulty in defining the phenotype (Thapar et al., 1999). The phenotype is diverse, including patients with inattention, patients who are hyperactive/impulsive, and those with both. There may be a difference in the contribution of genes to the clinical phenotype.

Another problem to address is that for ADHD no biochemical tests or opportunities to support the diagnosis with imaging are available. Therefore, for children with ADHD, diagnostic information is based on reports of observations of behaviour in different contexts. By convention, in order to meet DSM-IV criteria for ADHD, symptoms need to be present in at least two of three settings (home, school, work) (Shaffer et al., 2000). Agreement between various informants such as parents and teachers is low, ranging between 0.30 and 0.50 (Achenbach, McConaughy, and Howell, Achenbach et al.; Ferdinand et al., 2003). Variation in the child's behavior across different situations, and differences in the way different observers judge the child's behavior, are two possible sources of cross-informant variance (van der Ende, 1999). By combining information from both parents and teachers, the validity of the diagnosis of ADHD has been found to improve (de Nijs et al., 2004; Mitsis et al., 2000; Verhulst et al., 1994). The first question we address is whether the use of two informants is helpful in genetic studies. Second we addressed the question whether familial aggregation is similar for the three phenotypic subtypes of ADHD.

7.2 Methods

Study Population

This study was conducted within the framework of the program Genetic Research in Isolated Populations (GRIP). Approximately 150 individuals founded this population in the Southwest of the Netherlands in the middle of the 18th Century. The population is characterised by minimal migration ($< 5\%$) and rapid growth (700 inhabitants in 1848 and 20,000 inhabitants at present). For this population the genealogical records are available since 1750. The GRIP population has proved to be suitable to study complex diseases such as type 1 and type 2 diabetes mellitus (Aulchenko et al., 2003; Vaessen et al., 2002). For this study, two paediatric neurologists, who obtained referrals from this genetically isolated village, asked all their patients diagnosed with ADHD to participate in this study (n=49; 22% female). Thirty-three (67.3%) patients and their parents agreed to participate.

This programme has obtained approval of the Medical Ethical Committee. All parents provided informed consent for themselves and for their children. Children over the age of eleven co-signed the informed consent.

Psychiatric Assessment

The Dutch version of the National Institute of Mental Health Diagnostic Interview Schedule for Children (NIMH DISC or DISC)-IV was used to assess DSM-IV diagnoses (Ferdinand and van der Ende, 2000; Shaffer et al., 2000). Psychologists and psychology students trained by the authors of the Dutch DISC-IV administered the DISCs. The training schedule used was similar to the schedule used by the authors of the original English version, at Columbia University, New York. To obtain information regarding a wide range of current DSM-IV Axis 1 diagnoses, parent DISCs (DISC-P) were administered during face-to-face contacts, at a community general health centre or in a children's hospital. Furthermore, lifetime ADHD symptoms were also assessed with the DISC-P. Teachers were interviewed with the ADHD section (current, not lifetime) of the teacher DISC (DISC-T) via telephone. The child version of the DISC (DISC-C) was not applied since most of the children included in our sample were too young (< 11 years of age). To assess the presence of diagnoses besides ADHD, the following diagnoses were assessed with the DISC-P: social phobia, separation anxiety disorder, specific phobia, agoraphobia, generalised anxiety disorder, panic disorder without agoraphobia, panic disorder with agoraphobia, obsessive compulsive disorder, posttraumatic stress dis-

order, major depressive disorder, dysthymia, bipolar disorder, oppositional disorder and schizophrenia.

Phenotypic subgroups (inattentive, hyperactive/impulsive, and combined) of ADHD were formed based on application of the DSM-IV criteria that had been assessed with the DISC. Current ADHD diagnoses were based on information from parents and teachers. Two types of ADHD diagnoses were derived: (1) 'based on one informant', and (2) 'based on two informants'. A diagnosis of ADHD based on one informant was applied when either parent or teacher scored six or more criteria positive for the inattentive, hyperactive or combined phenotype, while the other informant scored less than three criteria positive. A diagnosis of ADHD based on two informants was applied when one informant scored six or more criteria of one of the ADHD subgroups positive and the second informant scored three or more criteria positive. The threshold of '3 criteria positive' was chosen arbitrarily for the purpose of the present study. DSM-IV does not provide explicit rules for the number of criteria that need to be positive in 2 settings to obtain an ADHD diagnosis. It merely states that symptoms have to be present in at least 2 settings. If a child did not fulfil criteria for current ADHD with the DISC-P, lifetime information from the DISC-P was used to obtain a lifetime diagnosis of ADHD, based on parent information.

Genealogical information

Genealogical information comprising the name, date, and place of birth of parents, grandparents and great-grandparents was collected during a home interview. This genealogical information was extended up to 22 generations using municipal and church registers and data from a large genealogy database holding genealogical information on 60,000 individuals from this region in the Netherlands (Vaessen et al., 2002).

Statistical analysis

The relationship between two patients was expressed as the kinship coefficient. This is the probability that variation in the genome of a patient is identical by descent to a randomly drawn allele at the same locus of another patient. For example the kinship coefficient is 0.25 for sib-pairs, and 0.125 for cousins, meaning that the probability of a random allele genotyped in a sib-pair or cousin-pair to be identical by descent is 0.25 and 0.125 respectively. Kinship coefficients were calculated for all pairs of patients with PEDKIN, using all information contained in the genealogical database (Zwetselaar, 2003).

Furthermore, mean kinship coefficients as well as Inbreeding coefficients were computed for each subgroup.

The null hypothesis of no differences between kinship coefficients of two subgroups was tested using a statistic (Z) as outlined in the appendix. This statistic Z is based on the difference between the means of the logarithm of the kinship coefficients.

To assess whether the number of patients connected to a common ancestor and the number of patients derived of consanguineous marriages is larger in particular subgroups than expected based on the population structure respectively, 100 random sets of controls were sampled from the pedigree.

7.3 Results

Of the thirty-three patients who agreed to participate, two were excluded because their genealogy could not be worked up. Baseline characteristics of the remaining study population and the co-morbidity found are presented in Table 7.1. Five children did not fulfil criteria for any of the definitions of ADHD used in the present study; these were excluded from further analyses. In the remaining group of twenty-six ADHD patients, the mean age at the time of the study was 10.1 years, and 23.1 % of patients were female. Oppositional disorder (54%) and specific phobias (27%) were the most prevalent co-morbid diagnoses. Eleven patients fulfilled the DSM-IV criteria for the combined type of ADHD, twelve for the predominantly inattentive, and three for the predominantly hyperactive/impulsive type.

Based on genealogical information, fifteen out of twenty-six patients (58%) could be linked to one common ancestor within ten generations (Figure 7.1). In nine patients the inbreeding coefficient was higher than 0.001 (range 0.001 - 0.027). The parents of seven patients were related within four to seven generations (patients 1, 5, 8, 9, 10, 12, 15; Figure 7.1).

The mean kinship coefficient was highest for children with the inattentive subtype of ADHD and lowest for those with the hyperactive/impulsive subtype (Table 7.2). Children with the inattentive phenotype were significantly more closely related than those with the combined type ($p < 0.01$). All of the patients with a consistent diagnosis of the inattentive subtype were connected to a common ancestor, which is significantly higher (p=0.028) than expected based on the structure of the population. Eighty-one percent of these patients derive of consanguineous marriages which is also significantly increased (p=0.015). We further found that children with a diagnosis of ADHD confirmed by two informants (mean kinship coefficient 0.0029) were signif-

icantly more closely related than children in whom the diagnosis was only confirmed by one informant (mean kinship coefficient 0.0005; p=0.03).

TABLE 7.1: Baseline characteristics of the study sample and co-morbidities

	All	ADHD ever	No ADHD
Number of subjects	31	26	5
Mean age at examination (range)	10 (6-16)	10 (6-16)	10 (8-13)
Females (%)	22.6	23.1	20.0
Co-morbidity			
Social phobia	2	2	0
Separation anxiety disorder	4	4	0
Specific phobia	8	7	1
Agoraphobia	3	3	0
Generalised anxiety disorder	1	1	0
Panic disorder with agoraphobia	1	1	0
Obsessive compulsive disorder	2	2	0
Oppositional disorder	15	14	1
Conduct disorder	2	2	0

Co-morbidities are based on DISC-P. Selective mutism, panic disorder without agoraphobia, posttraumatic stress disorder, major depressive disorder, dysthymia, bipolar disorder, and schizophrenia were not present in any of the patients.

TABLE 7.2: Mean kinship coefficient in ADHD phenotypes*

ADHD phenotype	Number of patients	Kinship coefficient
Inattentive	12	0.0046
Hyperactive/impulsive	3	0.00002
Combined	11	0.0030

**Based on DSM-IV criteria.*

7.4 Discussion

There are two important findings in this study. First, we found that adding diagnostic information of a second informant results in a group of patients who are genetically more closely related than patients in whom the diagnosis is based on one informant. This finding indicates that a consistent diagno-

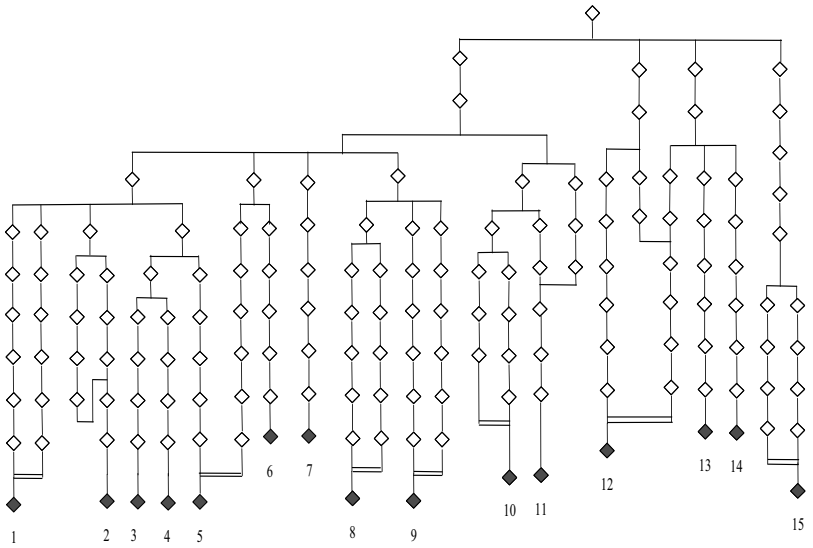

FIGURE 7.1: *Pedigree of the kindred. The symbol on top represents the common ancestor. Filled symbols indicate individuals affected with ADHD. The double line denotes a marriage between parents with a shared ancestor. A diamond symbol has been used to mask the sex of the patients, in order to protect patient confidentiality.*

sis of ADHD, confirmed by a second informant, is more suitable for future gene-finding studies. Second, we showed that children with the inattentive subtype of ADHD are in our population genetically more cluster related than those with the combined type. We confirmed previous studies which found that ADHD clusters in families (Faraone et al., 2001). We found, however, that patients with the inattentive phenotype were more closely related. Using extensive data-based genealogical information of the patients included in this study, we also found evidence of inbreeding. The presence of inbreeding strongly suggests that recessive genes are involved. In those which the diagnosis was confirmed by two informants as well as those with the combined phenotype, inbreeding was significantly increased in comparison to the control group. The chances that two similar recessive mutations are transmitted to a child are therefore much more likely when they come from the same ancestor in a pedigree with so-called "loops", than in an out-bred pedigree with non-related parents. So far, only genes with a dominant effect have been considered in the aetiology of ADHD.

A major limitation of our study is the small sample. In order to use information on genealogy we had to restrict our study to an isolated population for which we have genealogical data available. This has limited the number of patients eligible for the study. Nevertheless, the relation between the number of informants and the distance of relationship between patients was found to be statistically significant, even with this small sample size. The advantage of working with an isolated community is that we have detailed information on genealogy, which is not available in the general population. The loops identified in seven of fifteen patients who were linked to a common ancestor further suggest the involvement of a gene with a recessive effect. We have previously shown that, in this population with inbreeding, homozygosity mapping is a powerful approach in detecting genes with recessive effects (Bonifati et al., 2003). Of note is the fact that in our study the mean kinship coefficients for ADHD are relatively high. In the same genetically isolated population we studied Alzheimer's disease, which is known for its strong genetic clustering. The mean kinship coefficient for the Alzheimer's disease patients (0.0003) was found to be smaller than in any of the ADHD-subgroups (Roks et al., 2001). This suggests that the ADHD children in this population are closely related, making it suitable for future genetic studies.

Several studies addressed the question whether the number of informants confirming the ADHD diagnosis would improve the validity of the diagnosis (Achenbach, McConaughy, and Howell, Achenbach et al.; Ferdinand et al.,

2003). Their findings indicate that scores of informants from different settings (e.g., home, school) may differ, either due to a different behavior of the child in these surroundings, or to differences in the interpretation of the child's behavior by the informants. Combining this unique contribution of each informant may yield a more consistent diagnosis, which also may better discriminate ADHD from other psychiatric disorders, such as conduct disorder (Crystal et al., 2001; Mitsis et al., 2000). Various studies assessed associations between type of informant (parent or teacher) and heritability of ADHD (Martin et al., 2002; Thapar et al., 2000; Todd et al., 2001). Thapar et al. found that a common genetic factor underlies both the parent-rated and teacher-rated ADHD symptoms (Thapar et al., 2000). However, they also found that additional specific genetic factors might contribute to the ADHD symptoms as rated by the teacher. Also Martin et al. concluded that ADHD diagnosed by using parent and teacher information showed a high degree of heritability (Martin et al., 2002). They suggested, however, that different genes might underlie the symptoms reported by parent versus teacher.

Another complicating factor in the search for genes involved in ADHD may be that phenotypic subtypes show differences in heritability (Neuman et al., 2001; Todd et al., 2001), as seen by our finding of the closer genetic relationships in children with inattentive and combined subtypes compared to those with the hyperactive/impulsive subtype. Also our study shows that the use of these subtypes, instead of viewing all subtypes as one single disorder, may provide the best opportunity to find genes involved in ADHD (Neuman et al., 2001; Todd et al., 2001).

In conclusion, our data showed that patients with the inattentive phenotype of ADHD were more closely related. By adding phenotypic information of a second informant a genetically more homogeneous group may result suitable for gene finding studies.

7.5 Appendix

Statistic Z to test the null hypothesis of no difference in mean of kinship coefficients between two groups.

Let d_{ij} be the natural logarithm of the kinship of pair(i, j) in group of size n_k . Let μ_k, σ_k^2 and γ_k be the mean, the variance and the covariance of two pairs with one subject in common, respectively. To test the null hypothesis $\mu_1 = \mu_2$

the following statistic Z is proposed

$$Z = \frac{\hat{\mu}_1 - \hat{\mu}_2}{\sqrt{var(\hat{\mu}_1) + var(\hat{\mu}_2)}},$$

with $\hat{\mu}_k$ the mean of $\frac{n_k(n_k-1)}{2}$ kinship coefficients in group k. The variance of $\hat{\mu}_k$ depends also on the covariance γ_k and is given by

$$var(\hat{\mu}_k) = \frac{2\sigma_k^2 + 4(n_k - 2)\gamma_k}{n_k(n_k - 1)}$$

for $k = 1, 2$. The variance $var(\hat{\mu}_k)$ is estimated by replacing σ_k^2 and γ_k by their estimators. The distribution of Z under H_0 is approximated by a standard normal distribution.

SUMMARY