Am. J. Hum. Genet. 59:431–436, 1996

Complete Paternal Isodisomy for Chromosome 8 Unmasked by Lipoprotein Lipase Deficiency

Pascale Benlian,[1,2,3] Luc Foubert,[1,2] Eric Gagné,[1] Lynn Bernard,[1] Jean Luc De Gennes,[2] Sylvie Langlois,[1] Wendy Robinson,[1] and Michael Hayden[1]

[1]Department of Medical Genetics, University of British Columbia, Vancouver; and [2]Department of Endocrinology and Metabolic Diseases, Hôpital Pitié, and [3]Department of Molecular Biology, Hôpital Saint Antoine, Paris

Summary

Uniparental disomy (UPD)—the inheritance of two homologous chromosomes from a single parent—may be unmasked in humans by the unexpected appearance of developmental abnormalities, genetic disorders resulting from genomic imprinting, or recessive traits. Here we report a female patient with familial chylomicronemia resulting from complete lipoprotein-lipase (LPL) deficiency due to homozygosity for a frameshift mutation in exon 2 of the LPL gene. She was the normal term product of an unremarkable pregnancy and had shown normal development until her current age of 5.5 years. The father (age 33 years) and the mother (age 24 years) were unrelated and healthy, with no family history of stillbirths or malformations. The father was a heterozygous carrier of the mutation, whereas no mutation in the LPL gene was detected in the mother. Southern blotting did not reveal any LPL gene rearrangement in the proband or her parents. The proband was homozygous for 17 informative markers spanning both arms of chromosome 8 and specifically for the haplotype containing the paternally derived LPL gene. This shows that homozygosity for the defective mutation in the LPL gene resulted from a complete paternal isodisomy for chromosome 8. This is the first report of UPD for chromosome 8 unmasked by LPL deficiency and suggests that normal development can occur with two paternally derived copies of human chromosome 8.

Introduction

Uniparental disomy (UPD), defined as the inheritance of chromosome homologues from only one parent, has become increasingly recognized, as a result of the use of DNA polymorphisms in family analyses (Engel 1995). This genetic phenomenon results from meiotic nondisjunction events or from postzygotic trisomy or monosomy "rescue"—i.e., the elimination of a supplementary chromosome or the duplication of a univalent chromosome. Depending on the timing of nondisjunction, the two chromosomes observed in offspring may be identical over the entire length (isodisomy), indicative of a postzygotic error, or may show partial or complete heterozygosity (heterodisomy), indicative of a meiotic origin.

UPD has been documented in humans for 15 autosomes, as well as the XY chromosomes (Ledbetter and Engel 1995), and may be identified in association with various chromosome abnormalities, genetic syndromes related to genomic imprinting, or autosomal recessive disorders. However, UPD does not always manifest with a phenotype. For example, some cases of UPD, which have been ascertained through an isochromosome, have been associated with a completely normal phenotype. On the other hand, uniparental inheritance of imprinted genes at specific loci may lead to well-defined syndromes, such as the Beckwith-Wiedemann syndrome (chromosome 11) (Junien 1992) or the Angelman and Prader Willi syndromes (chromosome 15) (Robinson et al. 1993), or to an isolated growth retardation such as seen in the Russell Silver syndrome (chromosome 7) (Kotzot et al. 1995). UPD may also result in the reduction to homozygosity of an autosomal recessive mutation or the father-to-son transmission of an X-linked disease. UPD for chromosome 8 has not been reported, and nothing is known concerning the developmental consequences of inheriting both chromosomes 8 from a single parent.

The gene encoding lipoprotein lipase (LPL) maps to human chromosome 8p22 (Sparkes et al. 1987). More than 50 mutations have been reported as a cause of LPL deficiency in different populations (Hayden et al. 1991). LPL hydrolyzes triglycerides from circulating chylomicrons and very-low-density lipoproteins, which release fatty acids that are taken up by muscle cells for energy consumption or by adipocytes for storage (Brunzell 1995). Patients with mutations on both LPL genes usu-

Received February 14, 1996; accepted for publication May 3, 1996.

Address for correspondence and reprints: Dr. Pascale Benlian, Department of Medical Genetics, University of British Columbia, 2125 East Mall, Vancouver, British Columbia V6T 1Z4. E-mail: pascale@ulam.generes.ca

0002-9297/96/5902-0020$02.00

ally manifest with abdominal pain associated with fasting chylomicronemia during childhood. During episodes of severe hypertriglyceridemia (plasma triglycerides >1,000 mg/dl) eruptive xanthomatosis, hepatosplenomegaly, and lipemia retinalis may be observed, sometimes complicated by acute pancreatitis. However, growth and morphological, neurological, and mental development are entirely normal in these patients.

Here we describe a patient with LPL deficiency due to paternal isodisomy for chromosome 8 containing a mutation in the LPL gene. The finding that this patient was developmentally normal suggests that paternal copies of chromosome 8 may be sufficient for normal growth and development.

Subjects and Methods

Case Report

The proband was a female child of healthy parents of French and Spanish ancestry, aged 33 years (father) and 24 years (mother), respectively. There was no history of stillbirth or malformations in either side of the family. In the mother, this first pregnancy was uneventful. At 26 wk gestation, ultrasound analysis revealed normal fetal morphology, activity, and dimensions (biparietal and abdominal diameters of 65 mm and 60 mm, respectively; femur length of 48 mm) and normal placental position and size. The mother went into preterm labor at 35 wk gestation. A breech presentation necessitated a cesarean section resulting in the birth of a developmentally normal child (weight = 3,250 g; height = 49 cm; APGAR score = 3 at 1 min, 10 at 5 min, 10 at 10 min).

Chylomicronemia was discovered at 1 mo of age, after an episode of acute pancreatitis. The child was treated with a low-fat diet (<15% fat of daily energy intake) complemented with medium-chain fatty acids. This has resulted in relief from abdominal pain or acute pancreatitis, until her current age of 5.5 years. Fasting plasma triglycerides, analyzed yearly, have ranged between 610 and 2,200 mg/dl ($N < 150$). Her height and weight growth curves have been normal: height (H) = 0.73 m, weight (W) = 8.6 kg (25th percentile) at age 1 year; H = 0.86 m, W = 11.4 kg (25th percentile) at age 2.5 years; H = 0.95 m, W = 13 kg (25th percentile) at age 3.5 years; H = 0.98 m, W = 14 kg (25th percentile) at age 4.5 years; and H = 1.03 m, W = 15.5 kg (25th–50th percentile) at age 5.5 years. In addition, she has had normal intellectual, social, and developmental milestones.

Biochemical Studies

Fasting plasma cholesterol and triglyceride concentrations were determined enzymatically (Buccolo and David 1973; Allain et al. 1974), and HDL cholesterol was measured by heparin-manganese precipitation (Warnick and Albers 1978). Blood samples were collected for LPL functional assays after an overnight fast, before and 10 min after an intravenous injection of heparin (50 IU/kg body mass). LPL activity was measured on 100-fold diluted plasma by a radiolabeled glycerol tri[^{3}H]oleate emulsion as described by Babirak et al. (1989). LPL mass in pre- and post-heparin plasma was measured by an enzyme-linked immunoabsorbant assay method using the monoclonal antibodies 5D2 and 5F9 as described by Iverius et al. (1985).

DNA Analysis

Genomic DNA was extracted from blood leukocytes by a phenol-chloroform method, in the proband and in her relatives. PCR amplification of individual exons of the LPL gene was followed by SSCP (Gagné et al. 1994). When a gene variant was detected, the corresponding PCR product was sequenced directly and after subcloning by use of previously reported procedures (Monsalve et al. 1990). Southern blots were performed on genomic DNA by use of three different enzymes (*Eco*RI, *Pst*I, and *Sac*I) and LPL gene cDNA probe HLPL26 (Langlois et al. 1989). Nine LPL gene polymorphisms were analyzed. The VNTR (Zuliani and Hobbs 1990) was detected on 8% acrylamide minigels stained with ethidium bromide. RFLPs (*Bst*NI, *Hae*III exon 3 and exon 8, *Hin*dIII, *Mnl*I, and *Pvu*II) were analyzed after enzymatic cleavage of PCR products (Hata et al 1990; Gotoda et al. 1992; Gagné et al. 1994; Benlian et al. 1995); 5′ and 3′ microsatellites were analyzed after autoradiography of labeled PCR products run on denaturing sequencing acrylamide gels (Wood et al. 1993).

A panel of 15 microsatellites of chromosome 8 with a PIC >.75 and located outside the LPL locus (Cox Matise et al. 1994) were analyzed by PCR and revealed by silver staining of nondenaturing acrylamide gels. In addition, 11 polymorphisms of the apo B gene on chromosome 2p (Ludwig and Mac Carthy 1990), the CAG triplet repeat region of the HD gene on 4p (Goldberg et al. 1993), 8 polymorphisms of the LDL-receptor gene on chromosome 19p (Pereira et al. 1995), and apo E isoforms on chromosome 19q (Hixson and Vernier 1990) were analyzed.

Cytogenetic Analysis

Karyotypes were performed on cultured blood lymphocytes in the proband and her parents. A total of 13–19 metaphases were analyzed.

Results

Homozygosity for a Mutation of the LPL Gene

Undetectable plasma LPL mass and activity resulted in significant hypertriglyceridemia in the proband (table

Table 1

Plasma Lipids and LPL Functional Assays of the Proband

Measure	Value
Plasma Lipids:	
Total cholesterol (mg/dl) ($N < 200$)	150–550
Triglycerides (mg/dl) ($N < 150$)	610–1120
HDL cholesterol (mg/dl) ($N > 45$)	14
LPL Assays:	
Mass (ng/liter)	0
Activity (IU/ml)	0

1) No gross gene rearrangement of the LPL gene was evident (not shown). SSCP analysis revealed a band shift and an absence of the normal bands in exon 2 (fig. 1*A*). DNA sequencing identified a frameshift mutation resulting from an insertion of an "A" at nucleotide 183 (codon Glu35), leading to a downstream premature stop codon at residue 62 (fig. 1*B*). The putative protein product would lack all of the highly conserved domains necessary for lipid binding and for catalytic activity of the normal enzyme. This mutation was detected on both alleles in the proband. Moreover, each of the nine genetic polymorphisms analyzed within the LPL gene (fig. 2) revealed homozygosity for a single LPL gene haplotype.

Analysis of the Parents

No gross rearrangements were detected in either parent (not shown). SSCP analysis of exon 2 revealed that the homozygous variant in the proband was present in a heterozygous fashion in the father and was not found in the mother (fig. 1*A*). Sequence analysis revealed that the father was heterozygous for the frameshift mutation, whereas the mother had a normal LPL gene (fig. 1*B*). Segregation analysis using LPL gene polymorphisms spanning the entire locus revealed that the homozygous haplotype present in proband was inherited from the father, who was heterozygous for this haplotype (fig. 2). In contrast, this haplotype was not observed in the mother. Moreover, analysis of the family over three generations revealed that the mutation and the haplotype

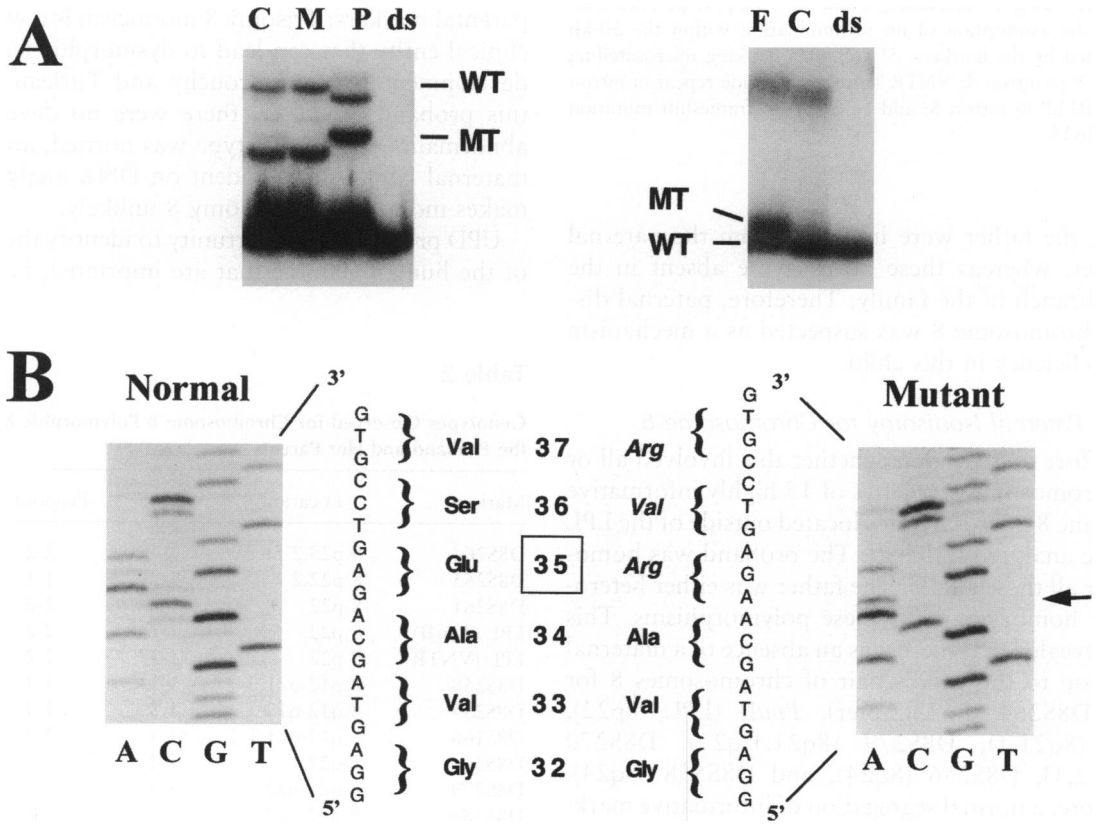

Figure 1 Mutation of the LPL gene in the proband and her parents. *A*, SSCP analysis of exon 2 on 10% polyacrylamide gels, at 4°C (*left*) and at room temperature (*right*). C = control subject; F = father; M = mother; P = proband; ds = double-strand DNA; WT = wild-type allele; and MT = mutant allele. *B*, DNA sequence obtained after subcloning of PCR products of exon 2 from the proband and his parents. The mother was homozygous and the father was heterozygous for the normal allele (*left*). The proband was homozygous and the father was heterozygous for the mutant allele (*right*). The mutation (*arrow*) at residue Glu35 leads to a frameshift with a stop codon at residue 62.

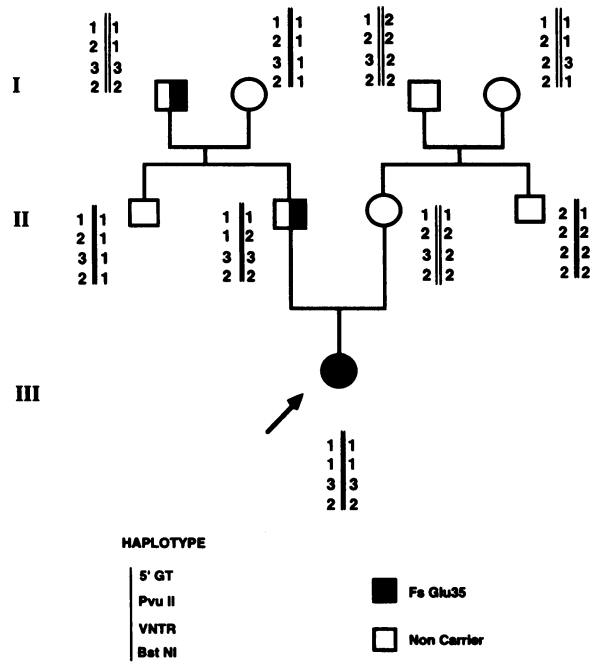

Figure 2 Segregation analysis of polymorphisms at the LPL locus. Only markers informative in the family are shown. Alleles are numbered according to decreasing band size. Haplotypes are constructed on the assumption of no recombination within the 30-kb region spanned by the markers. 5′ GT = 5′ flanking microsatellite; *Pvu*II = RFLP in intron 6; VNTR = tetranucleotide repeat in intron 6; *Bst*NI = RFLP in intron 8; and Fs Glu35 = frameshift mutation at residue Glu35.

present in the father were inherited from the paternal grandfather, whereas these alleles were absent in the maternal branch of the family. Therefore, paternal disomy for chromosome 8 was suspected as a mechanism for LPL deficiency in this child.

Complete Paternal Isodisomy for Chromosome 8

In an effort to determine whether this involved all or part of chromosome 8, a series of 15 highly informative chromosome 8 microsatellites located outside of the LPL locus were analyzed (table 2). The proband was homozygous for all these alleles. The father was either heterozygous or homozygous for these polymorphisms. This analysis revealed that there was an absence of a maternal contribution to the child's pair of chromosomes 8 for markers D8S264 (8p23.2-pter), *Pvu*II (LPL, 8p22), D8S260 (8q21.1), D8S279 (8q21.1-q22), D8S270 (8q21.3-22.1), D8S256 (8q24), and D8S558 (8q24). Furthermore, a normal segregation of informative markers was observed from both parents on chromosomes 2p, 4p, 19p, and 19q. Finally, no chromosomal abnormality was observed on the karyotype analyzed on blood lymphocytes from the proband or from her parents. Therefore, a complete paternal isodisomy for chromosome 8 was identified as the cause of a reduction to homozygosity of a frameshift mutation in exon 2 of the LPL gene, leading to LPL deficiency in a developmentally normal child.

Discussion

While exploring the molecular basis for LPL deficiency, we made the unusual observation of a homozygous mutation inherited only from the father and identified an example of complete paternal isodisomy for chromosome 8. This is the first example of LPL deficiency caused by this mechanism, and, in addition, this is the first example of UPD for chromosome 8.

Several mechanisms leading to UPD are possible. Complete homozygosity, as in the present case, is generally assumed to be indicative of a postzygotic chromosomal duplication event and has been observed to account for a small proportion of UPD(15) (Robinson et al. 1993) and of trisomies 8, 13, 18, and 21 (Antonarakis et al. 1993; Fisher et al. 1995; Robinson et al. 1995, 1996). However, lack of meiotic I recombination followed by a meiotic II error is also a theoretical possibility that cannot be excluded without analysis of grandparental markers. Trisomy 8 mosaicism is a well-known clinical entity that can lead to dysmorphic features and developmental delay (Grouchy and Turleau 1983). In this proband, however, there were no developmental abnormalities, the karyotype was normal, and no faint maternal bands were evident on DNA analysis, which makes mosaicism for trisomy 8 unlikely.

UPD provides an opportunity to identify those regions of the human genome that are imprinted, i.e., that ex-

Table 2

Genotypes Observed for Chromosome 8 Polymorphic Markers in the Proband and Her Parents

Marker	Location	Mother	Propositus	Father
D8S264	8 p23.2-pter	1-4	2-2	2-3
D8S265	8 p22.2	1-2	1-1	1-1
D8S261	8 p22	1-2	2-2	2-3
LPL (*Pvu*II)	8 p22	1-1	2-2	2-2
LPL (VNTR)	8 p22	1-2	2-2	2-2
D8S258	8 p12-p21.3	1-1	1-1	1-1
D8S255	8 q12-q13	1-3	1-1	1-2
D8S166	8 q13-q21.1	1-1	1-1	1-1
D8S260	8 q21.1	2-2	3-3	3-1
D8S279	8 q21-q22	3-3	1-1	1-2
D8S286	8 q21-q22	...	1-1	1-2
D8S528	8 q21.3	...	2-2	2-1
D8S270	8 q21.3-22.1	1-3	2-2	2-1
D8S256	8 q24	2-2	1-1	1-1
D8S284	8 q24	1-2	1-1	1-2
D8S558	8 q24	3-3	2-2	2-1

hibit a parental specific pattern of expression (Ledbetter and Engel 1995). In fact, many of the documented UPD cases refer to regions that lead to specific syndromes or to growth retardation as a result of imprinted genes or loci. Genes reported as imprinted in humans or other mammals play a regulatory role in growth or during early development (Hall 1990). Many loci encoding growth factors, oncogenes, peptidic hormones, or nuclear factors are located on human chromosome 8 (McKusick 1995). However, syntenic regions of the mouse genome do not seem to be imprinted (Cattanach and Jones 1994). Moreover, that the proband had LPL deficiency as the only abnormality associated with normal growth and development until age 5.5 years strongly supports the contention that genes located on human chromosome 8 are not paternally imprinted.

Because no cases of maternal UPD have yet been reported, we have not excluded that some regions of chromosome 8 may be maternally inactivated. However, we suggest that paternal copies of chromosome 8 may be sufficient for normal development in humans.

Acknowledgments

This work was supported by grants from INSERM (91CN45) and the French Ministry for Research and Universities (92166) to P.B. and from grants from the Medical Research Council of Canada to M.R.H. L.F. was recipient of a grant from Comité Français de Coordination des Recherches sur l'Artériosclérose et le Cholestérol. P.B. is recipient of a grant from the Fondation pour la Recherche Médicale and of a fellowship from the International Atherosclerosis Society. E.G. is a British Columbia and Yukon Heart and Stroke Foundation student. We wish to thank Suzanne Clark, Ian Forsythe, Linda Kwong, Jean Pierre Lagarde, and Leila Zekraoui for excellent technical assistance. We are indebted to Dr. Jacqueline Roux, from the Department of Pediatrics, and Dr. Basco Tateossir, from the Department of Biochemistry, at Henri Duffaut Hospital in Avignon for their invaluable help.

References

Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC (1974) Enzymatic determination of total cholesterol. Clin Chem 20:470–475

Antonarakis SE, Avramopoulos D, Blouin J-L, Talbot CC, Schinzel AA (1993) Mitotic (somatic cell) errors cause trisomy 21 in about 4.5% of cases and are not associated with advanced maternal age. Nat Genet 3:146–150

Babirak S, Iverius PH, Fujimoto WY, Brunzell JD (1989) Detection and characterization of the heterozygote state for lipoprotein lipase deficiency. Arteriosclerosis 9:326–334

Benlian P, Etienne J, De Gennes JL, Noe L, Brault D, Raisonnier A, Arnault F, et al (1995) Homozygous deletion of exon 9 causes lipoprotein lipase deficiency: possible intron-Alu recombination. J Lipid Res 36:356–366

Brunzell JD (1995) Familial lipoprotein lipase deficiency and other causes of the chylomicronemia syndrome. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic basis of inherited disease, 7th ed. McGraw Hill, Highstown, NJ, pp 1913–1932

Buccolo G, David H (1973) Quantitative determination of serum triglycerides by the use of enzymes. Clin Chem 19: 476–482

Cattanach BM, Jones J (1994) Genetic imprinting in the mouse: implications for gene regulation. J Inherit Metab Dis 17:403–420

Cox Matise T, Perlin M, Chakravarti A (1994) Automated construction of genetic linkage maps using an expert system (MultiMap): a human genome linkage map. Nat Genet 6: 384–390

Engel E (1995) Uniparental disomy: a review of causes and clinical effects. Ann Genet 38:113–136

Fisher JM, Harvey JF, Morton NE, Jacobs PA (1995) Trisomy 18: studies of the parent and cell division of origin and the effect of aberrant recombination on nondisjunction. Am J Hum Genet 56:669–675

Gagné E, Genest J Jr, Zhang H, Clarke LA, Hayden MR (1994) Analysis of DNA changes in the LPL gene in patients with familial combined hyperlipidemia. Arterioscler Thromb 14:1250–1257

Goldberg YP, Andrew SE, Clarke LA, Hayden MR (1993) PCR method for accurate assessment of trinucleotide repeat expansion in Huntington disease. Hum Mol Genet 2:635–636

Gotoda T, Yamada N, Murase T, Shimano H, Shimada M, Harada K, Kawamura M, et al (1992) Detection of three separate DNA polymorphisms in the human lipoprotein lipase gene by gene amplification and restriction endonuclease. J Lipid Res 33:1067–1072

Grouchy de J, Turleau C (1983) Autosomal disorders. In: Emery AEH, Rimoin DL (eds) Principles and practice of medical genetics. Churchill Livingstone, Edinburgh, London, Melbourne, New York, pp 170–192

Hall JG (1990) Genomic imprinting: review and relevance to human diseases. Am J Hum Genet 46:857–873

Hata A, Robertson M, Emi M, Lalouel JM (1990) Direct detection and automated sequencing of alleles after electrophoretic strand separation: identification of a common nonsense mutation in exon 9 of the human lipoprotein lipase gene. Nucleic Acids Res 18:5407–5411

Hayden MR, Ma Y, Brunzell J, Henderson HE (1991) Genetic variants affecting human lipoprotein and hepatic lipases. Curr Opin Lipidol 2:104–109

Hixson JE, Vernier DT (1990) Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with *Hha*I. J Lipid Res 31:545–548

Iverius PH, Brunzell JD (1985) Human adipose lipoprotein lipase: changes with feeding and relationship to postheparin plasma enzyme. Am J Physiol 249:E107–E114

Junien C (1992) Beckwith-Wiedemann syndrome, tumorigenesis and imprinting. Curr Opin Genet Devel 2:431–438

Kotzot D, Schmitt S, Bernasconi F, Robinson WP, Lurie IW, Ilyina H, Méhes K, et al (1995) Uniparental disomy 7 in Silver-Russell syndrome and primordial growth retardation. Hum Mol Genet 4:583–587

Langlois S, Deeb S, Brunzell JD, Kastelein JJ, Hayden MR

(1989) A major insertion accounts for a significant proportion of mutations underlying human lipoprotein lipase deficiency. Proc Natl Acad Sci USA 86:948–952

Ledbetter DH, Engel E (1995) Uniparental disomy in humans: development of an imprinting map and its implications for prenatal diagnosis. Hum Mol Genet 4:1757–1764

Ludwig EH, McCarthy BJ (1990) Haplotype analysis of the human apolipoprotein B mutation associated with familial defective apolipoprotein B100. Am J Hum Genet 47:712–720

McKusick VA (1995) Mendelian inheritance in man: catalogs of autosomal dominant, autosomal recessive, and X-linked phenotypes, 10th ed. Johns Hopkins University Press, Baltimore

Monsalve MV, Henderson H, Roederer G, Deeb S, Kastelein JJP, Peritz L, Devlin R, et al (1990) A missense mutation at codon 188 of the human lipoprotein lipase gene is a frequent cause of lipoprotein lipase deficiency in persons of different ancestries. J Clin Invest 86:728–734

Pereira E, Ferreira R, Hermelin B, Thomas G, Bernard C, Bertrand V, Nassif H, et al (1995) Recurrent and novel LDL receptor gene mutations causing heterozygous familial hypercholesterolemia in La Habana. Hum Genet 96:319–322

Robinson WP, Bernasconi F, Dutly F, Lefort G, Romain DR, Binkert F, Schinzel AA (1996) Molecular studies of translocations and trisomy involving chromosome 13. Am J Med Genet 61:158–163

Robinson WP, Bernasconi F, Mutirangura A, Ledbetter DH, Langlois S, Malcolm S, Morris MA, et al (1993) Nondisjunction of chromosome 15: origin and recombination. Am J Hum Genet 53:740–751

Robinson WP, Binkert F, Bernasconi F, Lorda-Sanchez I, Werder EA, Schinzel AA (1995) Molecular studies of chromosomal mosaicism: relative frequency of chromosome gain or loss and possible role of cell selection. Am J Hum Genet 56:444–451

Sparkes RS, Zollman S, Klisak I, Kirchgessner TG, Komaromy MC, Mohandas T, Schotz MC, et al (1987) Human genes involved in lipolysis of plasma lipoproteins: mapping of loci for lipoprotein lipase to 8p22 and hepatic lipase to 15q21. Genomics 1:138–144

Warnick GR, Albers JJ (1978) Heparin-Mn^{2+} quantitation of high density lipoprotein cholesterol: an ultrafiltration procedure for lipemic samples. Clin Chem 24:900–904

Wood S, Schertzer M, Hayden MR, Ma Y (1993) Support for a founder effect for two major lipoprotein lipase (LPL) gene mutations in French Canadians by analysis of GT microsatellites flanking the LPL gene. Hum Genet 91:312–316

Zuliani G, Hobbs HH (1990) Tetranucleotide repeat polymorphism in the LPL gene. Nucleic Acids Res 18:4958