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Mapping of Primary Congenital Lymphedema to the 5q35.3 Region

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Summary

Primary lymphedema is a chronic tissue swelling, most frequently of the lower limbs, resulting from deficient lymphatic drainage. The variability of the affected phenotype, incomplete penetrance, lack of large families, and possible genetic heterogeneity have hampered the identification of causative genes until now. We carried out a genomewide search, using a four-generation North American family with dominantly inherited primary congenital lymphedema (PCL), otherwise known as "Milroy disease," or "hereditary lymphedema type I" (MIM 153100). Linkage to markers from the 5q35.3 region in this and four additional, British families was established. A minimum of 79 directly scorable haplotypes (37 affected) in five families conspicuously segregated with the most telomeric region of 5q35.3, thus suggesting a major locus for PCL in this vicinity. No recombination was observed with D5S408 ($Z = 10.03$) and D5S2006 ($Z = 8.46$) with a combined multipoint score of 16.55. While D5S2073 and WIAF-2213 defined the upper centromeric boundary, no recombinants were obtained for the last telomeric marker of D5S2006. Four unaffected subjects were identified as gene carriers and provided an estimated penetrance ratio of .84 for this condition. A few of the positionally mapped genes in the 5q35 region that may potentially be involved in the etiology of this condition are *CANX, FGFR4, HK3,* and *hnRPH1*.

Introduction

Lymphedema is a chronic tissue swelling that is most commonly manifested in a limb. This condition results from impaired lymph drainage in the presence of normal capillary filtration. It may be either primary in type, which implies an intrinsic abnormality of the lymph-conducting pathways or, secondary in type, which implies that external factors such as radiotherapy, severe infection, or surgical excision have damaged lymph drainage routes (Mortimer 1995*a*). The three main consequences of lymphatic failure are swelling (lymphedema), infection (cellulitis), and, very rarely, cancer (Mortimer 1995*b*).

Lymphedema may occur as part of a well-recognized syndrome, in which the genetic defect may or may not be known—for example, in Turner syndrome or Noonan syndrome (Greenlee et al. 1993). Primary lymphedema presents in a variety of clinical patterns, suggesting different underlying etiologies (Browse and Stewart 1985). In the vast majority of families, the disease segregates in an autosomal dominant fashion, as it does in the pig model of congenital lymphedema (Van der Putte 1978). The expression of lymphedema is sex influenced and is much higher in females than in males, at 66% and 30%, respectively (Dale 1985). Most forms of primary lymphedema are thought to be caused by a congenital abnormality of the lymphatic system; however, onset of clinical symptoms in hereditary lymphedema type II (MIM 153200) usually occurs during or near either puberty or the menopause. True congenital lymphedema, or hereditary lymphedema type I (MIM 153100), usually presents either at, or soon after, birth and may be associated with other congenital malformations, such as distichiasis (Dale 1987; Kolin et al. 1991), congenital heart disease (Corbett et al. 1982), and hydrocele (Irons et al. 1996). Occasionally, primary lymphedema is both congenital and familial. The first reports, by Nonne (1891) and, in the following year, Milroy (1892), were of families in which the edema was present at birth.

Herein we describe the localization of the first lymphedema locus and provide evidence that primary congenital lymphedema (PCL), also known as "Milroy disease" (MIM 153100), maps to the 5q35.3 region in one large North American family and four British families.

Subjects and Methods

Ascertainment and Examination of Patients

During a 5-d period, 102 members (52 males and 50 females) of a single large four-generation North Amer-

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ican family of Irish descent (PCL-900; see fig. 1) were clinically examined by a single experienced observer (G.B.). A complete family history was obtained, and photographs of deceased subjects were examined. The clinical status of all examined affected subjects was documented. Diagnostic criteria included evidence of idiopathic, painless edema with characteristic skin thickening and associated hyperkeratosis and papillomatosis. Skin thickening was indicated by a positive Kaposi-Stemmer sign, which is a failure to pick up or pinch a fold of skin at the base of the second toe (Stemmer 1976). Twenty-milliliter EDTA blood samples, or buccal rubbings, were obtained from each individual, for DNA extraction. All samples were taken with informed written consent, either from the individual concerned or, in the case of a child <18 years old, from a parent. The study protocol and consent forms were approved by the University of Connecticut Health Center institutional review board. Skin biopsies and blood samples were also obtained from two affected individuals, for cell culture and karyotype analysis. A complete cytogenetic analysis of these two subjects did not reveal any obvious chromosomal abnormalities.

During the past 6 years, we have identified another four PCL families, from Britain (fig. 2), consisting of affected individuals in two (PCL-102), three (PCL-66 and PCL-100), or five (PCL-72) generations. DNA samples from a total of 17 affected (5 males and 12 females) and 18 unaffected subjects (12 meioses) were available for linkage study.

DNA and Linkage Study

DNA was extracted from peripheral blood and/or buccal cells (DNAzol; Molecular Research Center [Cincinnati] and Puregene), as described elsewhere (Moore 1994). A genomewide screen was performed with fluorescently end-labeled primers from the ABI Prism Linkage Mapping Set consisting of chromosome-specific marker loci selected from the linkage maps generated by Généthon (Weissenbach et al. 1992; Gyapay et al. 1994). Saturation mapping was performed by use of additional short tandem-repeat polymorphic (STRP) markers and single-nucleotide polymorphisms (SNPs). Amplification of the ABI Prism Linkage Mapping set was performed in multiplex PCR reactions using primer concentrations as suggested by the ABI manual. A total reaction volume of 7.5 μl was used, consisting of 30 ng of genomic DNA, 1 × PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl), 2.5 mM $MgCl_2$, 250 μM of each dNTP, and 0.3 U of *Taq* polymerase (Ampli*Taq* Gold; Perkin-Elmer). Amplifications were performed in a GeneAmp 9600 thermocycler (Perkin-Elmer), under the following conditions: initial denaturation at 95°C for 12 min; 10 cycles of denaturation at 94°C for 15 s, annealing at 55°C for 15 s (ramp time 2 min from 94°C to 55°C), and extension at 72°C for 30 s; and an additional 20 cycles of denaturation at 89°C for 15 s, annealing at 55°C for 15 s (ramp time 2 min from 89°C to 55°C), and extension at 72°C for 30 s, followed by a final extension at 72°C for 20 min. The amplified products were separated by electrophoresis on the ABI-377 DNA Sequencer (Perkin-Elmer), with GENESCAN (version 2.1) peak-calling software (Perkin-Elmer). The alleles were scored by the GENOTYPER (version 2.0) program (Perkin-Elmer).

Amplifications of chromosome 5–specific STRP markers were performed in a 25-μl reaction volume containing 100 ng of genomic DNA, 50 pmol of each primer, 210 μM of each dNTP, 50 mM KCl, 10 mM Tris-HCl (pH 8.8), 1.5 mM $MgCl_2$, 0.01% gelatin, and 0.5 U of *Taq* polymerase (Ampli*Taq;* Perkin-Elmer). Amplifications were performed in a GeneAmp 9600 thermocycler (Perkin-Elmer), under the following conditions: initial denaturation at 94°C for 2 min, 35 cycles of denaturation at 94°C for 15 s and annealing at 54°C for 30 s, and a final extension at 72°C for 2 min. The amplified products were separated by electrophoresis on 7% denaturing acrylamide gels, were visualized by silver staining (Bassam et al. 1991), and were photographed and subsequently genotyped.

For the two SNP markers of WIAF-616 and WIAF-2213 (Wang et al. 1998; WIBR/MITCGR Website), a single-strand conformational polymorphism (SSCP) assay was performed as follows: a 25-μl stop solution containing 95% formamide, 20 mM EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanole was added to the amplified fragments, and the reactions were denatured at 94°C for 5 min. Samples were run at room temperature on a 10% polyacrylamide (acrylamide:bisacrylamide ratio 37.5:1), 0.5 × Tris-borate EDTA gels at 9 W for 9 h, on a vertical electrophoresis unit (S2001) with a cooling system (GIBCO BRL). The gels were stained as described above. The exact sequence of the observed SSCP variations was determined by direct sequencing of representative polymorphisms on an ABI-377 DNA sequencer using dye-rhodamine chemistry. All genotypes either from the ABI linkage mapping set, the STRPs, or the two SNPs were entered into the Database Management System program (M. Sarfarazi, unpublished data) and subsequently were used to create data files for analysis by the LINKAGE programs (Lathrop and Lalouel 1984).

Results

Clinical Findings

A total of five families—one large North American (fig. 1) and four British (fig. 2)—were used in this study. The mode of inheritance in the North American family

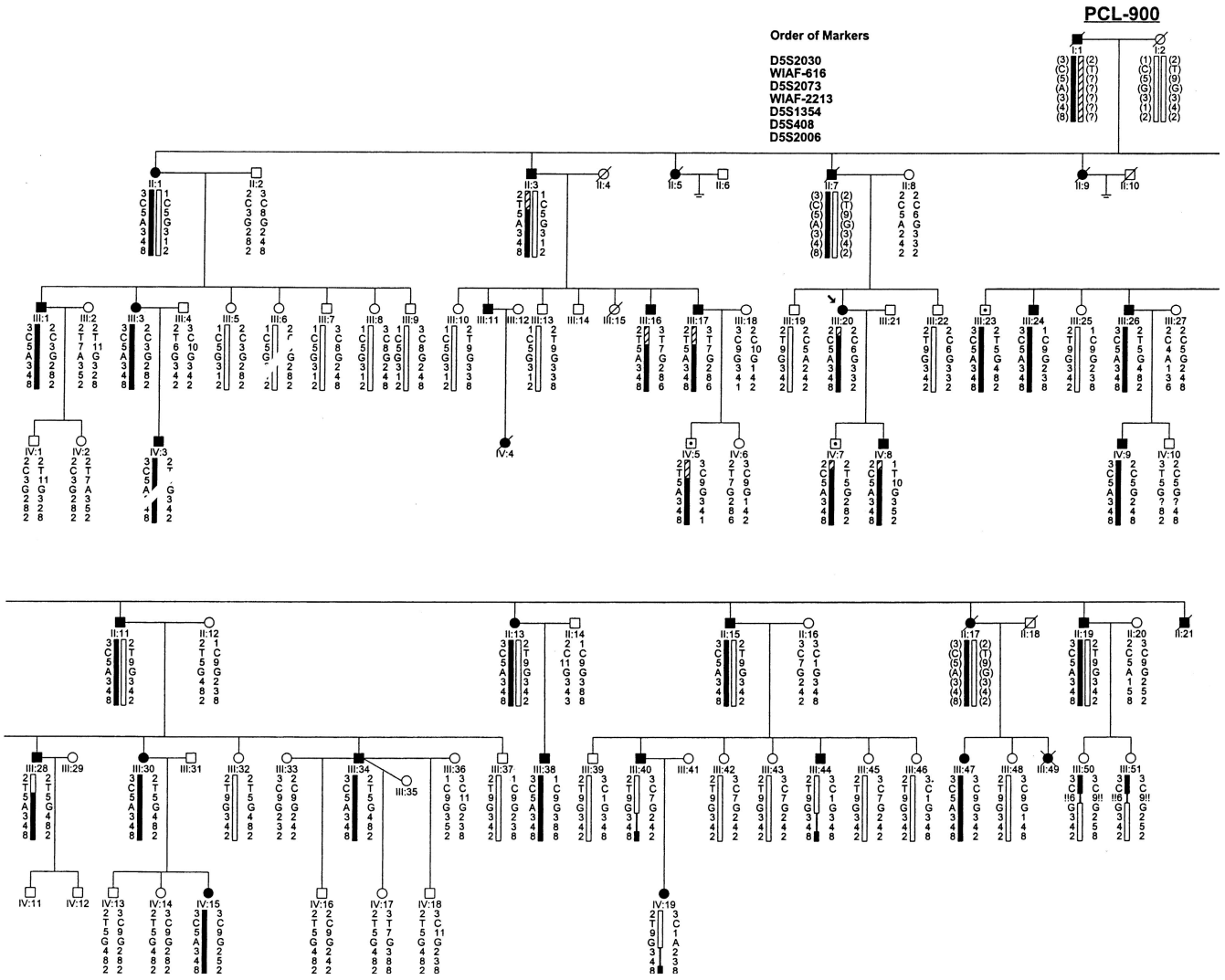

Figure 1 Pedigree structure and genotypic data for the North American family, PCL-900. The order of seven most telomeric polymorphic DNA markers on the 5q35.3 region are shown at the top of the pedigree. The inherited affected haplotypes are denoted by blackened bars, and the normal haplotypes are denoted by unblackened bars. The three normal males carrying the affected haplotypes are shown with a dot inside their symbols. Note that no telomeric recombination has been observed in this pedigree. However, two individuals (individuals III:40 and III:44) have recombined for the first four markers shown here; but they are not recombinants for the most telomeric marker of D5S2006. The exact site of recombination for the two markers of D5S1354 and D5S408 cannot be determined in these two individuals. Therefore, the PCL locus in this family maps below the SNP marker of WIAF-2213.

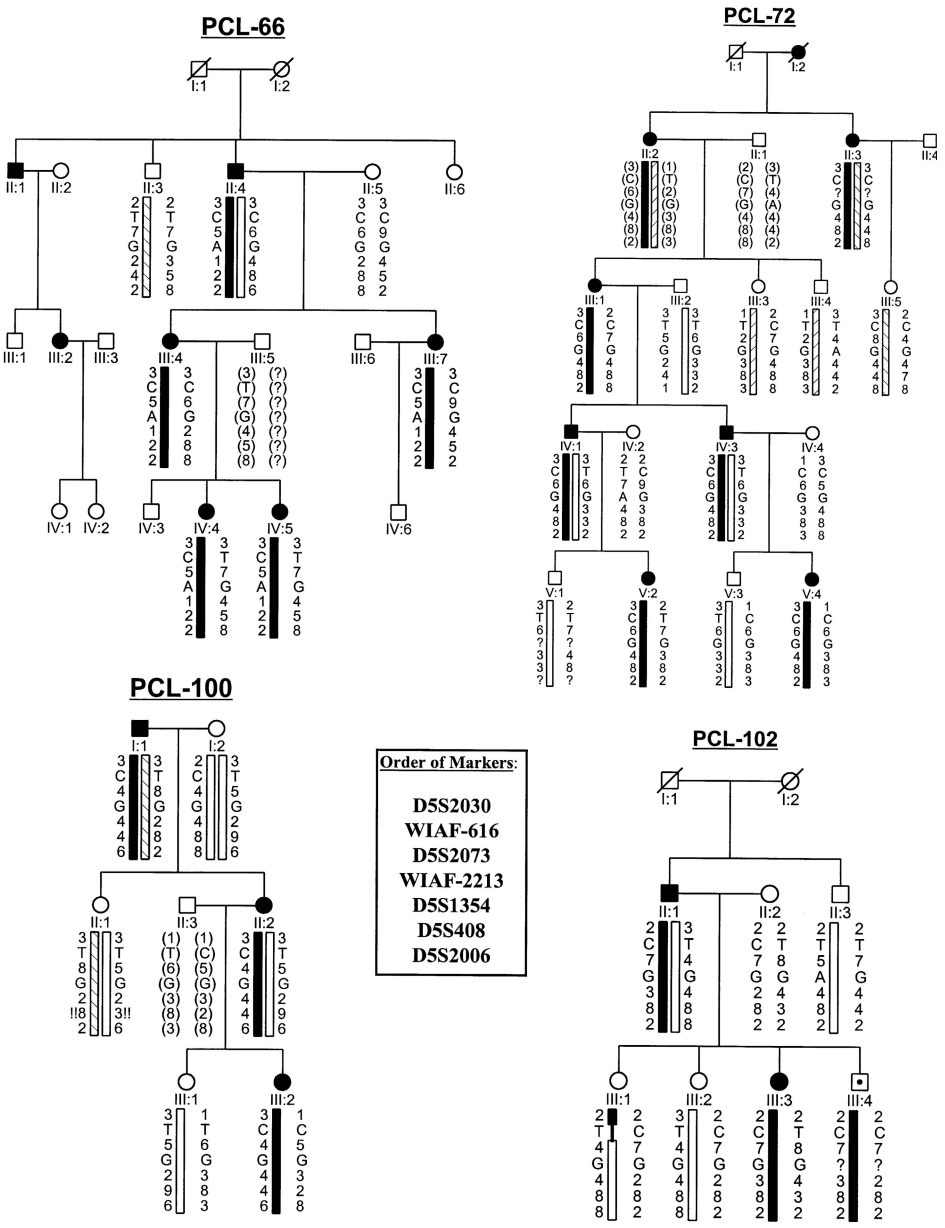

Figure 2 Pedigree structure of four PCL British families. The order of markers is shown. Haplotypes are denoted as in fig. 1. There are no affected recombinations for any of the markers shown in these pedigrees. Note that in PCL-102 individual III:4 has inherited the affected haplotype but is clinically unaffected.

was autosomal dominant, with no clinical evidence of reduced penetrance or of sex-influenced expression. Because of the reported lack of full penetrance in the dominant forms of congenital lymphedema (Milroy 1892), members of generation IV who had unaffected parents were also fully examined. Despite previously reported associations of lymphedema with hypothyroidism (Jones and Webb 1996), yellow nails (Wells 1966), and distichiasis (Falls and Kertesz 1964), no such clinical manifestations or other dysmorphic features were found. In generation II, all 11 siblings were classified as affected, on the basis of examination and/or history including photographic evidence. There was no historical or photographic evidence that the mother of these 11 subjects (individual I:2) was affected. However, her children reported a history of recurrent leg infections in their father (individual I:1), who was known within the family as "piano legs."

In this family, where presentation is invariably at birth, the clinical diagnosis is relatively straightforward;

however, there is marked variation in the degree of expression within the family. For example, congenital swelling of the right foot in a 6-year-old subject (fig. 1, individual IV:8) is illustrated in figure 3*A*. Marked swelling together with papillomatosis and nail changes in the left foot of a 70-year-old man (fig. 1, individual II:11) is shown in figure 3*B*. Asymmetrical bilateral leg swelling that has been present since birth in a 45-year-old individual (fig. 1, individual III:1) is presented in figure 3*C*. The right foot of the same subject (individual III:1), which is shown in figure 3*D*, demonstrates papillomatosis.

Eight of the affected family members gave a history of either fungal or bacterial infections, with one report that amputation of the foot had been suggested during the course of a particularly severe infection. This advice was rejected, and the limb eventually responded to antibiotic therapy. Three members of the family had undergone investigations using Evans blue dye injected into the subcutaneous tissues of the foot. Both individual II:13 and individual III:38 still had evidence of residual blue dye after 9 mo. Deceased individual II:5 also underwent radio-contrast lymphangiography, showing aplasia of lymphatic glands. All affected individuals re-

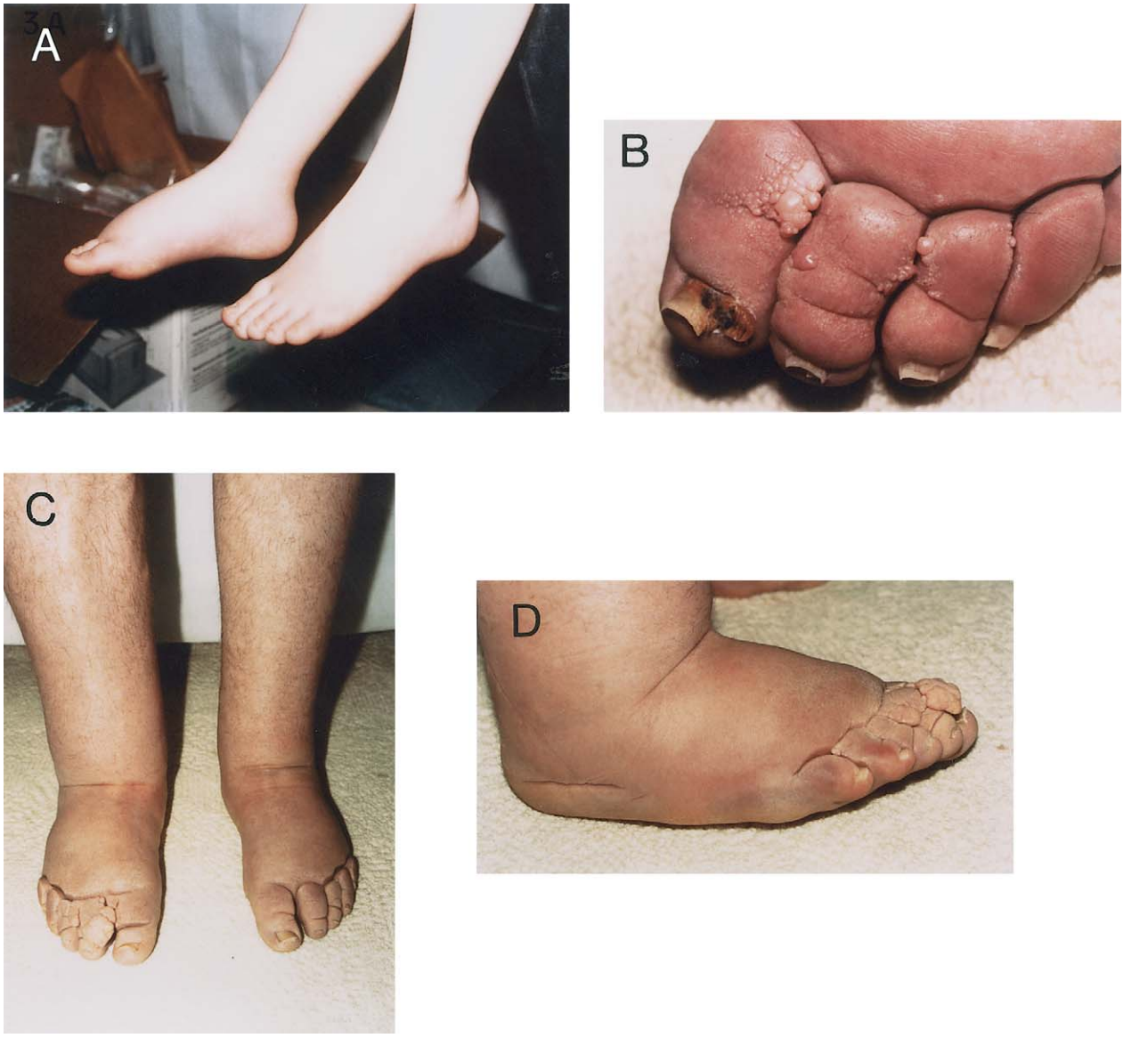

Figure 3 *A*, Swelling of right foot, since birth, in a 6-year-old child (fig. 1, individual IV:8). *B*, Marked swelling, with papillomatosis and nail changes, in a 70-year-old man (fig. 1, individual II:1). *C*, Bilateral swelling, since birth, in a 45-year-old man (fig. 1, individual III:1). *D*, Right foot of individual III:1, immediately after removal of compression hosiery, demonstrating papillomatosis.

Table 1

Combined LOD-Score Values, for Five PCL Families and Seven Polymorphic DNA Markers from the 5q35.3 Region

	Z AT θ =						Z_{max} (θ_{max}) IN	
MARKER	.00	.05	.10	.20	.30	.40	All Meioses	Affected Meioses
D5S2030	−∞	.48	2.49	3.31	2.67	1.35	3.31 (.19)	1.21 (.20)
WIAF-616	−∞	2.65	3.10	2.47	1.34	.46	3.10 (.10)	.09 (.37)
D5S2073	−∞	7.01	6.63	5.14	3.37	1.54	7.01 (.05)	1.51 (.07)
WIAF-2213	−∞	9.46	8.96	7.24	5.08	2.58	9.49 (.04)	1.34 (.11)
D5S1354	7.09	6.31	5.53	3.99	2.52	1.18	7.09 (.00)	2.72 (.00)
D5S408	10.03	9.80	8.96	6.94	4.70	2.32	10.03 (.00)	5.27 (.00)
D5S2006	8.46	8.00	7.24	5.35	3.28	1.31	8.46 (.00)	4.33 (.00)

ported that swelling had been present since birth and that the distribution had not altered with time; thus, those mildly affected at birth remained so throughout life. The degree to which individuals were affected ranged from single toes to the complete leg; however, there was no evidence of edema in hands, face, trunk, or sacrum. In 4 of the 19 affected individuals lymphedema was unilateral, and in 7 others the swelling was bilateral but asymmetrical in distribution, with the remaining 8 individuals being affected equally bilaterally. Severe papillomatosis was present in five individuals (four males and one female), with one giving a history of successful surgical removal of the growths 30 years previously. The Kaposi-Stemmer sign was positive in 15 of 19 affected individuals but in none of the unaffected family members. The presence of edema, which subsides readily with overnight elevation, suggests that the problem lies in excessive capillary filtration, rather than in reduced lymphatic drainage. Of 19 affected subjects, 10 reported that the swelling was not significantly reduced by overnight elevation of the limb whereas 9 reported varying degrees of incomplete reduction of swelling. Several individuals wore compression stockings, which were effective in containing the edema, whereas those who were more severely affected attended hospital for compression pumping to reduce limb size prior to being fitting for compression hosiery.

Four white English families with congenital lymphedema were also referred by interested clinicians, for linkage studies (fig. 2). Pedigree analysis showed no evidence of skipped generations; however, in two families (PCL-72 and PCL-66) males were less severely affected than females. Involvement in affected individuals ranged from swelling of only the toes to swelling of the entire leg. Five patients suffered unilateral lower-leg involvement only. There were no reports or evidence of edema other than that in the lower limbs. One individual (individual III:7 in PCL-66) had undergone attempted bilateral pedal lymphangiograms and bilateral inguinal lymphangiograms. Patent blue dye was injected into the webs of the feet. Exploratory incisions were made on the dorsum of each foot. No lymphatic vessels were located by either method. The groins were explored, and lymphangiograms were performed, which showed almost normal lymph vessels and nodes in the groin and in the iliac and lumbar regions. The diagnosis of peripheral lymphatic aplasia was made, and the patient was treated conservatively.

Linkage Analysis

For the purpose of positional mapping, we selected a group of 32 (21 affected individuals and their spouses and 4 normal subjects) from the North American family and scanned the genome randomly with the ABI Prism Linkage Mapping Set. Once a suggestion of linkage was found with the DNA markers D5S429 and D5S408, samples from 25 affected and 32 unaffected subjects were used for saturation mapping (fig. 1). An additional 11 markers from this region, including two new SNP markers (Wang et al. 1998), were used to genotype and construct the inherited haplotype in this kindred. As shown in figure 1, a total of 57 directly scorable meioses (25 affected individuals) conspicuously segregated with the three most telomeric markers. Inspection of haplotypes indicated that the gene for this kindred maps telomeric to the SNP marker WIAF-2213. Since the affected subject II:15 is not informative for D5S1354 and D5S408, neither the precise site of recombination in his children (individuals III:40 and III:44) nor the exact location of this SNP marker in relation to these two markers can be determined. However, both genetic-linkage mapping and radiation mapping data show that D5S1354 and WIAF-2213 are tightly linked to D5S408. In addition, our radiation mapping data for D5S2006 (not shown), as well as the published linkage data, clearly show that this marker is telomeric to both D5S408 and all other markers used in this study. The absence of recombination with marker D5S2006 in affected subject II:15 suggests that the newly identified PCL locus is tightly linked to this marker.

To test for genetic heterogeneity, we used four British

families with congenital lymphedema and established tight linkage in all families studied. No recombination was observed in the British families with any of the seven DNA markers genotyped (fig. 2). Table 1 shows the combined LOD score (Z) values for all five PCL families and seven DNA markers from the 5q35.3 region. The overall three-point scores for linkage between PCL and two DNA markers—D5S408 and D5S2006—are presented in table 2, for the North American family and for the four British families. There is overwhelming evidence for localization of the PCL locus to this region, with Z values of 3.10–10.03 for individual markers (table 1), as well as a combined haplotype score of 16.55 for the last two DNA markers, D5S408 and D5S2006 (table 2). Since, in the literature, there is evidence for incomplete penetrance in this condition (Dale 1985), we repeated the Z analysis for affected meioses only. Although the Z values decreased (table 1), they remained statistically significant, ranging from 5.27 (for D5S408) and 4.33 (for D5S2006) to 8.76 (for the haplotype of the two markers).

All clinically affected subjects within a family shared a common haplotype. Of the 44 clinically unaffected subjects genotyped, 4 males had also inherited the affected haplotype. Three of these individuals, ages 46, 7, and 9 years, respectively, belong to PCL-900 (fig. 1, individuals III:23, IV:5, and IV:7), and the fourth, age 5 years, belongs to PCL-102 (fig. 2, individual III:4). The three youngest individuals had no clinical signs of being affected, whereas individual III:23 demonstrated mild pedal edema and, in common with seven other examined affected male family members, reported a history of hydrocele. It is likely that these four males represent either incomplete or age-dependent penetrance, consistent with the previously reported low penetrance in males compared with females (Dale 1985). For the purpose of linkage analysis (tables 1 and 2), the clinical status of these four subjects was coded as "unknown."

Once linkage of PCL to the 5q35.3 region had been established, an estimate of penetrance was calculated for this phenotype. Three liability classes were defined—one for spouses, one for affected subjects, and one for at-risk individuals. Since DNA markers in different branches of these pedigrees were noninformative, we used a three-point multipoint score, between the PCL phenotype and the haplotype of D5S408 and D5S2006, to maximize the linkage information. The penetrance ratios for the at-risk individuals were .5–1.0, and their corresponding three-point scores were calculated. This analysis resulted in a maximum Z (Z_{max}) of 13.39 at a ratio of .16 (penetrance ratio .84).

Discussion

The findings reported in this paper indicate that the first locus for primary congenital lymphedema has been identified. Heterogeneity for the primary lymphedemas is probable because of both the variable age at onset and the differing clinical and lymphoscintigraphy findings in families with congenital, juvenile, and adult-onset lymphedema. In this study we used a four-generation North American family with PCL and mapped a locus to the 5q35.3 region. Three generations of this family had been examined almost 35 years ago (Beninson et al. 1967; Klemmer et al. 1997), at which time it was stated that 22 members of the F2 generation showed signs consistent with either obvious lymphedema or lymphatic dysplasia without clinical signs. Lymphatic dysplasia was diagnosed solely on the basis of the palpable thickening of the skin on the extremities. It is therefore noteworthy that, in our study, there are nine individuals in whom the clinical classification has changed from that in the originally published pedigree. In view of our clinical examination during 1998, it is apparent that none of those individuals originally thought to have lymphatic dysplasia have progressed to develop lymphedema, despite the passage of 34 years. This underlines the need for a simple diagnostic test, now available to this family as a result of our linkage study. Five of the nine individuals in whom there is a discrepancy have, among them, eight offspring. None of these offspring show any signs of lymphedema, which strengthens our clinical impression that their parents are unaffected. Although it is possible that those originally described as having lymphatic dysplasia may still develop lymphedema, there is no precedent for it in this large pedigree, where, to date, presentation invariably has been at birth. One interesting feature in this family is that all 11 siblings in generation II were affected. A possible explanation for this is that the father of these 11 individuals (individual I:1) was

Table 2

Three-Point Linkage Scores, for PCL Phenotype (in One American Family and Four British Families) and Combined Haplotypes of D5S408 and D5S2006, in All Meioses

	Z AT θ =									
SOURCE	.00	.05	.10	.15	.20	.25	.30	.35	.40	.45
American	12.61	12.01	11.05	9.95	8.74	7.45	6.06	4.59	3.03	1.40
British	3.94	3.90	3.58	3.16	2.70	2.21	1.72	1.24	.79	.38
Combined	16.55	15.91	14.63	13.11	11.44	9.66	7.78	5.83	3.82	1.78

affected as a result of a rare new mutation during the preembryonic period, such that all his spermatogonia carried the mutation, resulting in a single population of mutation-bearing sperm. An alternative explanation—that he was homozygous for the disease gene—is less likely, in view of his mild clinical signs.

The genetic linkage that was seen in this North American PCL family was subsequently confirmed in four British families. Taken together, these five families provide substantial evidence for localization of the PCL locus to 5q35.3. We observed no recombination with either D5S408 ($Z = 10.03$), D5S2006 ($Z = 8.46$), or their combined multipoint haplotype ($Z = 16.55$). When only the affected meioses were used in the analysis, Z values of 5.27 (D5S408), 4.33 (D5S2006), and 8.76 (for the haplotype of D5S408 and D5S2006) were obtained. We also used two newly described SNP markers from this region (Wang et al. 1998), both of which showed recombination in the affected subjects (fig. 1) and which therefore are outside the critical region of PCL. However, the closer SNP marker WIAF-2213 was sufficiently informative to yield $Z_{max} = 9.49$ at recombination fraction (θ) of .04 (table 1). This observation confirms the usefulness of this new type of polymorphism in mapping studies (Wang et al. 1998). Once linkage to this region had been established, we used a total of 44 normal meioses in these pedigrees and estimated a penetrance ratio of .84 for this condition. Construction and inspection of the inherited haplotypes (fig. 1) in the North American family confirmed that the PCL locus is flanked by two centromeric markers—D5S2073 and WIAF-2213—but no corresponding telomeric flanking marker has been identified. There is no recombination with D5S2006, the most telomeric known polymorphic marker in this region. Therefore, it is possible that PCL maps telomeric to all of the known existing markers. There is an obvious need for both development of additional polymorphic markers and establishment of a closely flanking marker, before any attempts can be made to clone the putative PCL gene.

The human-transcript map at the GeneMap'98 Website of the National Center for Biotechnology Information currently lists the gene for calnexin (*CANX*), the gene for EBI3-associated protein-p60, and 23 other expressed sequence tags that map between D5S408 and D5S2006. Other genes that loosely map to the 5q35 region are *NPM1* (for nucleophomin), *SNCB* (for synuclein beta), *GPRK6* (for G protein–coupled receptor kinase 6), *FGFR4* (for fibroblast growth-factor receptor 4), *LTC4S* (for leukotriene C4 synthases), *HK3* (for hexokinase 3), and *hnRPH1* (for heterogeneous nuclear ribonucleoprotein H1). The precise mapping of these genes in relation to D5S408 and D5S2006, as well as their possible role in the etiology of primary congenital lymphedema, remains to be determined.

Although no obvious candidate gene for protein components of the lymphatic wall maps to the 5q35.3 region, other possible modifying genes mapping to this or other regions may explain the reduced penetrance seen in some family members (Witte 1997). Linkage studies involving other families with congenital, juvenile, and adult-onset lymphedema will test for genetic heterogeneity and may provide evidence for phenotype-genotype correlation.

The phenotype studied here is deforming, with associated psychological as well as medical problems, all of which are treatable (Mortimer 1995*a;* 1995*b*). Presymptomatic screening of all at-risk first-degree relatives would be desirable to prevent complications, by early advice and treatment. Once identified as gene carriers, individuals at risk can be kept under regular surveillance and can be advised to (*a*) avoid infection, by maintaining strict hygiene of the feet, with early attention to fungal or bacterial infections; (*b*) avoid excessive weight gain; (*c*) exercise regularly; (*d*) use manual lymphatic drainage and either support hose or bandaging; and (*e*) avoid occupations that require standing for long periods. Such individuals may be at increased risk of lymphedema after surgery or irradiation (Tobin et al. 1993). Better treatment, based on improved understanding of the underlying molecular defect, is urgently required for this disease.

Acknowledgments

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

GeneMap'98, http://www.ncbi.nlm.nih.gov/genemap (for map locations of genes and markers)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for hereditary lymphedema types I [MIM 153100] and II [MIM 153200])

Whitehead Institute for Biomedical Research/MIT Center for Genome Research (WIBR/MITCGR), http://www-genome.wi.mit.edu (for SNP and marker information)

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