Genetic localization of an autosomal dominant leukodystrophy mimicking chronic progressive multiple sclerosis to chromosome 5q31

Christin M. Coffeen1, Catherine E. McKenna2, Arnulf H. Köppen3, Nikki M. Plaster1, Nicholas Maragakis4, Jason Mihalopoulos1, John D. Schwankhaus5, Kevin M. Flanigan6, Ronald G. Gregg7, Louis J. Ptácek1,2,6 and Ying-Hui Fu8,+

1Department of Human Genetics, 2Howard Hughes Medical Institute, 6Department of Neurology and 8Department of Neurobiology and Anatomy, University of Utah, Salt Lake City, UT 84112, USA, 3VA Medical Center and Department of Neurology, Albany Medical College, Albany, NY 12208, USA, 4Department of Neurology, Johns Hopkins University, Baltimore, MD 21287, USA, 5Neurology of Arkansas, Sherwood, AR 72120, USA and 7Departments of Biochemistry and Molecular Biology and Ophthalmology and Visual Sciences, University of Louisville, Louisville, KY 40202, USA

Received 26 November 1999; Revised and Accepted 17 January 2000

The hereditary leukodystrophies represent a group of neurological disorders, in which complete or partial dysmyelination occurs in either the central nervous system (CNS) and/or the peripheral nervous system. Adult-onset autosomal dominant leukodystrophy (ADLD) is a slowly progressive, neurological disorder characterized by symmetrical widespread myelin loss in the CNS, and the phenotype is similar to that of chronic progressive multiple sclerosis. We report clinical, neuroradiological and neuropathological data from the originally reported ADLD family. Furthermore, we have localized the gene that causes ADLD to a 4 cM region on chromosome 5q31. Linkage analysis of this family yielded a LOD score of 5.72 at $\theta = 0.0$ with the microsatellite marker D5S804. Genetic localization will lead to cloning and characterization of the ADLD gene and may yield new insights into myelin biology and demyelinating diseases.

INTRODUCTION

Hereditary leukodystrophies, the prototypes of dysmyelination, are rare disorders in which the loss of myelin is a primary condition; therefore, myelin loss does not result from secondary degeneration caused by neuronal disease. In these disorders, total or partial dysmyelination can occur in either the central nervous system (CNS) and/or the peripheral nervous system. Most hereditary leukodystrophies are either autosomal recessive or X-linked recessive, and age of onset typically is during infancy or childhood (e.g. Krabbe globoid cell leukodystrophy, metachromatic leukodystrophy and adrenoleukodystrophy) (1,2). The adult-onset autosomal dominant leukodystrophy (ADLD) presented in this paper is notable for the early autonomic abnormalities experienced by ADLD patients (3). Thus, ADLD appears to be a distinct disorder from the other leukodystrophies.

ADLD was first described in an American–Irish family (4). It is a slowly progressive and fatal neurological disorder, characterized clinically by autonomic abnormalities, pyramidal and cerebellar dysfunction and symmetrical demyelination of the CNS. The autonomic problems include bowel/bladder dysfunction,otence (in males), orthostatic hypotension and decreased sweating (5). Computed tomography (CT) scans and magnetic resonance imaging (MRI) studies indicate that the white matter abnormality begins in the frontal lobes of the brain and extends to the cerebellum (6). Affected individuals usually begin to exhibit neurological symptoms, such as loss of fine motor skills, in the fourth or fifth decades of life (3); however, autonomic abnormalities precede these symptoms by several years and are among the first to appear. A survival rate of 20 years is common, during which complete loss of voluntary movement is experienced (4).

Based on clinical diagnostic criteria of multiple sclerosis (MS) and prior to the advent of CT and MRI scans, 20 individuals in this family were misdiagnosed as having chronic progressive MS. However, several symptoms distinguish ADLD from MS. (i) ADLD patients exhibit early autonomic dysfunction, and such extensive autonomic abnormalities have not been noted in MS patients (4,7). (ii) Large families segregating a highly penetrant autosomal dominant MS allele have not been described. However, even though autopsy-verified MS has not been cited in more than three generations of a family, twin studies have shown a higher MS concordance rate in monozygotic (25.9%) as opposed to dizygotic (2.3%) twins, indicating that a genetic component is involved in MS susceptibility (8–10). (iii) CT/MRI scans illustrate a widespread symmetrical demyelination in ADLD patients, and MS demyelination is asymmetrical (4,6). (iv) MS is an inflammatory disorder, hypothesized to result from an autoimmune response directed against myelin proteins (11). Conversely, normal immunoglobulin levels have been noted in...
cerebrospinal fluid of one individual affected with ADLD, and no pathological indication of brain inflammation has been found, suggesting that ADLD is not an inflammatory disorder (4). Thus, ADLD is similar to, yet distinct from, chronic progressive MS.

In this paper, we present additional clinical data on the original ADLD family with the addition of a fifth generation not described previously (Fig. 1; kindred 2685). We also present neuropathological and neuroradiological data to characterize the disease further and demonstrate linkage of the ADLD gene in this family to a 4 cM region of chromosome 5q.

RESULTS

Clinical and neuroradiological findings in kindred 2685

Five generations of the ADLD pedigree are shown in Figure 1. Each affected member exhibits symmetrical demyelination of the white matter (Fig. 2) and phenotypic characteristics consistent with ADLD. Disease onset typically occurs during the fourth or fifth decade (mean age 40.5 ± 4.9 years, n = 16). The earliest symptoms usually involve abnormalities of the autonomic nervous system, such as bowel/bladder dysfunction, impotence, orthostatic hypotension and decreased sweating. These symptoms precede others by several years and are followed by loss of fine motor skills. Nerve conduction velocities performed on three affected individuals were normal. Upper motor neuron signs are also common phenotypic characteristics of this disorder. For example, spastic paralysis and posterior column dysfunction occur in 88% of these patients, and 81% exhibit Babinski signs. In addition to upper motor neuron dysfunction, ADLD patients also exhibit cerebellar signs. For instance, nystagmus is present in 69% of the individuals, and all patients experience ataxia.

Neuropathological findings

Some of the neuropathological findings in this leukodystrophy have been described (3,4,12). The gross and microscopic observations shown in Figure 3 were derived from the 21st individual in the fourth generation (Fig. 1, @). At the time of autopsy, the brain was divided in the midline, and one half was frozen for biochemical analysis. The other half was fixed in neutral buffered 4% formaldehyde solution. The grossly visible lesions closely matched those revealed by MRI, and they were most conspicuous in the white matter of the centra semiovalia and the cerebellar peduncles. Myelin loss (Fig. 3A) occurred in isolated and confluent patches, bearing some resemblance to progressive multifocal leukoencephalopathy. Microscopy showed greater loss of myelin than of axons; hence, the disorder meets standard criteria for a demyelinating disease. The patchy loss of myelin was confirmed by immunocytochemistry with antisera to the four major myelin proteins of the CNS [myelin basic protein (MBP) (Fig. 3B and C), proteolipid protein, myelin-associated glycoprotein (MAG) and cyclic nucleotide phosphodiesterase]. The white matter appeared vacuolated, and in contrast to MS, oligodendrocytes were abundant within the lesions (Fig. 3D and E). Also in contrast to MS, astrocytes were sparse, as revealed by immunocytochemistry for the glial fibrillary acidic protein (GFAP) (Fig. 3E). Immunostaining with antibodies to vimentin (Fig. 3F), and insulin-like growth factor 1 (Fig. 3G) showed intense reaction product in astrocytes. The processes of these astrocytes were abnormally beaded and foreshortened (Fig. 3E–G). Inflammatory infiltrates, activated microglia and macrophages were absent. There was no obvious neuronal pathology.
The deficit of GFAP was confirmed on extracts of the affected and grossly unaffected white matter. Quantitative SDS–PAGE and western blotting (Table 1) revealed a deficit in the GFAP in the normal-appearing white matter of this case of leukodystrophy, in contrast to the intact white matter of MS.

Genetic localization of the ADLD locus

An initial genome-wide search for linkage, using regularly spaced microsatellite markers, was performed in kindred 2685. In a subset of 19 family members (12 affected and 7 unaffected) (Fig. 1), the following three loci yielded positive LOD scores in the initial screening: D5S592 (LOD score = 2.38 at \( \theta = 0.05 \)), D21S1249 (LOD score = 1.39 at \( \theta = 0.0 \)) and D2S1248 (LOD score = 1.51 at \( \theta = 0.05 \)). Based on these results, markers flanking D5S592 on 5q were used to genotype all individuals in this family in order to test the hypothesis that the ADLD gene resides on this portion of chromosome 5. Linkage mapping of these flanking 5q markers demonstrated that the microsatellite marker D5S804 (LOD score = 5.72 at \( \theta = 0.0 \)) is completely linked to the disease allele (Table 2). Obligate recombinants were found with markers D5S467 and D5S2110 (Table 2; Fig. 1), defining a 4 cM region within which the ADLD gene resides.

Three additional markers, D5S2059, D5S642 and D5S649, maximize at \( \theta = 0.0 \) (Table 2) and are inherited as a haplotype with D5S804 in this family (Fig. 1). The calculated recombination distance [from the CEPH database (http://www.cephb.fr/cgi-bin/wdb/ceph/systeme/form)] between D5S804 and the Généthon marker D5S2059 is 0.00 with a LOD score of 27.64. Based on the marker order estimates from the Généthon human linkage map of chromosome 5 (13) and LOD score data (Table 2), markers in the region of the ADLD locus were ordered as in the haplotype of Figure 1.

Based on conservative affection criteria, the status of 18 individuals in kindred 2685 was determined as unknown (Fig. 1). Of these family members, five have inherited the complete ADLD haplotype from their respective affected parent (data not shown). Thus, it is predicted that these individuals eventually will develop ADLD.

DISCUSSION

Two neuropathological observations make the described leukodystrophy unique: preservation of oligodendroglia in the presence of subtotal demyelination and lack of astrogliosis. Eldridge et al. (4) commented on a possible relationship of this
leukodystrophy to a sporadic case of ‘diffuse sclerosis’ (14) and a possible variant of Pelizaeus–Merzbacher disease (15). Sponginess of the affected white matter and lack of astrogliosis were present in the diffuse sclerosis case (14), but the lack of a family history raises doubt about its identity as an example of the leukodystrophy described here. Autosomal dominant inherit-
ance, preservation of oligodendroglia and immunochemical detection of all major CNS myelin proteins also argue against an X-linked proteolipid protein deficiency, the hallmark of X-linked Pelizaeus–Merzbacher disease (16).

The autosomal dominant inheritance pattern and our linkage results distinguish ADLD from several more common leukodystrophies. Krabbe’s globoid cell leukodystrophy is an autosomal recessive trait, resulting from mutations in the gene encoding galactosylceramidase on human chromosome 1q (17–19). Metachromatic leukodystrophy is also an autosomal recessive disorder (1); the defect is caused by mutations in the lysosomal enzyme arylsulfatase A gene on human chromosome 22q13.31-qter (20,21). Adrenoleukodystrophy is an X-linked recessive disorder, which has been mapped to Xq28 (22). ADLD is genetically distinct from other leukodystrophies.

The patients reported here have similar symptoms to individuals suffering from chronic progressive MS (4,11). MS is an inflammatory disorder, probably resulting from an autoimmune response directed against myelin proteins (11). Several candidate genes, which have been mapped to the ~4 cM region between D5S467 and D5S2110, are involved with immune responses. They include members of the interleukin (IL) cluster on 5q31-q31.1, specifically IL-3, IL-4, IL-5 and IL-13 (23,24), and colony-stimulating factor-2. Interferon regulatory factor 1 and T cell transcription factor 1 are also located within this cluster and play a role in the immune response (25–27).

Thus, due to the similarity between the ADLD and chronic progressive MS phenotypes, we examined the 5′ untranslated and coding regions of several genes within this interleukin cluster, specifically IL-3, IL-5, IL-13 and colony-stimulating factor-2, for possible ADLD-causing mutations. However, no disease-causing mutations were found (data not shown). In addition, normal immunoglobulin levels in spinal fluid and the absence of pathological signs of brain inflammation have been found in post-mortem brain tissue (Fig. 3). These findings suggest that ADLD pathogenesis does not involve a direct autoimmune attack on myelin proteins. The presence of abundant oligodendrocytes within the lesions (Fig. 3D and E) argues against a developmental defect of this cell lineage. Instead, the ADLD phenotype may result from a mutation in a gene whose protein product is responsible for myelin synthesis, maintenance or regeneration. An alteration in myelin metabolism might result in a cumulative effect of degeneration as gene carriers age.

The white matter lesions resemble toxic demyelination caused by hexachlorophene (28) or cuprizone (29). The latter is of interest because it causes upregulation of insulin-like growth factor I in astrocytes during active demyelination (30) and thus resembles ADLD (Fig. 3G). The intense immunoreactivity for vimentin similarly indicates astrocytic activation, and glial hypertrophy seems to occur normally. However, glial proliferation (hyperplasia) was absent, giving this demyelinating disorder its unique neuropathological phenotype. The reason for the lack of this aspect of gliosis remains obscure.

In order to isolate the ADLD gene, we will continue to fine-map the region and to positionally clone the ADLD region on 5q31. In order to narrow the ADLD interval further, a search for additional polymorphic di- and tetranucleotide repeats on chromosome 5q31 is being conducted; using these new microsatellite markers, the recombinant individuals (Fig. 1) from this family will be genotyped in order to localize the recombinations better and to narrow the region. Also, a yeast artificial chromosome contig, spanning the ADLD interval between the markers D5S467 and D5S2110, exists in the Whitehead Institute/MIT Center for Human Genome Research database (http://www.genome.wi.mit.edu/), aiding in the positional cloning of this 4 cM region.

As a model for monogenic demyelination, the identification of the ADLD gene and its encoded protein could provide further insight into the molecular mechanisms of myelin assembly and maintenance. Subsequently, the study of this disorder could enhance our understanding of the cause and pathogenesis of non-Mendelian demyelinating diseases, such as MS.

**MATERIALS AND METHODS**

**Clinical evaluation of kindred 2685**

Because accurate diagnosis is critical for genetic linkage mapping, we have devised conservative criteria for diagnosing individuals as either ‘affected’ or ‘unaffected’. The status of first, second and third generation members of kindred 2685 was based on previously reported information (4). Members in the fourth and fifth generation were examined and classified as affected if they had characteristic MRI changes or orthostatic hypotension, upper motor neuron signs and ataxia. Individuals were considered unaffected if they: (i) were >40 years of age and had normal physical examination and MRI scan; (ii) were >60 years of age and had a normal physical examination; or (iii) were >75 years of age and asymptomatic. Those who did not meet either the conservative affected or unaffected criteria were classified as unknown. Figure 1 summarizes the status of each family member in kindred 2685.
Sample collection and DNA isolation
Anticoagulated venous blood samples were gathered from 43 individuals in kindred 2685. Patients signed a ‘Consent of Participation’ form, which was approved by the Institutional Review Board for Human Research at the University of Utah School of Medicine. High-molecular-weight genomic DNA was isolated from whole-blood lysate using a standard protocol outlined in the Puregene DNA Isolation kit (Genta Systems, Minneapolis, MN). Lymphoblastoid cell lines were transferred with Epstein–Barr virus as described previously (31).

Microsatellite marker analysis
Genetic examination of kindred 2685 began with an automated genome-wide scan. Highly polymorphic tetranucleotide and dinucleotide repeat markers, separated by 40–60 cM across the human genome, were chosen from the Utah Marker Development Group index linkage mapping set. The fluorescently labeled markers were used to amplify genomic DNA in total reaction volumes of 20 µl in an MJR PTC-200 thermocycler (MJ Research, Watertown, MA). The products were visualized on an Applied Biosystems (Foster City, CA) Model 373A and analyzed by the Genotyper peak-calling software.

This genome-wide scan yielded three chromosomal loci with LOD scores >1.0 at either θ = 0 or θ = 0.05 (see Results). Since the microsatellite marker D5S592 presented the highest LOD score in this genome scan, additional microsatellite markers surrounding this potential ADLD locus on chromosome 5q were examined by PCR amplification of genomic DNA sequences from the entire family. Forward primers (20 pmol) were end-labeled using T4 polynucleotide kinase and [γ-32P]dATP. Genomic DNA (40 ng) was amplified in 1.0x buffer (10 mM Tris–HCl pH 8.4, 40 mM KCl and 1.5 mM MgCl2), 50 µM dNTPs, 10 pmol of each primer (forward and reverse), 1 pmol of labeled markers were used to amplify genomic DNA in total reaction volume of 25 µl. PCR was performed under the following conditions: (i) one cycle at 94°C for 4 min; (ii) five cycles, each at 94°C for 20 s, 62°C for 20 s, 72°C for 40 s; (iii) 30 cycles, each at 94°C for 20 s, 60°C for 20 s, 72°C for 40 s; (iv) 72°C for 2 min 30 s; and (v) 4°C soak. After each PCR reaction, 20 µl of formamide dye (98% deionized formamide, 0.05% xylene cyanol and 0.05% bromophenol blue with 4 N NaOH) were added to each sample, and the samples were denatured for 4 min at 94°C. The products were electrophoresed through 5% denaturing polyacrylamide gels and visualized by autoradiography.

Linkage analysis
Pairwise two-point linkage analysis with MLINK of the LINKAGE program was utilized (32). Disease penetrance was set at 0.95, without a gender difference, and the normal and disease allele frequencies were set at 0.999 and 0.001, respectively. Individuals in the fourth and fifth generations of kindred 2685 were classified as either affected, unaffected or unknown for the purpose of linkage analysis (Fig. 1).

Immunohistochemistry
Formalin-fixed tissue samples of cerebral and cerebellar white matter were transferred into sodium phosphate-buffered sucrose solution (18%, pH 7.2). After an overnight infiltration at 4°C, 40 µm thick sections were prepared with a vibratome. Sucrose was removed by washing in water, and the sections were immersed in 95% ethanol to accomplish partial delipidization prior to incubation with antibodies to CNS myelin proteins. For the visualization of GFAP, vimentin and insulin-like growth factor, the ethanol step was omitted. Where applicable, ethanol was removed by washing in water. The sections were immersed in a 0.1 M solution of sodium metaperiodate. After 15 min, the oxidant was removed by washing with phosphate-buffered saline (PBS). Tissue aldehyde was then reduced by a 10 min incubation in a 5% solution of sodium borohydride in water, and after washing, tissues were made more permeable by immersion in a 5% solution of dimethylsulfoxide in PBS (pH 7.2). The sections were pre-incubated in a mixture of 10% normal horse serum in PBS that also contained 0.1% Triton X-100 (by volume). PBS containing 0.1% Triton X-100 and 1% normal horse serum (by volume) was used to dilute the following antibodies: polyclonal anti-proteolipid protein (33); polyclonal anti-MBP (a kind gift of Dr Marian Kies); polyclonal anti-MAG peptide (a kind gift of Dr James Salzer); polyclonal anti-vimentin (Sigma, St Louis, MO); polyclonal anti-insulin-like growth factor I (Upstate Biotechnology, Lake Placid, NY); monoclonal anti-cyclic nucleotide phosphodiesterase and anti-GFAP (Sternberger Monoclonals, Lutherville, MD). The sections were incubated overnight at 4°C under constant agitation. Immunoreactive sites were visualized by a standard procedure that involved a secondary biotinylated antibody to rabbit or mouse IgG (depending on the nature of the antibody) and the avidin–biotin–peroxidase complex method (34). The final reaction product was generated by incubation of the sections in a Tris-buffered solution (pH 7.6) of 0.1% diaminobenzidine and 0.02 hydrogen peroxide (by weight). Nuclear counterstaining was by brief immersion in Mayer’s hematoxylin. The sections were placed onto positively charged glass slides, dehydrated, and mounted in a xylene-soluble medium (Permount).

Quantitative western blots of GFAP
Samples of frozen gray and white matter were homogenized in water and lyophilized. The residues were extracted with six changes of a mixture containing 3 parts diethyl ether and 2 parts ethanol (1 ml/mg dry weight). After evaporation of the organic solvents, the delipidized material was dispersed in sample buffer (35) and heated at 100°C for 10 min. Samples of the solubilized tissues corresponding to 20 mg wet weight were electrophoresed on SDS–polyacrylamide gels (12% acrylamide and 1% cross-linker), electroblotted onto nitrocellulose membrane (36) and visualized immunchemically with anti-GFAP and the diaminobenzidine/hydrogen peroxide mixture (as for immunocytochemistry). The enzymatic reaction was allowed to proceed for 30 s. Standard amounts of bovine GFAP (Boehringer Mannheim, Indianapolis, IN) were electrophoresed in separate lanes and visualized on the same nitrocellulose blot. These samples constituted the basis for densitometric quantitation of GFAP in tissue samples (Table 2).

ACKNOWLEDGEMENTS
We would like to thank the families for their ongoing participation in this study. In addition, the authors wish to thank Dr Ric Hamsberger, Leslie Jerominski and the Genomics Core Facility.
at the University of Utah for technical assistance. Drs John Rose and Mahendra Rao provided valuable discussions and critical review of the manuscript. This investigation was supported, in part, by the Howard Hughes Medical Institute, NIH grant NS32711 (L.J.P.), a pilot grant from the MS society, and Public Health Service Research grant M01-RR00064 from the National Center for Research Resources.

REFERENCES


