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Genetics

Regional mapping of human chromosome 19: Organization of genes for plasma lipid transport (*APOC1*, *-C2*, and *-E* and *LDLR*) and the genes *C3*, *PEPD*, and *GPI*

(whole-arm translocation/somatic cell hybrids/genomic clones/gene family/atherosclerosis)

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ABSTRACT **We report the regional mapping of human chromosome 19 genes for three apolipoproteins and a lipoprotein receptor as well as genes for three other markers. The regional mapping was made possible by the use of a reciprocal whole-arm translocation between the long arm of chromosome 19 and the short arm of chromosome 1. Examination of three separate somatic cell hybrids containing the long arm but not the short arm of chromosome 19 indicated that the genes for apolipoproteins CI, CII, and E (*APOC1*, *APOC2*, and *APOE*, respectively) and glucose-6-phosphate isomerase (*GPI*) reside on the long arm, whereas genes for the low density lipoprotein receptor (*LDLR*), complement component 3 (*C3*), and peptidase D (*PEPD*) reside on the short arm. When taken together with previous studies, our results suggest the following physical gene map: pter–*LDLR*–*C3*–p13.2–*PEPD*–centromere–(*APOE*, *APOC1*, *APOC2*, *GPI*)–qter. In addition, we have isolated a single λ phage carrying both *APOC1* and part of *APOE*. These genes are tandemly oriented and are separated by about 6 kilobases of genomic DNA. Since previous family studies indicate tight linkage of *APOE* and *APOC2*, the apolipoprotein genes *APOC1*, *APOC2*, and *APOE* form a tight complex on the long arm of chromosome 19, suggesting the possibility of coordinate regulation.**

Human chromosome 19 contains genes involved in several familial diseases and, hence, has become a focus for genetic studies (1–3). Among these are genes for myotonic dystrophy (*DM*) and a form of neurofibromatosis (*NF1*) and several genes controlling lipoprotein structure and metabolism (1–12). Considering its small size, chromosome 19 has been assigned a large number of other genes, including complement component 3 (*C3*), peptidase D (*PEPD*), glucose phosphate isomerase (*GPI*), secretor (*SE*), the Lutheran blood group (*LU*), and a cluster of genes related to chorionic gonadotropin β subunit (*CGB*) (1, 13). However, the regional locations of these genes have been poorly defined.

We are particularly interested in genetic factors involved in plasma lipid transport and related diseases such as atherosclerosis. Lipids are transported through the circulation as noncovalently associated complexes with apolipoproteins (14). These complexes are secreted by liver and intestine, and their removal from the circulation is mediated largely by specific cell surface receptors. Genetic variations resulting in deficiencies or structural alterations of these apolipoproteins or receptors can predispose individuals to dyslipoproteinemias or premature atherosclerosis. The most striking example of this is familial hypercholesterolemia, which results from defects in the expression of the low density lipoprotein (LDL) receptor and is strongly correlated with atherosclerosis (15). Another relatively common dyslipoproteinemia, type III hyperlipoproteinemia, is associated with a structural variation of apolipoprotein E (apoE) (16). Also, a variety of rare apolipoprotein deficiencies result in gross perturbations of plasma lipid transport; for example, apoCII deficiency results in high fasting levels of triacylglycerol (17). The genes encoding human apolipoproteins have been mapped to three chromosomes: the gene for apoAII (*APOA2*) is on chromosome 1 (8, 18, 19); the genes for apoAIV (*APOA4*), apoAI (*APOA1*), and apoCIII (*APOC3*) are linked on chromosome 11 (20–22); and the genes for apoCI (*APOC1*), apoCII (*APOC2*), and apoE (*APOE*) are on chromosome 19 (refs. 4–6, 8; this paper). Also on chromosome 19 is the gene for the LDL receptor (*LDLR*) (refs. 7 and 23; this paper). The clustering of *APOC1*, *APOC2*, *APOE*, and *LDLR* on chromosome 19 raises the possibility of regulatory and evolutionary links between these genes. Consistent with this is evidence indicating that the various apolipoproteins have evolved from a common ancestral sequence (24, 25). In the present study, we have used somatic cell hybrids containing a translocation separating the chromosome 19 arms to further localize and orient *APOC1*, *APOC2*, *APOE*, *LDLR*, and three other chromosome 19 genes, *PEPD*, *C3*, and *GPI*. We have also confirmed the chromosome 19 locations of *APOC1*, *APOC2*, *APOE*, and *LDLR* by examining a human–mouse somatic cell panel with cloned probes for the genes. Finally, we have demonstrated tight linkage of *APOC1* and *APOE* by isolating a single genomic clone containing both genes.

MATERIALS AND METHODS

Cell Hybrids. A panel of 16 mouse–human somatic cell hybrids was derived by polyethylene glycol-mediated fusion of mouse B82 cells (GM 0347A) and human male fibroblasts (IMR91), using standard procedures (26–29). These clones were grown up in multiple dishes for DNA extraction and chromosome studies. Chromosome analysis (30) was done on a minimum of 30 Q-banded photographed metaphases per hybrid clone.

Fibroblasts carrying a reciprocal translocation between chromosomes 1 and 19 [t(1;19)(1p19q;1q19p)] were kindly provided by R. Bernstein (South African Institute for Medical Research, Johannesburg). These fibroblasts were fused with mouse B82 cells, and 30 independent clones were evaluated

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Abbreviations: apo, apolipoprotein; LDL, low density lipoprotein; bp, base pair(s); kb, kilobase(s). Other abbreviations are standard symbols for human genes (italic) or are based on those symbols (roman type).

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for the expression of human PEPD, GPI, PGM1 (phosphoglucomutase 1, a human 1p marker), and PEPC (peptidase C, a human 1q marker). Based on these results, 3 independent clones were identified that retained the 1p/19q chromosome. Cytogenetic analysis confirmed the presence of this chromosome and no other human chromosome 19 material in these clones. The three clones were grown in multiple culture dishes, and cytogenetic analysis of the pooled cells indicated that the 3 clones retained the 1p/19q chromosome nonselectively in 45%, 50%, and 60% of the cells analyzed.

Probes and Filter Hybridization. The human apoE cDNA clone pE301 (31) and the human apoCII cDNA clone pCII-711 (5) were provided by J. Breslow (Rockefeller University, New York). The human C3 cDNA clone pC3.11 was provided by G. H. Fey (Scripps Clinic, La Jolla, CA) (32). The bovine LDL receptor cDNA clone pLDLR-1 (33, 34) was provided by D. Russell, J. L. Goldstein, and M. S. Brown (University of Texas, Dallas). The human apoCI probe was a previously described (8) cDNA of 372 base pairs (bp). The cDNA inserts were isolated (35) and labeled with ^{32}P by nick-translation (36) or random priming (37) to specific activities of $0.3–3.0 \times 10^9$ cpm/μg. Genomic DNA was purified from isolated nuclei (38) by incubation in 10 mM EDTA, pH 8/0.2% $NaDodSO_4$ with proteinase K (600 μg/ml, Sigma) at 37°C for 24 hr followed by phenol extraction and ethanol precipitation. DNA from the hybrid clones was digested with the appropriate restriction enzyme, and 10 μg of each was electrophoresed in a 1.2% agarose gel in 40 mM Tris acetate/1 mM EDTA, pH 7.4, and transferred by blotting to nitrocellulose (35, 39). Hybridization with labeled probe ($5–10 \times 10^6$ cpm/ml) in 45% (vol/vol) formamide/4.6× SSC/0.1% polyvinylpyrrolidone/0.1% Ficoll/0.1% bovine serum albumin/ 20 mM NaH_2PO_4, pH 6.5/ denatured salmon sperm DNA (500 μg/ml)/10% dextran sulfate was for 12–16 hr at 42°C with shaking. The filters were then washed (20 min per wash with 0.1% $NaDodSO_4$) as follows: apoE probe, twice in 2× SSC and twice in 0.5× SSC at 55°C; apoCII, twice in 2× SSC and twice in 0.5× SSC at 65°C; apoCI and C3, twice in 2× SSC and twice in 0.1× SSC at 65°C; LDL receptor, twice in 2× SSC at 55°C and twice in 0.25× SSC at 60°C. (1× SSC is 0.15 M NaCl/15 mM sodium citrate, pH 7.0.)

Assays for GPI and PEPD. Electrophoresis of GPI (glucose phosphate isomerase, EC 5.3.1.9) was by the method of Detter *et al.* (40). Electrophoresis of PEPD (peptidase D, EC 3.4.13.9) was by the method of Lewis and Harris (41).

Screening of a Human Genomic Library. A library of human fetal liver DNA partially digested with *Hae* III and *Alu* I and cloned in the bacteriophage λ derivative Charon 4A (42) was screened by filter hybridization using random oligonucleotide-primed (37) apoCI and apoE cDNA probes. From 10^6 recombinants, 5 carrying apoCI cDNA and 3 carrying apoE cDNA were isolated and taken through rounds of secondary and tertiary plaque purification. One clone, λCIE, contained an insert that hybridized strongly to both probes. Phage DNA was prepared as described (43).

RESULTS

Hybrid Cell Panel and Hybrid Cells Containing Chromosome 19 Translocation. A human–mouse hybrid cell panel (Table 1) was constructed and used to confirm the locations of chromosome 19 genes. To map genes to regions of chromosome 19, we used hybrid cells containing a reciprocal whole-arm translocation between chromosomes 1 and 19 [46,XX,t(1;19)(1p19q)(1q19p)]. Three independent clones containing the 1p/19q chromosome were isolated. The metaphase chromosomes of one hybrid clone are shown in Fig. 1. Unfortunately, we were unable to isolate clones containing the reciprocal product of the translocation, 1q/19p, in the absence of a normal chromosome 19.

apoE. The chromosomal location of *APOE* was determined by examining a panel of human–mouse cell hybrids with a cloned human apoE cDNA probe. When the DNA was digested with *Eco*RI, the 2-kilobase (kb) human band was readily distinguishable from the 8-kb mouse band (Fig. 2). Partial examination of the panel was consistent with the chromosome 19 location of *APOE* (Table 1). Examination of three separate hybrid clones containing the 1p/19q chromosome indicated that *APOE* resides on the long arm (Fig. 2*a*).

apoCII. The chromosome 19 location of *APOC2* was confirmed by examining a panel of human–mouse hybrids with a cloned human apoCII cDNA (Table 1). DNA was restricted with *Eco*RI, and under the conditions employed only a human band at 3.8 kb showed significant hybridization (Fig. 2*b*). Examination of three hybrids containing the long but not the short arm of chromosome 19 indicated that *APOC2* resides on the long arm (Fig. 2*b*).

Table 1. Segregation of *LDLR*, *APOC1*, *APOC2*, and *APOE* with human chromosomes in cell hybrids

Hybrid	Human chromosome*																								Human gene†			
clone	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y	*LDLR*	*APOC1*	*APOC2*	*APOE*
84-2	+	+	−	+	+	+	+	+	−	−	(+)	+	−	+	+	+	+	+	+	+	−	+	−	−	+	+	+	
84-3	+	+	+	+	−	+	+	+	−	+	−	−	+	+	+	−	+	+	+	+	−	−	−	−	+	+	+	+
84-4	+	+	+	+	−	+	+	+	−	+	−	−	+	+	+	−	+	+	+	+	−	−	−	−	+	+	+	
84-5	−	+	+	−	+	(+)	−	+	−	−	−	−	(+)	+	+	−	+	−	+	−	+	+	+	+	+	+	+	
84-25	−	−	−	+	+	+	−	+	−	+	+	+	−	−	−	−	+	−	(+)	−	+	−	−	−	−	+	+	
84-26	+	−	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+
84-34	−	−	−	+	−	+	+	+	−	+	+	−	−	+	−	−	+	−	(+)	+	−	+	−	−	+	+	+	
84-38	+	−	+	+	−	+	+	+	−	+	−	+	+	−	+	−	+	+	+	−	−	+	−	−	+	+	+	
84-7	−	−	+	+	−	+	+	+	−	−	+	(+)	−	+	−	−	+	−	−	+	−	−	−	−	−	−	−	
84-20	−	+	+	+	(+)	−	+	+	−	−	+	+	+	+	(+)	+	+	+	−	+	+	+	+	−	−	−	−	
84-21	−	−	−	−	−	−	+	−	−	+	−	+	−	+	−	−	+	−	−	+	+	−	−	(+)	−	−	−	−
84-27	−	+	−	−	+	+	−	+	−	−	+	+	−	−	+	−	+	+	−	+	+	−	−	−	−	−	−	
84-30	−	−	+	+	(+)	+	−	−	−	+	−	(+)	+	+	−	−	+	+	−	(+)	+	+	−	−	−	−	−	−
84-35	−	−	+	+	−	+	+	+	−	−	−	+	−	+	+	−	+	+	−	−	−	−	−	−	−	−	−	
84-37	−	−	−	+	+	+	+	(+)	−	−	+	+	−	+	−	−	+	−	−	+	+	+	−	−	−	−	−	−
84-39	−	−	+	−	−	−	+	+	−	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−

*+ indicates presence of the human chromosome in >30% of metaphases analyzed; (+), presence of the chromosome in 10–30% of metaphases analyzed; and −, absence of the human chromosome. The chromosome 19 content of clones 84-25 and 84-34 was 15% and 25%, respectively.
†Presence of the respective gene sequence in the hybrid clone was determined by the presence (+) or absence (−) of the human band on Southern blots.

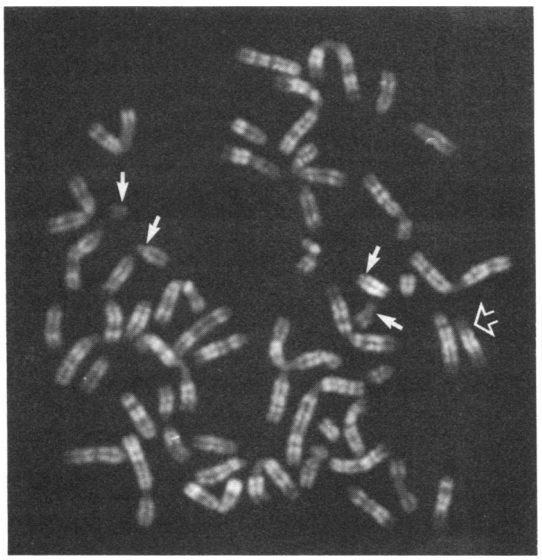

FIG. 1. A metaphase from a hybrid clone containing the 1p/19q chromosome (large arrow). Other human chromosomes in this cell are 13, 15, 17, and 22 (small arrows).

apoCI. The chromosome 19 location of *APOC1* was confirmed by examining a panel of mouse–human hybrids with a cloned human apoCI cDNA (Table 1). After DNA was restricted with *Hin*dIII, a human band at about 13 kb hybridized (Fig. 2*c*). Examination of hybrids containing the 1p/19q chromosome indicated that *APOC1* is on the long arm of chromosome 19 (Fig. 2*c*).

LDL Receptor. Previous evidence for the chromosome 19 location of *LDLR* was based on family studies of familial hypercholesterolemia and on examination of a panel of hybrid cells with antibody specific for the human LDL receptor (7, 23). We have confirmed the chromosome 19 location of *LDLR* by examining a human–mouse somatic cell panel with a cloned bovine LDL receptor cDNA probe (Table 1). For these experiments, DNA was restricted with *Pst* I, resulting in a 1.4-kb mouse band that was readily distinguished from the 2.5-kb human band (Fig. 2*d*). There was a single discordancy (clone 84-25) for the cosegregation of *LDLR* and chromosome 19. However, this clone had chromosome 19 in only 15% of analyzed cells and showed no hybridization. This, together with the fact that the probe is of bovine origin and thus shows relatively poor hybridization, explains this discordancy. The absence of the human band in hybrids containing the long but not the short arm of chromosome 19 indicated that *LDLR* resides on the short arm (Fig. 2*d*).

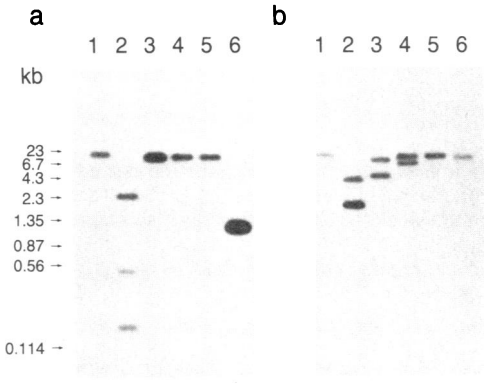

FIG. 3. Southern blot of phage λCIE restriction digests hybridized to apoE (*a*) and apoCI (*b*) cDNA probes. The enzymes used were *Xba* I (lanes 1), *Pst* I (lanes 2), *Hin*dIII (lanes 3), *Bgl* II (lanes 4), *Bam*HI (lanes 5), and *Eco*RI (lanes 6). Size markers are indicated at the left.

C3. The regional localization of *C3* on the short arm of chromosome 19 was confirmed by examining hybrids containing the long but not the short arm of chromosome 19 with a human C3 cDNA clone. When DNA was cut with *Hin*dIII, the hybrid cells exhibited the single mouse band at about 17 kb but not the two human bands at about 13 and 24 kb (Fig. 2*e*).

GPI and PEPD. Electrophoretic assays of the three hybrid clones containing the 1;19 translocation showed that GPI was expressed and PEPD was absent in each clone (data not shown), thus allowing assignment of *PEPD* to the short arm and *GPI* to the long arm of chromosome 19.

Isolation and Characterization of a Genomic Clone Containing APOC1 and Part of APOE. A single λ phage, λCIE, carrying both *APOE* and *APOC1* was isolated from a human genomic library constructed in Charon 4A (Fig. 3). The clone contains an insert of ≈18 kb that, by restriction mapping and blot hybridization, was shown to possess an intact *APOC1* gene but only the fourth exon and 3′ untranslated region of *APOE* (Fig. 4). The two genes are tandemly oriented and are separated by an intergenic region of at least 5 kb. *APOC1* spans up to 5 kb, although the precise location of the 5′ end within the 4-kb *Pst* I fragment has not been determined. Comparisons may be made with *APOC3*, which also contains a very large intron accounting for the bulk of the gene. All of the *APOC1* restriction fragments resulting from cleavage of λCIE can be detected in digests of human genomic DNA, but, in addition, there are numerous extra hybridizing bands not carried in this phage (data not shown). Preliminary data suggest that a second region of DNA that hybridizes to apoCI

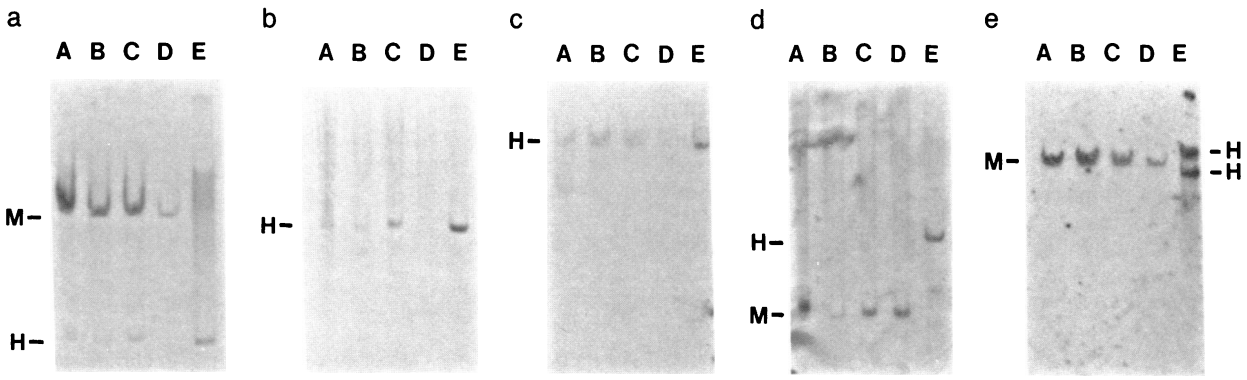

FIG. 2. Regional location on chromosome 19 of *APOE* (*a*), *APOC2* (*b*), *APOC1* (*c*), *LDLR* (*d*), and *C3* (*e*). Three separate cell hybrid clones containing the long arm of chromosome 19 (translocated to the short arm of chromosome 1) but not the short arm of chromosome 19 were identified. DNA from these hybrids (lanes A, B, and C) and from control mouse (lanes D) and human (lanes E) cells were restricted and analyzed by Southern blotting as discussed in the text. The human and mouse bands showing hybridization are marked H and M, respectively.

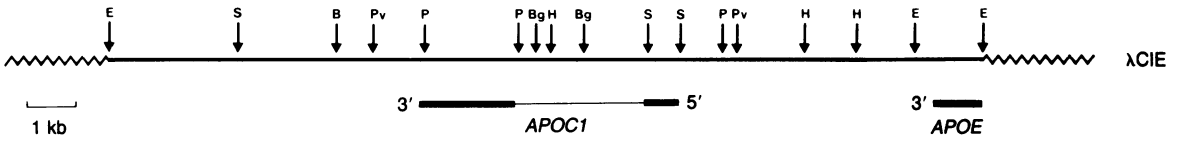

FIG. 4. Map of the *APOC1/APOE* region carried on phage λC1E. This clone contains ≈18 kb of genomic DNA. Those restriction fragments which hybridize to either apoCI or apoE cDNA are indicated by filled boxes. The relative orientation of the two genes was determined by hybridization using 5'- or 3'-specific cDNA subfragments. The *APOC1* coding sequences are separated by one very large intron (≈2.5 kb), and an additional one or two smaller introns exist in the 5' 700-bp *Sst* I fragment. The *APOE* coding sequence represents only the fourth exon and 3' untranslated region. Restriction sites: B, *Bam*HI; Bg, *Bgl* II; E, *Eco*RI; H, *Hin*dIII; P, *Pst* I; Pv, *Pvu* II; S, *Sst* I. Not all the sites for these enzymes are shown. The left and right arms of Charon 4A are indicated by zigzag lines.

cDNA probes is situated ≈8 kb further downstream of the apoCI gene described in this report. There may, therefore, be two apoCI genes. We do not know whether both are transcribed or if one is a pseudogene, but Knott *et al.* (44) have demonstrated the existence of two discrete apoC1 mRNA species in human liver.

DISCUSSION

In this study, we have examined the regional chromosomal locations of *APOE, APOC1, APOC2*, and *LDLR*, as well as three other chromosome 19 genes (*C3, GPI*, and *PEPD*). For the study, we used a naturally occurring translocation between chromosomes 1 and 19 [46,XX,t(1;19)(1p19q)(1q19p)]. Although the translocation appears to be whole-arm, the possibility that the breakpoint occurred near but outside the centromere cannot be ruled out. Somatic cell hybrids were constructed that contained the long arm but not the short arm of chromosome 19, and these were tested for the presence of seven chromosome 19 genes. The results confirm the chromosome 19 locations of *LDLR, APOE, APOC1*, and *APOC2*, and they indicate that *LDLR, C3*, and *PEPD* are located on the short arm, whereas *APOE, APOC1, APOC2*, and *GPI* are on the long arm. In addition, tight linkage of *APOE* and *APOC1* was shown by the isolation and characterization of a single genomic λ clone containing both genes.

With respect to the location and orientation of the lipoprotein genes and other genetic markers on chromosome 19, the following data have previously been reported. Pedigree analysis indicates that *APOE* and *APOC2* are tightly linked, with an overall lod score >4.0 at recombination fraction 0 (9, 45). Also tightly linked to the *APOE/APOC2* locus is the Lutheran blood group locus (*LU*) (3, 11). Using a restriction fragment length polymorphism for *APOE* in mice, the gene for apoE has been shown to be tightly linked to the locus for glucose phosphate isomerase on mouse chromosome 7 (46); thus, human *APOE* is probably also tightly linked to GPI. Family studies indicated that *APOC2* and *APOE* are linked to *C3*, with estimated male recombination fractions of about 25% and 15%, respectively (3, 4). Familial hypercholesterolemia, which shows synteny with *LDLR*, is also linked to *C3*, with a male recombination fraction of about 20%, but does not show linkage with *APOE* or *APOC2* (22, 45, 47, 48). Therefore, *C3* is apparently flanked by *LDLR* on one side and *APOE* and *APOC2* on the other. *PEPD* and *DM* are closely linked (no recombination in 14 opportunities) and *DM* shows about 6% recombination with *C3* (49, 50). *PEPD* is located between *C3* and *APOE/APOC2*, since it has a male recombination fraction of 9% with *APOE* and 14% with *C3* (11, 49, 51). The partial orientation and regional mapping of chromosome 19 genes has been carried out using various chromosome 19 translocations involving the terminal regions of the chromosome arms (see Fig. 5).

When taken together with previous family and cell hybrid studies, our results allow the following conclusions to be drawn: *LDLR* and *C3* are located within the region pter–p13.2, with *LDLR* about 20 centimorgans (cM) distal to *C3*. Loci *PEPD* and *DM* are located in the region p13–cen about 6 cM proximal to *C3*. Loci *APOE, APOC1, APOC2*, and *LU* are tightly linked in the region cen–q13, and this region likely also contains *GPI*. The distance between *C3* and the *APOE/APOC1/APOC2* locus appears to be about 25 cM. *CGB* is located in the q13.3–qter region of chromosome 19. These conclusions are summarized graphically in Fig. 5.

The tight linkage of the three chromosome 19 apolipoprotein genes is of interest for several reasons. In terms of family studies, it is noteworthy that polymorphisms identifying any of the genes should be useful in examining the inheritance of all three. Since the genes appear to have been derived from common ancestral sequences, this clustering undoubtedly reflects their common evolutionary history. In addition, the close proximity of at least *APOE* and *APOC1*, which are separated by about 6 kb of DNA, may have regulatory significance. With respect to this question, our preliminary studies of adult rhesus monkey tissues indicate that apoCI mRNA is present at moderate levels in spleen and macrophages but is absent in colon, pancreas, kidney, heart, and brain. On the other hand, previous studies (54, 55) have shown that apoE is abundantly expressed in many tissues, while the expression of the other apolipoproteins is largely restricted to liver and intestine. Another cluster of apolipoprotein genes, containing *APOA1, APOA4*, and *APOC3*, is

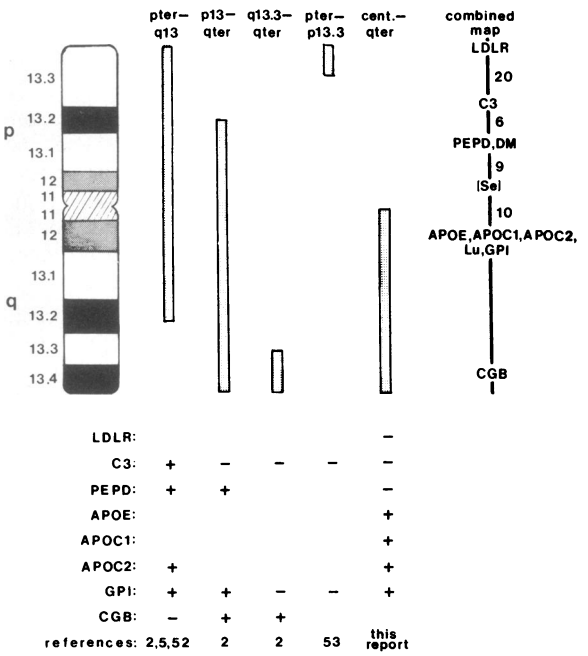

FIG. 5. Partial physical and genetic map of chromosome 19. The presence (+) or absence (–) of genes in regions of chromosome 19 isolated from translocation is given. The combined physical–genetic map is shown with male recombination frequencies. The physical locations of *DM* and *LU* are inferred from tight linkage to *PEPD* and *APOE*, respectively. The physical location of *SE* is unknown.

present on chromosome 11. A genomic clone containing both *APOA1* and *APOC3* was isolated and characterized, and the results indicated that the genes are oriented 3′ to 3′ and are separated by about 3 kb of intergenic DNA (56). This contrasts with the tandem (3′ to 5′) orientation of *APOE* and *APOC1* (Fig. 4).

That three apolipoprotein genes are clustered on chromosome 19 raises the possibility that genes for other apolipoproteins or other lipid transport functions may also reside there. Indeed, our preliminary findings suggest the presence of additional sequences hybridizing with apoC1 cDNA in the *APOC1/APOC2/APOE* cluster. We have recently identified cDNA clones for human apoB, and examination of a somatic cell panel indicates that *APOB* resides on human chromosome 2, unlinked to any of the three previously identified apolipoprotein loci (57, 58).

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