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Homozygosity and Linkage-Disequilibrium Mapping of the Syndrome of Congenital Hypoparathyroidism, Growth and Mental Retardation, and Dysmorphism to a 1-cM Interval on Chromosome 1q42-43

Ruti Parvari,[1] Eli Hershkovitz,[2] Adam Kanis,[3] Rafael Gorodischer,[2] Shlomit Shalitin,[4] Val C. Sheffield,[3] and Rivka Carmi[1]

[1]Genetics Institute and [2]Pediatric Department, Soroka Medical Center and the Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva, Israel; [3]Department of Pediatrics, Division of Medical Genetics, University of Iowa, Iowa City; and [4]Institute of Pediatric Endocrinology, Schneider Children's Medical Center of Israel, Petah Tikva, Israel

Summary

The syndrome of hypoparathyroidism associated with growth retardation, developmental delay, and dysmorphism (HRD) is a newly described, autosomal recessive, congenital disorder with severe, often fatal consequences. Since the syndrome is very rare, with all parents of affected individuals being consanguineous, it is presumed to be caused by homozygous inheritance of a single recessive mutation from a common ancestor. To localize the HRD gene, we performed a genomewide screen using DNA pooling and homozygosity mapping for apparently unlinked kindreds. Analysis of a panel of 359 highly polymorphic markers revealed linkage to D1S235. The maximum LOD score obtained was 4.11 at a recombination fraction of 0. Analysis of three additional markers—GGAA6F06, D1S2678, and D1S179—in a 2-cM interval around D1S235 resulted in LOD scores >3. Analysis of additional chromosome 1 markers revealed evidence of genetic linkage disequilibrium and place the HRD locus within an ~1-cM interval defined by D1S1540 and D1S2678 on chromosome 1q42-43.

Introduction

Hypoparathyroidism represents a range of clinical and biochemical syndromes characterized by parathyroid hormone (PTH) deficiency, hypocalcemia, and hyperphosphatemia. Although transient neonatal hypoparathyroidism is relatively common, congenital permanent hypoparathyroidism is rare. This latter condition may be isolated or associated with various syndromes and chromosome aberrations (Alon and Chan 1985). In addition to sporadic cases, X-linked recessive (Whyte and Weldon 1981) and autosomal dominant (Winter et al. 1983) forms have been reported, and the genes causing these forms have been mapped to Xq26-27 (Thakker et al. 1990) and 3q13 (Finegold et al. 1994), respectively. The later gene encodes the calcium-ion–sensing receptor that responds to, or "senses," extracellular calcium-ion concentrations in the parathyroid glands, kidney, and other tissues (Brown et al. 1995). Mutations in the calcium-sensing receptor were recently shown to be the cause of autosomal dominant hypoparathyroidism (Pearce et al. 1996) and of familial benign hypercalcemia (Pollak et al. 1993; Aida et al. 1995; Chou et al. 1995; Janicic et al. 1995; Pearce et al. 1995; Health et al. 1996). A donor splice-site mutation in the PTH gene, located on the short arm of chromosome 11, was associated with autosomal recessive hypoparathyroidism in one family (Parkinson and Thakker 1992). Hypoparathyroidism may be inherited as part of an autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy (APECED) or as part of the DiGeorge syndrome, in which developmental defects of the third and fourth pharyngeal pouches result in parathyroid and thymic aplasia with cardiac and facial anomalies. Genetic studies have mapped the DiGeorge syndrome to chromosome 22q11 (Carey et al. 1992; Driscoll et al. 1992), and the cause of APECED has been found to be mutations in the zinc-finger domains of a novel gene that may be a transcription regulator (Finnish-German APECED Consortium 1997; Nagamine et al. 1997). Hypoparathyroidism has also been reported in association with some rare syndromes, such as Zellweger syndrome (Alon and Chan 1985), Kenny-Caffey syndrome (Fanconi et al. 1986), and renal failure due to dysplastic kidneys, as well as with sensorineural deafness (Shaw et al. 1991; Bilous et al. 1992) with or without developmental delay. Thus, several genes in different locations

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Address for correspondence and reprints: Dr. Ruti Parvari, Genetics Institute, Soroka Medical Center, Beer-Sheva 84101, Israel. E-mail: ruthi@bguvms.bgu.ac.il

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are probably involved in both normal embryonic development of the parathyroid glands and maintenance of serum calcium homeostasis.

Two reports have been published concerning a new syndrome of hypoparathyroidism in patients of Arab origin (Sanjad et al. 1991; Hershkovitz et al. 1995). The syndrome consists of hypoparathyroidism, severe growth and mental retardation, and dysmorphic features (HRD), including microcephaly, deep-set eyes, flat nasal bridge, microphthalmia, small hands and feet, and abnormal dentition. No immunological abnormalities were observed. Normal karyotypes were found, and FISH analysis in several patients failed to disclose deletion at the DiGeorge locus in 22q11 (Hershkovitz et al. 1995). These findings in Saudi Arabian and Israeli Bedouin patients represent a unique syndrome of hypoparathyroidism that is clinically and genetically different from the well-known forms of hypoparathyroidism. The inheritance of this syndrome is compatible with the autosomal recessive model (MIM 241410). Syndromes that are similar but that include a reduced number of T lymphocytes (Richardson and Kirk 1990) and medullary stenosis of long bones (Khan et al. 1997) have also been described in Middle Eastern Arab patients.

Since HRD is a rare disorder, so far found only in Arab populations, it had been assumed that the disease is the result of homozygous inheritance of a recessive mutation from a common ancestor. In this study we undertook the localization of the HRD gene, using a DNA-pooling and homozygosity-mapping approach, in the previously reported Bedouin families (Hershkovitz et al. 1995), as well as in several newly ascertained families. Homozygosity mapping is based on the realization that patients with recessive diseases resulting from consanguineous marriages should have increased homozygosity, compared with random expectation, for markers adjacent to the disease gene (Smith 1953; Lander and Botstein 1987). Here we report the mapping of the HRD syndrome to a l-cM interval on 1q42-43 by means of homozygosity and linkage disequilibrium.

Subjects and Methods

Study Pedigrees

During the past 6 years, we have encountered 12 patients (6 females and 6 males; fig. 1) from six families with HRD (Hershkovitz et al. 1995). All were of Bedouin origin, and at least six were from the same clan. One extended family (pedigree C) had six affected, of whom two died before the initiation of the study. Another family (pedigree E) had two affected individuals, and four families had one affected each. Initially, eight patients were available, and DNA pooling was performed. During the course of the study, two new patients were born into pedigrees D and E. Although the various nuclear families denied any known genetic relationship between them, the rarity of the disease and the common ethnicity suggested a common ancestry.

All patients were affected with intrauterine and severe postnatal growth retardation (weight, height, and head circumference >4 SD below the means for age and sex). After being diagnosed, all patients were treated with a 1-α vitamin D_3/calcium supplement. Five patients have died. Pneumonia and sepsis were the cause of death in two children; the other three children died at home. Postmortem examinations were denied by the families; thus, the existence and state of the parathyroid glands in the patients could not be verified. All patients had the typical dysmorphic features described elsewhere (Hershkovitz et al. 1995) but no cardiac or other major malformation. Severe psychomotor delay was evident in early infancy. Undetectable or very low serum PTH levels in the presence of severe persistent hypocalcemia and hyperphosphatemia confirmed the diagnosis of permanent hypoparathyroidism. There was an absence of immunological and chromosomal abnormalities, and no medullary stenosis of long bones was found. Although the patients' phenotype resembles that of Kenny-Caffey syndrome, recently described in Bedouins from Kuwait (Khan et al. 1997), the absence of cortical thickening or overtubulation of long bones suggested that our patients have the unique syndrome of HRD first described, in Saudi Arabian patients, by Sanjad et al. (1991). Inheritance of the syndrome in the families reported here is compatible with the autosomal recessive mode.

Homozygosity Mapping and DNA Pooling

The study was approved by the Soroka Medical Center review board. DNA was prepared, by use of standard methods, from 10–20-ml samples of peripheral blood. Fifty nanograms of DNA from each individual was used in the pools. The genomewide screening was performed for two sets of pooled samples, as detailed in the Results section.

Microsatellite Markers and Genotyping

The short tandem-repeat polymorphic markers (STRPs) used were developed by the Cooperative Human Linkage Center (CHLC; Sheffield 1997) and purchased from Research Genetics. These markers are arranged in 150 groups of two to three markers that PCR amplifies together. PCR, gel separation, and silver-staining conditions were performed in accordance with methods described by Sheffield (1997). Regions with noninformative markers were filled with dinucleotide markers from the Weber version 8 genome screening set (Research Genetics). PCR conditions were as described above, except that 0.01 μCi of α-[^{32}P] dCTP was in-

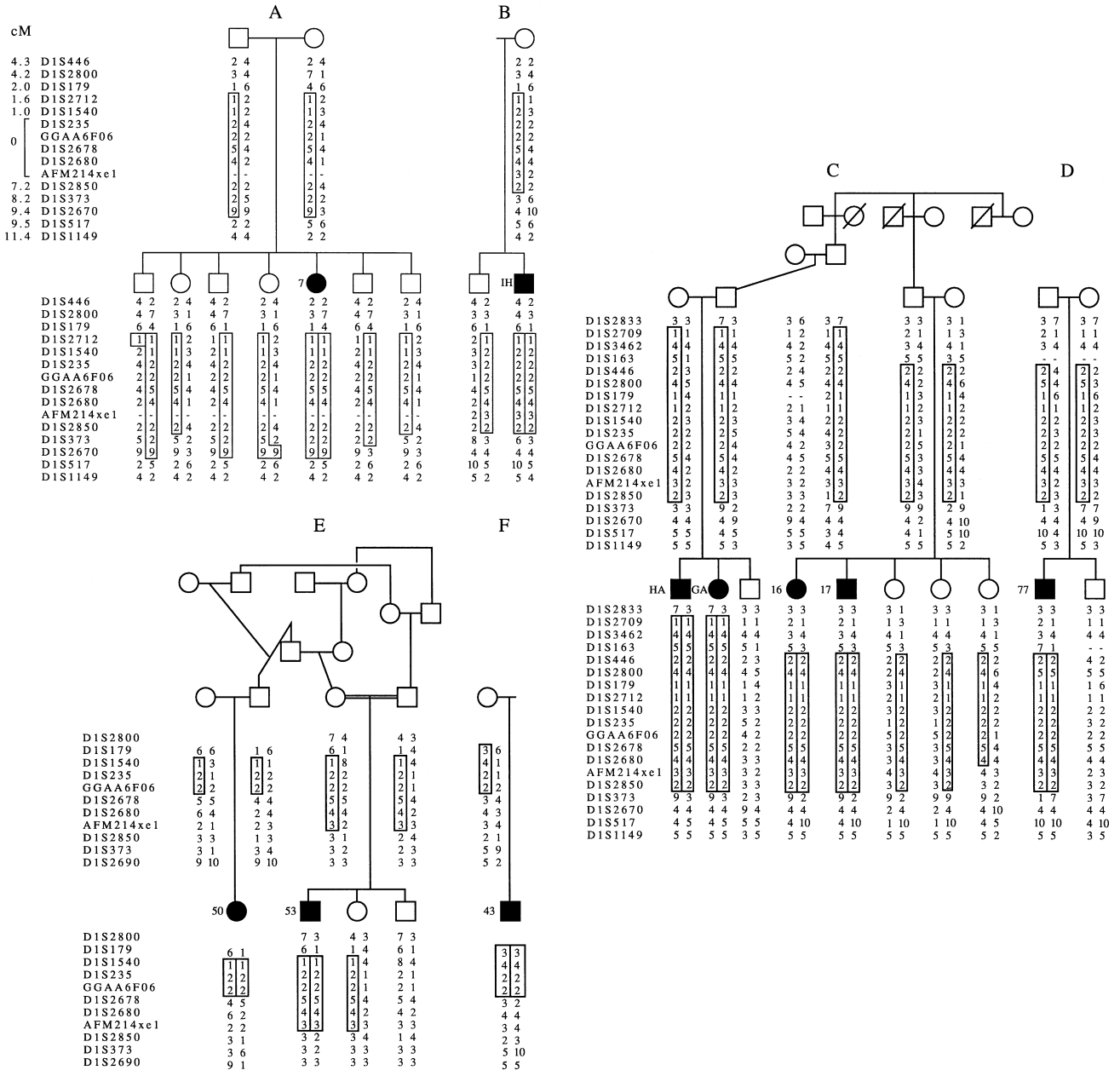

Figure 1 HDR pedigrees showing the recessive inheritance of the disease. The genotypes are presented under each individual analyzed in this study. The haplotypes showing homozygous inheritance near the HDR locus in each pedigree are boxed. The genetic distances between the markers are shown. Their distances and order are based on information derived from the recombination maps compiled by the Third International Workshop on Human Chromosome 1 (Chromosome 1 homepage).

cluded in the reaction, and PCR products were visualized by exposure of the gels to Kodak X-ray film overnight.

Linkage Analysis

Two-point LOD scores (Z) were computed by the LINKAGE and FASTLINK programs (Lathrop et al. 1984; Cottingham et al. 1993). A penetrance of 100% was assumed. Equal allele frequencies were assumed for each marker.

Results

A total of 353 STRPs distributed at ~10-cM intervals through all autosomes (Sheffield 1997) were first

Table 1

Pairwise LOD Scores between HDR and 17 Chromosome 1q42-43 Microsatellite Markers

Locus	Marker	Genetic Distance[a] (cM)	Z at θ = .00	.01	.05	.10	.20	.30	.40	Maximum Z	Maximum θ
D1S3642	ATA29C07	6.2	−∞	−.083	.01	.25	.26	.13	.02	.29	.15
D1S446	AFM238ya7	4.3	1.87	1.81	1.54	1.23	.66	.27	.06	1.87	.00
D1S2800	AFMb360zg1	4.2	−∞	2.89	3.03	2.65	1.69	.86	.30	3.09	.03
D1S179	mfd174	2.0	3.74	3.65	3.24	2.70	1.67	.84	.30	3.74	.00
D1S2712	AFMa349xc5	1.6	−∞	1.26	1.71	1.67	1.25	.73	.30	1.73	.07
D1S1540	UT1317	1.0	2.00	2.20	2.27	2.03	1.37	.77	.33	2.30	.03
D1S235	AFM203yg9	0	4.11	3.98	3.49	2.89	1.82	.98	.40	4.11	.00
	GGAA6F06	0	3.49	3.41	3.06	2.63	1.80	1.05	.45	3.49	.00
D1S2678	AFMa245wd5	0	3.18	3.35	3.30	2.92	1.98	1.11	.45	3.40	.02
D1S2680	AFMa247yg9	0	2.49	2.74	2.77	2.44	1.61	.88	.36	2.83	.03
	AFM214xe1	0	−.94	.13	.79	.94	.80	.49	.20	.94	.11
D1S2850	AFM088xe5	7.2	1.54	2.30	2.56	2.38	1.73	1.02	.43	2.57	.04
D1S373	UT492	8.2	−1.15	−.67	.10	.41	.50	.36	.16	.51	.17
D1S2670	AFMa230wh1	9.4	.30	.71	1.31	1.47	1.27	.85	.40	1.48	.11
D1S517	UT1570	9.5	.65	.92	1.31	1.37	1.11	.72	.34	1.38	.09
D1S1149	UT418	11.4	2.55	2.48	2.21	1.88	1.28	.75	.33	2.55	.00

[a] Distances from D1S235.

screened in two sets of pools. The first set was from the extended family (pedigree C; fig. 1) and included a pool of four patients and a pool of their four parents. The second set consisted of four nuclear families, each with one patient (pedigrees A, B, and D and patient 50 of pedigree E; fig. 1), and included a patient pool (four patients), a parent pool (seven parents), and a sibling pool (seven siblings). This strategy was used both to avoid type I error in case of genetic heterogeneity and to establish linkage in at least the largest family studied in which genetic homogeneity could be assumed. This first screening failed to find any clear linkage. Since some of the STRPs were not informative, the gaps of >5–10 cM were closed by use of markers from the CHLC/Weber 8th version genome-screening set. The addition of six more microsatellite markers revealed one intense band corresponding to allele 2 of D1S235 in the two patient pools, whereas the parent pool and the sibling pool showed several bands. Individual genotyping of each patient and of unaffected family members (including the newborn patients and their families) revealed that all 10 patients were homozygous for allele 2. The pairwise Z value for the HRD locus, with D1S235, is 4.11 at a recombination fraction (θ) of 0 (table 1).

To further refine the localization of the HRD interval, we analyzed all patients and relatives of families C and E for microsatellite markers flanking D1S235 (fig. 1). The markers cover a 21-cM interval on chromosome 1q41-44. D1S179 and D1S2678 define the centromeric and telomeric boundaries for HDR, respectively, showing heterozygous alleles in intrafamilial haplotypes (pedigree E, patient 50; fig. 1). These flanking loci are 4.2 cM apart (information derived from the recombination maps compiled by the Third International Workshop on Human Chromosome 1 and available at the Chromosome 1 homepage). The Z values obtained for the two large families (C and E) were 2.54 for D1S235 and 3.49 and 3.05 for the tightly linked GGAA6F06 and D1S2678, respectively. No individuals without hypoparathyroidism had the patients' homozygous phenotype, as can be seen in figure 1.

The interval containing the HRD gene could be further narrowed by means of a search for a shared haplotype in all affected individuals. The analysis of all 10 patients for haplotypes at the loci flanking D1S235 (fig. 2) is presented in figure 3. Since D1S235 and the nearby marker GGAA6F06 are homozygous for the same alleles in all HRD patients, we assumed that these alleles were in linkage disequilibrium with the HRD gene. Patients 50 and 43 showed heterozygosity at D1S2678, thus defining the telomeric border. Patients 7, 50, 53, and 43 show, at locus D1S1540, alleles that are different than those of the remaining patients, thus defining the centromeric border (fig. 3). The HDR interval is therefore confined between the flanking markers D1S2678 and D1S1540, an interval of 1 cM, on the basis of the recombination maps compiled by the Third International Workshop on Human Chromosome 1 (Chromosome 1 homepage).

Discussion

Homozygosity mapping is a powerful technique that is used to map genes responsible for recessive diseases and is applied mostly in large consanguineous pedigrees (Sheffield et al. 1995). Here we present the localization

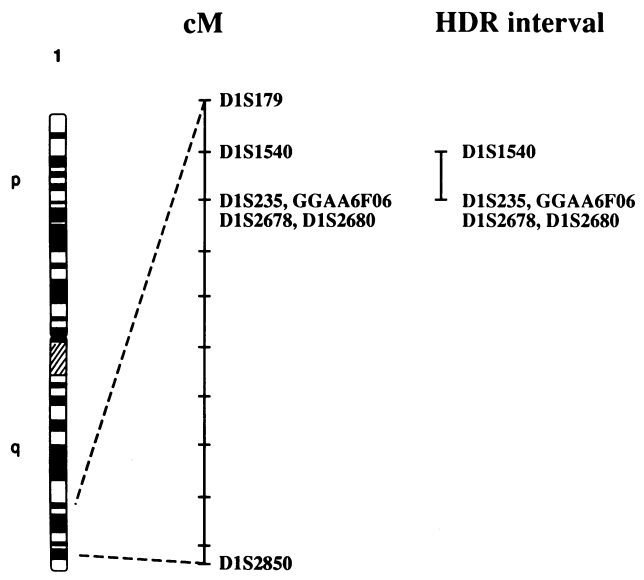

Figure 2 Genetic map of chromosome 1q42-43 region, showing the location of the HDR gene. The genetic distances between the markers, as well as their order, are based on information derived from the recombination maps compiled by the Third International Workshop on Human Chromosome 1 (Chromosome 1 homepage).

of the rare HRD gene, using this method in apparently unrelated families that belong to a relatively small Bedouin population, and thus demonstrate the use of this technique when a common ancestor is assumed. Evidence of linkage to this interval was provided by both significant *Z* values and homozygosity of markers across the disease interval. The fact that the families are not closely related necessitated screening of markers at intervals of 5 cM. The data on nuclear families aided in the narrowing of the HRD gene interval, thus revealing that all patients share the same alleles at only two tightly linked marker loci, D1S235 and GGAA6F06, with the flanking markers D1S2678 and D1S1540 defining a distance of 1 cM for the gene interval. It is highly plausible that both the mutation that we found and that of the Saudi Arabian patients represent one single HRD mutation that occurred generations ago in the Bedouin population migrating into the region of Israel and the Arab peninsula. Recently, the strength of using homozygosity and linkage disequilibrium even when large consanguineous families are not available was demonstrated by the mapping of the urofacial (Ochoa) syndrome to a 1-cM interval on chromosome 10 (Wang et al. 1997) and of the cerebellar ataxia locus to a small physical interval on chromosome 19p13.3 (Nystuen et al. 1996), in members of nuclear families from a large Columbian population and in an isolated population from the Cayman Islands, respectively. In all of these cases, the many meioses that occurred since the mutation appeared and spread from the common ancestor to the apparently unrelated families enabled a significant narrowing of the interval of search for the affected genes. The small interval of search for the HRD gene makes the task of its positional cloning feasible.

The gene for Chediak-Higashi syndrome was localized exactly at D1S235, flanked by markers D1S236 and D1S2680 (Barrat et al. 1996). However, this disorder bears no resemblance to HRD. Several additional genes were localized within the interval, including Rab escort protein-2 and inositol 1,4,5-triphosphate 3-kinase (Barrat et al. 1997), none of which appears a priori to be an obvious candidate for HRD. A syntenic mouse region exists on chromosome 13 (U.K. Human Genome Mapping Project). This region of the mouse genome does not contain any obvious candidate gene or a similar phenotype in the mouse (U.K. Human Genome Mapping Project). Forty-eight cDNAs (National Center for Biotechnology Information Human Gene Map) were placed in the 16-cM interval D1S446–D1S304, around D1S235. Fine mapping of these expressed sequence tags (ESTs) and examination of the expression pattern is a logical next step in defining which, if any, of these ESTs is responsible for HRD. A YAC contig of this region already exists (Barrat et al. 1996).

Identification of the gene that, when mutated, causes HRD may contribute to the understanding of the processes involved in the regulation of production and secretion of PTH. Since serum-ionized calcium closely regulates the production of the hormone, insight may be gained into the regulation of ionized calcium homeostasis as well. Indeed, in six kindreds diagnosed with autosomal dominant hypoparathyroidism, gain-of-func-

			C				E			
	7	IH	HA	GA	16	17	50	53	77	43
D1S1656	7 7	4 3	4 3	4 3	3 3	3 3				
D1S2833	1 7	7 3	7 3	7 3	3 3	3 3	6 7		3 3	
D1S2709	2 1	1 1	1 1	1 1	2 1	2 1	1 1		2 1	
D1S3462	1 4	4 4	4 4	4 4	3 4	3 4	4 4		3 4	
D1S163	1 5	7 5	5 5	5 5	5 3	5 3	- -		7 1	
D1S446	2 2	4 2	2 2	2 2	2 2	2 2	4 4		2 2	
D1S2800	3 7	4 3	4 4	4 4	4 4	4 4	4 4	7 3	5 5	
D1S179	1 4	6 1	1 1	1 1	1 1	1 1	6 1	6 1	1 1	3 3
D1S2712	1 1	1 1	1 1	1 1	1 1	1 1	1 1	1 1	1 1	1 1
D1S1540	1 1	2 2	2 2	2 2	2 2	2 2	1 1	1 1	2 2	4 4
D1S235	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2
GGAA6F06	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2
D1S2678	5 5	5 5	5 5	5 5	5 5	5 5	4 5	5 5	5 5	3 2
D1S2680	4 4	4 4	4 4	4 4	4 4	4 4	6 2	4 4	4 4	4 4
AFM214xe1	- -	3 3	3 3	3 3	3 3	3 3	2 2	3 3	3 3	3 4
D1S2850	2 2	2 2	2 2	2 2	2 2	2 2	3 1	3 2	2 2	2 3
D1S373	2 2	6 3	9 3	9 3	9 2	9 2	3 6	3 2	1 7	5 10
D1S2670	9 9	4 4	4 4	4 4	4 4	4 4	9 1	3 3	4 4	5 5
D1S517	2 5	10 5	4 5	4 5	4 10	4 10			10 10	
D1S1149	4 2	5 4	5 5	5 5	5 5	5 5			5 5	
D1S180	1 2	9 7	9 7	9 7	9 10	9 10				
D1S2785	8 7	1 1	1 1	1 1	1 3	1 3				
AFN151xb8	2 2	2 2	1 2	1 2	1 2	1 2				

Figure 3 Multilocus haplotypes for polymorphic markers near the HDR gene in 10 patients. The patients in the extended families are grouped, and the family to which they belong is indicated. The chromosomes showing homozygous alleles in patients are boxed.

tion mutations were recently found in the calcium-sensing receptor (Pearce et al. 1996). The growth and mental retardation observed in HRD patients was not reported in the various forms of isolated hypoparathyroidism. Apparently this is not caused by the hypoparathyroidism that per se still allows normal growth (Gorodischer et al. 1970), and it may represent a unique pleiotropic effect of the gene on fetal development and postnatal growth. The very similar Kenny-Caffey syndrome observed in Bedouins (Khan et al. 1997) might be caused by a different mutation in the HRD gene or might represent genetic heterogeneity for a similar phenotype.

Acknowledgments

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Electronic-Database Information

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

Chromosome 1 Home Page, http://linkage.rockefeller.edu/chr1/data/genmap/chr1-154.GIF (for a map of human chromosome 1)

Cooperative Human Linkage Center, http://www.chlc.org (for STRPs)

National Center for Biotechnology Information Human Gene Map, http://www.ncbi.nlm.nih.gov/cgi-bin/SCIENCE96/msrch2 (for cDNAs in 1q42-43)

Online Mendelian inheritance in man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for autosomal recessive hypoparathyroidism [MIM 241410])

U.K. Human Genome Mapping Project Resource Center, http://www.hgmp.mrc.ac.uk (for a mouse region syntenic to 1q43, http://www.hgmp.mrc.ac.uk/dhmhd-bin/hum-homology2.pl?1+q+42-43; and for gene or phenotype in the mouse, http://www.hgmp.mrc.ac.uk/dhmhd-bin/mous-id-look2.pl?13+A1-A2)

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