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A genome-wide linkage analysis of dementia in the Amish

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Abstract

Susceptibility genes for Alzheimer's disease are proving to be highly challenging to detect and verify. Population heterogeneity may be a significant confounding factor contributing to this difficulty. To increase the power for disease susceptibility gene detection we conducted a genome-wide genetic linkage screen using individuals from the relatively isolated, genetically homogeneous, Amish population. Our genome linkage analysis used a 407 microsatellite marker map (average density 7 cM) to search for autosomal genes linked to dementia in five Amish families from four Midwestern U.S. counties. Our highest two-point lod score (3.01) was observed at marker D4S1548 on chromosome 4q31. Five other regions (10q22, 3q28, 11p13, 4q28, 19p13) also demonstrated suggestive linkage with markers having two-point lod scores >2.0. While two of these regions are novel (4q31 and 11p13), the other regions lie close to regions identified in previous genome scans in other populations. Our results identify regions of the genome that may harbor genes involved in a subset of dementia patients, in particular the North American Amish community.

Keywords

Alzheimer's Disease; microsatellites; screen; chromosome 4; chromosome 10

Introduction

Alzheimer's disease (AD) is the leading cause of dementia in the elderly. There are over 4 million affected individuals in the U.S., a number projected to quadruple over the next 50 years as the population ages (Geldmacher and Whitehouse, Jr. 1997; Brookmeyer, Gray, and Kawas 1998). AD has a complex etiology with strong genetic and environmental determinants. While the genetics of early-onset autosomal dominant AD is not completely understood, overwhelming evidence suggests the involvement of at least three genes. The

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early-onset AD genes include the amyloid precursor protein (*APP* on chromosome 21) (St George-Hyslop et al. 1987; Goate et al. 1991), presenilin I (*PS1* on chromosome 14) (St George-Hyslop et al. 1992; Schellenberg et al. 1992; Van Broeckhoven et al. 1992; Sherrington et al. 1995), and presenilin II (*PS2* on chromosome 1) (Levy-Lahad et al. 1995; Rogaev et al. 1995). Our current understanding of the much more common late-onset Alzheimer's disease (LOAD), is limited to the role of one universally accepted candidate, the apolipoprotein E locus (*APOE*) on chromosome 19 (Pericak-Vance et al. 1991), which has been verified through numerous independent studies across multiple racial groups (Corder et al. 1993; Strittmatter and Roses 1995; Roses and Pericak-Vance 1997). The APOE-ε4 allele (approximately 16%) (Menzel, Kladetzky, and Asman 1983; Saunders et al. 1993) acts in a dose-related manner to increase risk for LOAD and decrease age-of-onset (Corder et al. 1993; Pericak-Vance et al. 1996). While the evidence for involvement of APOE is quite clear, it accounts for less than half of late-onset AD susceptibility and thus other genetic factors are likely to be involved (Corder et al. 1993). In this regard, multiple linkage screens have been conducted to elucidate additional regions harboring susceptibility genes for late-onset AD (Pericak-Vance et al. 1988; Pericak-Vance et al. 1997; Scott et al. 2003; Pericak-Vance et al. 1998; Zubenko et al. 1998a; Zubenko et al. 1998b; Rogaeva et al. 1998; Kehoe et al. 1999; Scott et al. 2000; Pericak-Vance et al. 2000; Myers et al. 2000a; Bertram et al. 2000; Ertekin-Taner et al. 2000; Myers et al. 2002; Mayeux et al. 2002; Blacker et al. 2003; Farrer et al. 2003; Holmans et al. 2005; Avramopoulos, Fallin, and Bassett 2005). While regions on chromosomes 9,10 and 12 are most consistently identified, candidate genes within those regions have yet to be clearly implicated in AD. A vast number of late-onset AD candidate gene studies, whether proposed by location or function, have been performed. However, none has provided convincing evidence for involvement in disease susceptibility (Reviewed in (Schellenberg, D'Souza, and Poorkaj 2000)).

One reason for the difficulty of replication across studies is likely to be locus heterogeneity. One method to avoid the problems of locus heterogeneity is to use a population drawn from a limited number of founders, such as the Amish. The North American Amish are a relatively isolated genetically well-defined homogeneous population, descended from groups of Anabaptists fleeing religious persecution in Europe (Jackson et al. 1968; Gingerich and Kreider 1986; Hostetler 1993; Kraybill 2001). The communities included in this study were founded as part of two main waves of immigration into the US. The first wave of immigration occurred in the early 1700s with Amish families initially settling in Pennsylvania with some migration to Ohio coinciding with the arrival of additional Amish families in the early 1800s. This second wave of immigration occurred throughout the 1800s with the majority of families settling in multiple counties in both Ohio and Indiana (Gingerich and Kreider 1986; Hostetler 1993; Agarwala, Schaffer, and Tomlin 2001). The families we studied are centered in Adams and the nearby Elkhart and LaGrange Counties in Indiana, and Holmes and surrounding counties in Ohio.

The Amish live very traditional lifestyles within their religious communities and do not accept more modern culture. These practices give rise to an overwhelming majority of marriages between members of the same Amish community due to strict adherence to religious and cultural practices. The Amish often have large sibships and maintain extensive genealogy records that permit estimations of IBD gene sharing to be accurately performed (Hostetler 1993; McKusick 1978; Johnson et al. 1997). These characteristics make the Amish society an excellent community to study the genetics of many complex genetic disorders like AD (Francomano, McKusick, and Biesecker 2003). Previous investigations within this community suggest a lower probable prevalence of cognitive impairment in individuals ≥ 65 years of age compared with the general population despite lower levels of formal education within the community (Rocca et al. 1991; Johnson et al. 1993; Johnson et al. 1997). Education is one of several factors including age, sex, head trauma, intelligence,

lifestyle, and environment hypothesized to play a role in Alzheimer's disease risk (Cummings et al. 1998; Fleminger et al. 2003). It has also been shown that the APOE-ε4 allele is not a major contributor to LOAD risk, at least within the Adams County Amish community (Pericak-Vance et al. 1996). However, work by Holder et al. in the Lancaster County, Pennsylvania Amish community reported a decreased prevalence of AD and a normal APOE- ε4 allele frequency compared with the general population (Holder and Warren 1998). While the contribution of APOE to dementia within the Amish community is unclear, there are likely other genes specifically involved in risk within this population. We hypothesize that the number of LOAD susceptibility genes contributing to Alzheimer's disease in the Amish will likely be smaller than in more heterogeneous populations. With this in mind, we conducted a genome-wide genetic linkage screen using extended Amish pedigrees ascertained within Adams and nearby Elkhart and LaGrange Counties in Indiana, and Holmes and surrounding counties in Ohio (Table 1).

Materials and Methods

A. Subjects

There were a total of 5 Amish pedigrees included in this study. Three families were from Elkhart and LaGrange Counties, Indiana, one extended family from Adams County, Indiana, and one extended family from Holmes and surrounding counties in Ohio. The extended pedigree from Adams County has been the subject of previous studies of dementia in the Amish (Pericak-Vance et al. 1996; Ashley-Koch et al. 2005). The vast majority of individuals within our families are Old Order Amish; however, some siblings are New Order Amish and a few have become Mennonites. Among the 115 individuals who were genotyped, 49 were classified either as affected (demented) or having a mild cognitive impairment (MCI) (unclear), while the remainder were classified as cognitively normal (unaffected) (Table 1). The study was undertaken after Institutional Review Board review and approval.

B. Clinical Evaluations

Members of the Indiana communities were first seen from 1991 to 1993 by trained interviewers. Participants were administered the Mini-Mental State Exam (MMSE) (Folstein, Folstein, and McHugh 1975). Possible scores range from 0 to 30. All individuals scoring 27 or greater were classified as cognitively normal/unaffected. Those scoring 23 or less were classified as cognitively impaired and labeled as probable dementia. Those who scored 24-26 had additional neuropsychological testing including the Dementia Rating Scale (DRS) (Mattis 1976), the Boston Naming Test (BNT) (Kaplan, Goodglass, and Weintraub 1976), and a reading subtest from the Wide Range Achievement Test-Revised (WRAT-R) (Jastak and Wilkinson 1984). Persons were categorized as having mild cognitive impairment (MCI) if their DRS score fell below an age-adjusted threshold.

Data collection occurred again in Indiana in 1996, 1997, and 2000 and in Ohio beginning in 1999. Field evaluations were conducted by a physician assistant or nurse practitioner, each with extensive experience in geriatric neurology and trained to administer screening neuropsychological instruments. When available, a number of Indiana individuals originally seen in 1991-1993 were re-evaluated through a more detailed examination. Additional members of their families were also seen for initial data collection. Each individual completed a Modified Mini-Mental State Examination (3MS) from which was generated scores for both the 3MS and the MMSE. Within the limits imposed by the Amish social setting, individuals to be evaluated and at least one close family member were interviewed to assess concern about forgetfulness (progression and/or severity) and to determine current level of abilities. A medical history was obtained to detect risk factors for vascular disease

or other possible causes of cognitive impairment. A brief and focused neurological examination was performed. Based on scores and data available at that time, each individual was assigned a clinical impression of dementia, unclear (MCI), or unaffected.

Beginning in 2002, participants underwent sequential screening and evaluation using methods employed in other similar studies of dementia in long-lived populations (Khachaturian, Gallo, and Breitner 2000). For some individuals this was the second or, rarely, third clinical evaluation. Individuals were first administered an adapted version (Tschanz et al. 2002) of the Modified Mini-Mental State Examination (3MS) (Teng and Chui 1987), which was developed for epidemiological studies. A 3MS score from 0 to 100 was calculated from the exam. A cutoff score of 86/87 was determined by other studies to be sensitive to early stage dementia and mild cognitive impairment (Khachaturian, Gallo, and Breitner 2000; Hayden et al. 2003). Those scoring 86 or less on the 3MS underwent further clinical evaluation including in depth neuropsychological assessment employing methods used in other similar studies of dementia (Tschanz et al. 2000)). This battery included the CERAD neuropsychological tests (Morris et al. 1989) (abbreviated Boston Naming Test (BNT), animal fluency, constructional praxis, word list learning and memory); additional measures of memory included the Logical Memory I and II from the Wechsler Memory Scale (Wechsler 1987) and the Benton Visual Retention Test (Benton 1992); executive control function was assessed with the Trail Making Test Part A & B (Reitan 1958) and the Symbol Digit Modalities Test (Smith 1973); and expressive language was evaluated with the Controlled Oral Word Association Test from the Multilingual Aphasia Examination (Benton, Sivan, and de Hamsher 1994).

Demographic factors that can modify test performance were also systematically gathered (years of education, occupational history) along with determination of lateral dominance using the Oldfield Handedness Questionnaire (Oldfield 1971). To determine pre-morbid intellect, the vocabulary test from the Shipley Institute of Living Scale was used (Shipley 1967). Mood symptoms were assessed using the Geriatric Depression Rating Scale (Clark and Ewbank 1996; Jamison and Scogin 1992). Functional impairment was determined via informant report using a rating form allowing calculation of a Clinical Dementia Rating Scale score (Hughes et al. 1982; Clark and Ewbank 1996). Focused neurological examinations were performed on each individual seen for the more extensive neuropsychological evaluation.

Though members of the Amish communities consult medical doctors, elderly individuals with significant decline in cognitive abilities are usually believed to be exhibiting normal aging. Additional laboratory tests (including brain imaging), on which to base a firm differential diagnosis of dementia, were often not performed and thus not available to researchers. Due to strong religious beliefs within the Amish community, autopsy confirmation of clinical diagnosis has not been possible in any family. Beginning in 2003, consensus case conferences were held at which all available clinical information from individuals in the families was presented. The case materials were reviewed by a physician assistant, a clinical nurse specialist, and two neuropsychologists (SRJ; KAWB). Each and every case was discussed and a consensus “final” diagnosis was determined. For analytical purposes, the cases were assigned to one of three consensus diagnoses: dementia (probable or possible AD), unclear (includes MCI), or unaffected. For each case, the consensus “final” was the same as the clinical impression that had been assigned previously. The only exceptions involved individuals who over the years of data collection had developed significant cognitive impairment. Such individuals effectively converted either from unaffected to unclear (MCI) or demented or from unclear (MCI) to demented.

C. Molecular analysis

Following informed consent, blood samples were collected from each individual and genomic DNA was extracted from blood using standard procedures. Cell lines have been initiated on most sampled individuals. All DNA samples were coded and stored at 4°C until used.

Markers were genotyped at both the Vanderbilt and Duke laboratories for all DNA samples. Laboratory personnel were blinded to pedigree structure, affection status, and location of quality control samples. Duplicate quality control samples were placed both within and across DNA sample plates and equivalent genotypes were required for all quality control samples to ensure accurate genotyping. At the Vanderbilt laboratory, marker primer sequences were obtained from the Genome Database (http://www.gdb.org/) or designed with Primer3 software (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi) and synthesized by Invitrogen Life Technologies (Carlsbad, CA). Amplification was performed in a PCR Express machine (ThermoHybaid, Needham Heights, MA) with the following conditions: 94°C-4 min.; 94°C-15 sec., AT-30 sec., 72°C-45 sec. (35 cycles); 72°C-4 min. PCR products were denatured for 3 min. at 95°C and run on a 6% polyacrylamide gel (Sequagel-6® from National Diagnostics, Atlanta, GA) for ~1 hr. at 75 W. Gels were stained with a SybrGold® rinse (Molecular Probes, Eugene, OR) and scanned with the Hitachi Biosystems FMBIOII laser scanner (Brisbane, CA). Marker genotyping at the Duke laboratory was performed using fluorescence imaging (Molecular Dynamics SI Fluorimager) and a semi-automated allele calling system (Vance and Othmane 1998).

Marker order and intermarker distance were determined using reference maps (http://research.marshfieldclinic.org/genetics/Map_Markers/maps/IndexMapFrames.html). The screen utilized 407 microsatellite markers with an average intermarker distance of approximately 7 cM.

Allele frequencies were calculated from the genotyped founders in each family. Hardy-Weinberg equilibrium calculations were performed for each marker and Mendelian inconsistencies were identified using PedCheck (O'Connell and Weeks 1998). Suspect genotypes were re-read by a different technician or re-run as necessary to reduce errors. All microsatellite markers were required to have >90% of possible genotypes to be included in the analysis. Verification of relationships between pairs of samples within families was performed using RELPAIR (Epstein, Duren, and Boehnke 2000).

D. Statistical analysis

Two-point parametric lod scores for the pedigrees were computed using SimWalk2 (Sobel and Lange 1996) assuming autosomal dominant and recessive models. The SimWalk2 program was chosen for these analyses due to its ability to handle the highly complex nature of these large consanguineous pedigrees. Computations were done using the Vanderbilt Multi-Processor Integrated Research Engine (VAMPIRE) cluster. Two analysis cases were defined depending on how the individuals classified as having a mild cognitive impairment (MCI) were treated. In case 1, MCI-diagnosed individuals were analyzed as having an unknown affection status and in case 2 these individuals were treated as affected. The disease allele frequency was assumed to be 0.001 and 0.01 for the autosomal dominant and recessive models, respectively. To assess the robustness of the interesting results obtained using SimWalk2, data for chromosomes 4 and 10 were re-run ten times using the Case 1 recessive and dominant models with different random number generator seeds. These data were compared with those reported in the initial screen.

Results

Fifteen markers spanning eleven chromosomes demonstrated a two-point lod score of ≥ 1.5 in either Case 1 or Case 2 (Table 2). Our best two-point lod score was observed at approximately 154 cM on chromosome 4q31. Here we observed a two-point lod score of 3.01 at marker D4S1548 under a recessive model, when treating the individuals with mild cognitive impairments as having an unknown affection status. Five additional regions (chromosomes 10q22, 3q28, 11p13, 4q28, 19p13) yielded two-point lod scores >2.0, and another nine produced two-point lod scores > 1.5 (Table 2). Furthermore, twelve other markers yielded two-point lod scores > 1.0 under case 1 or case 2 (Table 3).

These two-point results are not exact values, given that SimWalk2 employs the Markov chain Monte Carlo (MCMC) algorithm to consider configurations of the data related to their likelihood within our large extended and consanguineous pedigrees. To obtain a better approximation of the exact two-point lod score for our most promising regions of linkage, we ran ten simulations of the chromosome 4 (D4S1548 and D4S2394) and chromosome 10 (D10S2327) markers within the same dataset (Case 1, under both dominant and recessive models) using different random seeds within the SimWalk2 program. The resulting average, minimum value, maximum value, and standard deviations for each of these markers under both models are detailed in Table 4. For marker D4S2394 our screen score (2.12) obtained using the dominant model is below the average two-point score (2.38) obtained across ten additional simulations. Our results for this same marker under a recessive model are also below the simulation established average. In sharp contrast are the results of the nearby marker D4S1548 which initially has a reported two-point value of 3.01, but on the average is better estimated to be 1.54. On chromosome 10 at D10S2327 we report a two-point lod score of 2.42, but across an additional 10 simulations the average two-point lod score is 1.71. Although these two-point lod scores are only estimates and vary for the same marker across different simulations, we observe average two-point lod scores ≥ 1.5 for both regions on chromosome 4 and chromosome 10 (Table 4).

Discussion

Although late-onset AD linkage studies have pointed toward some common regions, such as chromosomes 9p (Pericak-Vance et al. 2000), 10q (Myers et al. 2000a; Bertram et al. 2000), and 12 (Pericak-Vance et al. 1997; Farrer et al. 2003), further localization and subsequent identification of a LOAD gene has been difficult. Confounding factors such as population heterogeneity may explain, in part, this disappointing outcome. The purpose of the present study was to perform a genome-wide screen for LOAD susceptibility genes in the isolated, and therefore presumptively genetically more homogenous, Amish population. Using 407 microsatellite markers, we report all markers demonstrating two-point lod scores ≥ 1.0 (Table 2 and Table 3) for our initial genomic screen analysis. Our highest two-point score was observed at chromosome 4q31 under a recessive model. Other highly suggestive regions included 10q22, 3q28 and 11p13.

Our highest lod score (3.01) was obtained at 154 cM near marker D4S1548. While this region of chromosome 4 is not entirely novel for linkage to Alzheimer's disease, it has not previously represented a region demonstrating the most suggestive evidence for linkage within a study population. Previous studies by Pericak-Vance et al. observed a two-point lod score of 1.32 at a nearby marker (D4S1629) on chromosome 4 (Pericak-Vance et al. 1997; Pericak-Vance et al. 2000). This marker maps only 4 cM (158 cM) from our current peak marker at D4S1548. Additionally, Blacker et al. report a two-point lod score of 1.9 at D4S1629 in their screen of 437 AD families comprising the total NIMH sample. While there is some overlap between the two samples, these are the only reports of linkage within this

region and there are currently no reported studies of LOAD candidate genes at 4q31. While we must take caution when interpreting these results given the complex genetic nature of our study population, this region of chromosome 4q31 may harbor a yet unconsidered susceptibility gene, whose effect is enriched within our genetically isolated Amish population.

Chromosome 10q is one of the most replicated regions seen in linkage studies of LOAD (Kehoe et al. 1999; Bertram et al. 2000; Ertekin-Taner et al. 2000; Myers et al. 2000b; Li et al. 2002; Blacker et al. 2003). These reports cover a wide interval of interest, from approximately 80-135 cM, suggesting that multiple distinct loci on 10q may be involved. Ertekin-Taner et al. presented evidence for linkage to ~80 cM on chromosome 10, when analyzing five extended LOAD pedigrees having an AD proband with extremely high plasma Aβ42 (amyloid β42 peptide) levels (Ertekin-Taner et al. 2000). This same group presented evidence for association to the SNPs within the positional and functional candidate gene *CTNNA3* (encodes α-T catenin a binding partner to β catenin) located within the linkage interval. However, Blomqvist et al. attempted to replicate the *CTNNA3* association findings of Ertekin-Taner et al. in Swedish and Scottish cases and controls, but found no evidence for *CTNNA3* involvement in their AD cases (Blomqvist et al. 2004).

Myers et al. initially generated a 3.83 multipoint lod score in the vicinity of marker D10S1225 (~80 cM) (Myers et al. 2000a) and in follow-up studies obtain a two-point lod score of 4.1 at marker D10S1211 (82 cM) (Myers et al. 2002). The closest linkage to ours comes from Blacker et al., who detected a multipoint lod score of 1.8 at marker D10S1432 (92 cM) (Blacker et al. 2003). Although both of these markers were run in our screen, neither demonstrated evidence for linkage in our study.

More distal linkage findings on chromosome 10q have also been seen. Bertram et al. observed a peak two-point lod score of 3.3 at marker D10S583 (115 cM) in their study of AD (Bertram et al. 2000). Li et al. detected a linkage peak (multipoint lod = 2.33) even more distal to our current peak, between markers D10S1239 and D10S1237 (~134 cM) affecting age-at-onset in both AD and Parkinson disease (Li et al. 2002). Additional work by Li and colleagues, within their region of linkage, detected significant association to *GSTO1* (glutathione *S*-transferase, omega-1) and *GSTO2* relating to age at onset in AD patients (Li et al. 2003). Presently there is substantial evidence indicating the involvement of chromosome 10q in late-onset Alzheimer's disease; however, definitive involvement of any specific locus across this large region has not been consistently replicated in additional studies.

Two other regions represent more novel findings for linkage to AD. These regions include chromosome 11p13 (D11S1392 at 43 cM) and chromosome 4q28 (D4S2394 at 130 cM). Though 11p is a novel region for linkage to AD, a nearby gene has been the focus of several candidate gene studies. At roughly 35 cM on chromosome 11p lies the *BDNF* (human brain-derived neurotrophic factor) gene, which may be involved in neuroprotection and neural development. It protects cholinergic neurons of the basal forebrain (Morse et al. 1993) and hippocampal neurons (Pringle et al. 1996) from induced death. In patients with AD, gene expression of *BDNF* has been shown to be reduced in hippocampal regions (Phillips et al. 1991). Kunugi et al. performed an association study between the C270T *BDNF* polymorphism and AD in a Japanese sample, and found that the frequency of individuals who carried the mutated allele (C-270T) was significantly more common in patients with late-onset AD than controls ($p = 0.00004$). They did not find a significant genotype distribution differential in early-onset AD and controls. In a similar study this same *BDNF* polymorphism was examined in a German sample, where the risk conferred by the T allele was found to be strongest in patients lacking the APOE-ε4 allele ($p = 0.015$)

(Riemenschneider et al. 2002). This finding is particularly interesting given that the Amish are believed to have less prevalence of the APOE-ε4 allele than the general Caucasian population (Pericak-Vance et al. 1996). The other linkage peak at chromosome 4q28 (D4S2394) may or may not be distinct from the peak at 4q31 (nearly 24 cM away). A noteworthy gene *MGST2*, microsomal glutathione *S*-transferase type 2, lies between these markers. Evidence previously mentioned with regard to the involvement of other glutathione *S*-transferase genes in AD, makes this a high priority candidate gene within this region.

Another novel peak of interest in our dataset is on chromosome 3q. We obtained a two-point lod score of 2.42 at marker D3S2398 (209 cM). One study used microsatellite markers to test for association with AD in a geographically distinct Finnish population descended from a small group of original founders (Hiltunen et al. 2001). Regions found in linkage disequilibrium with AD were followed up with additional microsatellite markers to obtain evidence for eight loci, including 3q28. One interesting candidate gene at the 3q28 location is *SST*, the gene encoding somatostatin. Somatostatin inhibits the release of growth hormone, insulin, glucagon, gastrin and secretin, and functions as a neurotransmitter in the central nervous system.

Our final marker (D19S586) with a two-point lod score ≥ 2.0 was located on chromosome 19p13 at approximately 33 cM. A recent study identified strong linkage evidence for late-onset AD at 19p13.2 using age-at-onset as a covariate (Wijsman et al. 2004). One interesting candidate at this location is the intercellular adhesion molecule-1 (*ICAM-1*). A study of *ICAM-1* found that the frequency of the EE genotype was significantly higher in AD patients ($p < 0.01$) (Pola et al. 2003).

In the interpretation of our results it is important to recognize that the Amish pedigrees analyzed are sparsely genotyped and complex, containing multiple marriage loops. Thus calculating exact likelihoods is computationally intractable and the use of other methods, such as the approximations obtained through SimWalk2, are the only viable alterative. The genotyped individuals are in the last generation and constitute only 115 of the 527 total individuals in the five pedigrees. The resulting size and complexity of these pedigrees, in addition to the number of ways in which genotypes can be inferred for missing individuals hampers the convergence of the SimWalk2 Markov Chain Monte Carlo analysis. To address this problem the maximum run-time settings recommended by the program developers were used to perform the analysis. Hence the computations, even on a large parallel computer, were very time-consuming. To partially assess the stability of the results obtained using SimWalk2, Case I data for chromosomes 4 and 10 were re-run an additional ten times with different random seeds. While the two-point lod scores differ between runs, we remain confident of these regions, given that the average two-point lod scores for both markers remained high (> 1.5).

While it is highly unlikely that all markers demonstrating a two-point lod score > 1.0 will prove to be true positive results, these data provide the initial groundwork for future study within this homogenous population. The difficult nature of analysis within these large extended pedigrees necessarily limits the conclusions that can be drawn from our current study. Future studies will attempt to refine linkage and test likely candidate genes within narrowed regions of interest. The regions on chromosome 4q and 10q are of particular interest given the strength of their results in the current study and the strength and consistency of previous reports. We continue to ascertain and expand our Amish family collection and efforts to fine map these regions are underway.

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Table 1

Demographics for the Amish Pedigrees

Family	County	Individuals Genotyped	Number of Affected Individuals[a,b]		Number of Individuals with Mild Cognitive Impairment		Case 1: Affected Relative Pairs (ARPs) based on MCI=U	Case 2: Affected Relative Pairs (ARPs) based on MCI=A
			Males	Females	Males	Females		
2073	Elkhart/LaGrange	42	6	8	0	3	91	136
2076	Elkhart/LaGrange	10	1	1	0	0	1	1
2083	Elkhart/LaGrange	3	1	1	1	0	1	3
605	Holmes	27	6	7	2	1	78	120
1683	Adams	33	4	5	1	1	36	55
	Total	115	18	22	4	5	207	315

[a] Age of examination range 64-100

[b] Age of onset range 58-100

Table 2

Microsatellite markers with two-point lod scores ≥ 1.50. Disease model: (D) dominant, (R) recessive

Chromosome	Map Position (cM)	Marker	Case 1: MCI=U	Case 2: MCI=A
1p21	137	D1S1631	1.29(R)	**1.83(R)**
2q31	185	D2S2978	0.90(R)	**1.94(R)**
3p14	79	D3S1766	1.39(D)	**1.78(D)**
3q26	177	D3S1763	1.51(D)	**1.69(D)**
3q28	209	D3S2398	1.89(D)	**2.16(D)**
4q28	130	D4S2394	**2.12(D)**	1.94(D)
4q31	154	D4S1548	**3.01(R)**	2.45(R)
6q23	129	D6S1040	1.03(R)	**1.94(R)**
7q36	163	D7S3070	1.34(R)	**1.74(R)**
10q22	101	D10S2327	**2.42(R)**	1.42(R)
11p13	43	D11S1392	**2.14(R)**	1.86(R)
14q24	76	D14S588	0.84(R)	**1.54(D)**
18q21	80	D18S858	1.62(D)	**1.88(R)**
19p13	33	D19S586	**2.06(D)**	1.82(D)
19q13	78	D19S246	0.75(R)	**1.57(R)**

Table 3

Microsatellite markers with two-point lod scores 1.50 > 1.00. Disease model: (D) dominant, (R) recessive

Chromosome	Map Position (cM)	Marker	Case 1: MCI=U	Case 2: MCI=A
1p31	102	D1S1665	**1.07(R)**	0.12(D)
2q36	227	D2S1363	**1.10(R)**	1.17(R)
3q13	124	D2S3045	0.68(D)	**1.00(D)**
3q28	216	D3S2418	**1.18(D)**	0.31(D)
4p15	51	D4S2632	**1.33(D)**	0.65(D)
4q34	182	D4S2417	0.25(D)	**1.21(D)**
5q14	98	D5S1725	**1.47(D)**	0.74(R)
7q21	91	D7S2204	**1.14(D)**	-0.29(R)
14q14	86	D14S53	**1.29(R)**	0.92(R)
19q13	59	D19S245	1.08(D)	**1.41(D)**
20p11	48	D20S477	**1.05(R)**	0.65(R)
22q11	14	D22S425	**1.38(D)**	1.22(D)

Table 4

Summary results of simulations of markers on chromosome 4 and 10 under Case 1. Disease model: (D) dominant, (R) recessive

Marker	Trial #1	Mean	MIN	MAX	Standard Deviation
D4S2394(D)	**2.12**	2.38	2.01	2.71	0.23
D4S2394(R)	1.69	2.02	1.27	2.89	0.52
D4S1548(D)	1.43	1.36	0.83	2.00	0.37
D4S1548(R)	**3.01**	1.54	1.07	2.27	0.60
D10S2327(D)	1.54	1.00	0.55	1.62	0.42
D10S2327(R)	**2.42**	1.71	1.08	2.42	0.43