Evidence for Linkage of Human Primary Systemic Carnitine Deficiency with *D5S436:* A Novel Gene Locus on Chromosome 5q

Yutaka Shoji,[1] Akio Koizumi,[2] Tsuyoshi Kayo,[2] Tomoaki Ohata,[1,2] Tsutomu Takahashi,[1] Kenji Harada,[1] and Goro Takada[1]

Departments of [1]Pediatrics and [2]Hygiene, Akita University School of Medicine, Akita, Japan

Summary

Primary systemic carnitine deficiency (SCD) is a rare hereditary disorder transmitted by an autosomal recessive mode of inheritance. The disorder includes cardiomyopathy, muscle weakness, hypoketotic coma with hypoglycemia, and hyperammonemia. In this study, we conducted a linkage analysis of a Japanese SCD family with a proband—a 9-year-old girl—and 26 members. The serum and urinary carnitine levels were determined for all members. The entire genome was searched for linkage to the gene locus for SCD, by use of a total of ~300 polymorphic markers located ~15–20 cM apart. In the family, there were two significantly different phenotypes, in terms of serum free-carnitine levels: low serum free-carnitine level (29.5 ± 5.0 μM; n = 14) and normal serum free-carnitine level (46.8 ± 6.2 μM; n = 12). There was no correlation of urinary free-carnitine levels with the low serum-level phenotype (putative heterozygote), but in normal phenotypes (wild type) urinary levels decreased as the serum levels decreased; renal resorption of free carnitine appeared to be complete in wild-type individuals, when the serum free-carnitine level was <36 μM. Linkage analysis using an autosomal dominant mode of inheritance of heterozygosity revealed a tight linkage between the disease allele and *D5S436* on chromosome 5q, with a two-point LOD score of 4.98 and a multipoint LOD score of 5.52. The haplotype analysis revealed that the responsible genetic locus lies between *D5S658* and *D5S434,* which we named the *"SCD"* locus. This region was syntenic with the *jvs* locus, which is responsible for murine SCD. Phylogenic conversion of the *SCD* locus strongly suggests involvement of a single gene, in human SCD.

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Address for correspondence and reprints: Dr. Akio Koizumi, Department of Hygiene, Akita University School of Medicine, Hondo 1-1-1, Akita 010-8543, Japan. E-mail: koizumi@med.akita-u.ac.jp

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Introduction

Primary systemic carnitine deficiency (SCD; MIM 212140) is a rare hereditary disorder that is transmitted by an autosomal recessive mode of inheritance (Roe and Coates 1995). It is a specific disease characterized by progressive cardiomyopathy, skeletal myopathy, hypoketotic hypoglycemic encephalopathy, and hyperammonemia. Furthermore, SCD is emerging as one of the causes of sudden infant death syndrome (Rinaldo et al. 1997).

The hallmark of SCD is low concentrations of carnitine in plasma and targeted tissues, with accumulation of lipid deposits and renal leakage of carnitine. The clinical symptoms are alleviated dramatically by oral administration of L-carnitine (Tripp et al. 1981) but, if the SCD is untreated, are precipitated into a crisis, with Reye-like syndrome or cardiac arrest, by even a minor episode of respiratory illness. The studies of carnitine uptake in vitro support the concept that SCD is due to a defect in the active transport of carnitine from extracellular fluid into the cell, in selected tissues, such as kidney, heart, muscle, and fibroblasts (Treem et al. 1988).

Mice with juvenile visceral steatosis (jvs) (Koizumi et al. 1988) have been suggested as a murine disease model for human SCD. In homozygous mice, the serum total- and free-carnitine levels were significantly lower than those in control mice. On the other hand, there was no decrease in urinary excretion of carnitines (Kuwajima et al. 1991). The linkage analysis located the responsible locus, *jvs,* to chromosome 11 (Nikaido et al. 1995); the putative syntenic region in the human genome is located on the q arm of chromosome 5.

As yet, the molecular basis of human SCD has not been explored. Linkage analysis would provide pivotal information leading to the identification of the gene responsible for SCD. The linkage analysis, however, is confronted with two major obstacles. First, in order to map the responsible locus, a large number of families would be required, owing to the mode of inheritance of an autosomal recessive trait, unless heterozygotes could be segregated by phenotype. Second, identification of het-

erogeneity of the disease phenotype has been suspect in human SCD (Roe and Coates 1995). One phenotype is an early age-at-onset type with recurrent attacks of Reye-like syndrome, with or without cardiomyopathy. Another is a late age-at-onset type with cardiomyopathy and without any recurrent episodes of Reye-like syndrome.

One solution to these difficulties is to conduct a linkage analysis of the family of a proband, with a clear clinical segregation of heterozygous subjects from the wild types. This approach appears to be feasible, since the parents of a proband who are heterozygous for the defective gene have been reported to have impaired carnitine transport that is 40%–50% of normal (Tein et al. 1990).

In this article, we report the results of a linkage analysis of a family with SCD. The expected difficulties were circumvented by the clear segregation of heterozygotes from wild types. A single responsible gene locus was assigned to 5q, which is in complete agreement with the syntenic region of the murine model *jvs* locus.

Subjects and Methods

The Patient and Study Pedigree

A girl (the proband; subject 20, fig. 1), the first child of unrelated parents, had complained intermittently of easy fatigue, vomiting, and abdominal pain, from 7 years of age. The proband was admitted to the Akita University Hospital when she was 8 years of age. She had unexplained fever, weakness, irregular respiration, and bradycardia and had lapsed into unconsciousness. She was found to have hepatomegaly and muscle weakness. The plasma or serum levels were as follows: ammonia at 179 μg/dl, asparatate aminotransferase at 275 IU/liter, alanine aminotransferase at 146 IU/liter, lactate dehydrogenase at 1,005 IU/liter, and creatine kinase at 1,261 IU/liter. Total- and free-carnitine levels in the serum were 4.6 and 3.6 μM, respectively, with a relatively high total urinary-carnitine concentration (225.7 μM). Dicarboxylic aciduria in the urine was absent, as determined by gas chromatography/mass spectrometry (GC/MS). A standard laboratory blood panel was taken for the proband, which included blood pH, blood gases, red blood cell count, and blood glucose levels. Background electroencephalogram activity revealed a pattern of predominantly δ waves and less θ waves, at admission. Echocardiograms showed left ventricular hypertrophy with normal left ventricular systolic function. The clinical symptoms gradually improved with intravenous glucose-drip infusion and disappeared within a few days. However, the hyperammonemia and extremely low carnitine concentrations in the serum were not alleviated by the treatment. The carnitine uptake was assessed in vitro by use of cultured skin fibroblasts from the proband and her parents, as described elsewhere (Stanley et al. 1991). The mean ($\pm$SD) uptake velocity (V) at 5 μM of extracellular carnitine was 0.12 $\pm$ 0.01 pmol/min/mg protein (triplicate determinations) for the proband, 0.64 $\pm$ 0.01 pmol/min/mg protein (triplicate determinations) for the parents (subjects 12 and 13), and 1.12 $\pm$ 0.31 pmol/min/mg protein (triplicate determinations) for the normal subjects ($n = 5$). The V_{max} of carnitine uptake for fibroblasts from the parents was 56% of that of the normal subjects (triplicate determinations, 0.95 $\pm$ 0.26 pmol/min/mg protein for the parents and 1.70 $\pm$ 0.82 pmol/min/mg protein for the normal subjects), but the Michaelis constants (K_m's) were comparable (triplicate determinations, 3.82 $\pm$ 1.30 μM for the parents and 3.42 $\pm$ 1.63 μM for the normal subjects). In the patient, the renal plasma threshold for free-carnitine excretion and the tubular reabsorptive maximum, per 100 ml glomerular filtrate, for free carnitine were 17 nmol/ml and 3.7 μmol/100 ml, respectively, which were lower than the reported normal values 46 $\pm$ 7.1 nmol/ml and 7.2 $\pm$ 0.44 μmol/100 ml (Engel et al. 1981).

This was the proband's first episode of Reye-like syndrome. There was no family history of sudden infant death syndrome, Reye syndrome, or unexplained neuralgic, cardiac, or muscle disease. The proband's two siblings (subjects 21 and 22) have not had such episodes.

On the basis of these findings, we diagnosed the proband as having SCD. Since the episode of illness, the patient has been treated with L-carnitine, 100–150 mg/kg/d taken orally, for 14 mo and has not suffered from any subsequent attacks. Initially, her body weight and height were below the first SD of the age-matched mean levels; since treatment began, her height and weight have been increasing and are now approaching average. Cardiac hypertrophy and muscular weakness also have improved significantly. Past medical-history interviews by physicians of the other family members turned up no reports of muscular weakness, cardiac symptoms, or other related symptoms.

Serum- and Urinary-Carnitine Determination

Participants were requested to refrain from eating meat at their most recent meal and to fast between the evening meal and the interview time. Fasting blood and urine samples were collected at the early morning interviews. In addition to the first sample collection, blood and urine samples were collected at two other times, from selected members whose initial serum total- and free-carnitine levels met the screening criteria. To set the screening criteria, we determined serum carnitine levels for 50 male and 50 female volunteers, whose ages were

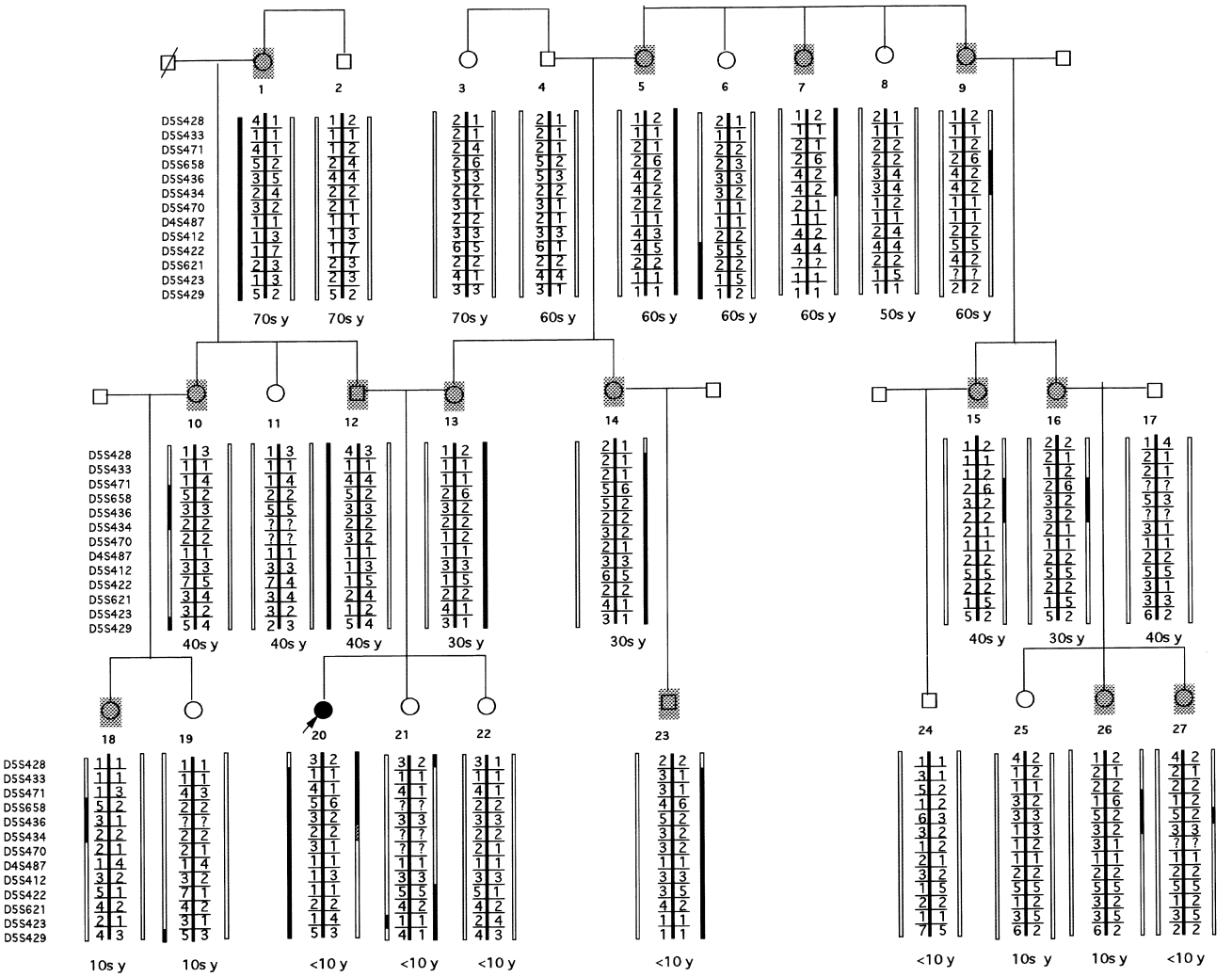

Figure 1 Haplotypes and recombinations in the study pedigree. The alleles at the microsatellite loci *D5S428*, *D5S433*, *D5S471*, *D5S658*, *D5S436*, *D5S434*, *D5S470*, *D5S487*, *D5S412*, *D5S422*, *D5S621*, *D5S423*, and *D5S429* are given for each individual. A question mark (?) indicates that no data were available. Haplotypes were optimized by use of the GENEHUNTER software, and the affected chromosomes in the grandparent generation are represented by blackened bars (subjects 1 and 5). A gray-shaded bar (subject 20) indicates that whether the region is from an affected or a normal chromosome was undetermined. No attempt was made to show crossovers on the normal chromosome. Circles denote females; squares denote males; the blackened circle denotes the proband; gray-shaded symbols denote heterozygotes; and the symbol with a slash denotes a deceased member of the pedigree. To protect privacy, ages were abbreviated as follows: "<10 y" for those younger than 10 years; "10s y" for teens; and "30s y" for those in their 30s, "40s y" for those in their 40s, and so on.

within the range of 1–75 years. These volunteers also were requested to refrain from eating meat at their most recent meal and to fast between the evening meal and the blood collection.

Serum and urinary carnitine levels were determined by an enzymatic cycling method using carnitine dehydrogenase (Takahashi et al. 1994). Serum was separated from the whole blood by centrifugation on the day of sample collection. Urine and serum were stored at −20°C until assayed. Total-, acyl-, and free-carnitine concentrations were standardized against creatinine concentrations in the urine.

Preparation of DNA and Marker Analysis

DNA also was isolated from the blood collected during the interview. DNA was extracted by use of a DNA isolation kit (DNA Purification System, Promega). A total of ~300 polymorphic markers, located ~15–20 cM apart, were selected on the basis of their polymorphic-

information content, garnered primarily from the genetic map from Généthon and the National Institute of Health–Center d'Etude du Polymorphism Humain. High-resolution mapping was conducted for the area covering the region syntenic with the mouse *jvs* gene (Nikaido et al. 1995), by use of a total of 13 polymorphic dinucleotide-repeat markers from *D5S428* to *D5S429*. These markers covered the entire cytogenic region from 5q13.1. to 5q35.1. Three different fluorescent dye–labeled primer pairs were prepared for visualization of the PCR products. PCR was performed in a 15-μl volume containing 50 ng of genomic template, 5.0 pmol of each primer, 0.3 U of DNA polymerase (Taq Gold, Takara), 300 μM of each dNTP, 1.5 μl of standard buffer, and 1.5 mM of $MgCl_2$. Amplification was performed in an MJ Research PTC-100 thermal cycler, at 95°C for 10 min followed by 30 cycles at 94°C or 89°C for 15 s, 55°C for 15 s, and 72°C for 30 s and a final extension at 72°C for 10 min. The amplified DNA products were analyzed by capillary electrophoresis on an ABI PRISM310 genetic analyzer (Applied Biosystems).

Linkage Analysis

Two-point linkage analysis was performed on a personal computer by use of version 5.2 of the MLINK program (Lathrop et al. 1984). Multipoint calculations were performed by use of the LINKAGE package (Lathrop et al. 1984) and the GENEHUNTER software (Kruglyak et al. 1996). The frequencies of the disease allele and the normal allele were assumed to be .0001 and .9999, respectively, and the observed frequencies in the family were assigned for the allele frequencies of microsatellite markers. In the analysis, we assumed a fully penetrant dominant phenotype, on the basis of heterozygosity. Since the linkage-analysis program GENEHUNTER limits the number of family members per pedigree, we separated the pedigree into three families, using a method described elsewhere (Terwilliger and Ott 1994). Robustness with respect to separation of the family was confirmed by testing different combinations of separation. The calculated results were compared with the results obtained by LINKMAP and were found to be essentially the same. We thus present multipoint calculations obtained by using the GENEHUNTER program.

Informed Consent

Twenty-seven members of the pedigree participated in the study and had signed informed-consent forms, in accordance with Akita University Hospital guidelines. We also obtained informed consent from the 50 male and the 50 female blood donors used for the determination of standard levels of carnitine, for the setting of the screening criteria. For individuals <14 years of age, we obtained informed consent from their parents or guardians.

Statistics

Serum carnitine values were presented as the means ± SDs. Statistical analyses, by ANOVA and regression analysis, were performed on a Macintosh computer by use of a statistical software package, STATIVIEW J4.5 (Abacus Concepts). A value of $P < .05$ was considered to be significant.

Results

Screening Heterozygotes

Serum carnitine levels in the population are shown in table 1. Although serum free-carnitine levels in males and females were statistically different, from a medical viewpoint, these differences were negligible. We thus determined common criteria for tentative assignment of heterozygotes, for both genders. The screening levels for heterozygotes were set as the mean − 2 SD. We did not use serum acylcarnitine for screening, because of a large SD for a relatively small mean for this value. The tentative screening criteria for putative heterozygotes were defined as serum total-carnitine levels <46 μM and serum free-carnitine levels <36 μM. Using these criteria, we classified individuals as putative heterozygotes (low-carnitine phenotype) or as having the wild-type phenotype (normal-carnitine phenotype), as shown in figure 1 and table 2. Note that the three repeat measures of serum total- and free-carnitine levels in putative heterozygotes never exceeded the screening level, except for those of subject 7. On the other hand, the levels in all the wild-type individuals were higher than the screening levels. Subject 7 was undergoing hemodialysis and had low serum free-carnitine levels but relatively high levels of acylcarnitine. We collected blood samples from this individual 1 d before hemodialysis.

Renal handling of carnitine also was evaluated in pu-

Table 1

Serum Carnitine Levels of 50 Males and 50 Females, for Setting of the Heterogeneity Criteria

	Concentration of Serum Carnitine[a] (mM)		
Sex	Total	Free	Acyl
Male	61.0 ± 6.9	51.3 ± 5.6*	9.8 ± 4.4
Female	59.6 ± 5.9	48.4 ± 6.10	11.8 ± 4.2

[a] Determined by the enzymatic cycling method. Values are means ± SDs.

* $P < .05$, by ANOVA.

Table 2

Segregation of Serum Total- and Free-Carnitine Levels, with Haplotypes at *D5S658, D5S436,* and *D5S434*

Subject	Sex	Concentration of Serum Carnitine[a] (μM)			Haploytype[e]
		Total[b]	Free[c]	Acyl[d]	
Putative heterozygote:					
1	Female	42.1 ± 3.3	33.9 ± 1.4	8.2 ± 1.9	532/254
5	Female	44.0 ± .2	33.2 ± 1.8	10.8 ± 1.6	622/244
7	Female	46.2 ± 1.3	24.0 ± 1.9	22.8 ± .5	622/244
9	Female	34.0 ± .9	27.1 ± .7	6.9 ± .2	622/244
10	Female	35.4 ± .3	30.7 ± .3	4.7 ± .0	532/232
12	Male	40.0 ± 1.0	35.1 ± .2	4.9 ± .8	532/232
13	Female	36.6 ± 2.4	28.1 ± 3.3	8.5 ± .8	622/232
14	Female	40.9 ± 1.6	33.7 ± 1.9	7.2 ± .3	622/552
15	Female	27.4 ± 1.5	18.7 ± 1.3	8.7 ± .5	622/232
16	Female	27.3 ± 2.4	22.4 ± 2.2	5.4 ± .3	622/233
18	Female	33.2 ± 2.4	28.9 ± 2.8	4.4 ± .5	532/212
23	Male	43.5 ± .6	35.0 ± .4	8.5 ± .2	622/453
26	Female	36.4 ± .4	32.0 ± 1.4	4.4 ± 1.2	622/153
27	Female	34.0 ± 2.3	30.7 ± 1.2	3.3 ± 1.2	223/153
Wild-type phenotype:					
2	Male	64.0	55.5	8.5	442/242
3	Female	68.7	56.9	11.8	632/252
4	Male	70.4	56.0	14.4	232/552
6	Female	50.1	40.7	9.4	332/233
8	Female	60.8	50.2	10.6	244/233
11	Female	53.2	40.7	12.5	25?/25?
17	Male	50.8	45.1	5.7	?3?/?5?
19	Female	54.1	44.0	10.1	2?2/2?2
21	Female	59.5	44.5	15.0	?3?/?3?
22	Female	64.5	41.2	23.3	232/232
24	Male	51.0	43.7	7.3	232/163
25	Female	50.4	42.9	7.5	233/331

[a] Serum total-, free-, and acylcarnitine levels were compared between two groups. Values for putative heterozygotes are mean ± SD.

[b] For putative heterozygotes, mean = 37.2, SD 5.9. For wild phenotypes, mean = 58.1, SD 7.5. The former mean is significantly smaller than the latter ($P < .01$, by ANOVA).

[c] For putative heterozygotes, mean = 29.5, SD 5.0. For wild phenotypes, mean = 46.8, SD 6.2. The former mean is significantly smaller than the latter ($P < .01$, by ANOVA).

[d] For putative heterozygotes, mean = 7.7, SD 4.8. For wild phenotypes, mean = 11.3, SD 4.7. There is no significant difference between the two means.

[e] For the haplotype of each individual, refer to the pedigree shown in figure 1. An underlined haplotype indicates an affected chromosomal segment. A question (?) mark indicates that no data were available. The haplotype for the proband (subject 20) was 622/532.

tative heterozygotes and in those having wild-type phenotypes. Analysis revealed a unique relationship between serum free-carnitine levels and urinary excretion of free carnitine. Excretion of free carnitine (μmoles/g creatinine) increased linearly in wild types, whereas that of putative heterozygotes showed a large variation irrespective of lowered serum carnitine levels (fig. 2*A*). In the putative heterozygotes, free carnitine excreted in the urine remained high even at serum levels <36 μM, but in the wild types complete renal reabsorption was predicted to occur, by regression analysis, when serum concentrations were below this level. These data indicate that there is an apparent phenotypic heterogeneity in renal excretion of free carnitine, in relation to serum levels in the pedigree: there was apparent renal leakage in putative heterozygotes. In contrast, urinary acylcarnitine was found to be proportional to its serum levels in both heterozygotes and wild types (fig. 2*B*).

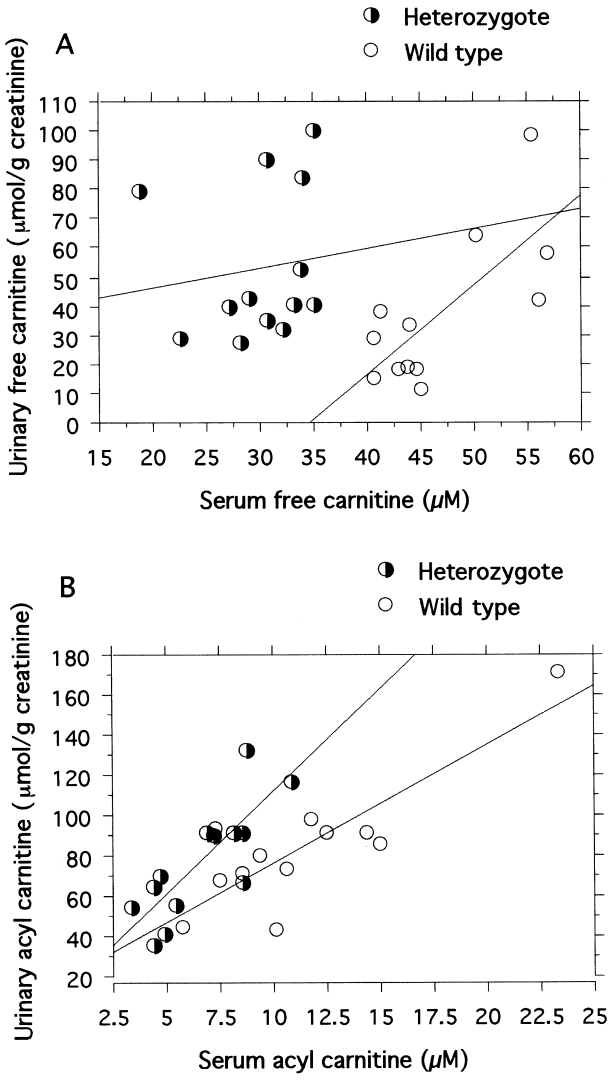

Figure 2 Relationship between serum and urinary free- and acylcarnitine levels in putative heterozygotes and wild-type subjects. Serum and urinary free- and acylcarnitine levels were determined as the mean of three determinations, for putative heterozygotes, whereas those for wild types were determined once. Subject 7 is on hemodialysis and was not included, since urine samples were not available. *A*, Serum free-carnitine levels (in μM; *X*-axis) and urinary free-carnitine levels (in μmol/g creatinine; *Y*-axis). The regression line for wild-type phenotypes was $Y = -106.4 + 3.1X$ ($r = .740$, $P < .05$), whereas that for heterozygotes was $Y = 30.4 + 0.77X$ ($r = .152$, $P > .05$). *B*, Serum acylcarnitine levels (in μM; *X*-axis) and urinary acylcarnitine levels (in μmol/g creatinine; *Y*-axis). The regression line for wild-type phenotypes was $Y = 17.4 + 5.9X$ ($r = .847$, $P < .01$), whereas that for heterozygotes was $Y = 9.6 + 10.2X$ ($r = .803$, $P > .01$).

Linkage Analysis

These phenotypic characteristics suggested that the phenotypic heterogeneity was inherited in an autosomal dominant mode (fig. 1). We therefore assumed an autosomal dominant mode of inheritance and a penetrance of 99% for the linkage analysis. A search of the entire genome revealed significant linkages with markers on 5q. The two-point LOD scores for the 13 microsatellite markers are shown in table 3: The highest linkage was observed between the disease allele and *D5S436,* with a LOD score of 4.98. Multipoint linkage analysis revealed large LOD scores between *D5S658* and *D5S434* (fig. 3), with a maximum LOD score (Z_{max}) of 5.52 at *D5S436.*

Since hemodialysis is known to reduce plasma free carnitine to subnormal levels (31–33 μM) and is known to elevate plasma acylcarnitine in wild-type patients (Golper et al. 1990), we also conducted linkage analysis after excluding a patient (individual 7) who was undergoing hemodialysis. Exclusion of this individual decreased two-point and multipoint LOD scores to 4.67 and 5.22, respectively, but did not change the peak position at *D5S436,* with minor changes of other values (data not shown).

The haplotype analysis of the affected chromosome helped to determine the location of the gene by revealing crossovers between the disease allele and the markers (fig. 1). In subject 27, double crossovers between the disease allele and *D5S658* and *D5S434* had occurred. Thus, on the basis of haplotype analysis, the gene responsible for SCD lies at *D5S436,* within a region between *D5S658* and *D5S434.*

Finally, we confirmed that serum total- and free-carnitine levels in heterozygous and wild-type subjects segregated clearly with haplotypes at *D5S658, D5S436,* and *D5S434* (table 2). All subjects whose serum free-carnitine levels were <36 μM shared one of two affected chromosomal segments with the proband, whereas none of the wild types shared these characteristics. This pattern of segregation is in complete accordance with the

Table 3

Two-Point LOD Scores between *SCD* Locus and 13 Marker Loci on 5q

	LOD Score at Recombination Fraction (θ) of							
Marker	.00	.01	.03	.1	.2	.3	Z_{max}	θ_{max}
D5S428	$-\infty$	−2.99	−1.68	−.54	−.17	−.04	−.04	.3
D5S433	.25	.25	.24	.22	.18	.14	.25	.0
D5S471	$-\infty$	−.51	.29	.82	.74	.48	.84	.13
D5S658	$-\infty$	3.42	3.71	3.52	2.72	1.72	3.73	.04
D5S436	4.98	4.89	4.70	4.04	3.01	1.91	4.98	.0
D5S434	1.78	1.77	1.73	1.53	1.16	.7	1.78	.0
D5S470	$-\infty$	−1.65	−.75	.08	.36	.36	.36	.3
D5S487	.24	.24	.24	.22	.18	.14	.24	.1
D5S412	$-\infty$	−3.85	−2.37	−.81	−.10	.12	.12	.3
D5S422	$-\infty$	−8.42	−5.6	−2.64	−1.17	−.5	−.5	.3
D5S621	$-\infty$	−1.91	−1.01	−.16	.17	.23	.23	.3
D5S423	$-\infty$	−8.66	−5.47	−2.31	−.92	−.37	−.37	.3
D5S429	$-\infty$	−7.29	−4.48	−1.6	−.31	.13	.13	.3

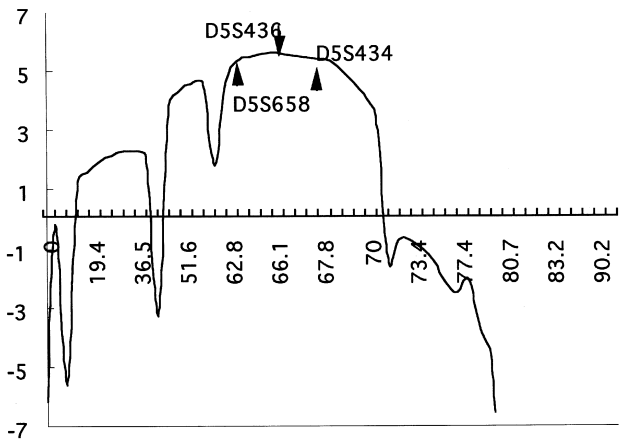

Figure 3 Fourteen-point linkage analysis between *SCD* locus and 13 marker microsatellite loci. Locus *D5S428* was selected as a starting point for the map. The Z_{max} and corresponding map location were 5.52 and 66.1 cM, respectively. The centromere is located to the left of the graph and is not shown.

presence or the absence of the defective gene, in this region.

Discussion

The gene for primary SCD in humans was assigned successfully to the long arm of chromosome 5 (5q31.1-32), by linkage analysis of a single pedigree of an affected family living in Japan. This is the first report on the location of the genetic locus for human SCD. Our results suggest that the carnitine-transporter gene lies on chromosome 5q in humans. We would like to name this locus *"SCD."*

The haplotype analysis of the pedigree demonstrated that there were no crossovers in a region flanking *D5S658* and *D5S434,* except in subject 27. Haplotype analysis of subject 27 indicated that *D5S436,* located at 5q31.1, was the closest microsatellite marker. The inheritance of these marker alleles is completely consistent with the phenotypes based on serum free-carnitine concentration and urinary excretion, autosomal dominant mode of inheritance, and full penetrance. Furthermore, it is quite interesting that the human assignment of *SCD* is in complete agreement with a region syntenic with the mouse *jvs* locus (Nikaido et al. 1995). The candidate gene, which codes for a carnitine transporter, is expressed in different tissues (heart, muscle, kidney, and fibroblasts) that play critical roles in SCD. The evidence that the genetic defect for *SCD* appears to be the same in evolutionarily distant mammals, such as humans and mice, not only implies that the common mechanism is phylogenetically conserved but also suggests that a single gene is involved in SCD in both species.

Heterogeneity as determined by clinical phenotypes often has been found to be suspect (Garavaglia et al. 1991; Roe and Coates 1995). The proband in this study was classified as a case of late age at onset, judging from her age at the first episode, but also could be classified as a case of early age at onset, from the viewpoint of symptomology. The age at onset easily may be affected by various factors, such as incidences of respiratory infections. Likewise, variation of symptoms also may be modified by the maturity of physiological reservoir capacities against stress. At present, we assume a single locus for SCD, despite apparent variations in clinically diagnosed onset.

In conclusion, this study gave definitive evidence for the genetic basis of SCD. Positional cloning of the carnitine-transporter gene will shed light on its physiological role, as well as on the molecular mechanisms of the disease. More importantly, the results of this study will enable physicians to conduct genetic counseling and heterozygote screening of families that have a history suggestive of SCD, a disease that is curable but that is often overlooked and, therefore, fatal.

Acknowledgments

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Electronic-Database Information

Accession number and URL for data in this article are as follows:

Online Mendelian inheritance in man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for SCD [MIM 212140])

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