Human Molecular Genetics, 1997, Vol. 6, No. 2 213–219

Homozygosity mapping of Alström syndrome to chromosome 2p

Gayle B. Collin, Jan D. Marshall, Lon R. Cardon[1] and Patsy M. Nishina*

The Jackson Laboratory, 600 Main Street, Bar Harbor, ME 04609-1500, USA and [1]Sequana Therapeutics, 11099 North Torrey Pines Road, La Jolla, CA 92037, USA

Received July 26, 1996; Revised and Accepted November 27, 1996

Alström syndrome is a rare autosomal recessive disorder characterized by pigmentary retinal degeneration, sensorineural hearing loss, childhood obesity, non-insulin-dependent diabetes mellitus, hyperlipidemia and chronic nephropathy. Features occasionally observed include acanthosis nigricans, hypogonadism, hypothyroidism, alopecia, short stature and cardiomyopathy. We report here the results of a linkage study in a large French Acadian kindred, as a first step in identifying the molecular basis of Alström syndrome. Evidence of a founder effect made it feasible to use a homozygosity mapping strategy to identify the chromosomal location of the Alström gene. In a genome-wide screen, haplotype sharing for a region on chromosome 2 was observed in all affected individuals. Two point linkage analysis resulted in a maximum lod score of 3.84 ($\theta = 0.00$) for marker *D2S292*. By testing additional markers, the disease gene was localized to a 14.9 cM region on chromosome 2p.

INTRODUCTION

Alström syndrome is an autosomal recessive disorder in which the earliest manifestations are pigmentary retinal degeneration, sensorineural hearing loss and childhood obesity (1,2). Chronic nephritis, non-insulin-dependent diabetes mellitus (NIDDM) and hepatic dysfunction occur in the final stages of the disease, between the second and fourth decade of life. Other features observed in some, but not all, affected individuals include acanthosis nigricans, male hypogonadism, hypothyroidism, alopecia, short stature and infantile cardiomyopathy (3–8). With the exception of studies showing normal chromosomal karyotypes in Alström patients (8,9), no attempt has been made to genetically define this syndrome.

A number of diseases with founder effects have been identified in the French Acadian population (10). This is due in part to the small number of individuals that were sent to colonize French Canada and the rapid expansion of the population thereafter (11). As a first step in determining the molecular basis for Alström syndrome, we began a linkage study in a large kindred of French Acadian ancestry. The identification of a common founder pair and the high degree of consanguinity in this kindred (average kinship coefficient = 0.01) allowed us to use the homozygosity mapping strategy to identify the chromosomal location of Alström syndrome (12). Assuming that the chromosomal region flanking the Alström gene was likely to be homozygous-by-descent, we performed a candidate gene and genome-wide search and identified a 14.9 cM interval on chromosome 2p segregating with Alström syndrome.

RESULTS

Candidate gene evaluation

Individuals with Alström syndrome and the mouse mutant tubby share marked phenotypic similarities including obesity, insulin resistance and retinal and cochlear degeneration. We hypothesized that tubby was a homolog of Alström and initially tested for linkage of Alström to human chromosome 11p15, the homologous human region to mouse chromosome 7 where *tub* maps (13). Additionally, linkage to homologous chromosomal regions of other mouse obesity genes (i.e. *fat*, *ob*, A^y and *db*) and to growth-associated candidate genes [i.e. growth hormone (GH), GH receptor and GH-releasing factor] were tested. No linkage was observed at any of the loci examined.

Homozygosity mapping of Alström to chromosome 2p

Consequently, we performed a genome-wide scan using the homozygosity mapping strategy with 226 polymorphic markers distributed at 15–25 cM intervals throughout the genome (14,15). To screen the genome rapidly, five affected individuals and one obligate heterozygote (carrier) were genotyped. A bias for homozygous alleles among the affected individuals was observed for seven markers on six chromosomes (Fig. 1). Subsequently, DNAs from parents and unaffected siblings were tested for each of these and nearby flanking marker loci. The only haplotype that co-segregated with the disease phenotype was a region on chromosome 2. To investigate further this apparent linkage to Alström syndrome, additional flanking markers were evaluated on all available DNAs from this kindred and haplotypes of family members were constructed (Fig. 2). Homozygosity for common alleles at markers *D2S136*, *D2S292*, *D2S2113*, *D2S327* and *D2S145* was observed in all affected individuals. The conserved founder haplotype for markers *D2S393*, *D2S136*, *D2S292*, *D2S2113*, *D2S327*, *D2S145* and *D2S286* appears to be

*To whom correspondence should be addressed

6-5-5-1-4-1-5, respectively. Ancestral recombination events in subjects 353 and 373 place the disease gene distal to *D2S286*, while an ancestral recombination event in subject 500 places the disease gene proximal to *D2S393*. Additionally, a maternal meiotic event between *D2S145* and *D2S286* (subject 372) further defines the proximal boundary of the Alström region. Altogether, these informative recombinational events delineate the Alström region to a 14.9 cM interval flanked by markers *D2S393* and *D2S286* (Fig. 3).

Statistical analysis

Pairwise linkage analysis between the Alström locus and the seven chromosome 2p markers was performed using the MLINK program of the LINKAGE 5.1 computer package (16). Although a founder effect is indicated by examination of >1600 individuals in this pedigree, inclusion of all relationships would have exceeded the capacity of the LINKAGE program. Additionally, identification of all consanguineous loops, especially in the earlier generations, may be incomplete. Therefore, linkage analysis was only done on those generations where most, if not all, genealogical relationships were established (Fig. 2).

The use of incorrect marker allele frequencies in linkage analysis may result in erroneous lod score calculations (17). Ideally, marker allele frequencies should be obtained from the same genetic population as the diseased individuals. In this study, pairwise analysis was completed using marker allele frequencies from unrelated individuals in the general population (GDB) and in the French Acadian population (Table 1). Although some variation in the frequencies of alleles was observed among the two populations, there was no significant effect on the calculated lod scores. All data included in this report, therefore, are based on the estimated French Acadian marker allele frequencies. Two point analysis indicated linkage of Alström syndrome to the chromosome 2 loci tested, with a maximum lod score of 3.84 (θ = 0.00) with marker *D2S292* (Table 2). We expect that the calculated lod scores are underestimated since two point and multipoint linkage analyses could not be performed with all genealogical relationships linking affected individuals to a common ancestor.

Table 1. Marker allele frequencies in French Acadians[a]

Marker	Allele	Size (bp)	Frequency	Marker	Allele	Size (bp)	Frequency
D2S136	1	91	0.06	*D2S327*	1	115	0.02
	2	95	0.36		2	121	0.23
	3	97	0.06		3	123	0.19
	4	105	0.02		4	125	0.23
	5	107	0.40		5	127	0.27
	6	109	0.10		6	129	0.04
					7	131	0.02
D2S145	1	248	0.69				
	2	262	0.02	*D2S393*	1	86	0.02
	3	264	0.02		2	88	0.06
	4	266	0.10		3	92	0.25
	5	268	0.00		4	94	0.19
	6	270	0.13		5	95	0.06
	7	274	0.04		6	96	0.38
					7	98	0.02
D2S286	1	136	0.38		8	100	0.00
	2	138	0.02		9	102	0.02
	3	140	0.17				
	4	142	0.04	*D2S2113*	1	166	0.11
	5	144	0.29		2	178	0.00
	6	146	0.06		3	184	0.04
	7	152	0.02		4	188	0.02
	8	156	0.02		5	192	0.32
					6	194	0.13
D2S292	1	180	0.33		7	196	0.08
	2	184	0.23		8	198	0.04
	3	186	0.06		9	200	0.08
	4	188	0.15		10	202	0.08
	5	190	0.17		11	204	0.06
	6	194	0.02		12	206	0.04
	7	196	0.04				
	8	202	0.00				

[a]Allele frequencies were estimated from 24 French Acadians.

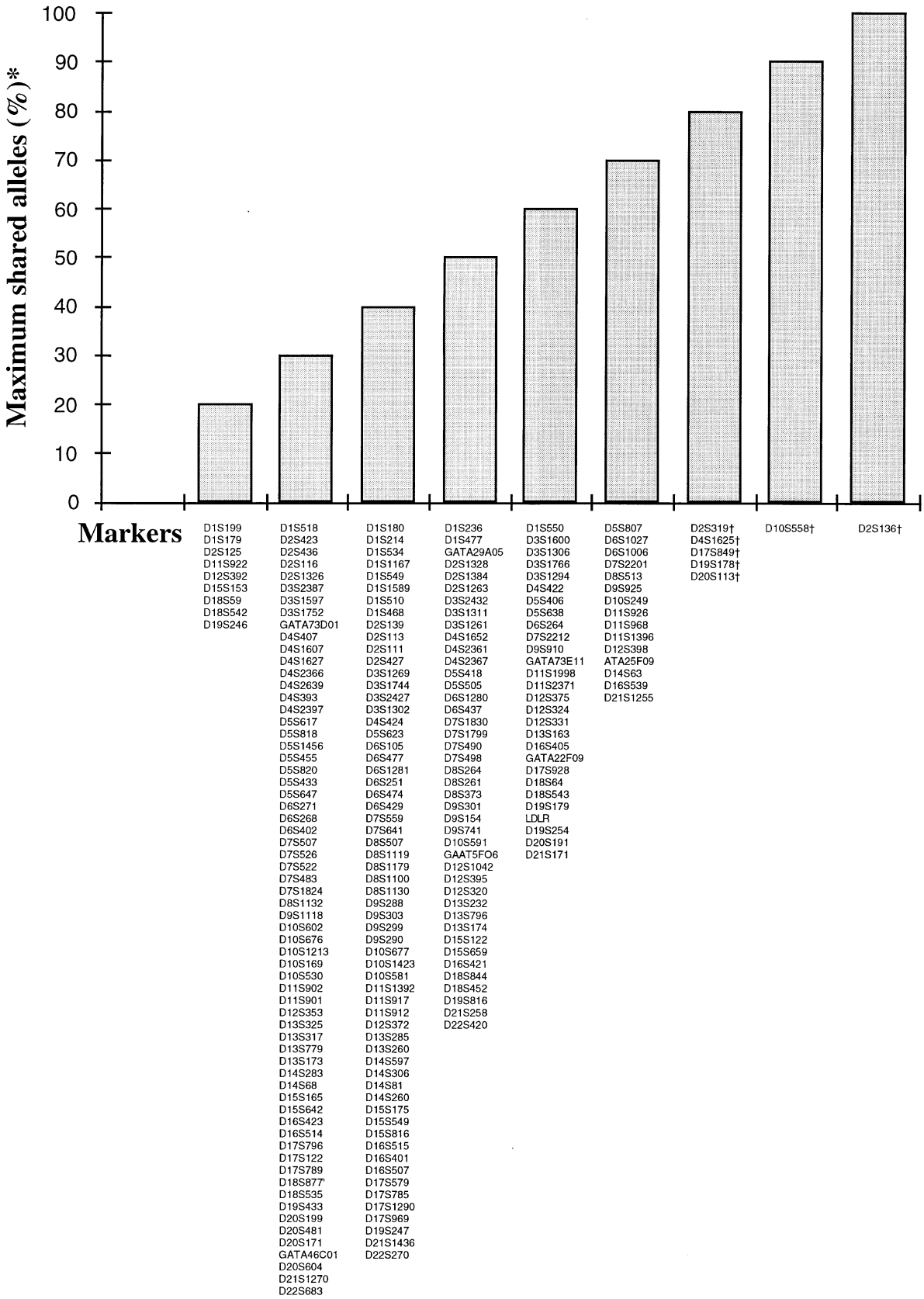

Figure 1. Allele sharing observed in a genome-wide scan among five individuals affected with Alström syndrome (*n* = 226 markers). *(% shared alleles = maximum number of shared alleles/total number of alleles). †The chromosomal region was examined further for linkage.

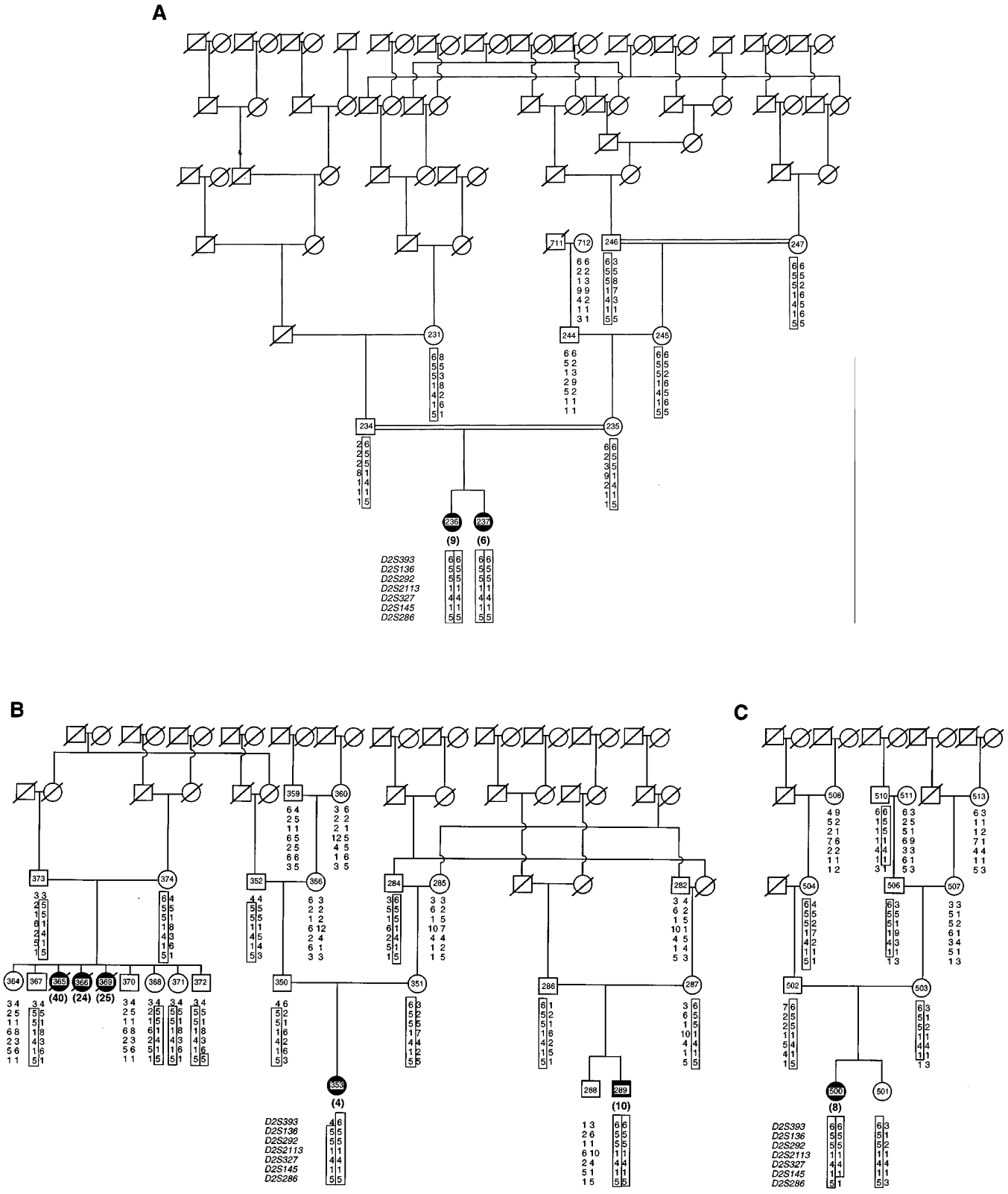

Figure 2. Three cohorts (**A**, **B** and **C**) of a large French Acadian kindred segregating for Alström syndrome. Filled symbols represent affected individuals and open symbols represent unaffected individuals. Slashed symbols indicate that an individual is deceased. Conserved haplotypes segregating with the Alström locus are boxed. All affected individuals are homozygous for a common haplotype defined by markers *D2S136*, *D2S292*, *D2S2113*, *D2S327* and *D2S145*. Ages of the affected individuals are indicated in parentheses. Clinical manifestations of subjects 236 and 237 and subjects 289 and 353 have been described previously in refs 4 and 6, respectively. Subjects 711 and 712 are not of French Acadian descent.

Table 2. Two point lod scores between Alström syndrome and chromosome 2 markers[a]

Marker	Recombination fraction							θ	Z_{max}
	0.00	0.01	0.05	0.10	0.20	0.30	0.40		
D2S393	–2.21	1.33	1.68	1.57	1.14	0.70	0.31	0.05	1.68
D2S136	2.49	2.43	2.19	1.91	1.39	0.91	0.45	0.00	2.49
D2S292	3.84	3.72	3.24	2.68	1.74	1.03	0.48	0.00	3.84
D2S2113	3.66	3.53	3.04	2.44	1.40	0.68	0.24	0.00	3.66
D2S327	3.25	3.14	2.73	2.25	1.46	0.90	0.44	0.00	3.25
D2S145	1.02	0.96	0.75	0.54	0.29	0.17	0.09	0.00	1.02
D2S286	2.23	2.22	2.06	1.78	1.21	0.73	0.34	0.00	2.23

[a]Lod scores were calculated using allele frequencies from the French Acadian population.

Table 3. Linkage disequilibrium

Marker	Allele	N(T)	N(U)	χ^2	*P*	θ_{obs}	$\theta_{control}$
D2S393	6 (96)	9/10	3/10	7.50	0.02	0.30	0.18
D2S136	5 (107)	10/10	0/10	20.00	<0.0001	0	0
D2S292	5 (190)	10/10	1/10	16.36	0.0001	0	0
D2S2113	1 (166)	10/10	2/10	13.33	0.001	0	0
D2S327	4 (125)	10/10	3/10	10.77	0.003	0	0
D2S145	1 (248)	10/10	6/10	5.00	0.09	0	0
D2S286	5 (144)	9/10	2/10	9.90	0.005	0.13	0.15

Frequency of transmitted (T) and untransmitted (U) chromosomes in affected individuals. Associated alleles and their sizes (bp) are presented, as well as estimates of the number of ancestral recombinants using unaffected frequencies from the observed untransmitted chromosomes (θ_{obs}) and the control sample ($\theta_{control}$). Probability values for the χ^2 statistics are derived from Fisher's exact test to accommodate the small sample sizes.

The region homozygous-by-descent on chromosome 2 was evaluated further for linkage disequilibrium. Simple contingency tables were used to compare the frequencies of associated alleles on parental transmitted (T) chromosomes and untransmitted (U) chromosomes. Fisher's exact test demonstrated a significant association between the alleles shared by Alström subjects and the transmitted parental chromosome at several markers tested (Table 3).

DISCUSSION

The characteristics of obesity, blindness and deafness have been observed in a number of childhood syndromes including Alström and Bardet–Biedl syndrome (18–20). Previous studies have demonstrated linkage of Bardet–Biedl to four independent loci: 3q, 11q, 15q and 16q (21–24). The mapping of Alström syndrome to chromosome 2p demonstrates that the two syndromes are genetically distinct. However, the overlapping phenotypes observed in Alström and Bardet–Biedl subjects suggests that the mutations may have arisen in common developmental pathways.

Goldstein and Fialkow (2) suggested that the molecular defect for Alström may be in a gene encoding a protein necessary for cell membrane integrity that is expressed in all affected end organs including the retina, neural elements of the ear, kidney, skin, testis and adipose. A defect in the architecture of the cell might explain the retinal, aural, renal, hepatic and cardiac muscle dysfunction and the resistance of tissue to recognize various hormones (e.g. insulin, vasopressin, gonadotrophin). The resulting insulin resistance could explain the obesity, acanthosis nigricans and hyperlipidemia. Membrane-related candidate genes mapping to 2p12–13 include *ADD2* (adducin-2) and *ANX4* (annexin IV) (25,26). Non-structural genes that lead to deafness and blindness in various human syndromes have also been identified recently. For example, mutations in the DNA-binding protein, HuP2 and its mouse homolog *Pax3*, which is expressed in early neurogenesis and necessary for normal neural development, have been identified as the cause of some forms of Waardenburg syndrome (27). Possible candidates for neural/endocrine and growth factors which map to the Alström minimal region include *GFPT* (glutamine-fructose-6-phosphate transaminase) and *TGFA* (transforming growth factor α) (28,29). As we refine the Alström interval and assemble a physical contig to facilitate the positional cloning of the disease locus, candidate genes mapping within the minimal region will be examined. In addition, the physical contig will be screened for tubby-like sequences, as the possibility still exists that a member of the tubby gene family may be responsible for the phenotypically similar Alström syndrome.

Although Alström syndrome is a rare genetic disorder, the characteristics of obesity, NIDDM and/or retinal and cochlear degeneration are commonly observed in the general population. The identification of the Alström disease gene may provide useful insight into the pathophysiology of these common diseases. In addition, characterization of the Alström gene and genes responsible for similar syndromes such as Bardet–Biedl should lead to a better understanding of the pathways necessary for normal development.

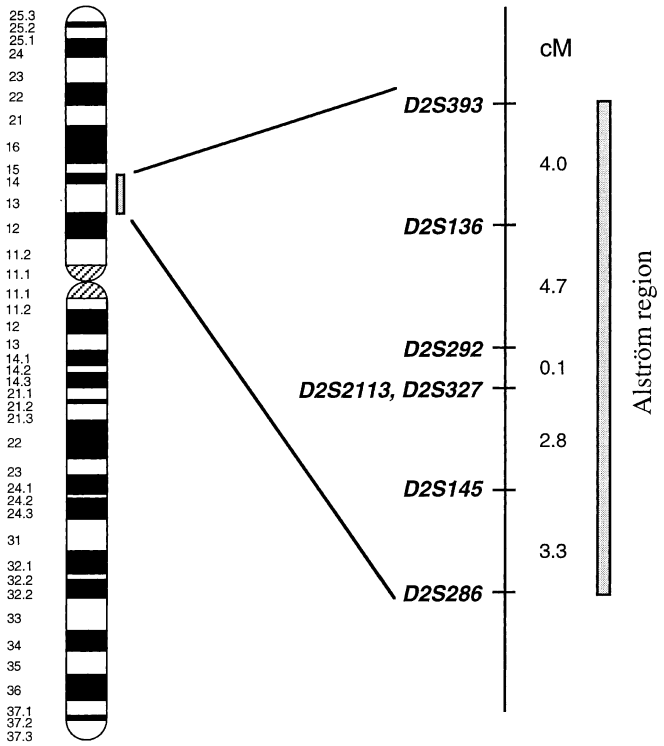

Figure 3. Genetic map of the chromosome 2p markers linked to Alström syndrome (15). Recombination distances are given in centimorgans (cM). *Ideogram obtained from GDB.

MATERIALS AND METHODS

Family material

The study was carried out in a large French Acadian kindred whose ancestry has been traced back to the mid-1600s in western Nova Scotia (J.D. Marshall *et al.*, in preparation). Extensive genealogical data were obtained for this kindred (>1600 individuals recorded). Thus far, we have identified one common ancestral pair for all parents of affected individuals. For parents of affected individuals, the average kinship coefficient is 0.010, ranging from 0.00001 to 0.03125. Thus, while some parents are as related as first cousins (once removed), on average, parents of affecteds are more distantly related than second cousins.

Five living subjects were clinically evaluated by a medical geneticist at the Izaak Walton Killam Hospital for Children (IWK), Halifax, N.S., Canada. All subjects were obese (BMI >95th percentile for age and gender) and displayed early retinopathy, sensorineural hearing loss and hyperinsulinemia. None of the subjects exhibited digital abnormalities or mental retardation. Limited medical records from three deceased subjects (#365, 366, 369) were obtained from IWK and Yarmouth Regional Hospital, Yarmouth, N.S., Canada. In addition to the clinical features described above, these deceased subjects developed NIDDM in their late teens and eventually died of renal failure.

Genotyping

Genomic DNA was isolated from peripheral blood by a standard protocol. Radiolabeled PCR reactions were performed as previously described (30). PCR products were separated on a 6% denaturing polyacrylamide gel and visualized by autoradiography. A total of 226 CHLC (Cooperative Human Linkage Center) and Genethon markers (Research Genetics), heterozygosity >70%, were used in the genome scan (14,15). Amplification of short tandem repeat polymorphisms (STRPs) was performed initially on one obligate heterozygote (carrier) and five affected individuals. Whenever an excess of homozygous alleles (>70% shared alleles) was observed in the affected individuals for a particular marker, the chromosomal region was examined further for linkage. Affected individuals, parents and unaffected siblings were genotyped with the marker and nearby flanking markers.

Statistical analysis

Pairwise lod score analysis was performed on all family members using the MLINK program of the LINKAGE 5.1 computer package (16). Because of the complexity in depth and inbreeding in this population (~30 consanguinity and marriage loops), linkage analysis could not be conducted using all familial relationships. Linkage analysis assumed an autosomal recessive mode of inheritance, full penetrance and a gene frequency of 10^{-6}. Linkage analysis assuming a gene frequency of 10^{-4} had negligible effects on the calculated lod score. Marker allele frequencies in the general population were obtained from Genome Data Base (GDB) and marker allele frequencies in the French Acadian population were calculated from 24 unrelated individuals. DNAs from unrelated French Acadians were isolated from individuals of French Acadian descent from the same geographical region.

As an alternative to classical linkage analysis, linkage disequilibrium tests were conducted to compare the frequencies of associated alleles on transmitted and untransmitted chromosomes as described in (31,32). Simple contingency tables (2×2) were employed to test the null hypothesis of no difference in allele frequencies between transmitted and untransmitted chromosomes. Fisher's exact test was used as a test of association in an attempt to provide a more valid test with the small size of available affected individuals.

A further attempt was made to estimate the average number of ancestral recombinants (θ) between the putative chromosome 2 mutation and each associated allele. Using the approach of (33), $y = x + (1 - x)e^{-\theta}$, where y and x represent the allele frequencies on affected and normal chromosomes, respectively. Estimates of θ were derived using sample frequencies of y and x. We note that these estimates of θ are likely to be poor due to the small number of chromosomes available for estimation of y and x. To partially alleviate the expected high degree of variability, estimates of x were calculated from two sources: the untransmitted chromosomes (x_{obs}) and the sample of 24 unrelated French Acadian controls ($x_{control}$). We have no other source for estimates of y; therefore, the corresponding θ values should be interpreted as only suggestive.

ACKNOWLEDGMENTS

We gratefully acknowledge the Alström family members for participation in this study, Dr Mark L. Ludman and colleagues for clinical evaluation of the patients, Carolyn S. Blake for recompiling the LINKAGE 5.1 program and Drs Barbara Knowles, Benjamin Taylor and Jürgen Naggert for review of the manuscript. This work was supported by the American Diabetes

Association, grant ADA528131 and the National Cancer Institute Support Grant, CA-34196.

REFERENCES

1. Alström, C.H., Hallgren, B., Nilsson, L.B. and Asander, H. (1959) Retinal degeneration combined with obesity, diabetes mellitus and neurogenous deafness. A specific syndrome (not hitherto described) distinct from Laurence–Moon–Biedl syndrome. A clinical endocrinological and genetic examination based on a large pedigree. *Acta Psychiatr. Neurol. Scand.*, **34** (Supplement 129), 1–35.
2. Goldstein, J.L. and Fialkow, P.J. (1973) The Alström syndrome. Report of three cases with further delineation of the clinical, pathophysiological, and genetic aspects of the disorder. *Medicine(Baltimore)*, **52**, 53–71.
3. Connolly, M.B. *et al*. (1991) Hepatic dysfunction in Alström disease. *Am. J. Med. Genet.*, **40**, 421–424.
4. Alter, C.A. and Moshang, T.J. (1993) Growth hormone deficiency in two siblings with Alström syndrome. *Am. J. Dis. Child.*, **147**, 97–99.
5. Millay, R.H., Weleber, R.G. and Heckenlively, J.R. (1986) Ophthalmologic and systemic manifestations of Alström's disease. *Am. J. Ophthalmol.*, **102**, 482–490.
6. Tremblay, F., LaRoche, R.G., Shea S.E. and Ludman, M.D. (1993) Longitudinal study of the early electroretinographic changes in Alström's syndrome. *Am. J. Ophthalmol.*, **115**, 657–665.
7. Warren, S.E. *et al*. (1987) Late onset dilated cardiomyopathy in a unique familial syndrome of hypogonadism and metabolic abnormalities. *Am. Heart. J.*, **114**, 1522–1524.
8. Michaud, J.L. *et al*. (1996) Natural history of Alström syndrome in early childhood: onset with dilated cardiomyopathy. *J. Pediatr.*, **128**, 225–229.
9. Rüdiger, H.W. *et al*. (1985) Impaired insulin-induced RNA synthesis secondary to a genetically defective insulin receptor. *Hum. Genet.*, **69**, 76–78.
10. McKusick, V.A. (1992) *Mendelian Inheritance in Man.* The Johns Hopkins University Press, Baltimore, MD.
11. Davignon, J. and Roy, M. (1993) Familial hypercholesterolemia in French-Canadians: taking advantage of the presence of a 'founder effect'. *Am. J. Cardiol.*, **72**, 6D–10D.
12. Lander, E.S. and Botstein, D. (1987) Homozygosity mapping: a way to map human recessive traits with the DNA of inbred children. *Science*, **236**, 1567–1570.
13. Coleman, D.L. and Eicher, E.M. (1990) Fat (*fat*) and tubby (*tub*): two autosomal recessive mutations causing obesity syndromes in the mouse. *J. Hered.*, **81**, 424–427.
14. Murray, J.C. *et al*. (1994) A comprehensive human linkage map with centimorgan density. Cooperative Human Linkage Center (CHLC). *Science*, **265**, 2049–2054.
15. Dib, C. *et al*. (1996) A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature*, **380**, 152–154.
16. Lathrop, G.M., Lalouel, J.M., Julier, C. and Ott., J. (1985) Multilocus linkage analysis in humans: detection of linkage and estimation of recombination. *Am. J. Hum. Genet.*, **37**, 482–498.
17. Kruglyak, L., Daly, M.J. and Lander, E.S. (1995) Rapid multipoint linkage analysis of recessive traits in nuclear families, including homozygosity mapping. *Am. J. Hum. Genet.*, **56**, 519–527.
18. Klein, D. and Amman, F. (1969) The syndrome of Laurence–Moon–Bardet–Biedl and allied diseases in Switzerland. Clinical, genetic, and epidemiological studies. *J. Neurol. Sci.*, **9**, 479–513.
19. Hauser, C., Rojas, C., Roth, A., Schmied, E. and Saurat, J.H. (1990) A patient with features of both Bardet–Biedl and Alström syndromes. *Eur. J. Pediatr.*, **149**, 783–785.
20. Dyer, D.S., Wilson, M.E., Small, K.W. and Pai, G.S. (1994) Alström syndrome: a case misdiagnosed as Bardet–Biedl syndrome. *J. Pediatr. Ophthalmol. Strabismus.*, **31**, 272–274.
21. Sheffield, V.C. *et al*. (1994) Identification of a Bardet–Biedl syndrome locus on chromosome 3 and evaluation of an efficient approach to homozygosity mapping. *Hum. Mol. Genet.*, **3**, 1331–1335.
22. Leppert, M. *et al*. (1994) Bardet–Biedl syndrome is linked to DNA markers on chromosome 11q and is genetically heterogeneous. *Nature Genet.*, **7**, 108–112 .
23. Carmi, R. *et al.* (1995) Use of a DNA pooling strategy to identify a human obesity syndrome locus on chromosome 15. *Hum. Mol. Genet.*, **4**, 9–13.
24. Kwitek-Black, A. *et al*. (1993) Linkage of Bardet–Biedl syndrome to chromosome 16q and evidence for non-allelic genetic heterogeneity. *Nature Genet.*, **5**, 392–396.
25. White, R.A., Angeloni, S.V. and Pasztor, L.M. (1995) Chromosomal localization of the beta-adducin gene to mouse chromosome 6 and human chromosome 2. *Mamm. Genome*, **6**, 741–743.
26. Tait, J.F. *et al*. (1992) Chromosomal mapping of the human annexin IV (ANX4) gene. *Genomics*, **12**, 313–318.
27. Tassabehji M. *et. al.* (1993) Mutations in the PAX3 gene causing Waardenburg syndrome type 1 and type 2. *Nature Genet.*, **3**, 26–30.
28. Whitmore, T.E., Mudri, S.L., and McKnight, G.L. (1995) Physical mapping of the human glutamine:fructose-6-phosphate amidotransferase gene (GFPT) to chromosome 2p13. *Genomics*, **26**, 422–423.
29. Tricoli, J.V. *et al*. (1986) The gene for human transforming growth factor alpha is on the short arm of chromosome 2. *Cytogenet. Cell Genet.*, **42**, 94–98.
30. Collin, G.B. *et al*. (1997) Physical and genetic mapping of novel microsatellite polymorphisms on human chromosome 19. *Genomics*, **37**, 125–130.
31. Puffenberger, E. G. *et al.* (1994) Identity-by-descent and association mapping of a recessive gene for Hirschsprung disease on human chromosome 13q22. *Hum. Mol. Genet.*, **3**, 1217–1225.
32. Nystuen, A. *et al.* (1996) A cerebellar ataxia locus identified by DNA pooling to search for linkage disequilibrium in an isolated population from the Cayman Islands. *Hum. Mol. Genet.*, **5**, 525–531.
33. Cox, T. K. *et al.* (1989) Mapping of the cystic fibrosis gene using putative ancestral recombinants. *Am. J. Hum. Genet.*, **45**, A136.