Genome-Wide Linkage Scan Maps ETINPH Gene to Chromosome 19q12–13.31

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Key Words

Essential tremor-idiopathic normal pressure hydrocephalus · ETINPH gene · Linkage analysis

Abstract

Objective: Essential tremor (ET), one of the most common neurological disorders, comprises uncontrollable tremor, usually in the upper extremities. Idiopathic normal pressure hydrocephalus (iNPH) features ventricular enlargement in the absence of elevated intracranial pressure; its three cardinal symptoms are gait impairment, incontinence, and dementia. iNPH is among the most common medical problems in the older population. To date, the genetic etiologies of ET and iNPH remain largely elusive. **Methods:** We previously identified a large kindred in which the affected members developed ET in adolescence or young adulthood and iNPH when elderly, in that case called ETINPH. Employing two different genotyping microarrays and two- or multipoint linkage analysis strategies, genome-wide linkage scans were performed. **Results:** We mapped the ETINPH locus to chromosome 19q12–13.31. Fine-mapping in this region revealed a maximum two-point lod score of 2.8 at rs2023865 and a maximum multipoint lod score of 17.9 at rs9304878. By haplotype construction, a (conservatively) approximately 17-cM critical region from SNP rs11084582 to SNP rs7258420 was defined. This region contains several neuronal genes that

constitute tantalizing etiological candidates for ETINPH.

Conclusion: The importance of uncovering the genetic etiology of this disorder is irrefutable, as such a discovery could offer valuable insights into ET, iNPH, and related neurological conditions.

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Introduction

With the prevalence of essential tremor (ET) as high as 5–6% of the population [1, 2], ET represents the most common movement disorder interfering with activities of daily living [1, 3]. In some studies it is as much as 20-fold more prevalent than Parkinson's disease [2–11]. Epidemiological research provides evidence that ET is often familial [7, 10, 12–15]. This finding likely led to initial descriptions of ET as 'familial tremor' [8, 9, 16, 17].

Idiopathic normal pressure hydrocephalus (iNPH) has its onset in geriatric years and is characterized by gait impairment, incontinence, and dementia. Anatomically, iNPH patients feature ventricular enlargement in the absence of elevated intracranial pressure. While about 40% of the congenital hydrocephalus cases are believed to have a possible genetic basis, no generally accepted genetic etiology for iNPH has been reported in the literature [18]. Tremor has occasionally been reported as a

Fig. 1. The pedigree of a five-generation ETINPH family. Blood samples have been collected from the numbered individuals. Circles represent females; squares represent males. Black symbols are affected individuals, white symbols are normal individuals, and gray symbols are individuals with unknown status. For the genome scans, the two individuals in parentheses (II-2, II-3) comprise the founding members of each split pedigree branch. Underlined individuals are persons added to the opposite pedigree for the subsequent overlapped split linkage analysis.

symptom of iNPH but is usually considered to be a Parkinsonian-type tremor [19–22].

In a previous report [23], we identified a five-generation kindred that bridged the gap between ET and iNPH, as multiple members of this family presented with ET in the adolescent or young adult years and three with additional iNPH when elderly. This disorder was named essential tremor-idiopathic normal pressure hydrocephalus (ETINPH). We hypothesized that the same neuronal gene was responsible for both the ET and the iNPH aspects of ETINPH. However, at the time, the identity and location of this putative gene were unknown. Using several different methods of linkage analysis, we have now identified the location of the gene down to a segment of chromosome 19. In this critical region, we have also identified several neuronal genes that constitute tantalizing candidates for this putative gene.

Subjects and Methods

Subjects

All research subjects are related to each other within a large kindred in which ET segregated as an autosomal dominant trait within the span of five generations. While the onset age of ET clusters in the early teens, the onset of iNPH occurs in affected subjects older than 65 years (3 affected subjects in the second generation). The recruiting process, clinical diagnostic criteria, and sample collection have been reported previously [23].

DNA Genotyping

To ensure robust results, two different single nucleotide polymorphism (SNP) genotyping microarrays were used: the Nsp array containing 262,000 SNPs from the GeneChip® Human Mapping 500K Array Set (Affymetrix) and the LINKAGE-12 panel containing 6,090 SNPs (Illumina). The genetic localization and allele frequencies of each SNP and the relevant genetic maps for candidate loci came from publicly available databases or were pro-

vided by the manufacturers (Affymetrix and Illumina). Both arrays share some identical SNPs but with a relatively low concordance rate, which provides a great QC for both genotyping and platform. All SNP microarrays were genotyped at Yale Keck genomic core facility using standard protocols provided by the manufacturers. After the raw genotyping data had been generated from different platforms, the highest quality SNPs were further selected based on criteria such as minor allele frequency of 1%, internal control markers, Mendelian consistency, and genotyping call rate of at least 93% as manufacture recommended, using Genotyping Console and Bead Studio programs for Affymetrix and Illumina platforms, respectively.

Linkage Analysis

Prior to linkage analysis, the linkage utility softwares Pedcheck and Mega2 were used to check the genotyping data and transform the linkage file formats from different linkage analysis programs, respectively [24, 25]. During the genome-wide scan, a model-dependent and a nonparametric linkage analysis of dense SNPs arrays were performed using sparse gene flow tree algorithms as implemented in Merlin [26], with a splitting approach due to the large size of the pedigree [27]. For fine-mapping, with the parameters of an autosomal dominant trait with a rare disease frequency of 0.0001 and full penetrance [23], a two-point model-dependent linkage analysis of the full pedigree was performed using the genotype/phase elimination algorithms implemented in LODLINK in the S.A.G.E. package [28], and a multipoint linkage analysis was performed using Markov chain-Monte Carlo approaches implemented in lm_lods in the Morgan package [29].

Results

After carefully reviewing the medical condition of each participant based on our clinical criteria, we determined that further medical evaluation was needed for two individuals previously diagnosed as ‘affected’, and redefined their status as ‘unknown’ before this analysis to ensure the conservativeness of linkage analysis (fig. 1), re-

Fig. 2. During genome scan using Merlin, the peak of lod scores on chromosome 19 increases from the split pedigree analysis (**a**) to reach almost 3.0 in the overlapped split pedigree analysis (**b**) in Affy chips, indicating a potential candidate site for the ETINPH locus.

sulting in subtle changes compared to our previously described pedigree [23]. We performed a genome-wide linkage scan with two split pedigrees (the II-2 branch and II-3 branch, fig. 1) first, using the linkage program Merlin. Merlin is the best program to handle linkage analysis with high-density SNPs, but it can only handle around 14 individuals at its maximal capacity. In both model-dependent and nonparametric analyses with Merlin, five peaks on chromosomes 10, 16, 17 and 19 with significantly large lod scores were found using Affymetrix chips (Affy chips); only two peaks on chromosome 10 and 19 with large lod scores were found using Illumina chips. While the locations of the peaks on chromosome 10 found in the Affymetrix and Illumina chips did not overlap, the locations of the peaks on chromosome 19 did.

Next, we performed another round of genome-wide linkage scan with two overlapped split pedigrees (the II-2 pedigree with two more affecteds from the II-3 branch, and the II-3 pedigree with one more affected from the II-2 branch). In Affy chips, only two peaks (chromosome 16 and 19) remained with increased lod scores in both model-dependent and nonparametric analysis. In Illumina chips, both peaks on chromosome 10 and 19 defined in the previous analysis remained and had increased lod scores in model-dependent and nonparametric analysis. The peak on chromosome 19 still represented the only common region in this round of analysis, with a maximum model-dependent lod score of 2.4 (fig. 2). Therefore, we concluded that the genetic locus of ETINPH is in this segment of chromosome 19.

Fine-mapping using LODLINK and two-point model-dependent linkage analysis for the full pedigree revealed significant lod scores among SNPs in this interval (more than 20 SNPs with maximum two-point lod scores around 2.0), with their recombination fractions (θ) at zero. A maximum two-point lod score of 2.8 was found at rs2023865. This finding in combination with haplotype construction places the ETINPH gene in an approximately 17-cM critical interval from rs11084582 to rs7258420 on chromosome 19q12–13.31 (fig. 3). Among 407 SNPs from the Nsp array in the 17-cM interval (approximately 13.2 Mb), only 29 SNPs with an average genetic distance of 0.605 cM were chosen in order to perform the multiple linkage analysis in an attempt to limit the potential inflation in the multipoint lod score due to the possibility of linkage disequilibrium. In multipoint linkage analysis using Markov chain-Monte Carlo approaches, 9 out of 10 runs in *lm_lods* generated positive results with a lod score >3.0 . Two maximum multipoint lod scores reached 17.9 (rs9304878), further corroborating the linkage of the ETINPH gene to this interval (fig. 3).

Discussion

Through this genome-wide linkage screening, we have mapped the ETINPH locus to chromosome 19q12–13.31. The current interval is relatively large (approximately 17 cM) based on our conservative approach. In a next stage, more powerful next-generation genomic sequencing

Fig. 3. Graph of chromosome 19 coordinates versus two-point lod scores generated from LODLINK (dashed line) and the multipoint lod scores maximized from *lm_lods* (solid line).

techniques will be applied to sequence the entire interval which will lead to rapid cloning of the ETINPH gene. Within the current large interval, we have selected several potential candidate genes whose products are required for the proper functioning of the nervous system. As such, mutations in these genes represent possible explanations for the pathology seen in ETINPH.

The first of these genes is ATP1A3. This gene codes for an isoform of the alpha subunit of N,K-ATPase that is expressed in the nervous system. A mutation in ATP1A3 has been found to cause the autosomal dominant disorder called rapid-onset dystonia-parkinsonism [30]. In this disease, patients between the ages of 14 and 45 years develop involuntary spasms of the extremities, bradykinesia, dysarthria, dysphagia, and postural instability [31–33]. Several features of this disease resemble ETINPH. Most obviously, both feature a central movement disorder. Additionally, the age at onset of rapid-onset dystonia-parkinsonism parallels that of ET in ETINPH, and both disorders are inherited in an autosomal dominant manner. These intriguing similarities suggest a possible link between ET and parkinsonism, as

has been suggested previously [34]. There is a possibility that different mutations of ATP1A3 that produce varying degrees of function loss may lead to different clinical manifestations.

Another noteworthy finding within the chromosomal segment identified by linkage is a collection of genes whose malfunction may perturb the biochemical pathway that has been implicated in Alzheimer's disease. Among these genes is presenilin enhancer 2 (PSENEN). The protein product of PSENEN enhances the transcription of presenilin, a component of gamma-secretase. Gamma-secretase is involved in the cleavage of amyloid precursor protein (APP); inappropriate cleavage of APP can cause Alzheimer's disease. Indeed, prior research has shown that mutations in presenilins and APP can cause familial forms of Alzheimer [35]. In addition to PSENEN, amyloid beta A4 precursor-like protein 1 (APLP1) resides within the identified segment of chromosome 19. APLP1 is structurally homologous to APP [36]; this structural similarity may imply that a mutation of APLP1 could engender Alzheimer-like pathology. Disruption of either the same or a similar biochemical pathway as Alzhei-

mer's disease could possibly account for the features of ETINPH that resemble Alzheimer's disease, including dementia and enlarged ventricles.

In addition to the aforementioned candidate genes, other genes that directly impact the nervous system reside in the chromosomal segment 19q12–13.31. CAPN12, a cysteine protease, contributes to synaptic plasticity [37]. LGI4 is highly expressed in the brain, and mutations of genes in the same family have been associated with epilepsy [38]. MAG encodes for a protein in myelin that inhibits neurite outgrowth and plasticity [39]. DPF1 is the gene for a neural-specific zinc finger protein [40]. CIC is a repressor of transcription that may influence CNS development; high CIC expression has been found in CNS tumors [41]. ETHE1 and PRX are both located within 19q12–13.31, but the neurological sequelae of mutations in these genes (ethylmalonic encephalopathy and Deferine-Sottas syndrome, respectively) are already well documented [42, 43].

In summary, although we have selected several candidate genes in the interval to discuss, it has to be empha-

sized here that the phenotypic spectrum of ETINPH does mostly not overlap with any of the clinical manifestations caused by those genes. It is our hope that identification of the ETINPH gene will help us to understand the molecular mechanism of how the shared pathophysiological pathway contributes to both diseases. A better understanding of this common pathophysiological pathway will further aid in our understanding of other related neurological conditions. Our current molecular cloning effort will definitely give us a clear answer as to the genetic etiology of ETINPH.

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