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Linkage of c-Harvey-ras-1 and INS DNA Markers to Unipolar Depression and Alcoholism is Ruled Out in 18 Families

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Summary. Eighteen families informative for c-Harvey-ras-1 and INS DNA markers were tested for linkage to unipolar depression and alcoholism. No evidence of linkage was found between these DNA markers and the disorders observed in the families. This study fails to replicate the Old Order Amish Study and suggests that a significant degree of genetic heterogeneity may be present among psychiatric disorders.

Key words: c-Harvey-ras 1 – INS – Depression – Alcoholics – Linkage

Introduction

Unipolar depression and alcoholism are two of the most common psychiatric disorders. Family and twin studies suggest that both are genetically transmitted (Allen et al. 1974; Cook and Winokur 1985; Gerson et al. 1982; Kaij 1960). Although most major mental disorders appear to be hereditary the mode of transmission and gene or genes for these illness are still unknown. C-Harvey-ras I and INS are two loci on the short arm of chromosome 11 that may be mrkers for affective disorder. Evidence for this, however, is limited to a single study that has not yet been replicated (Detera-Wadleigh et al. 1987; Egeland et al. 1987; Gill et al. 1988; Hodgkinson et al. 1987; Wesner et al., in press).

The search for genetic markers is complicated by the fact that mental disorders that are transmitted genetically may show incomplete or partial penetrance. That is to say some carriers of the genotype for a mental disorder may never express the disorder or may express something that is mild and not recognized as a mental disorder. A recent twin study of schizophrenia suggests that discordant monozygotic twin pairs transmit the disease gene to their offspring with equal frequency confirming the presence of unexpressed genotypes (Gottesman and Bertelsen 1989).

Mental disorders may also show variable or alternative expressivity. For example, a carrier may express an entirely different phenotype,making that individual appear to have a different unrelated illness. Family studies of unipolar depression have found high rates of alcoholism in first-degree relatives suggesting that alcoholism may be an alternative expression of the affective disorder genotype (Winokur 1972).

Family studies of unipolar depression by Winokur subdivide and classify cases of illness based on family history (Winokur 1982). Unipolar probands with first-degree relatives affected only with unipolar disorder are said to have familial pure depressive disease. Unipolar probands with first-degree relatives affected with alcoholism, antisocial personality, or unipolar disorder are referred to as having depression spectrum disease. Cases of unipolar disorder where there is no first-degree relative with unipolar disorder, alcoholism or antisocial personality are called sporadic depressive disease. This subdivision is an attempt to address the matter of heterogeneity in unipolar depression. Classifying unipolar depression on the basis of family history appears to produce three distinct forms of depression that have differing clinical characteristics. Familial pure depressive disease and depression spectrum disease appear to breed true and thereby suggest that they are separate genetic illnesses.

So far in genetic linkage studies the affected phenotype has been defined solely on individual diagnosis. If relatives are affected with other disorders, they may or may not be considered a part of the spectrum of affected. No study so far has included criteria beyond individual diagnosis to define the affected phenotype. This study utilizes Winokur's familial classification system as a means of separating what may be two separate illnes-

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ses, namely familial pure depressive disease and depression spectrum disease.

This study is also an attempt at replicating the Amish study by Egeland et al. (1987). In that study affective disorders which included bipolar and unipolar illness were found to be linked to c-Harvey-ras I and INS on 11p. If linkage to these markers could be demonstrated in our families, then perhaps both populations are demonstrating the same illness.

Methods

Twenty-four pedigrees containing unipolar depression and/or alcoholism were selected from a series of 38 collected by Tanna and Winokur. All families in this series were identified through either the University of Iowa Psychiatric Hospital or the Veterans Administration Hospital in Iowa City. Fourteen of the original 38 pedigrees were excluded from this study owing either to insufficient pedigree data or lack of sufficient blood samples for analysis. Each available member of the family was personally interviewed by an experienced psychiatric research assistant using a semi-structured interview. This interview has been used in several other genetic studies and is described elsewhere (Baker et al. 1971; Tanna et al. 1977; Winokur et al. 1971). Blood samples were also collected at the time of interview. Diagnoses were made by two clinical psychiatrists (VLT and GW) using the Feighner criteria (Feighner et al. 1972). Diagnoses of psychiatric illness were made without knowledge of genotypes.

Genomic DNA was extracted from buffy coats separated from whole blood samples collected in tubes containing anticoagulant. Genotypes were ascertained by digesting 10 μg DNA with the following endonucleases: Bam HI, TaqI, PvuII, and RsaI. All endonucleases with the corresponding buffers were purchased from Gibco/Bethesda Research Laboratories. DNA fragments were electrophoresed on 0.8% argorose gels at 40 mA for 16 h. Gels were stained with ethidium bromide for visual inspection and rinsed once in 1 × TPE for 10 min. The DNA was transferred to a nylon membrane (zetabind, Cuno) using a modified Southern method. This modification consisted of using 0.4 N sodium hydroxide as the transfer buffer rather than a sodium chloride/ sodium citrate solution. Hybridization was carried out with the DNA probes H-ras I (PEJ 6.6, A.T.C.C.) to Bam HI and TaqI digests and INS (PHINS 310, A.T.C.C.) to the PvuII and RsaI digest. The TaqI blots were also labeled with ^{32}P-labeled TGF alpha 925 as a quality control method for complete digestion. The hybridization procedure used in all cases was that of Feinberg and Vogelstein using a kit (Amersham Corporation) and was performed for 24 h at 42°C. Specific activity of all blots was approximately 10^8 CPM/μg DNA. The blots were then washed 3 times for 5 min each at room temperature in 2 × SSC, 0.1% SDS to remove residual hybridization solution and diminish background. This was followed by three washings at 55°C (twice for 20 min and once for 10 min) in 0.1 × SSC/0.1% SDS. The blots were autoradiographed using Kodak XAR-5 film and DuPont Lightening Plus intensifying screens at −80°C for at least 2 days.

Of the 24 pedigrees used, 17 were informative for H-ras and 16 were informative for INS. In total, 18 pedigrees were informative for either H-ras, INS, or both. Seven pedigrees were classified as familial pure depressive disease (FPDD) and the remaining 11 met criteria for depression spectrum disease (DSD). One hundred and sixty-three individuals comprised the 18 informative pedigrees and of those we were able to genotype 144. There were 86 females and 77 males. Seventy-three individuals received a psychiatric diagnosis. Fifty-one met criteria for unipolar depression. Nineteen meet criteria for alcoholism. Two met criteria for substance abuse other than alcoholism and one was diagnosed as a phobic disorder. No one received a diagnosis of antisocial personality and no one received more than one psychiatric diagnosis. The mean age (SD) at interview for all subjects was 45.2 ± 16.8 years with a range of 16–84. The mean age of onset for the unipolars were 35.6 ± 16 years with a range of 14–80. The mean age of onset for alcoholism could not be accurately calculated because the age of onset could not be determined in 11 of the 19 cases. Using only the eight cases of alcoholism where the age of onset was known, the mean onset was 27.9 ± 7.0 years with a range of 18–41. For the purposes of linkage analysis the age of onset for those whose age of onset was not known was set at 25. This age corresponds to the average age of onset for alcoholism in clinical research samples and is close to the mean age of onset seen in our sample (Cook and Winokur 1985).

The computer program M-LINK of the LINKAGE package was used to calculate two-point lod scores between disease and marker loci (Lathrop et al. 1985). Four penetrance models were established and applied assuming autosomal dominant transmission. In penetrance model 1 four liability classes were used and correspond to the penetrance model used by Egeland in the old-order Amish study (Egeland et al. 1987). The four liability classes have the following cut-off ages: 19, 24, 29, and those 30 and older. Penetrance values for the four age groups were 0.10, 0.27, 0.56, and 0.63 respectively. In models 2, 3, and 4a single liability class was used. Penetrance values were as follows: 0.63 (model 2), 0.85 (model 3), and 1.0 (model 4). The penetrance values used in models 2 and 3 were also taken from old-order Amish study. In the case of model 2 the penetrance value used is the maximum estimated penetrance for bipolar affective disorder for the entire old-order Amish sample. The value used in model 3 is the maximum estimated penetrance for bipolar affective disorder in the pedigree tested for linkage to H-ras and INS. Model 4 assumes complete penetrance of the disease gene. Gene frequency for unipolar depression and alcoholism was set at 0.01.

Selecting penetrance models for linkage analysis of psychiatric disorders is difficult, if not impossible, since the is no way of determining the exact value for any mental disorder at his time (Kennedy et al. 1988). In a family study of unipolar illness Dorzab et al. (1971) reported that the cumulative percent ill was 19% by age 19, 39% by age 29, and 54% by age 39. These figures roughly correspond to the penetrance values used in model 1 but are slightly lower. Using very low estimates of penetrance may result in lod scores close to zero. Obtaining meaningful lod scores with low penetrance values requires a high number of informative families each contributing a small amount to the score. The models used here are borrowed from earlier genetic research and are based on population estimates from those studies. It is understood that these estimates may not accurately reflect the true penetrance of the disease gene in this population, but our models do cover a wide range of penetrance values and for that reason are probably fair to use.

Only the diagnoses of unipolar depression and alcoholism were taken as affected. Three affected phenotypes were used in the analysis: (1) unipolar depression and alcoholism taken together as affected; (2) unipolar depression alone (alcoholism considered unaffected); and (3) alcoholism alone (unipolar depression considered unaffected). FPDD families and DSD families were analysed together as well as separately with all four penetrance models and all three affected groupings. This produced six separate phenotype groupings based on individual diagnosis and familial classification. A total of 24 separate two-point linkage runs were performed for each DNA marker assuming autosomal dominant transmission.

Multi-point analysis was performed using the LINKMAP program of the LINKAGE package (Lathrop et al. 1985). The disease locus was moved across a fixed map of H-ras and INS set 3 c*M* apart. Male/female ratio of recombination was 1.0. All four penetrance models were used in the multi-point analyses.

Results

Table 1 presents two-point lod scores between all informative pedigrees combined (FPDD and DSD; $n = 17$)

Table 1. Two-point lod scores between all informative families combined (Familial pure depressive disease and depression spectrum disease: $n = 17$)

Affected phenotype	Penetrance model[a]	Recombination fraction 0.00	0.05	0.20	0.40
Unipolar illness only (alcoholism considered unaffected)	1	−2.55	0.24	0.85	0.17
	2	−3.58	−0.23	0.69	0.14
	3	−7.83	−1.79	0.48	0.16
	4	−∞	−6.19	−0.19	0.12
Unipolar illness and alcoholism	1	−2.56	−0.03	0.98	0.23
	2	−3.72	−0.63	0.79	0.21
	3	−8.28	−2.59	0.60	0.29
	4	−∞	−7.22	0.21	0.38

[a] Penetrance model 1 = four liability classes with the following values: 0.10, 0.27, 0.56, 0.63
Model 2 = single liability class with penetrance set at 0.63
Model 3 = single liability class with penetrance set at 0.85
Model 4 = single liability class with penetrance set at 1.0

Table 2. Two-point lod scores between informative depression. Spectrum disease families ($n = 11$) and H-ras at 11p

Affected phenotype	Penetrance model[a]	Recombination fraction 0.00	0.05	0.20	0.40
Unipolar illness only (alcoholism considered unaffected)	1	−1.60	0.30	0.71	0.15
	2	−2.17	−0.05	0.55	0.11
	3	−5.47	−1.34	0.33	0.12
	4	−∞	−4.76	−0.27	0.06
Alcoholism only (unipolar illness considered unaffected)	1	−0.17	0.31	0.37	0.06
	2	−0.13	0.32	0.38	0.06
	3	−1.54	−0.30	0.45	0.10
	4	−∞	−3.03	0.31	0.15
Unipolar illness and alcoholism	1	−1.61	0.03	0.84	0.21
	2	−2.31	−0.45	0.65	0.18
	3	−5.92	−2.14	0.45	0.25
	4	−∞	−5.79	0.13	0.32

[a] Penetrance model 1 = four liability classes with the following values: 0.10, 0.27, 0.56, 0.63
Model 2 = single liability class with penetrance set at 0.63
Model 3 = single liability class with penetrance set at 0.85
Model 4 = single liability class with penetrance set at 1.0

and H-ras at 11p. Considering only unipolar depression to be the affected phenotype, tight linkage was ruled out for all penetrance models. The distance linkage could be ruled out from the marker locus varied between penetrance models but in most cases was less than 5 cM. Taking unipolar depression and alcoholism together as a single affected phenotype, tight linkage was also ruled out to H-ras for all penetrance models. The distance linkage oculd be ruled out from the marker locus varied between penetrance models and ranged from approximately 0.1 cM for model 1 to just over 10 cM for model 4.

Table 2 represents two-point lod scores between the informative depression spectrum disease pedigrees ($n =$ 11) and H-ras at 11p. Three affected phenotypes were used in the analysis: (1) unipolar depression alone (alcoholism considered unaffected); (2) alcoholism alone (unipolar depression considered unaffected); and (3) alcoholism and unipolar depression taken together as affected. In the DSD families tight linkage of the unipolar depression only phenotype to H-ras could be ruled out under penectrance models 2, 3, and 4. Considering only alcoholism as the affected phenotype, tight linkage to H-ras could be ruled out only for penetrance model 4. Taking both unipolar depression and alcoholism as affected tight linkage to H-ras could be ruled out under penetrance models 2, 3, and 4.

Table 3. Two-point lod scores between informative familial pure depressive disease families ($n = 6$) and H-ras at 11p

Affected phenotype	Penetrance model[a]	Recombination fraction 0.00	0.05	0.20	0.40
Unipolar illness only (alcoholism considered unaffected)	1	−0.95	−0.06	0.14	0.02
	2	−1.41	−0.18	0.14	0.03
	3	−2.36	−0.45	0.15	0.04
	4	−∞	−1.43	0.08	0.06

[a] Penetrance model 1 = four liability classes with the following values: 0.10, 0.27, 0.56, 0.63
Model 2 = single liability class with penetrance set at 0.63
Model 3 = single liability class with penetrance set at 0.85
Model 4 = single liability class with penetrance set at 1.0

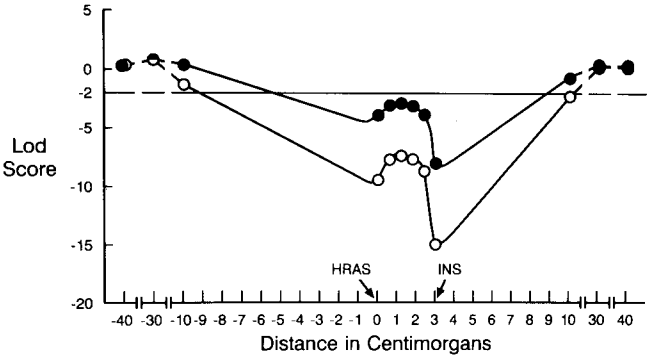

Fig. 1. Multipoint analysis of unipolar depression and alcoholism taken together as affected across a fixed map of HRAS and INS set 3 cM apart. ○ Penetrance 0.85; ● Penetrance 0.63

Table 3 presented two-point lod scores between the informative FPDD families ($n = 6$) and H-ras at 11p. Tight linkage was ruled out to H-ras for penetrance models 3 and 4.

The INS locus was informative for 16 families. Tight linkage of the INS locus to all affected phenotypes was ruled out (data not shown). No convincing evidence of linkage was observed for any affected group to either H-ras or INS for any recombination value and under any penetrance model.

Multi-point analyses are presented in the figures. Figure 1 represents the multi-point analyses of the unipolar depression and alcohlism combined phenotype across a fixed map of H-ras and INS set 3 cM apart. Figure 2

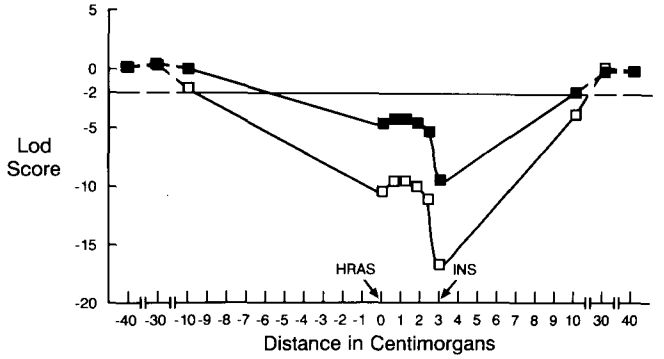

Fig. 2. Multipoint analyses of unipolar depression across a fixed map of HRAS and INS set 3 c*M* apart. □ Penetrance 0.85; ■ Penetrance 0.63

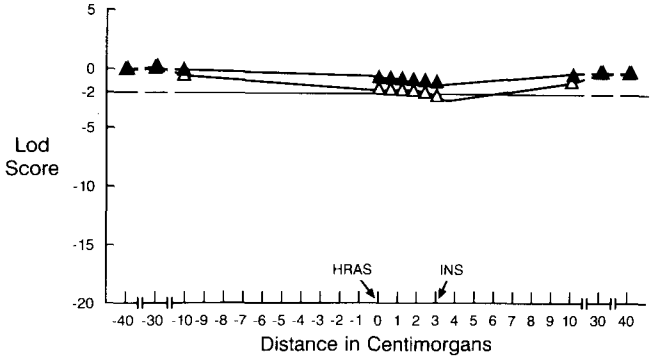

Fig. 3. Multipoint analyses of alcoholism across a fixed map of HRAS and INS set 3 c*M* apart. △ Penetrance 0.85; ▲ Penetrance 0.63

shows the same multi-point analyses of the unipolar depression alone phenotype and Fig. 3 shows the multipoint analyses of the alcoholism alone phenotype. Multipoint analyses of all three affected phenotypes under all four penetrance models failed to demonstrate any convincing evidence of linkage (Only penetrance models 2 and 3 are represented on the graphs.)

Discussion

This is the first DNA marker study to look specifically at unipolar depression and alcoholism. Although the results are negative this study may be better viewed from its methodology rather than the results alone. This is a first attempt using DNA probes to find a genetic marker for two common illnesses that may be genetically related but may also be separate and each heterogeneous. Available methods of psychiatric diagnosis fail to identify genetically homogeneous subtypes and this fact obviously complicates genetic linkage studies. Incomplete penetrance and alternative expression create methodological difficulties for DNA marker studies because they result in an unknown number of false negatives or unclassified genotypes. Partially or alternatively expressed genotypes are not recognized by present diagnostic methods and thereby frustrate attempts at linkage analysis.

Success in finding genetic markers for mental disorders will require creative methods of identifying the affected phenotype. It is apparent that individual diagnosis based on clinical symptoms and history will not suffice and additional criteria such as familial classification, response to treatment, hospitalization, number of episodes, age of onset, dexamethasone suppression test response, or possibly REM latency may improve the ascertainment of homogeneous family samples.

The present study does move beyond using individual diagnoses alone and includes a familial classification system that has been supported by clinical data. This system defines the affected phenotype by separating two types of unipolar depression based on the presence or absence of alcoholism in first-degree relatives. Since there is no way of knowing if this system identifies genetic isolates, our negative results may be due to faults in identifying the affected phenotype or that we did not look at the proper point on the genome.

Since bipolar and unipolar illness may be genetically related, one goal of our study was to attempt a replication of the old-order Amish study where affective disorder was shown to be tightly linked to H-ras and INS on the short arm of chromosome 11. Although the Amish study largely centered around bipolar illness, unipolar disorder was considered affected in that study and five cases of unipolar illness were included in their sample of affected persons. Since unipolar depression showed no evidence of linkage to H-ras or INS in this study, it suggests that our population is not manifesting the same illness as in the Amish study.

The techniques of molecular genetics are expanding at a rate faster than psychiatry's ability to recognize correctly the affective phenotype and correctly ascertain homogeneous family samples for linkage analysis. Soon it will be possible to survey the entire genome. Improving our ability to recognize the affected phenotype will allow molecular geneticists in psychiatry to fully exploit the rapidly expanding technology so that the gene or genes for all major mental disorders can be isolated and characterized.

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