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ORIGINAL INVESTIGATION

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Maternal lineages and Alzheimer disease risk in the Old Order Amish

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Abstract Old Order Amish, founded by a small number of Swiss immigrants, exist in culturally isolated communities across rural North America. The consequences of genetic isolation and inbreeding within this group are evident by increased frequencies of many monogenic diseases and several complex disorders. Conversely, the prevalence of Alzheimer disease (AD), the most common form of dementia, is lower in the Amish than in the general American population. Since mitochondrial dysfunction has been proposed as an underlying cause of AD and a specific haplogroup was found to affect AD susceptibility in Caucasians, we investigated whether inherited mitochondrial haplogroups affect risk of developing AD dementia in Ohio and Indiana Amish communities. Ninety-five independent matrilines were observed across six large pedigrees and three small pedigrees then classified into seven major European haplogroups. Haplogroup T is the most frequent haplogroup represented overall in these maternal lines (35.4%) while observed in only 10.6% in outbred American and European populations. Furthermore, haplogroups J and K are less frequent (1.0%) than in the outbred data set (9.4–11.2%). Affected case matrilines and unaffected control lines were chosen from pedigrees to test whether specific haplogroups and their defining SNPs confer risk of AD. We did not observe frequency differences between AD cases compared to controls overall or when stratified by sex. Therefore, we suggest that the genetic effect responsible for AD dementia in the affected Amish pedigrees is unlikely to be of mitochondrial origin and may be caused by nuclear genetic factors.

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Introduction

Contemporary Amish populations residing in North America are descendants of the Anabaptists from Switzerland (Hostetler 1993). The Anabaptist section of Protestant Christianity was founded in 1525 by followers who practiced adult baptism, nonresistance and separation of church and state (Kraybill 2001). Over a century, this religious group evolved into separate bodies including the Old Order Amish and Old Order Mennonites. Both religious isolates sought religious freedom within Alsace France and Rhineland-Pfalz (Palatinate) Germany during the late 1600s and early 1700s. War, famine and continued religious persecution throughout this European region forced the Anabaptists to immigrate to North America (Kraybill 2001). During the first wave of immigration in the early eighteenth century, approximately 500 Amish arrived in Pennsylvania and settled in several counties throughout the state. In the early nineteenth century, a number of these families migrated westward to Holmes County, Ohio. A second wave of European Amish, approximately 3,000, arrived in Pennsylvania from 1816 to 1880, but continued westward to Indiana (including Adams County) and Ohio (areas excluding Holmes County) to establish farming communities. The second wave of immigrants tended to settle in areas that were not concentrated with Amish from the first immigration wave. Decades later, families from the Holmes County settlements within Ohio moved to Elkhart County, Indiana and LaGrange County, Indiana (Hostetler 1993). Present communities within Holmes and Elkhart/LaGrange Counties are primarily descendants from the first immigration wave

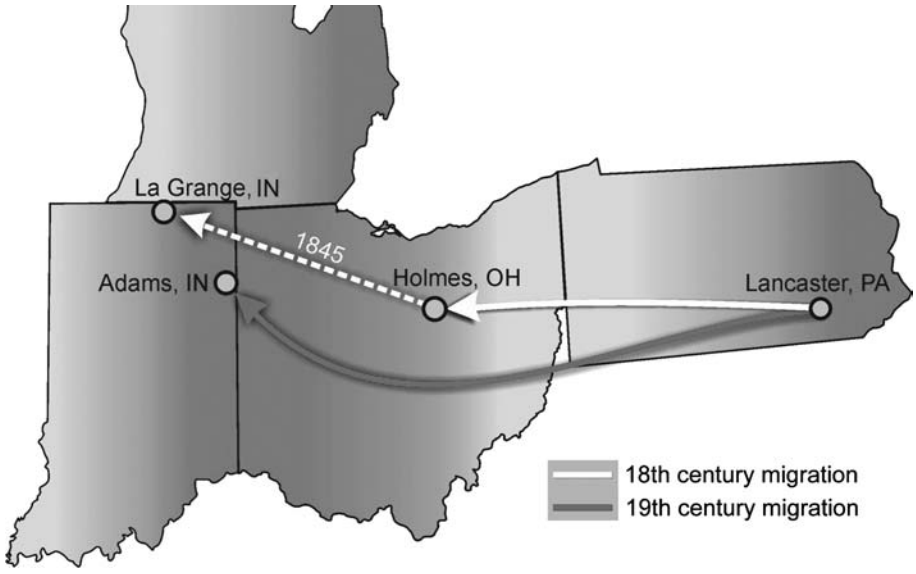

Fig. 1 The eighteenth century migration wave settled in Pennsylvania (Lancaster and surrounding counties). A subset of these families later moved west to Holmes County, Ohio. In 1845, some families from Holmes Co. moved to Elkhart/LaGrange Co., Indiana. The nineteenth century migration occurred between 1816 and 1880. Families from this second-wave established communities in Indiana including Adams County

of Swiss while Amish of Adams country are principally descendants from the second one (Fig. 1).

Founder effect and genetic drift have influenced the genetic architecture of the Amish population. Since settlement, the Amish have existed in small reproductively and culturally isolated kin groups throughout the United States. Social custom allows marriage only between the members of the Amish community. However, communities today are very much aware of the consequences of consanguineous marriages and actively avoid marriage between closely related kin group members. In effect, the religious and cultural practices of the Amish have created island populations by restricting gene flow into communities thereby increasing inbreeding within these groups. Inbreeding depression is evident by increased frequencies of many deleterious recessive alleles leading to an increased frequency of many Mendelian disorders (Francomano et al. 2003; Puffenberger 2003).

Similarly, disease prevalence of common complex disorders such as affective disorder, Parkinson disease, obesity and high blood pressure is elevated in families of affected individuals within specific Amish communities (Ginns et al. 1998; Hsueh et al. 2000; 2001; Racette et al. 2002). Alzheimer disease (AD) also demonstrates familial clustering in Amish families; however, in contrast to the other complex diseases noted, the prevalence of AD in the general Amish population is much lower than that of outbred populations (Haines et al. 1997; Holder and Warren 1998; Johnson et al. 1997; Pericak-Vance et al. 1996). AD is a primary form of dementia characterized by progressive degeneration of cortical and subcortical neurons that leads to substantial memory and cognitive decline. The etiology of late-onset AD (≥60 years) results from a combination of multiple genes plus environmental factors. The apolipoprotein E (APOE) gene is a major genetic risk factor that accounts for at least 30% of AD susceptibility (Corder et al. 1993). Therefore, the genetic factors responsible for the majority of AD cases have not yet been identified.

Mitochondrial dysfunction has been proposed as an underlying mechanism of AD pathogenesis due to reports of significant decrease of energy metabolism within AD brain tissue and platelets (Beal 1995). Furthermore, reduced activity of AD brain cytochrome oxidase (complex IV), the last enzyme within mitochondrial oxidative phosphorylation pathway (OXPHOS), has been observed in multiple studies (Cottrell et al. 2002; Kish et al. 1992; Maurer et al. 2000). Impairment of OXPHOS functioning and energy production is responsible for the increase in reactive oxygen species (ROS) generation, which leads to oxidative damage within neurons. Cellular injury is evident in the form of mitochondrial DNA (mtDNA) lesions, mt protein nitration and mt lipid peroxidation.

We recently reported that variations within a specific mitochondrial lineage (haplogroup U) may be involved in AD expression in outbred Caucasian Americans and that this effect is sex specific (van der Walt et al. 2003). mtDNA is a nonrecombining circular molecule with a high-mutation rate that is transmitted maternally. Due to the mutational process of mt genome, basal mutations have occurred throughout human evolution within lineages that are specific to geographic regions (Wallace et al. 1999). Therefore, mitochondrial lineages can be classified into ethnic-specific haplogroups by the combination of signature mtDNA sites (Torroni et al. 1992). Nine primary mt haplogroups have been identified in European populations (H, I, J, K, T, U, V, W, X) (Torroni et al. 1996; Torroni and Wallace 1994). MtDNA haplogroups have been used to follow movements of humans from early human expansions to recent migrations (Maca-Meyer et al. 2001; Torroni et al. 2001). Furthermore, haplogroup studies have also been used to explore disease association (Hofmann et al. 1997; Kalman et al. 1999; Kok et al. 2000; van der Walt et al.

Table 1 Description of Amish pedigrees

Community	Pedigree	#generations	#nuclear families	#ind	#founders	#ascertained ind
Adams	A	9	31	109	29	51
Adams	B	2	2	9	4	4
Elkhart/LaGrange	A	9	46	132	42	42
Elkhart/LaGrange	B	4	5	20	6	10
Elkhart/LaGrange	C	2	1	4	2	2
Elkhart/LaGrange	D	2	1	5	2	3
Elkhart/LaGrange	E	2	1	5	2	3
Holmes	A	13	93	218	61	28
Holmes	B	7	80	209	72	50
Total			260	711	220	193

2003) and longevity within populations (De Benedictis et al. 2001; Niemi et al. 2003; Rose et al. 2001a, b).

The goal of this study is twofold: firstly, to characterize the mitochondrial genetic diversity found within pedigrees of the Ohio and Indiana Amish communities through haplogroup typing; and secondly, to assess whether mitochondrial haplogroups or haplogroup-defining SNPs confer risk to AD in these communities.

Subjects and methods

Family ascertainment

Amish families ascertained for dementia are derived from three separate communities (Table 1). One community is composed of Amish families from the first migration wave residing in three contiguous counties —Elkhart and LaGrange Counties in northern Indiana and St. Joseph County in Southern Michigan. A second community is located in Holmes County, Ohio. The third community consists of descendants from the second immigration wave and is located in Adams and Jay Counties in Indiana. For the purpose of this study, we will refer to the three communities by the names of Elkhart/LaGrange, Holmes and Adams. Informed consent for clinical evaluations and blood draws for DNA isolation was obtained from all participating individuals under protocols approved by the Duke University Medical Center and Vanderbilt University Medical Center Institutional Review Boards (IRB). Cognitive impairment was screened using either the Mini-Mental State Examination (MMSE) or the Modified Mini-Mental State Examination (3MS). For all possible dementia diagnoses, additional informant interviews were completed. Some subjects identified between 1993 and 2001 were seen on two or more occasions. Subjects with education-adjusted scores of less than or equal to 86 on the 3MS evaluated from 2002 to the present also completed a more thorough neuropsychological evaluation. Clinical diagnosis of AD was made according to NINCDS-ADRDA guidelines (McKhann et al. 1984). Individuals were diagnosed as affected (diagnosis of probable or possible AD), mild cognitive impairment (MCI; memory complaint but functioned independently), or unaffected (no history of cognitive impairment). Using all sources of information, experienced clinical staff (KWB (neuropsychologist), PCG (physician assistant), and LM (nurse clinician)) reached consensus diagnosis on all cases. Age at onset (AAO) was defined as the age at which onset of cognition and memory decline impeded daily activity as reported by the individual or family member. The average AAO for females is 81 years while AAO for males is 75 years.

Pedigree construction and matriline identification

Pedigrees were constructed using participant information, which included family names and birth dates of parental and grand-parental family members. This information was cross-refered with existing family data using Progeny software to verify relationships of the new participants added into the study. To choose independent matrilines, we were able to follow lines of relation from 2 to 4 generations in the smaller pedigrees or from 7 to 13 generations in the large pedigrees. For the case-control study, matrilines were considered "affected" if one member within a matriline was diagnosed with dementia, MCI or had family history of dementia. Similarly, control lines were identified in the pedigrees if no members within the line were affected. Additional controls were identified as unrelated married-in spouses and unrelated Amish from families that were not ascertained for this dementia study. One individual of each matriline was chosen at random for case-control haplogroup analysis (Fig. 2).

Genotyping

Mitochondrial SNPs

Genomic DNA was isolated from whole blood samples by the Duke University Center for Human Genetics DNA Banking core and Vanderbilt University Center for Human Genetics Research DNA Resources core using Puregene (Gentra Systems, Minneapolis, MN,

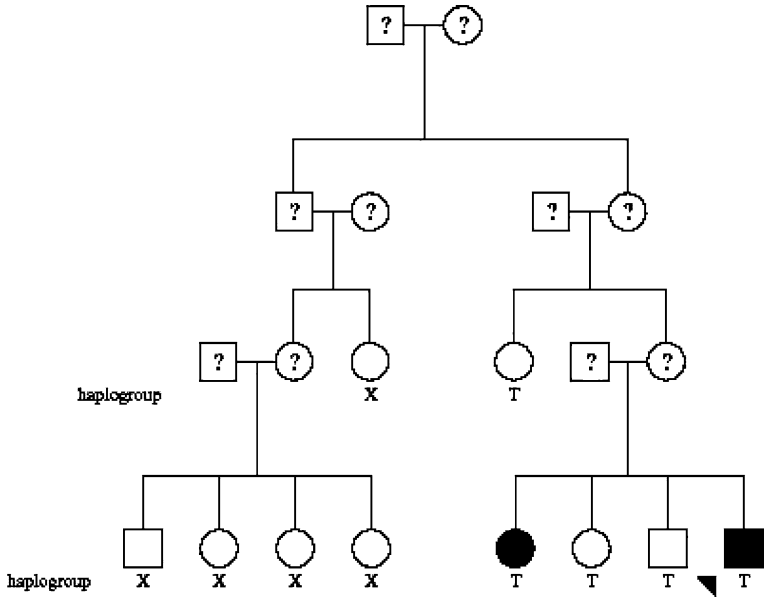

Fig. 2 This simplified Amish pedigree is composed of both an X haplogroup matriline and a T haplogroup matriline. Two individuals are affected (*black circle and square*) in T haplogroup lineage therefore therefore we identified this line as affected for the case-control analysis

USA). European haplogroup defining mitochondrial SNPs ($n = 11$) were genotyped using the Taqman allelic discrimination method. High-throughput genotyping was carried out in a 384 well format on the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). 2.6 ng of DNA was distributed in each well using a Hydra HTS Workstation microdispensing system (Robbins Scientific, Sunnyvale, CA, USA). Probes and primers for each SNP were designed using ABI Prism Primer Express software Version 2.0 (Applied Biosystems). Probes were either designed incorporating a black-hole quencher reporter (Integrated DNA Technologies, Inc., Coralville, IA, USA) or a minor groove binding molecule (MGB) (Applied Biosystems).

Each reaction mixture consisted of 3 μl of master mix (0.2 U/μl Taqman Universal PCR Master Mix, 0.9 ng/μl of each forward and reverse primer and 0.2 ng/μl of each probe) was dispensed by a MultiProbe2 204DT (Packard Instruments, Shelton, CT, USA). The amplification reaction was conducted on an ABI Dual 384-well GeneAmp PCR System 9700 utilizing the following program: 50°C for 2 min; 95°C for 10 min; 95°C for 15 s and 62°C for 1 min, repeated for 40 cycles; and held at 4°C upon cycling completion. Data were generated on an ABI Prism 7900HT Sequence Detection System (SDS) and analyzed using the SDS version 2.0 software.

Statistics

Data were stored and managed using the Pedigene system (Haynes et al. 1995). Haplogroup frequencies within Amish kin groups were estimated from direct counts. Fisher's exact tests (two-sided) were used to identify significant frequency differences of haplogroups and SNPs between case and control groups. Statistical significance was declared at $\alpha = 0.05$. All statistical analyses were performed using SAS software release 8.1 (SAS Institute Inc, Cary, NC, USA).

Table 2 Mitochondrial haplogroup frequencies in Amish communities

Independent matilines Haplogroup	Caucasian (n = 340)		Amish Overall (n = 95)		Indiana Amish				Ohio Amish Holmes Co. (n = 40)	
					Adams Co. (n = 22)		Elkhart/La Grange Co. (n = 33)			
	n	Freq	n	Freq	n	Freq	n	Freq	n	Freq
H	134	39.4	30	31.6	7	31.8	9	27.3	14	35.0
I	11	3.2	1	1.1	0	0.0	0	0.0	1	2.5
J1	38	11.2	1	1.1	0	0.0	0	0.0	1	2.5
K1	32	9.4	2	2.1	0	0.0	2	6.0	0	0.0
T	36	10.6	34	35.8	10	45.5	9	27.3	15	37.5
U	41	12.1	13	13.7	0	0.0	5	15.2	8	20.0
V	10	2.9	0	0.0	0	0.0	0	0.0	0	0.0
W	5	1.5	0	0.0	0	0.0	0	0.0	0	0.0
X	5	1.5	5	5.3	2	9.1	3	9.1	0	0.0
Other	28	8.2	9	9.5	3	13.6	5	15.2	1	2.5

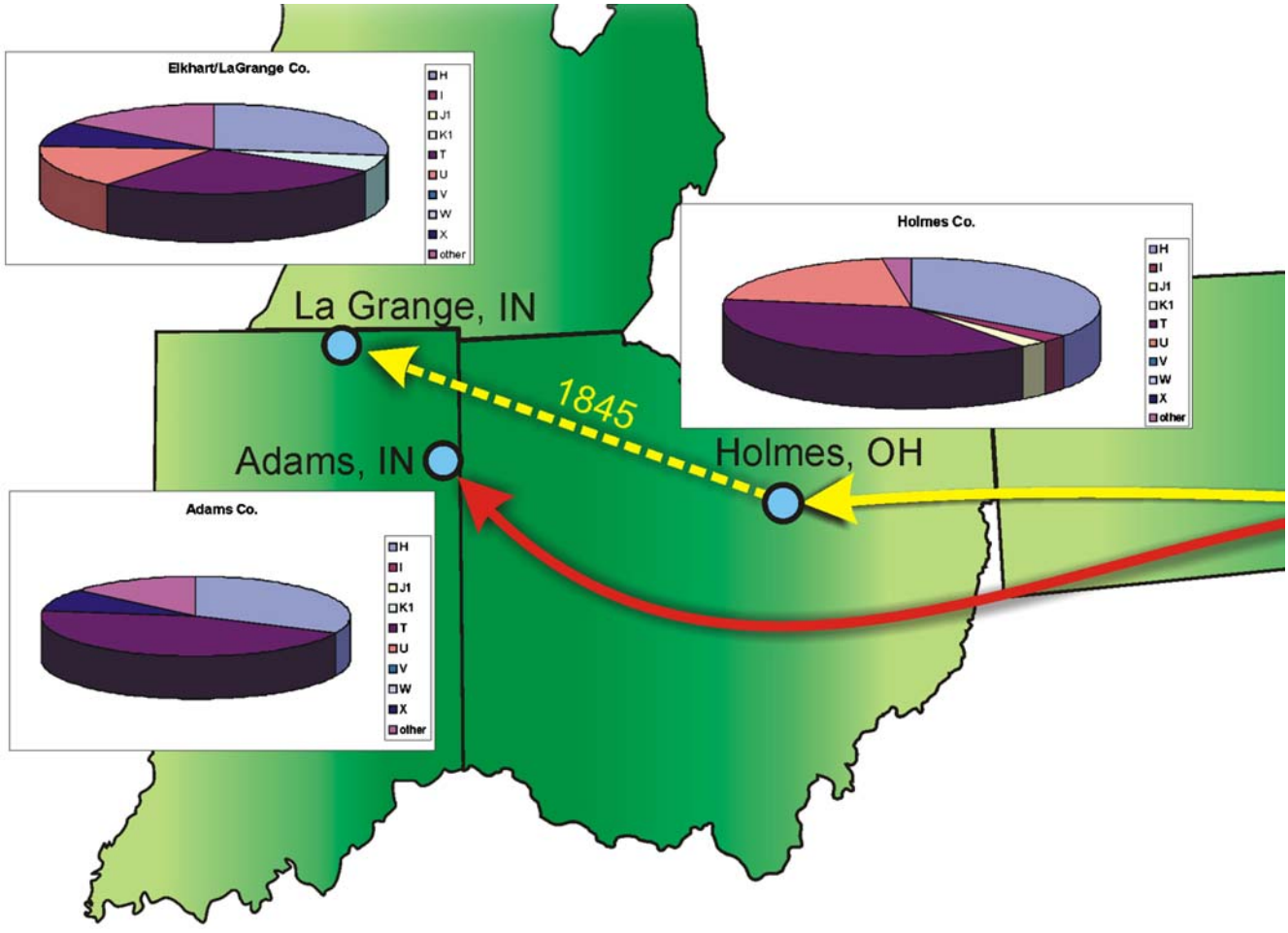

Fig. 3 Families from Adams County consisted of: H(31.8%), T(45.5%), X(9.1%), other (13.6%); families from Elkhart/LaGrange were composed of: H(27.3%), K(6.0%), T(27.3%), U(15.2%), X(9.1%), other (15.2%); families from Holmes County contained: H(35.0%), I(2.5%), J(2.5%), T(37.5%), U(20.0%), other(2.5%)

Results

A total of 228 individuals (124 females, 104 males) were genotyped for 11 mt polymorphisms. All related individuals were removed from the data set to reveal 95 independent maternal lines distributed within nine Amish pedigrees in the Indiana and Ohio Amish communities. Furthermore, we observed seven out of the nine major European haplogroups in the Amish pedigrees examined (Table 2). Families analyzed in this study were composed of a minimum of two matrilines to a maximum of six lines. Haplogroup frequencies observed within maternal lines were significantly different from an outbred Caucasian sample ($P = 0.0000001$). Haplogroup T is the most frequent overall in the matrilines (35.4%) while observed in only 10.6% in Europeans. Furthermore, haplogroups J and K are less frequent (1.0%) than in the European data set (9.4–11.2%). Haplogroup H is the most common mitochondrial lineage found in outbred

Table 3 Haplogroup frequencies in Amish AD case and control matrilines

Haplogroup	Control		AD		AD + MCI	
	n	%	*n*	%	*n*	%
H	14	35.0	9	26.5	12.0	30.0
I	1	2.5	0	0	0.0	0.0
J	1	2.5	0	0	0.0	0.0
K	1	2.5	1	2	1.0	2.5
T	15	37.5	11	32.4	14.0	35.0
U	4	10.0	6	17.7	6.0	15.0
X	2	5.0	3	8.8	3.0	7.5
Other	2	5.0	4	11.8	4.0	10.0

Table 4 Fisher's exact test (two-sided *P*-values) for haplogroups and SNPs

Comparison	All Case Control		Females Only		Males Only	
	AD	AD + MCI	AD	AD + MCI	AD	AD + MCI
European haplogroups	0.833	0.942	0.149	0.352	0.478	0.546
H	0.460	0.641	0.068	0.261	0.517	0.740
T	0.811	1.000	0.220	0.248	0.372	0.556
U	0.326	0.513	0.175	0.228	0.461	0.701
Haplgroup defining SNPs						
7028tc	0.460	0.641	0.068	0.261	0.517	0.740
10398ag	0.624	0.616	0.291	0.256	0.489	1.000
12308ag	0.352	0.542	0.400	0.439	0.287	0.461
1719ga	1.000	1.000	0.656	0.689	0.348	0.348
4580ga[a]	NA	NA	NA	NA	NA	NA
8251ga[b]	1.000	1.000	1.000	1.000	NA	NA
9055ga	1.000	1.000	1.000	1.000	0.489	1.000
13368ag	0.811	1.000	0.220	0.248	0.372	0.556
13708ag	1.000	1.000	0.260	0.471	0.137	0.137
16391ag	0.499	0.494	1.000	1.000	1.000	1.000
3010ag	0.124	0.116	0.248	0.131	1.000	1.000

NA not available
[a]All cases and controls had "g" allele for s4580ga
[b]All male cases and controls had "g" allele for s8251ga

Caucasian populations and found at similar frequencies among Amish communities.

Stratification of Amish families by county demonstrates that certain haplogroups are shared among groups while others are not (Fig. 3). For example, all three communities share haplogroups (H, T, X, other); however, two families from Elkhart/LaGrange community carry the K haplogroup while Holmes Co. families carry haplogroups I and J.

Matrilines were classified as "case" or "control" lines to test whether specific haplogroups and SNPs confer risk of AD in the Amish population. Many of the control individuals were married into the pedigrees and assumed "unrelated" to that pedigree since they were not linked through pedigree analysis. We did not observe frequency differences between the AD cases compared to controls overall or when stratified by sex (Table. 3, 4). We obtained a similar finding when MCI individuals were added into the case group.

Discussion

The Amish population of North America was founded by a small number of Swiss immigrants and has been studied extensively for Mendelian recessive diseases. Recently, genetic studies have utilized genetically isolated populations to map complex disease traits since they often display homogenous phenotypes, exist in the same environment and, most importantly, have large multigenerational pedigrees that can be used to track disease alleles (Arcos-Burgos et al. 2002). Indeed, the Amish population is a prototypical group to study complex disease for the reasons mentioned above. This investigation has provided an initial screen of the mitochondrial genetic composition of the Ohio and Indiana Amish and examined the role of mt variations with AD risk.

We observed 95 independent matrilines within the pedigrees examined and identified 7 out of the 9 major European haplogroups. A founder effect, continued isolation of the communities, and subsequent genetic drift has increased the frequency of many of the Caucasian haplogroups compared to the outbred American population. The frequency data also revealed that the Holmes County and Elkhart/LaGrange County kin-groups founded by the first immigration wave of the eighteenth century have greater haplotypic diversity (six haplogroups in each) than in Adams County kin-group (four haplogroups), which are descendants from the second immigration. Of course, additional haplogroups may exist but were not sampled in this study since we have not yet sampled all pedigrees from the Ohio and Indiana Amish populations.

The haplogroup frequency data also indicate that there may be a genetic substructure among the three Amish communities according to the haplogroup diversity. This observation is in agreement with the results from studies of blood-group frequencies, which demonstrated population substructure among the Lancaster County, Pennsylvania, Holmes County, Ohio and Elkhart/LaGrange Counties Indiana groups (Juberg et al. 1971; McKusick et al. 1967). Furthermore, previous studies have shown that increased frequencies of rare recessive disorders appear to be specific to individual Amish communities. For example, hemophilia B is highly common in Holmes County but nearly absent in Lancaster County and Elkhart/LaGrange Counties (McKusick 2000). Together, these studies suggest that Amish communities founded by the separate immigration waves are genetically distinct. This information is critical for the successful mapping of complex disease genes in the Adams, Elkhart/LaGrange and Holmes isolates.

In contrast to the difference we observed between the communities, we observed no differences in the mitochondrial haplogroups between AD case and control matrilines. Mitochondrial dysfunction, as a result from mtDNA mutations, deletions or polymorphisms, has been implicated in several neurodegenerative disorders (Giacchetti et al. 2004; van der Walt et al. 2003). We previously reported that polymorphisms that lie within the haplogroup U lineage may increase susceptibility of AD in males but decrease risk in females (van der Walt et al. 2004). These data suggest that the etiology of AD in the Amish, like the Amish communities themselves, may be distinct from the outbred Caucasian population. Another explanation for the negative finding is that the small sample size (40 cases including AD + MCI and 40 controls) may have precluded the statistical power to detect small or moderate effects. A power calculation demonstrated that we would have sufficient power to detect only large effects (e.g., RR > 3.5). Thus SNPs or haplogroups with small or moderate effects would have been missed in our study.

Our results are unable to correlate AD expression in the Ohio and Indiana Amish to a specific mitochondrial haplogroup as we have previously reported in an outbred Caucasian population. Therefore, we suggest that the genetic effect in AD is most likely caused by nuclear factors in these Amish communities. We are currently conducting a genome-wide mapping study on these pedigrees to identify possible chromosomal regions that influence AD susceptibility in each community.

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