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The Molecular Genetics of Pediatric Lipid Disorders: Recent Progress and Future Research Directions[1]

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ABSTRACT. Over the last 10 years, the explosion of molecular biology and molecular genetic techniques have allowed major advances in the diagnosis and management of a wide variety of human disorders. These range from accurate and simple screening for carriers of thalassemia (Old JM, Varawalla NY, Weatherall DJ: Lancet 2:834–837, 1990) to the use of preimplantation diagnosis of embryos at risk for untreatable congenital defects (Monk M, Holding C: Lancet 1:985–988, 1990) and the development of gene therapy for treatment of disorders such as adenosine deaminase deficiency (Sharp D: Lancet 1:1277–1278, 1991). These same molecular techniques have also been applied to pediatric lipid disorders with some notable successes, both in their diagnosis and understanding the mechanisms of the resulting pathology, including the recent experiments (Wilson JM, Grossman M, Wu CH, Chowdhury NR, Wu GY, Chowdhury JR: J Biol Chem 267:963–967, 1992) that have led to proposals to treat homozygous familial hypercholesterolemia by gene therapy. The purpose of this review is to detail this molecular genetic progress for two of the disorders that result in disturbed triglyceride metabolism in infants, lipoprotein lipase deficiency and apo CII deficiency, and four disorders that lead to disturbed cholesterol levels in infancy, abetalipoproteinemia, hypobetalipoproteinemia, familial defective apo B, and familial hypercholesterolemia. We will also address the question of how knowledge of the mutation causing the defect in a particular patient could be clinically useful and highlight areas of research for the future. (*Pediatr Res* 34: 403–415, 1993)

Abbreviations

FH, familial hypercholesterolemia
LPL, lipoprotein lipase
FDB, familial defective apo B100
PCR, polymerase chain reaction
SSCP, single-strand conformational polymorphism
TG, triglyceride
RFLP, restriction fragment length polymorphism
ABL, abetalipoproteinemia
HBL, hypobetalipoproteinemia
LDL-R, LDL-receptor

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MOLECULAR TECHNIQUES FOR IDENTIFICATION OF MUTATIONS

The genes for most of the apolipoproteins, enzymes, and receptors that are involved in lipid metabolism have now been cloned, and their DNA sequence and arrangement of introns and exons determined and published (reviewed in 1 and 2). The control of expression of these genes is very complex and must be coordinated in response to a number of environmental challenges, rapidly in the postprandial state and more slowly in adaptation to hormonal changes, for example, at puberty or during pregnancy. Molecular details of these control processes are not yet fully understood, but excellent progress has already been made (3). Taken together, this information provides the framework for the identification of the mutations occurring in different patients with pediatric lipid disorders.

Several methods have been published that allow rapid comparison of the sequence of specific fragments of DNA from different individuals amplified *in vitro* by PCR (4). The first uses chemical cleavage of mismatched bases in the duplex formed between two heterologous DNA fragments after hybridization (5). This is a slow but robust technique that has been used successfully to look for mutations in the apo B gene (6). Because it is based on chemical methods, the technique is able to detect all mismatched bases irrespective of sequence (7), and individuals can be identified who are heterozygous for any sequence difference compared with the normal "probe" DNA, which is radiolabeled with ^{32}P deoxycytidine triphosphate. Fragments of DNA of about 500–600 bp give good results, but for longer fragments the yield of amplified DNA is reduced, and cleaved mismatches within 50–60 bp of the ends of the DNA fragments may not be detected (as a size reduction) on gel fragments of over 500–600 bp. This can be overcome by using amplifying oligonucleotides that produce fragments that overlap by 100 bp.

A second approach is the use of denaturing gradient gel electrophoresis (8), which again appears to detect all possible mutations. A recent report has used this method to screen the promoter region of the LDL-R for mutations in FH patients, but none were identified (9). The third method is called SSCP and is based on the fact that single base changes result in conformational changes in single-stranded DNA that can be detected as a different pattern of migration on a polyacrylamide gel (10). DNA fragments are labelled by inclusion of ^{32}P deoxycytidine triphosphate in the amplification mixture and subsequent detection by x-ray film. The advantage of this method is that it is rapid and does not use toxic chemicals or require a hybridization step, but it is not yet clear whether it detects all single base changes. The frequency of such "false negatives" has been reported to be low in one study (11), but this probably depends on the specific sequence or base composition of the DNA being studied, and the procedure may not be equally efficient for all genes or all exons of the same gene. To attempt to overcome this problem, different SSCP gels are run with varying conditions of tempera-

ture and gel porosity. As with chemical cleavage of mismatched bases, the ability to detect differences in the migration pattern is reduced in fragments over 300–400 bp. Over the last year, we have used this method to detect sequence changes in the apo B and LPL gene (12), and an example of this method to detect mutations in the 3′ half of exon 4 of the LDL-R gene is shown in Figure 1 (13). DNA samples were available from patients with five different mutations in this part of the gene, including a 3-bp and a 2-bp deletion, and three different single base substitutions. All of these gave a pattern of fragments distinguishable from that seen with normal DNA.

Once a sequence change in a gene has been identified by these methods, direct sequencing of amplified DNA can be used (14) to determine the precise change and its potential effect on the function of the protein. This may be confirmed by expression of the protein *in vitro* and appropriate assays of function. The next stage would be to develop screening methods to identify relatives of the proband or individuals in other families who are carriers of the mutation. A number of PCR-based methods are available for such screening that do not require radiolabeled probes, but rely instead on fluorescence (15) or chemoluminescence (16). This can be carried out directly where the mutation creates or destroys a restriction enzyme site in the gene by separating the different sized amplified DNA fragments by gel electrophoresis (17), or by the amplification refactory mutation system method using differential amplification oligonucleotides (18, 19). Alternatively, sequence differences can be detected in a few hours by allele-specific oligonucleotide melting with, for example, biotinylated oligonucleotides and streptavidin conjugated with horseradish peroxidase and an appropriate chromogenic system (20). Reagents for such detection systems are commercially available; the labeled probe is stable for many months and thus is suitable for use in a routine diagnostic laboratory. For mutations that occur with low frequency, a "pooling" strategy may be appropriate. Small volumes of whole blood (or DNA isolated by rapid methods from Guthrie blood spots) can be pooled, for example, in batches of 10, and these batches are then pooled in a cumulative fashion. Using PCR methods, the detection of one variant allele in 100 (*i.e.* 50 samples pooled) has been reported (21), and in the future new methods may result in greater sensitivity. Such pools of DNA can be efficiently screened for many rare mutations in different genes, and the relevant individuals can be easily identified by rescreening the subpools.

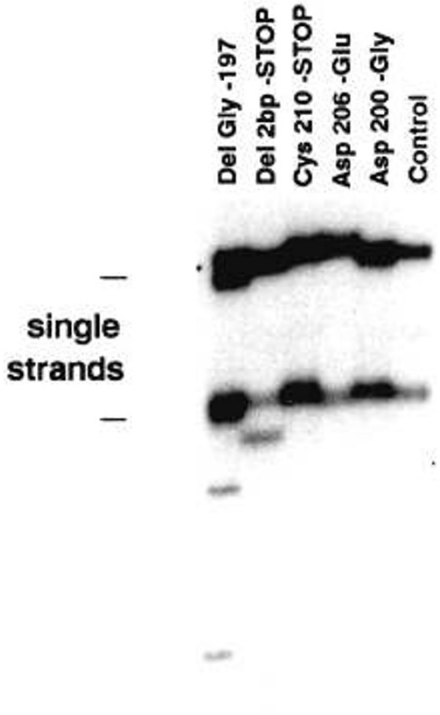

Fig. 1. SSCP gel to detect mutations in the 3′ half of exon 4 of the LDL-R gene. A different pattern of bands is seen in all patients with known mutations in exon 4, compared with the control.

MUTATIONS AFFECTING PLASMA TRIGLYCERIDE LEVELS

LPL. LPL is a heparin-releasable enzyme, bound to glycosaminoglycan components of the capillary endothelium, with a central role in lipid metabolism (22, 23). LPL is found in a variety of tissues, including muscle, adipose tissue, and macrophages. It is a glycoprotein and is active as a dimer of two identical subunits each of approximate molecular weight 60 000. LPL, which has an essential requirement for an apoprotein cofactor apo CII, hydrolyzes TG in large TG-rich lipoproteins (chylomicrons and VLDL) (reviewed in 22, 23). The three dimensional structure of human LPL has been deduced by comparison with the x-ray crystallography data obtained from the highly homologous human pancreatic lipase (24). The amino terminal two thirds of the protein contain the catalytic triad in a mostly parallel β-sheet structure, with the catalytic triad residues Asp_{156}, His_{241}, and Ser_{132} (25, 26) being buried in a hydrophobic pocket covered by a surface loop comprising residues 238–262, which is rotated away as part of the interaction of the enzyme with the lipid substrate. The carboxy-terminal domain consists mainly of antiparallel β-sheet structures and is thought to have a major function in lipid binding (27). The region that interacts with the apo CII cofactor is thought to be within the N-terminal portion of the protein, although the precise location has yet to be determined (28). There is evidence to suggest that binding to heparin is conferred by residues in the regions 290–300 (29).

The gene for LPL has been located to the short arm of chromosome 8 (30), and cDNA sequence and the gene structure have been elucidated (31–34). The gene spans about 30 kb and contains 10 exons coding for a 475–amino acid protein including a 27–amino acid signal peptide; exon 10 codes for the entire 3′ untranslated region. Several RFLP in the LPL gene have been reported including a *Pvu*II RFLP in the intron between exons 6 and 7 (35) and a *Hin*dIII RFLP in the intron between exons 8 and 9 (36). Neither of these RFLP alters any amino acids of the enzyme. One polymorphism that does lead to a change in the amino acid sequence is a C to G transversion at nucleotide 1595 of the cDNA sequence (37) (the Ser_{447}–Stop substitution) causing production of a protein truncated by two amino acids. Current evidence suggests this truncated protein maintains normal enzyme activity (38, 39) but may have altered lipid binding properties and thus substrate specificity (38).

In recent years, over 30 nonsense or missense mutations or deletion/rearrangements have been described in the gene for LPL (40–46, reviewed in 47) in patients with chylomicronemia syndrome (23) (type I hyperlipidemia). This disorder is characterized by extreme plasma levels of TG resulting in "creamy" plasma and pancreatitis and is usually diagnosed in childhood. In the absence of TG hydrolysis, chylomicrons are not converted to remnants, and thus cannot be recognized by specific lipoprotein receptors and consequently accumulate in the plasma. The phenotype is caused occasionally by a defect in apo CII, the essential cofactor for LPL, but more usually by homozygosity for defects in LPL itself (23). The amino acids of the catalytic triad of the enzyme are encoded in exons 4–6 of the gene and, as shown in Figure 2, many of the reported mutations are clustered in these exons and appear to effect the conformation of the predicted secondary structure or the hydrophobic nature of the pocket surrounding these important residues. Many of the patients reported to date are compound heterozygotes, but this

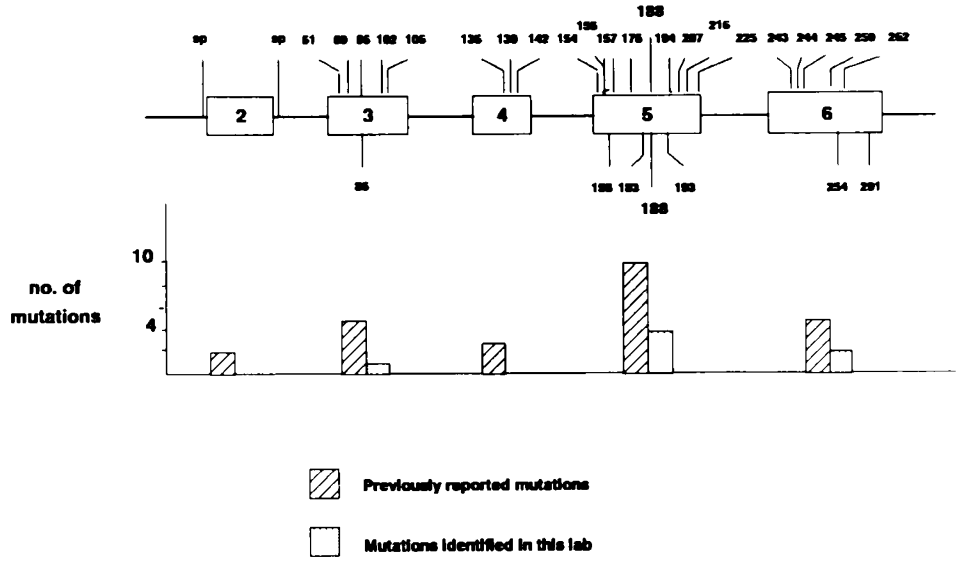

Fig. 2. Map of the LPL gene showing approximate positions of the previously reported mutation and those detected in the work of Mailly *et al.* (12) in patients from the United Kingdom and other European countries. The *boxes* represent the exons of the LPL gene. Exons are numbered 1 (the 5′ most exon) to 10 (the 3′ most exon), with only 2–6 shown.

is not the case in those populations where a single mutation occurs frequently because of a "founder" effect, for example, in patients of French-Canadian origin where a Pro_{207}-Leu change is common (45). Interestingly, one mutation that results in the substitution of Gln_{188} with Glu has been found in patients from many different countries, including those from Europe, Asia, and the Indian subcontinent (46). This suggests that this mutation is of ancient origin, possibly predating the divergence of the major ethnic groups. We have been examining a group of 20 patients with type I hyperlipidemia from the United Kingdom and Europe by using the SSCP method to screen exons 2–6 for mutations (12). To date, out of the 40 mutant alleles in this group of patients, a mutation has been identified in 27 alleles, and with the exception of the Gly_{188}-Glu mutation, all occurred only in a single family. None of the mutations have been reported previously, and their effect on lipase activity is being determined in expression studies. Overall in this sample, the Gly_{188}-Glu mutation has been found in 12 of 40 mutant alleles, but among Caucasian patients only, it explained 12 of 26 mutant alleles, or roughly 50%. It would seem likely that this mutation is therefore the most common mutation in patients in the United Kingdom and may be present in the general population at an appreciable frequency. The prevalence of type I hyperlipidemia is hard to estimate, but it is at least as common as homozygous FH (one per 1 000 000), suggesting a carrier frequency for mutations causing LPL deficiency of roughly one per 500 in the general population. If 50% of these defects are caused by the Gly_{188}-Glu mutation, it is possible that the frequency of this single defect may be as high as one per 1000 individuals.

Clinical relevance and future research. Although there have now been a large number of reports of specific defects in different patients with type I hyperlipidemia, there has been no attempt to date to compare the severity of hyperlipidemia or clinical symptoms in these different individuals. Our preliminary evidence from the sample of 20 patients suggests those with the Gly_{188}-Glu mutation have the typical pattern found in this disorder with severe symptoms, often showing signs of pancreatitis shortly after birth and developing xanthomas, with plasma TG levels of 20 mmol/L or greater. They respond well to appropriate dietary treatment, with resolution of all clinical symptoms. Biochemical studies on postheparin plasma from these individuals and *in vitro* expression studies (46) have shown that this mutation results in LPL dimers that are extremely unstable, with the result that the patient has virtually undetectable levels of LPL mass and activity. By contrast, there are other mutations that have been reported in patients with type I that have a much less severe effect on lipase activity. For example, one of the patients we have examined is homozygous for a novel mutation that changes Ala_{158} to Thr, and this individual shows about 10% of normal postheparin lipase activity (12), which is significantly higher than the activity found in the patient with the Gly_{188}-Glu mutation. Interestingly, neither of the affected children in the family has ever reported pancreatitis or xanthomas and their hyperlipidemia has been easily resolved by moderate adherence to dietary recommendations. In the future, as more information is known about the relationship between specific mutations and their effect on lipase function, it is likely that more differences will be reported on the effect of different mutations, and this information may be useful in clinical management.

One of the important aspects of LPL deficiency is whether carriers for a mutation in the lipase gene have an elevated risk of developing familial combined hyperlipidemia. This is a disorder first described in the early 1970s with an estimated prevalence in the general population at least two or three times higher than that of carriers for FH and a strong association with increased risk of coronary artery disease (48, 49). It is characterized by elevated cholesterol or TG in a proband and relatives, with the primary mechanism thought to be overproduction of apo B–containing lipoproteins from the liver (50, 51). To date, the molecular defects underlying this disorder are not understood, although in some patients, mutations in the apo AI-CIII-AIV gene cluster have been implicated (52, 53). Several lines of evidence suggest that low lipase activity, either genetic or acquired, may be involved in causing the combined hyperlipidemia pattern in at least a subset of individuals. The first comes from investigations of the relatives of probands with type I hyperlipidemia, where it was found that carriers of the Gly_{188}-Glu mutation were prone to hypertriglyceridemia, with high plasma levels of apo B and low HDL cholesterol concentrations. The effect of the mutation was modulated by age, with the largest effects being noted in individuals over 40 y (54). Similar results have been reported from the large French-Canadian kindred with the Pro_{207}-Leu change (45). The second line of evidence comes from studies in patients with familial combined hyperlipidemia, where LPL mass and activity have been measured with accurate assays. Roughly a third to a fifth of these individuals have levels of activity and mass below the 10th percentile for their age and gender (55, 56), suggesting heterozygosity for an allele that codes

for a defective LPL enzyme. This might predispose an individual to a defective clearance of TG-rich lipoproteins, which could develop into the full pattern of combined hyperlipidemia when other environmental or genetic factors result in VLDL overproduction or LPL down-regulation, which saturates this reduced clearance system. Two recent reports have suggested possible mechanisms to explain how low LPL levels could result in overproduction of apo B–containing lipoprotein from the liver, but the precise mechanism is not yet established (57, 58).

There still remain several unresolved questions about the mechanism of the association between factors that cause an individual to have low levels of LPL activity and the subsequent development of hyperlipidemia. However, the data so far suggest the strong possibility that individuals who are carriers for a mutation in the LPL gene may benefit from an early identification of such a risk, followed by appropriate counseling on lifestyle changes that would help them to reduce their subsequent risk, and more detailed monitoring so that if they should develop hyperlipidemia at a later age they could be given appropriate therapeutic advice. Thus, although being a carrier for a mutation in the LPL gene does not require immediate pediatric treatment, such information may be useful in adulthood for the individuals identified and may be of immediate use for their relatives.

Apo CII. Apo CII is a 79–amino acid protein that acts as a cofactor for the activation of LPL (reviewed in 59). The gene spans 3.32 kb (60) and is part of the apoprotein gene cluster on chromosome 19 together with apo E, apo CI, and the apo CI pseudogene (61). Apo CII, apo E, and apo CI show strong sequence homology and a similar intron/exon arrangement reflecting their evolution from a common progenitor (62). Three functional domains of apo CII have been elucidated by studies on synthetic peptides and proteolytic fragments (59) and by studies on the expression of normal and mutated variants of human apo CII in a bacterial system (63). The lipid-binding domain, between residues 14 and 50, consists of three amphipathic α-helices that anchor the molecule to the lipoprotein surface. The LPL-activation domain consists of residues 55–65 at the carboxy-terminal third of the protein, whereas the LPL-binding domain is residues 65–79, on the basis of the predicted amphipathic helix structure of this part of apo CII (59) and including the charged terminal tetrapeptide.

In some individuals with type I hyperlipoproteinemia, LPL is normal and the disorder is caused by a defect in apo CII (24, 64, 65). Apo CII deficiency is extremely rare, with a frequency considerably lower than LPL deficiency, but the clinical expression of the two are very similar, both being characterized by massive fasting chylomicronemia and recurrent pancreatitis. The pattern of inheritance of the clinical phenotype of apo CII deficiency is recessive, with obligatory heterozygotes having half normal apo CII levels but normal lipid levels. To date, 14 apo CII–deficient families have been identified worldwide and, as shown in Table 1, the mutation in eight families has now been elucidated. The defect can be caused either by frameshift mutations due to insertions or deletions, by base substitutions that create a premature stop codon or alter a donor splice site, or by a single base change in the initiation codon (72). All these mutations have a profound effect on either the structure or the amount of apo CII in the plasma. To date, no single amino acid substitution leading to loss of apo CII function has been reported, and in all but two families (73) the mutations are unique.

Clinical relevance and future research. So far there are no data to suggest that different mutations in apo CII might be associated with different clinical consequences or that the dietary management of type I hyperlipidemia caused by apo CII deficiency should be different from that caused by LPL deficiency, although there is general agreement that type I caused by apo CII deficiency is usually associated with milder symptoms (23). This may be in part because, even in the complete absence of apo CII, LPL still has some residual activity (27, 59), and thus some lipoprotein metabolism occurs. However, the extreme rarity of apo CII deficiency makes any such comparison difficult and any definite conclusion must await further data. There is also little evidence to indicate any strong association between the carrier status for apo CII deficiency and hyperlipidemia. One study reporting recently on a large kindred from Toronto (74) indicated that carriers for apo CII deficiency who are also heterozygous for the apo E4 allele of apo E that is associated with elevated levels of plasma lipids have significantly higher levels of TG and lower HDL cholesterol than relatives with apo CII deficiency and other apo E isoforms. Although this is a very informative example of how genetic variations at two different loci, coding for proteins that are both involved in lipid metabolism, may interact to determine an individual's plasma lipid levels, the low frequency of the apo CII deficiency makes this not of immediate clinical relevance.

Table 1. *Mutations in apo CII gene causing type I hyperlipoproteinemia*

Variant	Mutation	Effect on apo CII
Apo $CII_{Toronto}$[66]	T deletion → frameshift	74–amino acid protein—last
	Leu_{75} → stop	6 residues abnormal
Apo $CII_{St.\ Michael}$[67]	Single base insertion codon[66] or → frameshift[67]	96–amino acid protein Residues 71–96 abnormal
Apo $CII_{Hamburg}$[68]	G → C/intron 2 donor splice defect	No protein synthesized
Apo $CII_{Nymegen}$[69]	G deletion → frameshift Val_{18} → stop	No protein synthesized
Apo CII_{Padova}[70]	C → A/Tyr_{37} → stop	Truncated protein 36 amino acids long
Apo CII_{Bari}[71]	C → G/Tyr_{37} → stop	Truncated protein 36 amino acids long
Apo CII_{Paris1}[64]	A → G/Initiation Met_{22} → Val	No protein synthesized
Apo CII_{Paris2}[64]	C → T/Arg_{19} → stop	No protein synthesized
Apo $CII_{Venezuela}$[73]	C deletion codon 2 or 24	No protein detected
Apo CII_{Japan}[73]	C deletion codon 23 or 24	No protein detected

MUTATIONS AFFECTING LEVELS OF PLASMA CHOLESTEROL

Apo B. Apo B is the sole protein component of LDL and therefore the principal cholesterol-transporting protein. The mature apo B100 protein is 4536 amino acids long (75) and is secreted from the liver and found in VLDL, intermediate density lipoproteins, and LDL. Amino acid sequence comparisons with the receptor-binding domain of apo E (76) and from studies with MAb that inhibit binding of LDL to the LDL-R (77) suggested that the region encompassing amino acids 3359–3780 is responsible for the interaction with the receptor. There is a second, shorter form of apo B (B48) that is associated with chylomicrons and secreted from the intestine. The apo B48–containing chylomicron remnants are rapidly cleared from the circulation, whereas the apo B100 containing particles are partially metabolized to LDL that has a relatively long half-life in the circulation. The receptor binding region of apo B is absent from apo B48, and it has been suggested that this may have evolved to ensure the rapid delivery of the remnant particles of dietary lipid to the liver mediated by apo E binding to the LDL-R or the remnant receptor.

There is a single apo B gene on chromosome 2 (p23–p24) (78). The gene contains 29 exons spanning 43 kb (62), and its intron/exon organization is very different from that of the other apoprotein genes, suggesting a different evolutionary history. The intriguing question of how both the liver and intestinal forms of apo B are synthesized from the same gene has now been elucidated. The intestinal apo B48 form of apo B has been shown to be colinear with the amino terminal half of apo B100 and

terminates at isoleucine$_{2152}$ of apo B100 as the result of the introduction in the mRNA of a C→U transition at nucleotide 6666 that creates a stop codon and results in protein termination (79). This apo B mRNA editing mechanism is under tissue-specific and developmental regulation (80). Because apo B48 lacks the sequence that is required for interaction with the LDL-R, the differential expression of the two forms of apo B is important in directing the metabolism of lipoproteins.

ABL. Two primary disorders of lipoprotein metabolism exist, characterized by a complete absence or deficiency in apo B–containing lipoproteins. These are ABL and HBL (75). ABL is inherited as an autosomal recessive disorder, with heterozygous parents having normal lipid levels, whereas affected individuals have trace amounts of apo B–containing lipoproteins in the plasma and this is associated with the clinical symptoms of fat malabsorption, acanthocytes, retinitis pigmentosa, and muscular neuropathies (75). Most of the neuropathies are caused by the deficiency of fat-soluble vitamin A and E transportation by the chylomicrons, and once identified, these individuals can be treated very successfully by appropriate dietary measures and vitamin supplements (81). Mutations in the apo B gene would be an obvious possibility for causing this disorder, but because there is a single apo B molecule per LDL particle, it can be postulated that defects in the apo B gene would be expressed in a codominant fashion, with half the circulating LDL having the normal apo B protein and half having the abnormal variant of apo B. Thus, the recessive inheritance of ABL suggests strongly that the mutation may be in a gene other than that for apo B. In addition, a study carried out on liver biopsies from two patients with ABL showed that apo B mRNA could be easily detected, and that levels were 8-fold enhanced (82) and immunohistologic techniques have shown that apo B accumulates within both hepatocytes and enterocytes in this disorder (83, 84). This suggests that the defect is most likely in a gene involved in posttranslational modification or secretion of apo B. Genetic confirmation of this, ruling out the apo B gene as a candidate, was obtained by cosegregation studies such as those shown in Figure 3. For a recessive disorder caused by a mutation in the apo B gene, all affected children in a family should have the same genotype at the apo B locus, having inherited the same defective allele from each of their parents, as shown in family I. The results obtained from several families each with two affected children showed the pattern demonstrated in family II with no cosegregation of the apo B gene with ABL (85, 86), thus confirming that defects in another gene must be the cause of the disorder. One possibility is that the defect is in a "chaperon" protein that is involved in translocation of the apo B protein from the cystolic compartment or through the Golgi apparatus, where the lipoprotein particle is assembled. The identification of the gene or genes involved in this process will provide valuable insights into the mechanisms

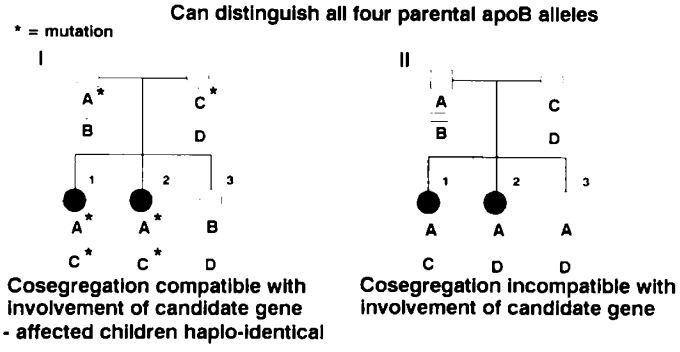

Fig. 3. Example pedigrees of families with ABL. *A*, *B*, *C*, and *D* are parental apo B haplotypes. *Black symbols*-affected. Data from family I would support the possibility of mutations in the apo B gene as being involved in causing ABL. In family II, the two affected children 1 and 2 have inherited different apo B haplotypes, and this excludes cosegregation of HBL and the apo B gene. Data adapted from Talmud *et al.* (85).

that control secretion of lipoproteins from the liver and intestine, both in this disorder and in individuals in the general population.

HBL. In contrast with ABL, HBL is inherited as a codominant disorder with heterozygotes having total cholesterol, LDL cholesterol, and apo B levels below the 5th percentile. Cosegregation studies have supported the hypothesis that the mutation is in the apo B gene (87). All mutations identified to date result in truncated proteins and most have been initially detected at the protein level using PAGE of plasma or LDL to estimate protein size. Further identification and sizing of truncations have been achieved with immunoblots and binding with MAb with well-defined epitopes. In a recent screening carried out on blood donors in St. Louis, the frequency of familial HBL was estimated to be less than 0.01% (88). In the homozygous form, patients have trace levels of apo B–containing lipoproteins and the clinical characteristics of ABL. More than 15 truncated apo B mutations causing HBL have now been characterized at the molecular level (Table 2). A map of the apo B gene showing the position of some of the mutations that lead to truncated proteins is shown in Figure 4. Except in the case of apo B25, the result of a deletion of the entire exon 21, all the truncated forms reported to date are caused by C→T transitions or base deletions. In addition, all the reported mutations have been unique to the kindreds they have been identified in and no two unrelated families have had the same mutation.

These truncated apo B species have helped define the functional domains of apo B, namely the lipoprotein assembly, lipid binding, and receptor binding regions. Where the mutation creates a protein greatly reduced in size, for example, the truncated apo B species apo B25 (89) and apo B29 (90), no plasma apo B was detected. It has been suggested that these small truncated species lack a domain necessary for lipoprotein association and stability. The amino acid residues in the amino terminus of the protein are hydrophilic and would not bind well to lipid. The truncated species apo B31 (91) and apo B37 (92) are associated with small amounts of TG-rich lipoproteins and occur primarily in the HDL density range; this reflects the small amount of associated lipid and not an HDL-type particle. These conclusions have been confirmed by *in vitro* expression studies (101, 102). Taken together these results suggest that the hydrophobic amino acids found between apo B31 and apo B39 are necessary for assembly and secretion of apo B–containing lipoproteins. Thus, there appears to be a critical size that the protein must excede to associate with lipid, with truncated apo B proteins smaller than apo B31, although detected in the plasma, being very unstable and rapidly degraded after secretion. Apo B46 (94), apo B50 (95), and apo B54.8 (88) are found primarily in the VLDL density range, although apo B46 is seen in LDL and HDL ranges and apo B54.8 in the LDL density range. The larger apo B species, apo B86 (99), apo B87 (100), and apo B89 (103) are found in a similar density range as full-length apo B100. Thus, the truncated apo B proteins help define the region in the amino terminal end of the protein important in the association with lipids (Table 3, Fig. 4).

The low levels of these truncated forms in the plasma are the result of several different mechanisms. Most of the truncated apo B proteins lack the portion of the protein that binds to the LDL receptor; thus an increase in receptor-mediated catabolism is unlikely to be an explanation for the low concentrations of apo B, and it is probable that the instability of the mutant lipoproteins is the major contribution to their low levels. The larger truncated proteins apo B87 and apo B89, which do encode the primary LDL-R binding domain, provide evidence of secondary binding domains on apo B for the LDL-R. In the case of the apo B89, degradation was increased in cultured fibroblasts compared with apo B100 (103), and in addition there was enhanced clearance of apo B89 in LDL turnover studies performed in rabbits (104). This suggests that apo B89 has an increased affinity for the LDL-R, thus explaining the low concentration of apo B89 in the plasma of the original patient (103).

Table 2. *Characteristics of truncated apo B species associated with HBL*

	Mutation	Predicted no. amino acids	Density fractions
Apo B25[89]	Deletion exon 21	1085	None
Apo B29[90]	C → T Arg_{1036} → stop	1305	None
Apo B31[91]	Deletion G_{4480} → frameshift	1425	HDL + infranatant
Apo B37[92]	Deletion AACA → $frameshift_{5391-5394}$	1728	VLDL, LDL, HDL
Apo B39[90]	Deletion G_{5591} → frameshift	1799	VLDL, LDL
Apo B40[93]	Deletion $TG_{5693-5964}$ → frameshift	1829	VLDL, LDL, HDL
Apo B46[94]	C → T Arg_{2058} → stop	2057	VLDL, LDL, HDL
Apo B50[95]	C → T Gln_{2252} → stop	2251	VLDL
Apo B54.8[88]	C → T Arg_{2486} → stop	2485	VLDL, LDL
Apo B55[96]	C → T Arg_{2495} → stop	2494	VLDL, LDL
Apo B61[97]	Deletion 7 bases → $frameshift_{8525-8561}$	2784	VLDL, LDL
Apo B67[98]	Deletion $nucleotide_{9327}$ → frameshift	3040	VLDL, LDL
Apo B86[99]	Deletion $nucleotide_{11840}$ → frameshift	3896	VLDL, LDL
Apo B87[100]	Deletion $nucleotide_{12032}$ → frameshift	3979	VLDL, LDL
Apo B89[93]	Deletion nucleotide G_{12032} → frameshift	4039	VLDL, LDL

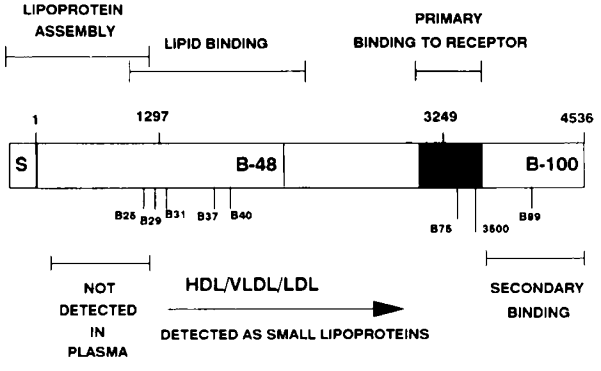

Fig. 4. Schematic map of the apo B cDNA identifying reported mutations that lead to HBL. The *black box* depicts the proposed LDL-R binding domain. *S* shows the signal peptide of the protein.

Other truncations in this region of the protein have been reported with a similar increase in receptor-mediated catabolism (100). Thus, the absence of the carboxyl-terminal 500 amino acids, which are hydrophobic and firmly embedded in lipid, may result in the release of the constraint put on the primary receptor binding domain or make more available a secondary region around amino acids 4001–4019 (105), resulting in an increased catabolism of the LDL.

Clinical relevance and future research. There is some evidence to support the view that different mutations in HBL are associated with different clinical consequences. The extent of the severity appears to be related to the size of the truncated apo B protein and whether it can associate with lipid; for example, the patient with homozygous HBL, homozygous for an apo B25, presented with symptoms of ABL (89). However, several compound heterozygous patients (92, 103) were asymptomatic, and in these cases at least one truncated apo B protein was capable of associating with lipid, thus participating in fat-soluble vitamin transportation. Further research is required to explore whether there are different clinical consequences in later life associated with these different classes of truncations, for example the onset of neuropathy or retinopathy (96). It is still unclear, however, why some heterozygous HBL patients have LDL levels below the 5th percentile and not at the 50th percentile despite the presence of one normal apo B100 allele. Some insight comes from LDL turnover studies in HBL patients. LDL turnover studies performed on a patient with apo B55 revealed a reduced LDL synthetic rate (5.5 mg/dL/d) compared with the normal range of 10–15 mg/dL/d (106), confirming results from an earlier study (107) that showed a reduced LDL synthetic rate and a reduced synthetic rate of VLDL-apo B in two HBL patients,

Table 3. *Summary of mutations in LDL-R gene in 200 patients with FH in United Kingdom*

Type of mutation	No. of unrelated patients	Comments	Reference
Deletions/rearrangements detected by Southern blotting	9	4/9 same as and 3/9 similar to previously detected mutations	151, 152
		1/9 insertion complementary to previously described deletion	152
Glu_{80} → Lys (exon 3)	5	Probably same allele	150
Exon 4 mutations			
Deletion of Gly_{197}	6	Common in Lithuanian Jews	149
Deletion of 2 bp in codons 206/207	5	Same allele in all; "English" defect	149
Cys_{210} → stop	1	Irish/Scottish patient	149
Asp_{206} → Glu	3	Same as common Afrikaner	146
Ser_{156} → Leu	1	Same as Puerto Rico, but recurrent mutation	154
Asp_{200}-Gly	1	New: English origin	13
Pro_{664} → Leu (exon 14)	5	Recurrent mutation	153
Total	36	(18% of mutant allele)	

where the molecular defect is unknown. These data support the view that a reduced synthetic rate of VLDL occurs in these patients and thus the synthetic rate of LDL containing both the truncated and the normal apo B100 may be reduced. Apo B synthesis is constitutive in liver cells, and overall the output of apo B is controlled by changes in the mRNA translational efficiency, or more likely by changes in degradation of intracellular apo B in response to endogenous factors such as the level of plasma fatty acids or postprandially (108). Thus, it is possible that in the hepatocytes of the heterozygous HBL patients, although mRNA coding for both the truncated and normal apo B proteins are translated, the truncated species interferes with the assembly of apo B100 into normal lipoproteins, leading to an overall reduction in secretion of apo B–containing lipoproteins. Further research to explore such mechanisms should lead to a better understanding of the control of secretion of apo B–containing particles from the liver.

FDB. It is well known that defects in the LDL-R that destroy function lead to FH. Similarly, hypercholesterolemia could be caused by defects in the apo B gene that would reduce binding to the LDL-R and lead to raised cholesterol levels, and the name FDB has been proposed for such mutations (109). A number of laboratories have carried out systematic searches, using molecular biology techniques, to identify patients who have mutations in the apo B gene that would alter receptor binding. To date, there is only one form of FDB and that is caused by a mutation that substitutes codon Arg_{3500} with Gln (110), thus it is called the apo B3500 mutation. Originally observed in a patient who showed reduced clearance of autologous LDL compared with clearance of LDL from a normal donor (111), the mutation was identified as a G→A substitution altering codon CGG to CAG (112). This single amino acid change reduces binding of the LDL containing apo B-Gln_{3500} resulting in the accumulation of such LDL (109), whereas LDL-Arg_{3500} is cleared with normal efficiency. In some patients, it has proved possible to separate the two species of LDL using binding to MAb (113). Purified LDL-Gln_{3500} has 5% normal receptor binding affinity and thus accumulates *in vivo*; in an FDB heterozygote, as much as 70% circulating LDL will be LDL-Gln_{3500}. The mechanism of the effect has been elucidated by studying the differential binding of MAb and using carbon-13 nuclear magnetic resonance (114), which showed that the six lysine residues within the region of amino acid 3500 have altered ionization constants in the presence of Gln_{3500}. Lysine residues are known to be involved in the binding of apo B100 to the LDL-R, and the substitution of Arg_{3500}→Gln, by a large effect on the conformation of the surrounding area of the protein, alters the microenvironment of the receptor binding domain.

To date, only individuals who are heterozygous for the mutation have been identified (115). Individuals with FDB have been identified in the United States, Canada (110), Austria (116), United Kingdom, and Denmark (117), Germany (118), and Italy (119), but not in Finland (120). Haplotype analysis using apo B gene polymorphisms has shown that in all cases reported to date the mutation is identical by descent, and thus all FDB carriers have a common ancestor (121, 122). The occurrence of the mutation in ethnic groups, other than Caucasians, has not yet been reported. Rough estimates of the frequency of the mutation in the general population made on the basis of the frequency found in lipid clinic patients are in agreement, ranging from one per 500 (117) to one per 700, (118), but to date, no systematic study to screen for the mutation in the general population has been carried out. The apo B3500 mutation appears to be the most common single gene defect causing hypercholesterolemia, and although other apo B mutations causing FDB may be identified in the future, it is likely that apo B3500 will be the most common.

The mutation was originally identified in a patient who was moderately hypercholesterolemic (111), but three recent studies have screened patients with a clinical diagnosis of FH and found that roughly 3% of these patients were heterozygous for the apo B3500 mutation (117, 118, 123). Where the patients have been analyzed further, this occurred in individuals who had LDL-R activity within the normal range (124). This mutation can be associated with severe hypercholesterolemia, tendon xanthomas, and a family history of premature coronary artery disease, and it is therefore clinically indistinguishable from FH caused by receptor defects. Furthermore, as with defects in the LDL-R gene, the effect of the apo B3500 mutation on plasma lipid levels can be expressed in children (Fig. 5), with several families (124) now reported with children under the age of 5 y having markedly elevated LDL cholesterol levels.

Clinical relevance and future research. The range of expression of the mutation is of major clinical interest. All carriers of the mutation have LDL that binds with reduced affinity to the receptor, but the absolute levels of LDL cholesterol in the blood and thus the future risk of developing coronary artery disease will depend on the interaction between other genetic and environmental factors. In a recent review of all the published biochemical and clinical information on 70 FDB carriers (125), it was clear that the rate of development of coronary artery disease in this disorder was similar to that reported in FH patients, with a faster rise in males than females. Over 85% of carriers have total and LDL cholesterol levels over the 95th percentile for their age and gender, but unlike FH patients, TG and HDL cholesterol levels are within the normal range. This is presumably because the metabolism of the TG-rich lipoprotein, mediated through apo E and the normal LDL-R, is not affected by the apo B mutation. However, the information regarding the range of clinical severity of the disorder is biased, because to date the majority of patients have been identified by screening individuals attending lipid clinics, many of whom had a clinical diagnosis of FH. This raises the possibility that the mutation may not always be associated with such severe clinical consequences, and in support of this, a review of the published data on plasma lipid levels of relatives (125) shows that some have levels within the normal range for their age and gender. Presumably environmental factors, and variation at other gene loci, will contribute to these individual differences and may possibly interact specifically with the apo B mutation. Although no such data on range of expression is yet available for any of the mutations in the LDL-R gene causing FH, *a priori* it is likely that a similar range will be seen. It is therefore important to identify FDB carriers

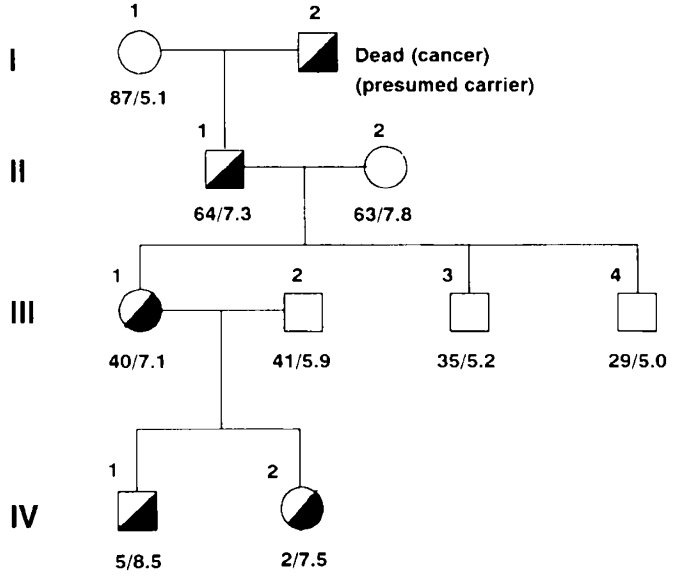

Fig. 5. Pedigree of a four-generation family with apo B3500. Data shown are age and cholesterol levels (mmol/L) below each member. Generation I-2 is deceased and is a presumed heterozygote. *Half-filled symbols*-heterozygote for FDB. Data adapted from Myant *et al.* (124).

from the general population to allow an unbiased estimate of the risk of hyperlipidemia and atherosclerosis associated with this defect. Only then can accurate information be given to carriers and appropriate therapeutic management strategies started.

It is still not clear what is the best drug therapy to offer FDB patients. Most conventional drug therapies for FH rely on up-regulation of the LDL-R to enhance clearance of LDL, but the LDL-Gln cannot be cleared by this route and may still accumulate. The atherogenic potential of such accumulating defective LDL is not understood, and it is possible that LDL-Gln may be susceptible to oxidation and it thus may accumulate in foam cells and promote atherosclerosis. Studies have been carried out on FDB patients to examine the responsiveness to drugs (126–128), and although plasma levels of LDL do fall in most patients, it is possible that this is caused mainly by a reduction in plasma levels of LDL-Arg. Thus, for FDB patients, treatment with antioxidants or a combination treatment may prove a better therapy, and if this were the case, using genetic tests to distinguish hypercholesterolemia caused by a receptor or a ligand defect would be of major importance.

LDL-R defects in FH. FH is a common inherited disease showing an autosomal dominant pattern of inheritance (129). It is characterized clinically by elevation in the concentration of LDL cholesterol in blood, tendon xanthomata, and an increased risk of myocardial infarction. FH is present in 5–10% of individuals under the age of 55 y in the United Kingdom and the United States who develop coronary artery disease (130, 131), and is therefore the best understood single-gene cause of hyperlipidemia and thus atherosclerosis risk. Based on the estimated population frequency of carriers of one per 500, there are more than 100 000 FH heterozygous individuals in the United Kingdom, of which probably less than 3000 have been identified to date. Once identified, the hyperlipidemia of these patients is responsive to treatment by diet and drugs (132, 133), and such treatment reduces subsequent morbidity and mortality (134). Children who have inherited two defective alleles of the LDL-R (homozygous FH, but usually compound heterozygous for two different defects) represent one per 1 million of the population. In these children, there is usually little useful lowering of plasma LDL cholesterol levels in response to diet or drugs, and many suffer a major coronary event in the first or second decade of life, but life expectancy can be extended by appropriate treatment (135, 136). Current treatment is usually plasma exchange or LDL apheresis (137), but patients may alternatively be treated by transplantation of a donor liver, possibly in conjunction with a heart transplant (138).

FH results from different genetic defects in a cell surface receptor that normally controls the uptake of plasma LDL (129, 139). Five classes of mutations at the LDL-R locus have been identified on the basis of phenotypic behavior of the mutant protein (140). Class I mutations fail to produce any immunoprecipitable protein (null alleles). Class II mutations encode proteins that do not fold properly after synthesis and are blocked, either partially or completely, in transport between the endoplasmic reticulum and the Golgi complex (transport defective alleles). Class III mutations encode proteins that are synthesized and transported to the cell surface but fail to bind LDL normally (binding-defective alleles). Class IV mutations encode proteins that move to the cell surface and bind LDL normally, but are unable to cluster in clathrin-coated pits and thus do not internalize LDL (internalization-defective alleles). Class V mutations encode receptors that bind and internalize ligand in the coated pits, but fail to discharge the ligand in the endosome and fail to recycle to the cell surface (recycling-defective alleles). These different classes of mutations are caused by defects scattered over the entire LDL-R gene in the case of the first two classes, and mutations in the ligand binding region, the cytoplasmic domain, and in the epidermal growth factor precursor homology domain for classes III–V, respectively.

The cloning of the human LDL-R gene (141) has made it possible to study FH using DNA technology. There have been at least 40 different mutations of the LDL-R gene characterized at the DNA level (140), and they have given valuable insights into the function of the different domains in the LDL-R. Many more mutations are likely to be found, and Hobbs *et al.* have postulated from the number of homozygous FH patients they have investigated that in the Dallas collection alone there could be as many as 183 mutant alleles, although only the minority have been characterized at the DNA level so far (140). Several studies have been published demonstrating that within a geographically or culturally isolated population, or where a large proportion of people are related by descent because of migration, there may be a single mutation causing FH in many of the patients (142–148). In the United Kingdom, where there is a very heterogeneous population, it is unlikely that any mutations will be present at a high frequency in FH patients. As part of a collaborative project to study mutations in FH patients in the United Kingdom, we have recently discovered that two small deletions in exon 4 of LDL-R gene (Table 3) are each present at a frequency of 2–3% in a group of 200 FH patients in London (149). It has also recently been shown that a mutation identified in exon 3 of the gene was present in 2% of the London sample, but 15% of a sample of patients from Manchester (150). It is thus possible that even in a heterogeneous population, such as in the United Kingdom, the frequencies of some mutations may be relatively high in certain local areas. Our findings to date in this sample from London are that 3% have the apo B3500 mutation (117) and 5% of patients have a gross deletion (151, 152), whereas in a further 12% a small deletion or a single base mutation has been detected in exon 3, 4, or 14 (13, 150, 151, 153, 154). Thus, with six DNA tests the specific defect causing FH can be determined in roughly 20% of patients from the London sample. These results are extremely encouraging, although either many additional mutations must be found or a different strategy must be developed if DNA methods are ever to become a useful adjunct to classic screening methods. However, the techniques are now available for examining the entire LDL-R gene from a patient with suspected FH in a rapid and accurate way, for example using the SSCP method shown in Figure 1, and once the mutation has been identified, it is likely that this information will be of use in both the management of the patient and identification of relatives.

Clinical relevance and future research. There are a number of clinically important questions about FH that have not yet been answered, and can be addressed in the next few years using the molecular techniques now available.

DNA tests for identification of FH in relatives. Recently, several overview analyses of the results of published trials of diet and drug therapy to lower lipids in middle-aged high-risk patients, have suggested the possibility of harmful side effects in some individuals (155–157). These side effects include increased frequency of violent deaths and suicides in the treated group, and it has been suggested that precipitous reduction in plasma lipid levels in middle age may alter membrane physiology in the brain, and thus affect behavior (155). However, no study has suggested that lifelong low plasma cholesterol levels are associated with such risk (158), and this strengthens the argument for early identification of children with FH and early commencement of lipid-lowering treatment. Furthermore, it is well accepted that atherosclerosis develops in youth, as has been shown recently in a study of postmortem material from young individuals (159). Early identification of children carrying LDL-R gene defects will enable life-style changes, dietary therapy, or, where necessary, drug therapy such as resins to be started, and studies have shown that early commencement of such treatment is associated with better compliance (160). Several studies have shown that measures of total cholesterol or LDL cholesterol alone do not allow unequivocal diagnosis of FH in 10–15% of cases, even in the children of a parent with FH (161, 162). We have also recently shown that some children whose lipid levels are initially within

the normal range for their age and gender show a greater than average rise in lipid levels over time, to a point where it is evident that they have inherited the LDL-R gene mutation (163). Although the frequency of this problem is unknown, there is no doubt that an unequivocal DNA test would be very useful, both to allay fears and to identify children for whom dietary advice and appropriate therapy should be started. When the mutation is known for a patient, DNA tests will give an unequivocal result that can be obtained within 1–2 d.

It has been suggested that a monocyte or lymphocyte assay may be a useful tool for identifying individuals with defective LDL-R function, and there are several reports of such methods (164–166). However, there are considerable technical difficulties with these approaches that prevent their application for routine screening at the present time. In particular, as with all other tests used for diagnosis of FH, there is still overlap between the values obtained for some "normal" individuals and patients with a defect in the receptor, whereas a genetic approach to identify LDL-R defects gives an unequivocal result. At least 12 RFLP of the human LDL-R gene have now been reported (167, 168), and the usefulness of such polymorphisms depends on the relative frequency of the alleles and the degree of linkage disequilibrium between the alleles of the different polymorphisms. Using Southern blotting methods and the enzymes *Nco*I, *Apa*LI, *Puv*II, and *Stu*I (169), over 80% of patients with FH in London are heterozygous for at least one of the RFLP, showing an overall heterozygosity index of 0.7 with a combination of *Ava*II and *Nco*I being the most useful pair (polymorphism information content 0.61). However, the *Apa*LI and *Puv*II variable sites are located in the 5-kb long intron 15, and so far it has not proved possible to amplify this intron using primers for exon 15 and 16 (unpublished observation). Recently, we have repeated this study for six RFLP detectable by PCR methods using the enzymes *Sfa*NI, *Taq*I, *Stu*I, *Hin*cII, *Ava*II, and *Nco*I. For these RFLP, the combination of *Hin*cII and *Nco*I gives the highest pairwise polymorphism information content value of 0.68, and with the addition of the *Sfa*NI RFLP, 85% of patients in the sample were heterozygous for at least one polymorphism (170). Recently, variable copy dinucleotide repeat polymorphisms have been reported in the LDL-R gene, which have been detected using an 8% denaturing polyacrylamide gel (171) or by the SSCP method (10). Although technically more difficult than the PCR methods, these polymorphisms should also be useful for family studies.

With the availability of many RFLP of the LDL-R gene, the main factors determining the success of diagnosis by a family cosegregation approach are uncertainty in the diagnosis caused by individual differences in genetic background or environmental factors, unavailability (due to premature death) of affected relatives to determine phase, and the confounding problem of nonpaternity. In situations of doubt, paternity can be confirmed using genetic tests (172), and a cosegregation study of families of patients with FH from Munich has recently shown that diagnosis was possible in all cases where samples from four or more relatives were available (173), although this depends on the pedigree structure. The usefulness of such DNA diagnosis has been confirmed by in a recent study from Finland in which relatives of patients with a known mutation in the LDL-R gene were investigated (174). Based on unequivocal DNA diagnosis, 15% of the relatives were misclassified using only adult lipid data to determine hypercholesterolemia (lipid levels above the 95th percentile). This misclassification was reduced using age- and gender-specific lipid values, but 5% of relatives were still incorrectly classified as "FH" or "normal" by lipid values alone. In the next few years, it is increasingly likely that the problems of diagnosis by family studies will be avoided by the identification of specific mutations in the LDL-R gene. Until these become routine, unequivocal identification of carriers and noncarriers in the relatives of FH patients may be aided by cosegregation studies using RFLP (175, 176).

Differences in symptoms. In different FH patients, there is a great deal of variation in the levels of untreated plasma lipids (177) and in the age of onset of coronary artery disease (178). In a recent publication from the UK FH register, strong evidence was obtained for differences in risk of coronary artery disease in different patients, with some individuals developing disease at a very early age, whereas those who had survived past 50 y had a standardized mortality ratio that was only slightly higher than in the general population (179). It has also been shown that the age of onset of coronary artery disease aggregates in families (180), and although it is possible that this may be caused by the presence of environmental or other genetics factors such as genes for higher levels of Lp(a) (181, 182), it cannot be ruled out that different mutations in the LDL-R gene may be the cause. A recent report from South Africa suggested that the Val_{408}-Met mutation is associated with more severe clinical symptoms than the other common mutations in this population, the Asp_{206}-Glu and the Asp_{154}-Asn mutations (183). If such an association could be established for other mutations, more active therapeutic strategies could be recommended to patients and their relatives who had inherited a mutation associated with a greater risk.

Differences in response to diet or drugs. Several studies have reported that there is a great variation in the fall in total and LDL cholesterol levels when patients with heterozygous FH are treated with drugs such as resins or hydroxymethylglutarate-CoA reductase inhibitors (133, 184). One possibility is that these differences are caused by genetic variation at other loci, and several genes have been shown to be related to baseline lipid levels or response to therapy in patients with FH (185–187). However, given the genetic heterogeneity of the patients recruited for these studies, one can postulate that the variation may be caused by the presence of different mutations in the LDL receptor gene in different patients. This has not been studied in FH patients with well-characterized mutations in the LDL receptor gene, so it is not known whether different mutations in the receptor gene are responsible for any of the observed variation in drug response. The main mechanism of reducing LDL cholesterol with hydroxymethylglutarate-CoA reductase inhibitors is because of a reduced residence time of the LDL cholesterol caused by up-regulation of the normal LDL receptor. The observed variability in response in different patients could thus be mediated through the presence of a partially functioning mutant that augments the novel receptor or a partially inhibiting mutant receptor that interferes with the function of the normal protein. Alternatively, the variability could be caused by a differently functioning normal receptor in different patients (188–191). Recently, a small study has presented evidence to support the suggestion that there may be mutation-specific differences in response (192), and if such differences could be confirmed it would obviously be useful in patient management.

SUMMARY

Techniques for molecular biology are extremely powerful and have allowed the precise molecular defect to be identified in a number of pediatric patients with disorders of plasma TG and cholesterol metabolism. In very few situations to date has this information been useful to the pediatrician in decisions about the management of the child, but for disorders such as FH or type I hyperlipidemia caused by LPL deficiency, this may be possible in the future if different mutations are found to be associated with better response to a particular therapy or are associated with a different risk for developing disease in later life. For rare disorders that show a recessive pattern of inheritance, the identification of relatives who are carriers is not of great benefit, because the possibility of these marrying another carrier is too low to be of major concern. However, for "codominant" disorders, where carriers have a greater risk of developing clinical symptoms in adulthood, screening relatives to identify carriers will be of major benefit; this applies mainly to the disorders of FH, FDB, and LPL deficiency. For each of these, carriers may

represent one of 500 in the general population and random or opportunistic screening would be feasible, but a case-finding strategy is likely to be most appropriate to identify individuals who have a specific major-genetic predisposition to hyperlipidemia and thus coronary artery disease. However, before such screening can be started, much more information will be required about the relationship between a specific mutation and the resulting plasma lipid levels that occur in a range of environmental situations, such as different diets, smoking, or drug therapy, as well as the possibility of interaction between the mutation and other ameliorating or exacerbating genetic factors. Only then can appropriate advice be given to an individual about the subsequent long-term risk of developing coronary artery disease. Once such information is available, there will still be ethical dilemmas, and the application of such diagnostic techniques to the relatives of children will require a sensitive collaborative approach from molecular biologists, molecular geneticists, and particularly from pediatricians.

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