Apolipoprotein E Genotype and Neurological Disease Onset in Niemann–Pick Disease, Type C1

Rao Fu,1,2 Nicole M. Yanjanin,2 Matthew J. Elrick,3 Christopher Ware,3 Andrew P. Lieberman,3 and Forbes D. Porter2*

1School of Basic Medical Sciences, Peking University, Beijing, China
2Section on Molecular Dysmorphology, Program in Developmental Endocrinology and Genetics, NICHD, NIH, DHHS, Bethesda, Maryland
3Department of Pathology, University of Michigan Medical School, Ann Arbor, Michigan

Manuscript Received: 1 August 2011; Manuscript Accepted: 3 March 2012

Niemann–Pick disease, type C1 (NPC1) is a lipid storage disorder that results in progressive neurological impairment. The NPC1 phenotype is extremely variable and at the individual level is likely influenced by other genetic traits. In addition to residual function of NPC1 protein, we hypothesize that modifier genes, as frequently observed with other autosomal recessive diseases, influence the NPC phenotype. The NPC1 phenotype includes progressive dementia, and the NPC pathology has some overlap with the pathology of Alzheimer disease (AD). Thus, we examined apolipoprotein E (ApoE) and microtubule-associated protein tau (MAPT) polymorphisms in a cohort of 15 NPC1 patients with well characterized longitudinal disease progression. Although we did not find any correlations between disease severity and tau polymorphisms, we found significant associations between ApoE polymorphisms and phenotypic severity. Specifically, ApoE4 and ApoE2 alleles were associated, respectively, with increased and decreased disease severity in this cohort of NPC1 patients. These data support the hypothesis that ApoE may play a role in modulating NPC1 neuropathology.

How to Cite this Article:

INTRODUCTION

Niemann–Pick disease, type C (NPC) is a lysosomal storage disorder with a wide spectrum of clinical symptoms that are a consequence of dysfunction of either the NPC1 or NPC2 protein. Understanding the factors that contribute to this phenotypic variation will provide insight into pathological mechanisms and potential therapeutic interventions. NPC is caused by mutations of either NPC1 or NPC2. To date, 244 NPC1 and 18 NPC2 mutant alleles have been reported (http://npc.fzk.de/). The majority of NPC cases, and the cases in this report, are due to mutation of NPC1. The NPC1 phenotype is variable in both age of neurological onset and symptoms [Yanjanin et al., 2010]. Although there currently is no good assay to quantify NPC1 function, it is likely that residual NPC1 function accounts for a large degree of this phenotypic variability.

However, residual NPC1 function does not explain phenotypic variability within family and between individuals with the same genotype [Lossos et al., 1997]. Modifier genes frequently contribute to the phenotype in autosomal recessive disorders. For example, monoamine oxidase type B (MOAB) is a modifier gene in phenylketonuria [Ghozlan et al., 2004], and polymorphisms of both mannose-binding lectin2 (MBL2) and transforming growth factor beta 1 (TGFβ1) influence pulmonary outcome in cystic fibrosis [Accurso and Sontag, 2008]. This study was conducted to investigate the impact of potential modifier genes on NPC severity.

Although NPC1 is a distinctly different disease than Alzheimer disease (AD), common pathological processes may contribute to both disorders. These include aberrant cholesterol metabolism [Mann et al., 2004] and increased amyloid-β (Aβ) [Yamaguchi et al., 2001]. AD is pathologically characterized by amyloid plaques and neurofibrillary tangles [Tiraboschi et al., 2004]. Neurofibrillary tangles are a common feature of NPC1, and their immunohistochemical and ultrastructural appearances are identical to those of neurofibrillary tangles in AD [Suzuki et al., 1995]. A major

© 2012 Wiley Periodicals, Inc.

Grant sponsor: NIH; Grant numbers: NS063967, NS065662.
*Correspondence to: Forbes D. Porter, MD, PhD, Program in Developmental Endocrinology and Genetics, NICHD, NIH, DHHS, Bldg. 10, Rm. 9D42, 10 Center Dr., Bethesda, MD 20892. E-mail: fdporter@mail.nih.gov
Article first published online in Wiley Online Library (wileyonlinelibrary.com); 28 September 2012
DOI 10.1002/ajmg.a.35395
component of amyloid plaques in AD is Aβ protein generated due to aberrant processing of amyloid precursor protein (APP). Consistent with overlapping pathological processes, abnormal APP processing has been observed in cultured NPC1 neurons [Jin et al., 2004], and levels of Aβ protein are significantly elevated in cerebral spinal fluid from NPC1 patients [Mattsson et al., 2011]. Although the characteristic amyloid plaques that are a cardinal finding in AD are not observed in NPC, Saito et al. [2004] reported the presence of diffuse plaques in 3/9 NPC patients. Of particular note, the ApoE genotype for these three patients was ApoE4/ApoE4.

Based on the pathological overlap of NPC1 and AD we explored the hypothesis that the NPC1 phenotype could be modulated by polymorphisms of apolipoprotein E (APOE) or microtubule-associated protein tau (MAPT). APOE encodes a lipoprotein that is highly expressed in the brain and plays a major role in central nervous system cholesterol homeostasis [Han, 2004]. ApoE is polymorphic with three major isoforms (ApoE2, ApoE3, and ApoE4). These isoforms differ from each other only by a Cys to Arg amino acid substitution at positions 112 or 158 [Zuo et al., 2006]. ApoE3 accounts for approximately 64% of alleles [Eisenberg et al., 2010], and is considered the "neutral" ApoE genotype. ApoE4 is associated with increased risk of AD [Bignall, 1993] and impaired cognitive function [Asada et al., 1996]. In contrast, ApoE2 may be protective against AD in some populations [Tyrrell et al., 1998].

MAPT encodes a protein, tau, that promotes and stabilizes microtubules [Weingarten et al., 1975]. The MAPT haplotype H1 is a risk factor for AD [Shaw-Smith et al., 2006]. In this study, we correlated ApoE and MAPT genotypes with neurological disease onset and found that ApoE4 and ApoE2 alleles were associated, respectively, with early and later neurological disease onset.

**MATERIALS AND METHODS**

**Study Subjects**

This study was approved by the Eunice Kennedy Shriver National Institute of Child Health and Human Development Institutional Review Board. DNA or cell lines were available for 15 of the 19 patients for whom longitudinal disease progression had previously been characterized by chart review [Yanjanin et al., 2010]. DNA of H12 was only available for APOE but not MAPT genotyping. To determine the "age of neurological onset", the progression curve derived from the total score was extrapolated to the x-axis (age) by linear regression. Based on the calculated age of neurological onset, patients were ordered from earliest to latest onset. A similar analysis was also performed using the subset of the total score derived from the memory and cognition subcores. Two of the patients had no change in their subcores over multiple visits. H10, with an E3/E4 genotype, had a subscore of 3 stable from 10 to 18 years old; and H12, with an E3/E3 genotype, had a subscore of 7 stable from 26 to 32 years old. In order to include these patients in the analysis, we determined the mean (0.44 points/year) progression slope for these two domains based on data from the other 13 patients. We then used this value combined with the latest age for which data were available to rank these patients. We felt that this conservative estimate was more appropriate than exclusion of these data.

**APOE and MAPT Genotyping**

APOE genotype was determined by PCR Restriction Fragment Length Polymorphism (PCR-RFLP) assay using a previously published protocol with minor modifications [Hixson and Vernier, 1990]. Briefly, PCR was used to amplify a 218 bp fragment of genomic DNA which included APOE codons 112 and 158. The PCR was carried out with the outer primer sequences (APOE-F: 5’-CGGCGTGTTCAAAGGAGCTGC-3’ and APOE-R: 5’ GGCCCT-GGTACACTGCCAGG-3’), amplifying a 218 bp DNA product. The PCR was performed in a 50 μl reaction mixture containing 100 ng gDNA, 50 pmol/μl primer, 10× RAB PCR buffer with MgCl2 (Roche Applied Science, Indianapolis, IN), 100 mM dNTPs, 5 U/L Taq polymerase, and 5 M Betaine. One cycle of denaturation at 94°C for 4 min, was followed by 35 cycles at 94°C for 45 sec, 64°C for 45 sec, and 72°C for 45 sec. The reaction was concluded with a 10 min extension step at 72°C. An aliquot of the PCR-amplified product was digested with restriction enzyme HhaI overnight at 37°C. For genotype analysis, the small fragment-sized cleavage products of HhaI (48, 72, 81, and 91 bp) were electrophoresed through 20% polyacrylamide gels which were stained with ethidium bromide and visualized with ultraviolet illumination.

MAPT genotype was determined by PCR amplification based on a previously reported method [Baker et al., 1999]. The PCR reaction utilized the forward primer sequence 5’-GGAAGACGTT-TCTCAGTATGTCG-3’ and reverse primer 5’-AGGGAGCGTT-GCTCAGGCTC-3’ to detect a 238 bp deletion in the H2 haplotype, yielding products of 484 or 245 bp for the H1 or H2 haplotype, respectively. The reaction was carried out in a 20 μl reaction mixture containing 50 ng DNA and final concentrations of 500 nM of each primer, 1X PCR buffer containing 1.5 mM MgCl2, 250 μM dNTPs, and 2 U Taq polymerase (Roche). Denaturation at 95°C for 4 min was followed by 35 cycles of 95°C for 30 sec, 60°C for 30 sec, and 72°C for 45 sec; then an additional cycle of 95°C for 30 sec, 50°C for 30 sec, and 72°C for 45 sec; and a final extension at 72°C for 8 min. PCR products were electrophoresed through 2% agarose gels and visualized by ethidium bromide staining and ultraviolet illumination.

**Statistic Analysis**

Statistical analysis was performed using Graph Pad Prism software Version 5. The Mann–Whitney Test was used for statistical comparisons of ranked data. A nominal value of P < 0.05 was defined as indicating a significant change. Error bars on graphs represent standard error of the mean (SEM).

**RESULTS**

APOE genotyping was conducted on 15 DNA samples from patients for whom longitudinal neurological progression had previously been characterized by chart review [Yanjanin et al., 2010]. This cohort of patients includes five females and 10 males, whose severity score at initial evaluation range from 1 to 27, with a mean of 9 and a median of 7. Initial evaluation age of these patients ranges from 1 to 38, with a mean of 14 and a median of 12. We previously showed that disease progression was linear, and the rate
of progression was independent of age in this group of patients. Thus, to rank patients by onset we extrapolated the progression curve to the x-axis. These values ranged from 0.5 to 35.5 years, and we refer to this value as the calculated age of neurological onset. The 15 NPC1 patients were ranked in order from earlier to later neurological disease onset; and the number of patients with childhood onset (0–12 years old), adolescent onset (13–18 years old), and adult onset (>18 years old) were 10, 3, and 2, respectively. 

APOE genotype was determined by PCR-RFLP (Fig. 1A) and APOE genotypes for these 15 subjects are shown in Figure 2A. Of note, ApoE4 and ApoE2 alleles are clustered in earlier and later onset cases, respectively. Figure 2B compares the calculated age of neurological onset for patients with and without an ApoE2 allele and patients with and without an ApoE4 allele. NPC1 subjects with an ApoE4 allele had a significantly earlier onset (3.6 ± 0.8 years) than those without an ApoE4 allele (15.5 ± 3.64 years; P < 0.05), and subjects with an ApoE2 allele had a significantly later onset (19.3 ± 0.8 years) than those without an ApoE2 allele (7.6 ± 2.7 years, P < 0.001).

Because memory and cognition are major issues characterizing the dementia observed in NPC1, we also ranked this cohort of NPC1 patients based on the combined score of these two subscores (Fig. 3A). In this method, NPC1 patients appear to have calculated age of neurological onset ranging from −7.5 to 33.2 years. Negative values result from extrapolation to the x-axis and only represent a tool to rank patients with regards to severity. Though not statistically significant, we observed that patients with an ApoE4 allele and ApoE2 allele appeared to have a trend of earlier and later onsets, respectively. Comparing the calculated age of onset values utilizing the cognitive and memory subdomains (Fig. 3B), patients with at least one ApoE4 allele had an earlier mean age of onset (3.9 ± 1.5 years) compared to patients without an ApoE4 allele (12.2 ± 4.3 years). However, this was not significant (P = 0.09). In contrast, patients with an ApoE2 allele had a later calculated age of onset compared to patients without an ApoE2 allele (Fig. 3B) (6.5 ± 3.0 vs. 15.8 ± 1.7 years, P = 0.07). MAPT genotyping for a group of 14 NPC1 patients is shown in Figure 4. No significant correlations between disease onset and MAPT genotype were observed when ranking based on the total score (Fig. 4A) or the memory and cognition subscore (Fig. 4B).

DISCUSSION

AD and NPC1 have some common pathological overlap including increased levels of both A\(\beta\)40 and A\(\beta\)42, and the presence of neurofibrillary tangles. The neurofibrillary tangles reported in NPC are immunologically and ultrastructurally identical to those observed in AD [Suzuki et al., 1995], and Mattsson et al. [2011] have recently shown that both A\(\beta\)40 and A\(\beta\)42 are abnormally increased in CSF from patients with NPC1 disease.

ApoE4 is the most common genetic risk factor for AD [Bignall, 1993]. The mechanism by which ApoE4 increases the risk for AD is not entirely understood [Vergese et al., 2011]. However, a number of pathological mechanisms, which also may influence pathological progression of NPC1 disease, have been proposed. ApoE isoforms differentially modulate amyloid \(\beta\) metabolism including production and clearance, and thereby may contribute to \(\beta\) toxicity [Huang et al., 2011; Vergese et al., 2011; Ye and Zhang, 2012]. ApoE isotype may influence other pathological mechanisms that could play a role in both NPC1 and AD. ApoE4 has been reported to impair synaptic plasticity [Chen et al., 2010], and ApoE4 isoform appears to influence the development of neurofibrillary tangles [Montine et al., 1999]. In this latter context, it is notable that Saito et al. [2004] observed increased neurofibrillary tangles in brain tissue from NPC patients who were homozygous for the ApoE4 allele. In this study we demonstrate a correlation between APOE genotype and neurological disease onset in a 15-patient cohort of NPC1 patients for whom we have both longitudinal disease progression data and DNA. Our observation that an ApoE4 allele is associated with earlier neurological disease onset is consistent with the pathological case series by Saito et al. [2004] that found that an ApoE4 allele was associated with more severe neuropathological findings. This observation is consistent with ApoE being a modifier gene that contributes but does not determine to an earlier onset of neurological symptoms in NPC1 patients. The major determinant is likely residual NPC1 function, and one would expect that other genes also influence the pathology of NPC1 disease. A limitation of both of these studies is the small number of cases. Unfortunately this is a limitation that is inherent to the study of rare genetic diseases. However, when combined, these studies suggest that ApoE may play a role in modulating NPC neuropathology and that specifically an ApoE4 allele may increase the severity of neurological disease in NPC1. It will also be interesting to determine if ApoE genotype

![FIG. 1. Apolipoprotein E (APOE) and microtubule-associated protein tau (MAPT) genotyping. A: APOE genotype was determined by PCR/Restriction Fragment Length Polymorphism (PCR-RFLP) analysis. APOE alleles were identified by the DNA fragmentation pattern (ApoE2: 67 and 91 base pairs; ApoE3: 48 and 91 base pairs; ApoE4: 48 and 72 base pairs). B: MAPT genotype was determined by PCR. H1 and H2 haplotypes correspond to DNA fragments of 484 base pairs and 245 base pairs, respectively.](image-url)
FIG. 2. Correlation of Apolipoprotein E genotype and neurological disease onset. A: NPC1 patients were ranked based on calculated age of neurological onset and corresponding ApoE genotype is shown. ApoE genotypes including an ApoE4 allele are in red and those including an E2 allele are in green. B: Significant differences in the calculated age of neurological onset were observed for NPC1 patients with and without an ApoE2 allele (**P < 0.001) and NPC1 patients with and without an ApoE4 allele (*P < 0.05). Calculated age of neurological disease onset was earlier for patients with an ApoE4 allele and later for patients with an ApoE2 allele.

FIG. 3. Correlation of Apolipoprotein E genotype and onset of cognitive and memory deficits. A: NPC1 patients were ranked based on memory and cognition scores. ApoE genotypes including an E4 allele are in red and those including an E2 allele are in green. *Ranking was estimated for these two subjects. B: NPC1 subjects with at least one ApoE2 allele had a later age of onset compared to patients without an ApoE2 allele, although this different was not significant (P = 0.09). Similarly, mean age of onset was later in patients with at least one ApoE4 allele with a P-value of 0.07.
contributes to the severity of cholestatic liver disease that is frequently observed in the neonatal period. Although confirmation will be required after longitudinal progression has been quantified in larger cohorts of patients, identifying potential genetic modifiers in NPC1 could provide immediate insight into pathological processes that would be amendable to therapeutic intervention.

ACKNOWLEDGMENTS

This work was supported by the intramural research program of NICHD and a Bench-to-Bedside award from the Office of Rare Disease. This work was also supported by grants from the NIH (NS063967 to A.P.L. and NS065662 to M.J.E.). We acknowledge the support from the Ara Parseghian Medical Research Foundation and Dana Angel’s Research Trust.

REFERENCES


Mattsson N, Zetterberg H, Bianconi S, Yanjanin NM, Fu R, Mansson JE, Porter FD, Blennow K. Gamma-secretase-dependent amyloid-beta is...


