Brief Genetics Report

Molecular Analysis of Berardinelli-Seip Congenital Lipodystrophy in Oman

Evidence for Multiple Loci

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Congenital generalized lipodystrophy (CGL) is a rare disorder characterized by the absence of body fat and insulin resistance and accompanied by other features, including acanthosis nigricans, organomegaly, hyperandrogenism, and diabetes. We have examined case subjects from 11 families in Oman with CGL. All subjects were the progeny of consanguineous marriages; therefore, a homozygosity mapping strategy was used to investigate the reported loci, 11q13 and 9q34. Three subjects could be linked to 11q13, and mutations were found within the seipin gene. An additional eight subjects were linked to 9q34, but the locus was in a 9-cM interval with no known microsatellites, so further fine mapping was not possible. However, two sibships (four subjects) did not map to either locus, raising the possibility of more than two lipodystrophy loci within the Oman population. *Diabetes* 51:1291–1293, 2002

Congenital generalized lipodystrophy (CGL; Berardinelli-Seip syndrome MIM 269700) is a rare disorder that is autosomal recessive in inheritance. CGL is characterized by the almost complete absence of body fat deposition and is usually evident at birth or during early infancy. In addition, the syndrome often includes the following features: organomegaly, muscle hypertrophy, acanthosis nigricans, hyperandrogenism, hyperlipidemia, and hyperinsulinemia or insulin-resistant diabetes. Garg et al. (1) mapped a locus for CGL to 9q34 (*BSCL1*), a 9-cM interval between markers D9S1818 and D9S1826, but the gene is not yet identified at this locus. Of the 17 pedigrees analyzed, 2 showed no linkage to this locus, suggesting that CGL was genetically heterogeneous. Recently, CGL (*BSCL2*) in nine pedigrees from two geographical clusters, Lebanon and Norway, was mapped to 11q13 between markers D11S4076 and D11S480 (2). Magré et al. (2) identified a gene, designated the seipin gene, within this locus and demonstrated functional mutations within this gene in their CGL cohort. Twenty additional families of various ethnicities were analyzed, and the disease was found to cosegregate with either 9q34 or 11q13.

We have identified 16 subjects with CGL from 11 consanguineous sibships in Oman originating from three different geographical areas (groups A, B, and C) (Fig. 1). The evaluation of the main clinical and biological characteristics of subjects revealed no obvious differences between subjects in groups A and B, but a variable expression of the disease in group C. Affected individuals in group C had lipodystrophy and muscle hypertrophy as common features but did not show significant developmental delay, acanthosis nigricans, or childhood-onset insulin resistance. In addition, all individuals in group C and none in groups A and B were affected by cardiomegaly and hypertrophic pyloric stenosis. Group C also showed reduced exercise tolerance, percussion myoedema in skeletal muscle (the production of a dimple in the muscle after pressing on the surface), and disturbance of cardiac rhythm and cardiomyopathy. Detailed clinical findings are described elsewhere (3). As all subjects were the offspring of consanguineous marriages, we decided to use a homozygosity mapping approach to investigate the genes involved in these forms of CGL. This approach was based on the premise that in the event of a rare recessive disease, the affected progeny of a consanguineous mating will have inherited two identical copies of the disease gene from a common ancestor and therefore should be homozygous for the defective allele.

The 11q13 locus was initially investigated for homozygosity using microsatellite markers D11S4191, D11S4076, CA10, CA9, D11S1883, and PYGM and five SNPs within *BSCL2*. In group A, subjects A1, A2, A3, and A4 were homozygous for the microsatellites flanking *BSCL2* and the SNPs within the gene. These families were examined for additional microsatellites at the *BSCL2* locus to determine the extent of the homozygosity at this locus (Table 1). For A3, the flanking homozygous markers are CA10 and

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CGL, congenital generalized lipodystrophy; SNP, single nucleotide polymorphism.

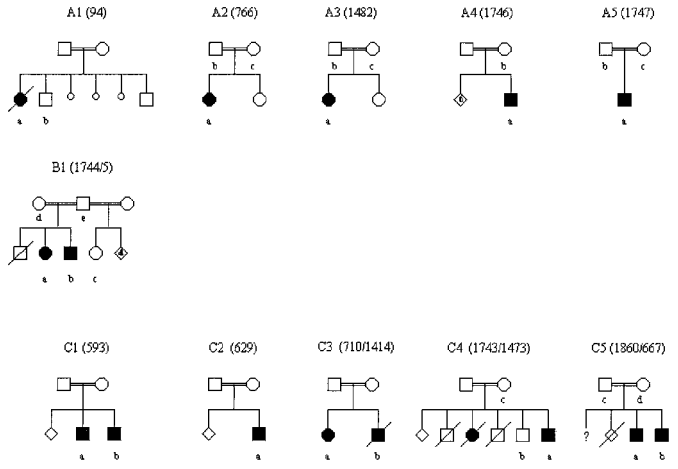

FIG. 1. Pedigrees showing lipodystrophy subjects analyzed in this study. The families are divided into three groups depending on their geographical origin in Oman. Groups A and B have a similar phenotype, whereas group C has a phenotype that varies from the classical description of CGL (see text). Samples available for analysis are denoted by lower case letters under their symbol on the pedigree. Additionally, the deceased, marked unaffected in pedigrees C4 and C5, died early in life and are thought to have suffered from CGL from family descriptions, although no clinical diagnosis was made. For families A4 and B1, the numbers inside the diamonds indicate the number of normal sibs of unknown sex.

D11S4113, which are about 6.5 cM apart. This is of interest when considering the *BSCL1* locus in these families (see below). Subject A4 was homozygous for the microsatellites and for the five single nucleotide polymorphisms (SNPs) listed in Table 1 but was found to be heterozygous for an SNP in intron 1 [1 (+62 → +64) GGG/deletionGGG]. A5 was homozygous for the five seipin SNPs, as were both parents, and A5 was heterozygous for the microsatellites D11S4076–D11S1883 (Table 1). Subject A4 had a small homozygous interval, but the internal polymorphism in the seipin gene indicated that the homozygosity in this subject was probably coincidental and not indicative of the presence of a causative gene. No mutation was found in this family, but the possibility exists that the intron 1 polymorphism was a de novo event, and there was a noncoding mutation in the seipin gene in this pedigree, as the mutation screen (see below) did not cover noncoding regions.

TABLE 2
Status of microsatellites and SNPs at the seipin locus in all families

Family	Chromosome 9
A1	Het
A2	Het
A3	Het
A4	Hom
A5	Hom D9S1818, Het D9S1826
B1 sib a	Hom
B1 sib b	Het
C1 sib a	Hom D9S1826, Het D9S1818
C1 sib b	Hom D9S1826, Het D9S1818
C2	Hom D9S1818, Het D9S1826
C3 sib a	Hom D9S1826, Het D9S1818
C3 sib b	Hom D9S1826, Het D9S1818
C4	Hom
C5 sib a	Het
C5 sib b	Het

Het, heterogeneous; hom, homogeneous.

Mutation analysis of the seipin gene in all affected individuals revealed that subjects from families A1 and A3 had a novel homozygous mutation in exon 7 that would give rise to a stop codon (W259X). Subject A2 had a novel mutation in the acceptor splice site of intron 6 (cDNA: 1016/-2 A→C). A mutation previously found near this splice site (cDNA:1016/-3) in a family originating from Turkey was demonstrated to cause skipping of the next exon, exon 7 (2). Therefore, it is likely that the mutation we identified at the adjacent base, which belongs to a strictly conserved sequence for RNA splicing, causes exon 7 to be skipped. No mutations were detected in the other samples from group A. It is interesting to note that although these subjects, A1, A2, and A3, reside in the same geographic area of Oman, two different mutations within the seipin gene were observed, suggesting that these individuals do not share a common ancestor from whom the lipodystrophy-causing allele was inherited.

Moreover, although all families from group A are from the same area in Oman and patients present with a similar phenotype, at least two loci are involved. In families from groups B and C, haplotype analysis indicated that the disease was not linked to *BSCL2*, as all sibships showed

TABLE 1
Status of D95188 and D951826 in all families

Microsatellites	A1	A2	A3	A4	A5	B1	C1	C2	C3	C4	C5
D11S4191	4,4	3,3	7,7	6,7	7,7	5,6	6,7	4,4	5,6	6,6	2,5
D11S1765	2,2	1,1	2,2								
D11S4076	1,1	2,2	2,4	5,5	1,2	1,3	2,3	2,3	1,2	1,4	1,4
CA10	7,7	7,7	7,7	3,3	1,8	4,5	2,7	3,5	3,4	4,5	3,5
CA9	1,1	1,1	1,1	1,1	1,2	1,1	1,1	1,2	1,1	1,1	1,2
D11S1883	3,3	3,3	3,3	3,3	1,5	2,4	4,5	1,2	2,2	2,2	2,3
Pygm	4,4	3,4	4,4	2,5	3,4	5,6	1,3	3,7	7,7	1,3	2,5
D11S4113	1,1	1,1	1,1								
D11S4136	3,3	1,1	2,3								
Seipin SNP											
Int3/+11	TT	TT		GG	GG	GT	GT		GG	GG	GT
Int5/+69	AA	AA	AA	GG	GG	AG	AG	GG	GG	AG	AG
Int5/−49	CC	TT	CC	CC	CC	TC	TC	CC	CC	CC	TC
Int7/−50	TT	TT	TT	TT	TT	TG	TT		TG	TT	TT
Ex9/65	GG	GG	GG	AA	AA	AG	AG	AA	AA	AA	AG

heterozygosity for the interval examined on chromosome 11. No mutations were detected in any of these samples on analysis of the seipin gene.

Homozygosity at markers flanking the 9q34 locus was investigated in the Omani families and is shown in Table 2. It was found that subjects A4, A5, C1, C2, C3, and C4 could map to this locus. However, the majority of subjects were only homozygous for either D9S1818 or D9S1826, and in subject C4, who was homozygous for both markers, this is uninformative because her mother and unaffected brother were similarly homozygous. The distance between these microsatellites at the 9q34 locus is 9 cM; thus, it is possible that recombination could have occurred within this chromosomal region, especially when considering the smaller interval found at the *BSCL2* locus for A3, as described above. Unfortunately, no microsatellite markers have been identified between D9S1818 and D9S1826 that could be used to obtain further mapping information; therefore, only the discovery of new markers internal to the existing interval will enable further analysis of this locus. Neither family C5 nor B1 were compatible with linkage to *BSCL1*. The similarity in phenotype of Group C does not make it unreasonable to assume that the *BSCL1* locus may not be involved in any of these subjects, but such an assumption is not certain. Even if this is so and CGL in all individuals in Group C is produced by mutations in an unidentified gene, family B1 appears unlikely, by virtue of its phenotype, to map to the same locus.

Other candidates for the lipodystrophy locus in the Omani subjects have been considered. Using the ABI LMS v2 markers, homozygosity mapping has been performed on chromosomes 1q, 2p, 18p, and 20q, the locations of *LMNA*, *LPIN1*, *LPIN2*, and *LPIN3*, respectively. Mutations in *LMNA* have previously been reported in subjects of autosomal dominant familial partial lipodystrophy (Dunnigan type) (4–6) but not in CGL patients (7). The LPIN genes were considered because lipodystrophy is a component of the fld mouse model phenotype; the fld mouse was demonstrated to have a mutation in the mouse Lpin1 gene that is highly expressed in adipose tissue (8). We examined linkage to these loci in all samples and neither sibship B1 nor C5 demonstrated homozygosity at these loci.

In conclusion, novel mutations in the seipin gene (W259X and cDNA:1,016/-2 A→C) were identified in three of five CGL subjects in group A of the Omani samples. Interestingly, the haplotypes and mutations observed in these subjects were not identical, suggesting that although group A shared a similar phenotype and the same geographical origin, the lipodystrophy-associated locus was not inherited from a common ancestor. Group B and C subjects appear to be unlinked to *BSCL2*. The majority of subjects unlinked to 11q13 showed homozygosity at one flanking marker of the 9q34 locus. As the interval between these markers is large, we cannot exclude the possibility that subjects A4, A5, C1, C2, C3, and C4 were linked to this locus and underwent recombination within the interval. However, the shared and distinct phenotype of all families in group C strongly suggests that they may share a common genetic etiology that is distinct from family B1. It therefore appears that there are two or more novel loci for autosomal recessive CGL in the Omani population.

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