Gene mapping and chromosome 19

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SUMMARY Chromosome 19 is currently the most fully mapped of the smaller chromosomes, with about 40 loci assigned to it (HGM8). Major inherited disorders on this chromosome include myotonic dystrophy and familial hypercholesterolaemia. Other loci include five blood groups, a cluster of apolipoprotein genes, and the receptors for insulin and polio virus. A number of cloned genes and random DNA sequences identify polymorphisms which, together with blood group and other protein polymorphisms, have been used to establish a framework for ordering the loci and estimating genetic distances. Hybrid cell lines allow loci to be assigned to one of eight different regions and a detailed genetic map of the chromosome will be possible in the near future.

Chromosome 19 is currently the best mapped of the smaller human chromosomes, with more than 30 distinct gene loci assigned to it, quite apart from anonymous DNA fragments. It also has the longest mapping history of any autosome, although this has only become apparent in the past four years, with the assignment of an already well established linkage group to the chromosome. For both these reasons, and on account of the important diseases involving it, chromosome 19 can be seen as a paradigm for the mapping of the human autosomes.

The first detected genetic linkage in man was that reported between the Lutheran and Lewis blood groups by Mohr in 1951.^{1,2} Although this was later reinterpreted as linkage between Lutheran and the Secretor locus, Lewis was subsequently shown to form part of this linkage group.³

As early as 1965, these loci revealed another fundamental aspect of genetic linkage when Cook⁴ showed a marked sex difference in the Lu-Se recombination rate, with female recombination twice that seen in males.

An additional 'first' was the localisation of a serious autosomal genetic disorder to this linkage group. Mohr's original work included data on myotonic dystrophy, using Danish families previously studied and reported by Thomsen.⁵ The data

suggested, though not conclusively, linkage with Lu and this was subsequently confirmed by the studies of Renwick *et al*⁶ and Harper *et al*.⁷ Thus, by 1972, a well defined group of linked loci had been identified, containing a major genetic disorder and showing a clear sex difference in recombination rate. The data had even been analysed by a multipoint computer programme to give most likely order.

Despite all these advances, the linkage group remained unassigned for a further 10 years, until linkage was shown for both Lu and Se with the complement component 3 (C3).⁸ This extended the linkage group significantly, since C3 was already known to be linked to the disorder familial hypercholesterolaemia.⁹ Even more importantly, it provided a chromosomal assignment for the entire group, since hybrid cell studies had localised the C3 gene to chromosome 19.¹⁰ Thus, the early work on what are now regarded as 'classical' chromosome 19 markers was carried out in complete ignorance of the chromosomal localisation.

Inherited disorders and other major loci (table 1)

MYOTONIC DYSTROPHY

As already mentioned, this was the first human autosomal disorder for which genetic linkage to another marker was demonstrated. Since no basic

TABLE 1 Major genetic loci on chromosome 19.

	Polymorphic
Genetic disorders	
Myotonic dystrophy (DM)	
Familial hypercholesterolaemia (FHC)	+
Mannosidosis (α mannosidase)	
Blood groups	
Lutheran (Lu)	+
Lewis (Le)	+
Secretor (Se)	+
H antigen	
Landsteiner-Wiener (LW)	+
Enzymes	
Peptidase D (PEPD)	+
Glucose phosphate isomerase (GPI)	
Lysosomal DNase	
Creatine kinase, muscle type (KMM)	
Phosphoglycerate kinase 2 (PGK2)	
Other proteins	
Complement C3	+
Insulin receptor (INSR)	+
LDL receptor (LDLR)	+
Apolipoprotein E (APOE)	+
Apolipoprotein C1 (APOC1)	
Apolipoprotein C2 (APOC2)	+
Chorionic gonadotrophin, beta subunit (CGB)	
Luteinising hormone, beta subunit (LHB)	
Ferritin light chain (FTL)	

protein or cellular defect is yet known for the condition, its localisation has remained dependent on family studies, a major drawback for a relatively uncommon disorder. The linkage with Se, Le, and Lu, though fully established, remains rather uncertain as regards precise distance and order of loci, largely because they are not expressed in cultured cells, show dominance, and are not as polymorphic as is desirable. The clinical application of these markers in prediction has been correspondingly limited, even though Se is detectable prenatally.^{11 12} Hopes that the C3 linkage might be more useful in this respect were raised by the discovery of a C3 gene probe showing a number of polymorphisms,¹³ but while this locus shows moderately close linkage with myotonic dystrophy in male meioses, there is frequent recombination in females. The precise localisation of the myotonic dystrophy gene has required the development of additional DNA probes as described more fully below.

MANNOSIDOSIS

This rare lysosomal storage disease, due to deficiency of the enzyme α -mannosidase was localised to chromosome 19 by means of rodent-human cell hybrid studies on this enzyme.¹⁴ Subsequent work has shown the locus to be in the central region of the chromosome (p13→q13),^{15 16} but since the enzyme is not polymorphic and no specific gene probe has yet been isolated, nothing is known about its linkage

relationships with other diseases or markers on the chromosome.

NEUROFIBROMATOSIS

This requires a brief mention as a disorder thought to be on chromosome 19 that has now been excluded. Close linkage with myotonic dystrophy was considered possible on the basis of coinheritance of the disease in two kindreds,^{17 18} with a total lod score exceeding 3. However, study of neurofibromatosis families with polymorphisms known to be linked to myotonic dystrophy (Se, C3, and APOC2) has given such strongly and uniformly negative results¹⁹ that other mechanisms are required to explain the association.

FAMILIAL HYPERCHOLESTEROLAEMIA (FHC)

This important disorder, one of the major identifiable genetic causes of early coronary heart disease, follows autosomal dominant inheritance with occasional severely affected homozygotes recorded. Family studies have shown definite though loose linkage between this condition and C3.²⁰ Since results for other markers in the linkage group, such as Se and Lu, have been negative, this suggested that the FHC locus might be on the opposite side of C3 to the other markers.

Studies on familial hypercholesterolaemia (FHC) have been greatly helped by the identification of defects in the low density lipoprotein (LDLR) receptor as the cause.²¹ The gene for this receptor has recently been cloned,²² and this has allowed more detailed studies to be carried out on both the physical localisation in cell lines and on the linkage relationships. These show the LDL receptor to be on the short arm (p13.2→p13.1).²³ The question as to whether the locus is distal or proximal to the C3 locus remains uncertain, with linkage data from FHC families favouring the former,²⁴ but hybrid cell lines suggesting that it is proximal.²⁵

Apolipoproteins E, C1, and C2

It is now known that a group of at least three loci determining apolipoproteins exists on chromosome 19, quite separate from the locus for familial hypercholesterolaemia. Apolipoprotein E (APOE) shows a protein polymorphism which was found to be linked to C3 by family studies.²⁶ The APOC2 gene has been cloned²⁷ and also shows polymorphisms which suggest close linkage with APOE^{28 29}; neither are measurably linked to FHC or the LDL receptor. The original reports showed no recombination between APOE and APOC2, but recombinants have now been detected both with the APOC2 probe³⁰ and in a large kindred with

APOC2 deficiency,³¹ so the loci may be around 3 cM apart. Gene probes for both APOE and APOC1 also exist, but currently show no polymorphisms. However, direct molecular studies have shown the two DNA sequences to be only about 15 kb apart.³²

Both APOC2 and APOE are now known to be closely linked to myotonic dystrophy. Shaw *et al*³³ showed a distance of 4 cM between DM and APOC2, with a lod score of 7.8, and this has been confirmed by two other groups,^{34 35} bringing the total lod score to over 20. APOE has been shown to have around 10% recombination with myotonic dystrophy in a large French-Canadian kindred.³⁶

Blood group loci

The blood groups that form a linkage group on chromosome 19 have been the subject of extensive studies over many years, though there are some doubts as to the interpretation of some of the early data. In addition to Secretor, Lewis, and Lutheran, there is also the 'H' locus determining ABO specificities, as well as the newly recognised Landsteiner-Wiener (LW) group,³⁷ giving a total of five loci. To what extent their coexistence on chromosome 19 reflects functional or evolutionary factors is not clear at present. Until the underlying genes are cloned it will not be easy to determine their precise physical distances and ordering.

Enzyme and other protein loci

The importance of the complement C3 locus, which shows polymorphisms at both the protein and the DNA level, in mapping chromosome 19 has already been mentioned. The only enzyme on chromosome 19 that currently shows significant polymorphism is peptidase D,³⁸ and even this polymorphism is so infrequent as to seriously limit its use in gene mapping. Cook *et al*³⁹ suggested the possibility of linkage with Se, but it was not until PEPD had been located on chromosome 19 by hybrid cell studies that its linkage relationships were reassessed. O'Brien *et al*⁴⁰ showed close linkage with myotonic dystrophy in two families (no recombinants in 14 opportunities). Unfortunately, no subsequent myotonic dystrophy families have proved informative, and the precise ordering in relation to other loci is uncertain, though recombination has been shown between PEPD and APOC2.

Non-polymorphic enzymes include glucose phosphate isomerase (GPI),⁴¹ one form of phosphoglycerate kinase (PGK2),⁴² the muscle specific form of creatine kinase,⁴³ and a lysosomal DNase,⁴⁴ as well as cytochrome p450, the gene for which has

been cloned and localised to the long arm of chromosome 19.⁴⁵ Among the non-enzymatic proteins is the B subunit of chorionic gonadotrophin and luteinising hormone⁴⁶ (the gene has been cloned but no polymorphisms detected) and the ferritin light chain⁴⁷ (the heavy chain is separately located on chromosome 11).

An important gene recently characterised and assigned to chromosome 19 is that for the insulin receptor.⁴⁸ This gene is located relatively distally on the short arm and shows polymorphisms with several restriction enzymes. Its relationship to different forms of diabetes has yet to be defined. It might also have been considered as a 'candidate gene' for myotonic dystrophy, in which hyperinsulinism is a consistent finding, but the different regional localisation and the occurrence of recombination between the loci in families rule this out (D J Shaw *et al*, unpublished data).

Cell surface receptors (table 2)

Several cell surface receptors are coded for by genes on chromosome 19; those already mentioned include the low density lipoprotein receptor (LDLR) and the insulin receptor (INSR). The receptor for poliovirus is located on chromosome 19,⁴⁹ as are the baboon virus M7⁵⁰ and RD114 virus receptors.⁵¹

There are at least three monoclonal antibodies which have been raised to cell surface antigens encoded by chromosome 19.^{52 53} These loci, mostly less well defined than those discussed above, may well prove to be of considerable functional importance, but their relationships are as yet not well understood.

Repair loci

Two genes, ERCC1⁵⁴ and RCC,⁵⁵ which correct defective DNA repair in Chinese hamster cells have been assigned to chromosome 19.

TABLE 2 Other genetic loci on chromosome 19.

Gene	Regional localisation (where known)
Poliovirus receptor (PVR)	
Cell surface antigen MSK 19	
Cell surface antigen MSK 20	
Cell surface antigen MER 5	q13.3
DNA repair gene (RCC)	
DNA repair gene (ERCC-1)	q13.2→q13.3
RD114 virus receptor (RDRC)	
Baboon virus M7 receptor (M7V1)	
Cytochrome p450 (CYP1)	q13.2
Beta subunit of transforming growth factor (TGFB)	q13.1→q13.3

DNA polymorphisms

For chromosome 19, as for other chromosomes, the detection of restriction fragment length polymorphisms (RFLPs) based on DNA is now making a substantial contribution to the overall gene map, as well as to more specific studies of major loci, such as myotonic dystrophy. Some polymorphisms are detected by specific gene probes (for example, C3, APOC2, LDLR), while others are defined by anonymous DNA sequences localised to chromosome 19 by hybrid cell studies. Table 3 lists those RFLPs identified so far, together with their approximate localisation.

The first sequence to be isolated, 1J2, was produced from a flow sorted chromosome 19 and 20 enriched DNA library, while succeeding sequences have been isolated from a library constructed from the cell line 'WILF' mentioned below (probes p17-1 and p4-1), from a further flow sorted library (p BAM 47) (R Bartlett, 1985, personal communication) and from a cosmid library (cos 1-13).⁵⁶ These probes all show useful polymorphisms and, although linkage data are still preliminary for most of them, their physical localisation suggests that they are already sufficient in number to provide a framework for mapping most of the chromosome, especially when considered together with the classical protein polymorphisms.

Cytogenetic and hybrid cell evidence

The study of human chromosomal disorders, in particular small deletions and balanced transloca-

tions, has given valuable evidence on the localisation of specific genetic disorders, notable examples being the retinoblastoma locus on 13q and Duchenne muscular dystrophy on the short arm of X (p21). Chromosome 19 is morphologically a somewhat featureless chromosome, with a band structure that is considerably less distinctive microscopically than would appear from conventional diagrams. The predominantly pale staining with Giemsa may reflect the presence of a relatively large number of functional genes. Centromeric polymorphism can be recognised with C banding techniques and is beginning to be utilised in linkage studies,³⁵ though centromeric DNA probes are likely to be more specific in this respect.

Trisomy 19q has been reported,⁵⁷ but without features that can be related to known loci on the chromosome. So far, no deletions or monosomies have been available for molecular study. A number of instances of balanced rearrangements have been recorded, mostly without clinical abnormalities. Cell lines from such persons have formed the basis for constructing hybrid cell lines which are proving a valuable independent source of information on gene localisation and order.^{15 16}

Hybrid cell lines containing only one copy of chromosome 19 and lacking various specific regions of this chromosome have now been constructed and have been used as a panel both to localise specific genes and other markers to chromosome 19 and to give a regional localisation on it.

This type of evidence is particularly valuable for localising those loci that are not polymorphic and which thus cannot be studied by genetic linkage analysis. It is also an important independent approach for ordering loci already defined in linkage groups, especially where linkage data are equivocal or contradictory, and where the loci are infrequently separated by crossing over.

Using a panel of hybrid cell lines, Brook *et al*¹⁵ defined the direction of the C3-PEPD/DM-Lu linkage group and have placed a number of other loci in its framework. Subsequent work has extended the number of loci with a defined localisation and fig 1 shows the current situation. One point of uncertainty until recently has been the position of the centromere in relation to the various loci. Preliminary results using a cell hybrid line with a centromeric breakpoint, allowing a clear separation of loci on long and short arms, have now been reported,⁵⁸ and suggest a long arm localisation for APOC2. Since this locus shows clear linkage with C3 on the short arm, it seems likely that the C3-Se-DM-Lu linkage group spans the centromere, and that recombination in this region may be relatively infrequent.

TABLE 3 Cloned DNA sequences on chromosome 19.

	Regional localisation (where known)	Polymorphic
<i>Specific gene probes</i>		
C3	p13-3→p13-2	+
APOE	cen→q13-2	
APOC1	cen→q13-2	
APOC2	cen→q13-2	+
LDL receptor	p13-2→p13-1	+
Insulin receptor	p13-3→p13-2	+
Ferritin light chain	q13-3→q13-4	
CGB	q13-3	
Phosphoglycerate kinase 2		
Creatine kinase (muscle)		
Cytochrome p 450	q13-1→q13-3	+
<i>Anonymous DNA sequences</i>		
1J2 (D19S9)	p13-2→q13-2	+
Cos 1-13 (D19S11)	p13-2→cen	+
P Bam 34 (D19S6)	q13-3→qter	+
P.B. 47 (D19S5)	p13-1→cen	+
17-1 (D19S8)	p13-1→cen	+
4-1 (D19S7)	p13-2→q13-2	+
OLS (D19S4)

FIG 1 Regional localisation of chromosome 19 markers. The bracket on the right hand side indicates regions within which the precise order of markers is unknown.

More detailed localisation using hybrid cell lines has been attempted using a line 'WILF' which contains chromosome 19 as its major human component.²⁵ Using a series of subclones from this, each of which contains a different fragment of chromosome 19, it has been possible to identify a series of overlapping fragments that may give more detailed localisation than is currently possible using hybrids based on naturally occurring rearrangements. However, the possibility of internal rearrangements within the chromosome 19 fragments means that this type of evidence must be regarded with caution at present.

Direct cytogenetic studies of meiosis in chromosome 19 are few and so far confined to males.⁵⁹ A chiasma is usually seen on each arm, and the localisation of chiasmata is frequently more terminal than proximal, which would support the relative infrequency of recombination in the centromeric region, with a greater recombination between physically close loci nearer the ends of the chromosome. Direct observation of female meioses will be of considerable interest in view of the pronounced sex difference shown for loci on chromosome 19.

Genetic linkage distances on chromosome 19

By comparison with the structural map of loci shown in fig 1, the genetic linkage map of chromosome 19

must still be regarded as tentative. This is in part because of the limited size of most families, requiring the combination of numerous data sets and often the extrapolation of indirect genotype assignments to achieve the maximum information from the data. A further factor is the marked sex difference in recombination.

Sherman *et al*²⁴ have recently constructed a linkage map based on all available published data up to 1984. Although no single order could be considered definitive, they found a most likely linkage (from p to q) of: FHC-(Le-C3)-(LW-PEPD-DM)-Se-(APOC2-APOE-Lu). Fig 2 is an attempt to combine this with further data from DNA polymorphisms reported at the 1985 Human Gene Mapping Workshop, in particular, the strong evidence for close linkage between DM and APOC2, while table 4 gives details of some of the linkage distances between the various markers and myotonic dystrophy. The hybrid cell studies discussed earlier make it likely that the linkage group extends onto the long arm, rather than being confined to the short arm as suggested by Sherman *et al*.²⁴ There still remains considerable scope for rearrangement, but there is little doubt that the orientation and approximate sequence are now correct.

Sherman *et al*²⁴ found the overall recombination in female meioses to be three times that in males, suggesting that while the total genetic length of the chromosome in males is around 100 cM, it may approach 300 cM in females. This striking difference will also have practical implications for the use of linked markers in the prediction of genetic disorders such as myotonic dystrophy.

Computer programmes allowing multipoint linkage analysis of the data should help in establishing the relative order of the various loci,⁶⁰ particularly if the linkage data are tested on suitably structured reference families, such as those currently being

TABLE 4 Peak lod scores ($\hat{Z}$) and recombination fractions between DM and seven RFLPs.

		Male	Female	Combined
C3	θ	0.01	0.45	0.30
	$\hat{Z}$	2.69	-0.19	0.14
p17.1 (D19s8)	θ	0.05	0.10	0.10
	$\hat{Z}$	0.66	2.09	2.98
APOC2	θ	0.01	0.05	0.05
	$\hat{Z}$	1.06	5.63	6.52
p4.1 (D19s7)	θ	0.10	0.30	0.20
	$\hat{Z}$	1.56	0.41	1.47
Cos 1-13 (D19s11)	θ	0.25	0.45	0.30
	$\hat{Z}$	0.88	-0.06	0.54
pI J2 (D19s9)	θ	0.01	0.45	0.15
	$\hat{Z}$	1.89	-0.09	0.87
LDLR	θ	0.20	0.20	0.15
	$\hat{Z}$	0.53	0.48	1.65

made available from Paris and Utah. However, family studies remain an insensitive method of determining order for closely linked loci, and the precise order may well remain uncertain until direct methods of scoring large numbers of meioses become available.

Molecular approaches to isolation of the myotonic dystrophy gene

Until now, the techniques of molecular genetics have been used principally to construct gene probes for relatively well characterised proteins on chromosome 19, and to increase the availability of polymorphic markers for mapping in the form of anonymous cloned DNA sequences. For serious genetic disorders such as myotonic dystrophy, the information available on localisation of the gene now makes it potentially feasible to approach the isolation and characterisation of the gene itself, despite our present almost complete ignorance about the nature of its likely product.

Because of the limited accuracy of the linkage approach to human genetics, it will not be possible to define points nearer than a few million base pairs to a disease gene by family studies alone. Such lengths of DNA are far too large to clone by conventional techniques, and so new methodologies are being developed for the isolation and characterisation of million base pair DNA fragments. These include the use of somatic cell hybridisation⁶¹ to propagate human sub-chromosomal fragments, and a new form of gel electrophoresis involving periodic alterations in the electrical field direction, to analyse and isolate large DNA fragments (orthogonal field alteration gel electrophoresis).^{62 63}

Having isolated a length of DNA of a few million base pairs, within which the disease gene is thought to be located, a number of experimental approaches are possible. The fragment could be cloned as a 'mini-library' and a complete restriction site map constructed. Probes could then be selected from any site for further study. The positions of expressed gene sequences could be deduced by hybridisation with mRNA or by direct selection of protein coding regions.⁶⁴ Alternatively, RFLPs could be investigated for linkage disequilibrium with the disease. This population based approach should allow one to detect very closely linked markers in a way that is not possible with family studies. 'Candidate' disease genes could then be investigated in several ways: for example, to study differences in expression in various tissues from patients and normal control subjects; or to determine their complete base sequence and relate this to the structures of genes of known function; or the effect of introducing the

cloned gene into cultured cells or animal embryos could be studied. Any or all of these techniques, as well as others still being developed, could be applied to myotonic dystrophy, and, for that matter, to any of the many other human diseases whose molecular basis is still unknown.

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FIG 2 Observed recombination fractions and maximum lod scores (in parentheses) for various polymorphisms

LE	M: 0.50 (0.00) F: 0.02 (0.08)					
C3	M: 0.19 (1.74) F: 0.25 (1.50)	M: 0.04 (7.91) F: 0.21 (1.44)				
LW		M: 0.18 (0.78) F: 0.43 (0.00)	M: 0.01 (3.40) F: 0.20 (0.55)			
PEPD		M: 0.01 (0.09)	M: 0.21 (1.63) F: 0.32 (0.20)			
DM	M: 0.19 (0.53) F: 0.20 (0.48)	M: 0.50 (0.00) F: 0.50 (0.00)	M: 0.06 (3.73) F: 0.38 (0.21)		M: 0.00 (2.96) F: 0.05 (0.78)	
SE	M: 0.02 (0.08) F: 0.50 (0.00)	M: 0.32 (0.32) F: 0.50 (0.00)	M: 0.19 (4.17) F: 0.32 (0.36)	F: 0.50 (0.00)	M: 0.15 (1.99) F: 0.50 (0.00)	M: 0.03 (3.33) F: 0.06 (1.55)
APOE		M: 0.01 (0.18) F: 0.50 (0.00)	M: 0.17 (5.24) F: 0.41 (0.13)			F: 0.10 (7.12)
LU	M: 0.26 (0.82) F: 0.50 (0.00)	M: 0.50 (0.00) F: 0.50 (0.00)	M: 0.19 (2.23) F: 0.43 (0.09)	M: 0.04 (3.68) F: 0.34 (0.15)	M: 0.25 (0.49) F: 0.41 (0.01)	M: 0.50 (0.00) F: 0.10 (1.22)
APOC2	M: 0.50 (0.00) F: 0.30 (0.17)	M: 0.25 (0.05) F: 0.00 (0.87)	M: 0.26 (0.84) F: 0.31 (0.30)		M: 0.06 (3.17) F: 0.34 (0.11)	M: 0.00 (10.00) F: 0.03 (8.75)
IJ2	M: 0.01 (0.49) F: 0.50 (0.00)		M: 0.01 (0.68) F: 0.29 (6.38)			M: 0.01 (1.33) F: 0.50 (0.00)
17-1	M: 0.27 (0.05) F: 0.01 (0.28)		M: 0.01 (0.56) F: 0.18 (0.11)			M: 0.06 (0.66) F: 0.09 (2.00)
4-1	M: 0.50 (0.00) F: 0.50 (0.00)		M: 0.01 (1.65) F: 0.50 (0.00)			M: 0.10 (1.11) F: 0.29 (0.44)
	FHC/LDLR	LE	C3	LW	PEPD	DM

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chromosome 19. Based on data of Sherman *et al*²⁴ with additional data from HGM8.

M: 0.15 (2.37) F: 0.20 (1.52)					
M: 0.11 (4.50) F: 0.30 (0.99)	M: 0.03 (2.59) F: 0.01 (1.98)				
M: 0.00 (2.73) F: 0.05 (0.34)	M: 0.00 (2.40) F: 0.00 (3.91)	M: 0.00 (2.18) F: 0.00 (0.45)			
M: 0.50 (0.00) F: 0.50 (0.00)		M: 0.01 (0.60) F: 0.01 (0.86)	M: 0.25 (0.05) F: 0.10 (3.87)		
M: 0.50 (0.00) F: 0.18 (0.03)		M: 0.01 (0.29) F: 0.01 (0.58)	M: 0.01 (0.50) F: 0.01 (2.95)	M: 0.01 (0.13) F: 0.14 (0.16)	
M: 0.01 (0.02) F: 0.50 (0.00)		M: 0.01 (0.02) F: 0.50 (0.00)	M: 0.01 (1.02) F: 0.27 (0.15)	M: 0.14 (0.92) F: 0.07 (1.34)	M: 0.07 (0.61) F: 0.18 (0.06)
SE	APOE	LU	APOC2	IJ2	17-1

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