Localization of the Gene for Familial Laryngeal Abductor Paralysis to Chromosome 6q16

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Background: Vocal fold paralysis is a common cause of neonatal stridor. Although it is usually classified as idiopathic or iatrogenic in origin, a small subset of patients have a family history of this disorder, indicating a possible genetic cause.

Objective: To identify the genetic locus of the gene that causes familial laryngeal abductor paralysis.

Design: A standard nonorganic protocol was used to extract DNA from whole-blood samples. The DNA samples were quantified by DNA fluorometry, and the concentration of all samples was standardized at 40 ng/µL. A pooled DNA strategy was used to facilitate rapid polymerase chain reaction screening of markers in the Weber v8.0 genome screening set. Polymerase chain reaction screening of individual DNA samples was performed using possible linked markers initially identified as having an allele that appeared with a higher incidence in the affected vs the nonaffected pool. Statistical analysis of possible linkage was performed using the LINKAGE 5.1 set of linkage analysis computer programs.

Subjects: A family in which a form of familial laryngeal abductor paralysis segregates was ascertained. Whole blood samples were drawn from 40 participating individuals within this family after the subjects’ fully informed consent was obtained.

Results: Initial screening of the pooled DNA specimens revealed a band pattern for D6S1021 on chromosome 6q16, indicating an allele with a higher incidence in the affected vs the nonaffected pool. Two-point analysis of individual allele patterns confirmed linkage to D6S1021 with an lod score of 3.86 (θ=0.0) at a penetrance value of 0.8. Haplotype analysis with flanking markers defined a 5-centimorgan critical region between D6S283 and AFMA047YG1.

Conclusion: An autosomal dominant form of familial laryngeal abductor paralysis is linked to a 5-centimorgan region on chromosome 6q16 surrounding D6S1021.


Vocal Fold Paralysis (VFP) is a frequent cause of congenital stridor and airway obstruction. Among different laryngeal anomalies causing stridor, VFP is second only to laryngomalacia in frequency. Persistent hoarseness, dysphagia, and recurrent aspiration pneumonia are complications that may develop because of laryngeal immobility. When significant airway obstruction exists, it is a potentially life-threatening condition that usually requires a tracheostomy to provide an adequate airway. Traditionally, VFP has been classified as acquired (postintubation, injury from cardiothoracic surgery), neurologic (secondary to Arnold-Chiari malformation), or idiopathic. However, multigenerational kindreds have been described in which several persons are afflicted with congenital VFP, indicating a definite genetic component in these families.

In this study, we used linkage analysis on a large family with an autosomal dominant form of familial laryngeal abductor paralysis (FLAP) to identify a genetic locus for this specific form of hereditary vocal fold immobility.

Patients and Clinical Details

The family we studied displayed a dominantly inherited form of congenital laryngeal abduction paralysis, as previously described in a report by Manaligod and Smith. A summary of the family is presented below. In summary, 3 individuals in this family were identified who required tracheostomy soon after birth for bilateral vocal fold abductor paralysis. The propositus (individual 40) had absence of abduction of the vocal folds documented clinically and by laryngeal electromyography. The other 2 individuals (individuals 26 and 28) were sec-
SUBJECTS, MATERIALS, AND METHODS

PATIENTS AND SAMPLES

A family displaying a familial form of FLAP was ascertained through the University of Iowa Hospitals and Clinics, Iowa City (Figure 1). The study was approved by the institutional review board of the University of Kentucky, Lexington; appropriate informed consent was obtained from subjects. Consenting individuals were screened by a directed history and physical examination, focusing primarily on symptoms of stridor and cyanosis at birth or persistent hoarseness. Fully affected individuals also underwent flexible and rigid laryngoscopy and bronchoscopy as part of their clinical care. The propositus also underwent laryngeal electromyography.

GENOTYPING

Following a standard nonorganic protocol, DNA was extracted from 10 mL of whole blood from each consenting individual. We used the CHLC (Cooperative Human Linkage Center)/Weber v8.0 human screening set of short tandem repeat polymorphisms (STRPs) (Research Genetics, Huntsville, Ala) to complete a genome-wide screen. This screening set contains 386 polymerase chain reaction (PCR)–based primer pairs that densely cover the entire human genome with an average intermarker spacing of 10 centimorgans.

To facilitate rapid marker screening, we used a DNA pooling strategy. Initially, individual samples were diluted to a concentration of 40 ng/μL. A standard PCR reaction then was carried out using all individual DNA samples to verify that amplified STRPs were of equal intensity in all individuals. In cases of unequal amplification, concentrations were adjusted and the PCR repeated. Once equal DNA concentrations had been confirmed, affected and nonaffected sample pools were created by combining equivalent concentrations of the appropriate individual samples.

Rapid PCR screening of the reference panel of markers was carried out by visually comparing band intensities between the affected and nonaffected pools. For an autosomal dominant inheritance pattern, 50% of the alleles in the PCR sample from the affected pool are identical if a STRP is linked to the disease phenotype. This association means that one band will have a higher intensity among multiple bands of weaker intensities; in the nonaffected pool, in contrast, there will be multiple bands of similar intensities.

After possible linked markers were identified, PCR genotyping of individual samples was performed with the specific STRPs tightly linked to the candidate marker. All PCR products were separated by electrophoresis on a 6% denaturing acrylamide gel and visualized by autoradiography through the incorporation of phosphorus 33–labeled dATP (deoxyadenosine triphosphate) during PCR.

LINKAGE ANALYSIS

Two-point linkage analyses were carried out using the MLINK subroutine of the LINKAGE package computer programs, version 5.1. Maximum lod scores (Zmax) were calculated using the ILINK subroutine of LINKAGE 5.1. Allele frequencies were assumed to be equal for each marker, and recombination frequencies were assumed to be equal for both sexes. The frequency of the disease allele was arbitrarily set at 0.0001 because of its rarity.

An assumed penetrance level of 80% was initially used to calculate lod scores. Additional lod scores for marker D6S1021 were calculated at different penetrance levels as well, because the actual penetrance of this disorder is unknown. Multipoint linkage analysis was carried out using the LINKMAP subroutine of LINKAGE 5.1. Three-point rolling analyses were performed using adjacent markers and the disease locus, basing marker orders and genetic distances on the Genethon linkage map (Genethon, Evry France; available at: http://www.genethon.fr).

HAPLOTYPE ANALYSIS

Polymorphic markers that flank the linked marker by approximately 10 centimorgans were identified using the Human Physical Mapping Project database of the Center for Genome Research (Whitehead Institute for Biomedical Research, Cambridge, Mass; available at: http://www .genome.wi.mit.edu/) for cytogenetic locations of FLAP markers. These markers were used with PCR to screen the individual DNA samples in order to detect recombination events centromeric and telomeric to the initial identified marker to define the critical region that could contain the genetic locus for this disorder.

ond cousins of the mother of the propositus. Bilateral laryngeal abductor paralysis was documented in these 2 individuals by direct laryngoscopy and bronchoscopy prior to tracheostomy. In all 3 patients, laryngeal abduction appeared intact clinically. No other neurologic or autonomic abnormalities were evident on physical examination.

All 3 fully affected individuals displayed variable recovery of vocal fold movement over time. Individual 40 developed partial vocal fold abduction over the 2 years following his tracheostomy. At age 3 years, although he had only minimal vocal fold abduction, this patient underwent successful decannulation of his tracheostomy. Individual 26 showed gradual complete recovery of vocal fold movement over the first 4 years of life, resulting in decannulation at age 4½ years. Finally, individual 28 recovered only right vocal fold abduction; nevertheless, she underwent successful decannulation at age 2 years.

After the other family members were questioned, 4 individuals were identified who had symptoms of stridor and recurrent cyanosis during infancy; these individuals were classified as affected with decreased expression. In all 4 individuals, these symptoms gradually resolved over the first 3 to 5 years of life. One of these individuals, the maternal grandfather of the propositus, underwent video laryngoscopy, which showed no persistent vocal fold movement abnormalities. Of the 4 persons meeting these criteria, 2 were obligate carriers. Four other individuals who were obligate carriers were clini-
were significant at all penetrance levels between 0.2 and 0.99, and lod scores D6S1021 (in the chromosome 6q16 region), as a possible linked marker. Individual genotyping and linkage analysis (MLINK) performed with this marker produced a Zmax of 3.86 (θ=0.0) at a penetrance value of 0.8. Additional genotyping with flanking markers identified a second marker, D6S1546, that also produced a significant lod score (Zmax = 3.77, θ=0.0) (Table 1).

The Zmax scores for D6S1021 were calculated with penetrance levels between 0.1 and 0.99, and lod scores were significant at all penetrance levels between 0.2 and 0.99 (Table 2). Multipoint analysis with adjacent markers in the chromosome 6q16 region generated a maximum lod score of 4.25 between markers D6S1546 and D6S1021 (Figure 2).

HAPLOTYPE ANALYSIS

To identify the margins of the critical region linked to FLAP, additional STRP markers within 10 centimorgans of D6S1021 were used for haplotype analysis. A recombination at marker D6S1692 was noted for individual 28, marking the centromeric border of the critical region. Another recombination event was discovered with marker AFMA047YG1, involving individuals 32 and 40, marking the telomeric border of the region (Figure 1). Based on the Whitehead Institute physical map of chromosome 6, these markers define a 5-centimorgan region on chromosome 6q16 (Figure 3).

Vocal fold immobility is a common cause of stridor and airway obstruction in infants. In a review by Emery and Fearon,64% of patients with acquired cases of VFP (intubation or birth trauma) achieved some degree of spontaneous recovery over time, in contrast to only 29%
of patients with congenital cases (hereditary and idiopathic). In a similarly structured study, Daya and associates saw a slightly higher rate of recovery of vocal fold motion in idiopathic VFP (46% [12/26]). An interesting discovery of that report was the recovery of vocal fold motion in cases of idiopathic bilateral VFP as late as 11 years of age.

Vocal fold paralysis has been further differentiated based on whether the primary defect is in vocal fold abduction (glottic opening) or adduction (glottic closure). Plott originally described the former type of paralysis in a family with 3 brothers who had an X-linked recessive form of congenital laryngeal abductor paralysis and mental retardation (Online Mendelian Inheritance in Man; available at: http://www.ncbi.nlm.nih.gov/omim/; Plott syndrome, OMIM 308850). Subsequent reports of families with both laryngeal paralysis and varying degrees of mental retardation have supported an X-linked recessive inheritance pattern. A second syndrome, Gerhardt syndrome, is a form of FLAP with autosomal dominant inheritance and variable penetrance and expressivity (Gerhardt syndrome, OMIM 150260). Although mental retardation is not a common feature, subtle central neurologic abnormalities can be demonstrated in some kindreds.

The finding of additional neurologic deficits and other malformations in conjunction with laryngeal abductor paralysis is common. Pridmore et al described a family whose affected members had bilateral VFP and progressive distal spinal muscular atrophy. In another family, described by Boltshauser et al, affected persons had similar symptoms in addition to progressive sensorineural hearing loss. Other known syndromes of central neurologic impairment, such as Möbius syndrome, occasionally feature laryngeal paralysis if the vagus nerve is affected.

The most likely anatomic site of involvement in FLAP is the nucleus ambiguus. Genetic abnormalities that affect the development of this central nucleus also may affect those portions of the brain and brainstem that develop concomitantly. The result would be phenotypic

Table 1. Pairwise lod Scores for Chromosome 6q16 Markers and Familial Laryngeal Abductor Paralysis

<table>
<thead>
<tr>
<th>Chromosome Marker</th>
<th>0</th>
<th>0.01</th>
<th>0.05</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
<th>0.5</th>
<th>Maximum lod Score (Zmax)</th>
<th>Recombination Fraction (u)</th>
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<tbody>
<tr>
<td>D6S1692</td>
<td>-2.05</td>
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<td>0.34</td>
<td>0.52</td>
<td>0.54</td>
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<td>3.71</td>
<td>3.46</td>
<td>3.13</td>
<td>2.39</td>
<td>1.57</td>
<td>0.68</td>
<td>0</td>
<td>3.77</td>
<td>0</td>
</tr>
<tr>
<td>D6S1021</td>
<td>3.86</td>
<td>3.8</td>
<td>3.56</td>
<td>3.23</td>
<td>2.49</td>
<td>1.67</td>
<td>0.76</td>
<td>0</td>
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<tr>
<td>D6S1592</td>
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<td>1.12</td>
<td>1.23</td>
<td>1.21</td>
<td>0.99</td>
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<tr>
<td>D6S447</td>
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Table 2. Maximum lod Scores for D6S1021 and Familial Laryngeal Abductor Paralysis at Varying Penetrance Levels

<table>
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<th>Penetrance</th>
<th>Maximum lod Score (Zmax)</th>
<th>Recombination Fraction (u)</th>
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<tr>
<td>0.1</td>
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<td>0.99</td>
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Figure 2. Multipoint analysis of chromosome 6q16 markers and familial laryngeal abductor paralysis. Marker distances (in parentheses) are in centimorgans (cM) relative to marker D6S1021.

Figure 3. Chromosome 6 and putative marker order in the D6S1021 region.
heterogeneity and variability in the severity of the laryngeal paralysis.

In this study, we demonstrated statistically significant linkage between D6S1021 and the form of FLAP segregating in this family. This is the first definitively identified locus for any form of laryngeal dysfunction. Haplotype analysis narrows the critical region to a 5-centimorgan interval on chromosome 6q16 flanked by D6S1692 and AFMA047YG1. Twenty-four uncharacterized expressed sequence tags and several genes have been mapped to this area (GeneMap 99; National Center for Biotechnology Information, Bethesda, Md/International Radiation Hybrid Mapping Consortium) and to several genes, including KIAA0331, PREP (prolyl endopeptidase, an endopeptidase that cleaves neuropeptides), FKHR1 (forkhead Drosophila homolog rhabdomyosarcoma like 1), CDW52, ASP (apoptosis-specific protein, a protein specific for programmed cell death) sequences, and GPR6 (G protein–coupled receptor 6). Both PREP and ASP may have roles in neurological and developmental function and should be considered as particularly strong candidate genes for FLAP.

Future efforts to identify the specific gene mutation that causes this disorder should focus primarily on genes expressed in the central nervous system that show sequence homology to known genes implicated in neural development and differentiation. Because this family (as well as other described pedigrees) displays anticipation, a phenomenon caused by exansile trinucleotide repeats in diseases such as fragile X syndrome and Friedreich ataxia, the same process may underlie this disorder. Therefore, candidate genes within this interval that contain trinucleotide repeats should be considered strong possibilities for the FLAP gene at this locus.

Accepted for publication February 7, 2001.

This research was enabled by support from the Kentucky Children’s Miracle Network, Lexington.

We are extremely grateful to the family members who participated in this study.

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REFERENCES