CURRENT

NEUROLOGY

VOLUME 6

Edited by

Stanley H. Appel, M.D.

CURRENT

NEUROLOGY

VOLUME 6

Edited by

Stanley H. Appel, M.D.
Professor and Chairman
Department of Neurology
Baylor College of Medicine
Houston, Texas

YEAR BOOK MEDICAL PUBLISHERS, INC.
CHICAGO • LONDON

International Standard Serial Number: 0161-780X
International Standard Book Number: 0-8151-0229-1

Sponsoring Editor: Elizabeth Sugg
Manager, Copyediting Services: Frances M. Perveiler
Copyeditor: Francis A. Byrne
Production Project Manager: Max Perez
Proofroom Supervisor: Shirley E. Taylor

Contributors

James H. Abbs, M.D.
Speech Motor Control Labs
Department of Neurology
University of Wisconsin
Madison, Wisconsin

Stanley H. Appel, M.D.
Professor and Chairman
Department of Neurology
Baylor College of Medicine
Houston, Texas

Antonio V. Delgado-Escueta, M.D.
Professor of Neurology
Director, Comprehensive Epilepsies
VA Southwest Regional Epilepsy Center
West Los Angeles VA Medical Center
Los Angeles, California

David A. Greenberg, M.D., Ph.D.
Assistant Professor
Department of Neurology
University of California, San Francisco
Ernest Gallo Clinic and Research Center
San Francisco General Hospital
San Francisco, California

David A. Hafler, M.D.
Multiple Sclerosis Unit
Center for Neurologic Diseases
Brigham and Women's Hospital
Harvard Medical School
Boston, Massachusetts

Ann Henderson Tilton, M.D.
Assistant Professor of Neurology and Pediatrics
Louisiana State University
School of Medicine
New Orleans, Louisiana

Joseph Jankovic, M.D.
Associate Professor
Department of Neurology
Baylor College of Medicine
Houston, Texas

Marcel Kinsbourne, M.D.
Director, Department of Behavioral Neurology
Eunice Kennedy Shriver Center
Waltham, Massachusetts

David S. Lefkowitz, M.D.
Assistant Professor of Neurology
Department of Neurology
Bowman Gray School of Medicine
Winston-Salem, North Carolina

David N. Levine, M.D.
Assistant Professor of Neurology
Harvard Medical School
Director, Neurology Service
Spaulding Rehabilitation Hospital
Assistant Neurologist
Massachusetts General Hospital
Boston, Massachusetts

John Newsom-Davis, M.D.
Department of Neurologic Sciences
Royal Free Hospital
Institute of Neurology National Hospital
London, Great Britain

Roger N. Rosenberg, M.D.
Professor of Neurology
University of Texas Health Sciences Center at Dallas
Southwestern Medical School
Dallas, Texas

David B. Rosenfield, M.D.
Associate Professor
Stuttering Center, Department of Neurology
Department of Otorhinolaryngology and
Communicative Sciences
Baylor College of Medicine
Houston, Texas

Allen D. Roses, M.D.
Professor and Chief, Division of Neurology
Duke University Medical Center
Durham, North Carolina

Warren J. Strittmatter, M.D.
Associate Professor
Department of Neurology
Baylor College of Medicine
Houston, Texas

James F. Toole, M.D.
Walter C. Teagle Professor of Neurology
Department of Neurology
Bowman Gray School of Medicine
Winston-Salem, North Carolina

Howard L. Weiner, M.D.
Professor, Section of Neurology
Multiple Sclerosis Unit
Center for Neurologic Diseases
Brigham and Women's Hospital
Harvard Medical School
Boston, Massachusetts

Preface

Continuing advances in the neurosciences provide us with a better understanding of the biological processes underlying neurological disease, and thus bring us closer to an understanding of disease etiology and pathogenesis. Ultimately such advances should pave the way for meaningful therapies for many previously untreatable disorders. The continuing goal of the *Current Neurology* series is to place such advances into a meaningful clinical context with the ultimate goal of improving the quality of neurological care.

This year we emphasize the tremendous progress in neuromuscular diseases including the application of recombinant DNA technology to Duchenne muscular dystrophy and myotonic muscular dystrophy, and the application of immunological technique to myasthenia gravis and myasthenic syndrome. In addition, calcium channel blockers are comprehensively covered because of their potential applicability to so many different neurological conditions. The latest advances in epilepsy, stroke, multiple sclerosis, Alzheimer's disease, inherited disease, neurobehavior, speech, and the autonomic nervous system are also covered. All of these chapters should help the clinical neurologist remain abreast of the increasingly sophisticated approaches to neurological disease.

STANLEY H. APPEL, M.D.

Contents

CHAPTER 1

Myotonic Muscular Dystrophy and Duchenne Muscular Dystrophy

Allen D. Roses

PROGRESS TOWARD CLINICALLY USEFUL DIAGNOSTIC TESTS and identification of the genetic loci responsible for myotonic muscular dystrophy (DM) and Duchenne muscular dystrophy (DMD) using recombinant DNA techniques has been rapid.[1–3] An understanding of these advances requires familiarity with new experimental strategies. The more traditional scientific approaches looked for differences of specific measurements in muscle, nerve, fibroblast, or some other tissue from patients and controls. Associated with these data are attempts to determine whether any differences are directly related to the defect or are secondary consequences. These considerations are directly addressed by recombinant DNA research strategies.

One of the recombinant DNA research approaches involves genetic linkage.[4–6] This is most simply explained as an attempt to isolate segments of DNA from the genome that are so closely linked to the gene for the disease on the chromosome that they segregate along with it through meiosis. The closer the piece of DNA to the gene, the less the chance that a recombination event would occur between it and the disease locus during meiosis. Ideally, the fragment of DNA will be located so close to the disease locus that no recombination would be observed in a large number of meioses, allowing it to be used to track the disease through families with a very high degree of probability. In practice, these segments of DNA can be followed through large families because they can detect DNA polymorph-

0161-780X/86/0006-0001-0016-$04.00

isms called restriction fragment length polymorphisms (RFLPs).[7-8] The use of polymorphisms to determine parentage is a familiar concept to physicians. Blood groups, HLA types, and other familiar phenotypic polymorphisms, often called expressed markers, present no new territory. The concept of RFLPs is totally comparable except that segments of DNA detect polymorphic regions in the genome, rather than the phenotypic expression of a particular polymorphism, such as a blood type.

Restriction enzymes that cut DNA at defined base sequences will randomly cut DNA from each individual at different places depending on the differences in the sequence of their DNAs. Sequence differences are common along the genome and, for the most part, cause no known "abnormalities." If a particular segment of unique sequence DNA is made radioactive and is tested against the DNA of several individuals, it may detect differences in the number of sites at which a restriction enzyme cuts the DNA sequence in each individual. The differences would be detected as varying *lengths* of DNA on a gel or gel replica blot that separates DNA *fragments* according to size.[9] If no *restriction* enzyme sites were recognized by the enzyme in one individual, then one long piece of DNA would be detected (Fig 1). If the second individual's DNA contained a site recognized by the enzyme, two pieces would be produced. The inheritance of that site could then be mapped through a large pedigree by appropriately testing each individual in the family. Thus, the term restriction fragment length polymorphism refers to the differences in lengths of DNA fragments produced in the population by using particular restriction enzymes. Figure 1 illustrates the detection of a simple hypothetical polymorphism in three individuals.

Since DM is inherited as an autosomal dominant trait, it is essentially a polymorphic trait that can be mapped through families.[10-12] A family history and appropriate examination of relatives are used to diagnose and track DM through pedigrees. A linked RFLP would be associated with the DM locus so that it would be inherited through the family along with the disease locus unless a crossover event took place during meiosis between the two loci. If the loci can be demonstrated to be tightly linked so that, for example, a recombination event occurs only once in 100 meiotic events, then the DNA segment would be very useful as a tool for clinical diagnosis or prenatal diagnosis in those families where it can be shown to be segregating in an informative manner.

The DNA segment *needs not have anything at all to do with DM* but just happens to be located close to the disease locus on the chromosome and to be detectable. Thus, isolation of the particular piece of DNA linked to DM tells us *nothing* about DM but only serves to locate the gene for DM with a measurable degree of precision. Should this piece of DNA be so close to the disease locus that chromosome walking experiments could be performed, then the piece of DNA can serve as a starting point for experiments designed to determine the product of the gene responsible for DM.[13,14] Once the gene is identified, the product of that gene can be made and studied, the pathogenesis of the disease understood, and rational therapeutic strategies planned.

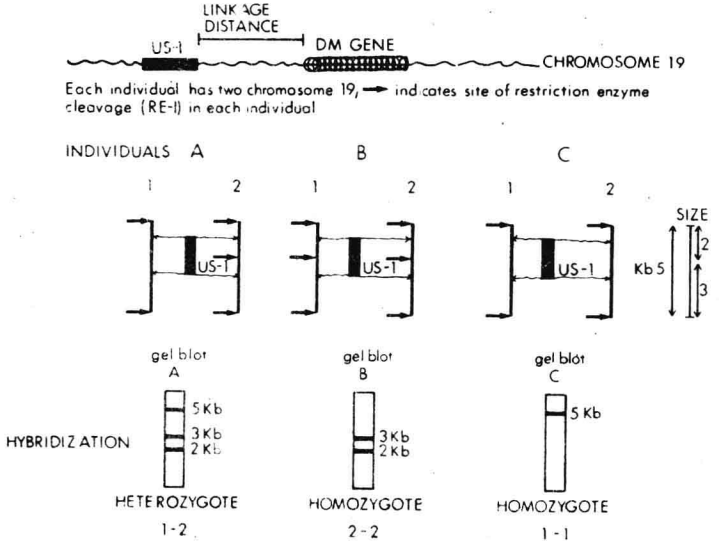

Fig 1.—Hypothetical RFLP detection method. In this illustration, US-1 is a random unique sequence selected from a chromosome 19 library and confirmed to be located on chromosome 19. DNA is obtained from the blood of three individuals, A, B, and C, and each sample is enzymatically digested using a hypothetical restriction enzyme, RE-1. There are two constant restriction sites that define a 5kb DNA fragment. Hybridization of the US-1 sequence radiolabeled as a probe can be detected by autoradiography of blots obtained from agarose gel electrophoresis of RE-1 digested DNA samples from each individual (see example of Southern blot in Figure 2). In addition, there is a variable site within the sequence of the probe itself, located between the two constant sites which, when present, yields two fragments of 2 kb and 3 kb instead of a single 5 kb fragment. Since each individual contains two chromosome 19 sequences, it is possible to have three combinations of this simple polymorphism. The heterozygous individual would have only one chromosome 19 with the variable RE-1 site and US-1 would detect all three possible fragments. If no variable sites were present, US-1 would detect only 5 kb fragments. If both chromosome 19 had the variable fragment, then US-1 would detect only 2 kb and 3 kb fragments. These combinations can be scored and the inheritance pattern of the variable site polymorphism produced by digestion with this particular restriction enzyme analyzed in large DM pedigrees.

Progress in the muscular dystrophies has been steady. DM has been assigned to a particular chromosome (chromosome 19), and multiple RFLPs have been isolated and are in the process of being tested. In the case of DMD, more specific experiments aimed directly at the genetic locus are underway and promise to identify the defect within a short time. Preliminary linkage studies are also in progress in oculopharyngeal muscular dystrophy, Emery-Dreifuss dystrophy, and inherited neuropathies.

MYOTONIC MUSCULAR DYSTROPHY

As of March, 1985, the locus for DM has been localized to the short arm of chromosome 19.[16–21] Highly purified preparations of chromosome 19 have been isolated, cut into fragments using restriction enzymes, and inserted into phage and plasmid vectors. These chromosome 19 libraries have been screened randomly and several unique sequences of DNA located on chromosome 19 have been isolated. Several of these detect RFLPs and are currently being tested for linkage to DM (Fig 2). In a short time, not only will tightly linked DNA probes be available but pieces of DNA that flank the DM locus will allow very accurate diagnosis in informative situations. These markers will provide the DNA limits within which the DM gene will be sought.

The linkage group around DM has been known for a long time. In 1954 Mohr first described the linkage of a polymorphism called Secretor to DM.[15] This was expanded so that not only Secretor, but the Lutheran and the Lewis blood groups were also linked to DM.[16–28] More recently, the polymorphism for the third component of complement (C3) was also linked to DM.[19–21] Since C3 is a key protein in immunology, it was purified, sequenced, and the mRNA isolated.[22, 23] Using a cDNA probe derived from the mRNA, the locus for C3 was found to be on chromosome 19 by use of somatic cell hybrids as well as by in situ hybridization studies.[21] Thus, the locus of DM is also on chromosome 19 since it is linked to C3. Linkage studies using both the protein polymorphism and several RFLPs from the C3 locus have determined that the C3 locus is approximately 12cM from DM.[24–25] Although useful for establishing the chromosomal locus, C3 is too far from DM to be satisfactory by itself for diagnosis and prenatal prediction since the probability of a crossover is 12%.

With this information, the strategy for identifying DNA segments tightly linked to DM became more clearly delineated. Only DNA sequences from chromosome 19 need be tested. Using flow microfluorimetry methods, highly enriched preparations of chromosome 19 were prepared. Recombinant libraries have been constructed and unique sequences isolated (Fig 3).[26, 27] Our laboratory isolated more than 100 unique sequences from flow-sorted chromosome 19-enriched libraries and is currently identifying the sequences located on chromosome 19 by testing hybridization to a panel of somatic cell hybrids that distinguishes chromosome 19 DNA. Each sequence found to be from chromosome 19 is then tested against a panel of random individuals' DNA cut with a number of different restriction enzymes to determine whether it detects polymorphisms. Each chromosome 19 polymorphism is then tested against a large number of individuals in several extensive DM pedigrees, and linkage is calculated using standardized methodology. Table 1 lists the currently known RFLPs from chromosome 19 as well as their sources. ApoCII has recently been tested for linkage to DM and has been found to be the most closely linked polymorphism. Data from Shaw et al. and Pericak-Vance et al. suggest that ApoCII is localized approximately 2-4 cM from the DM

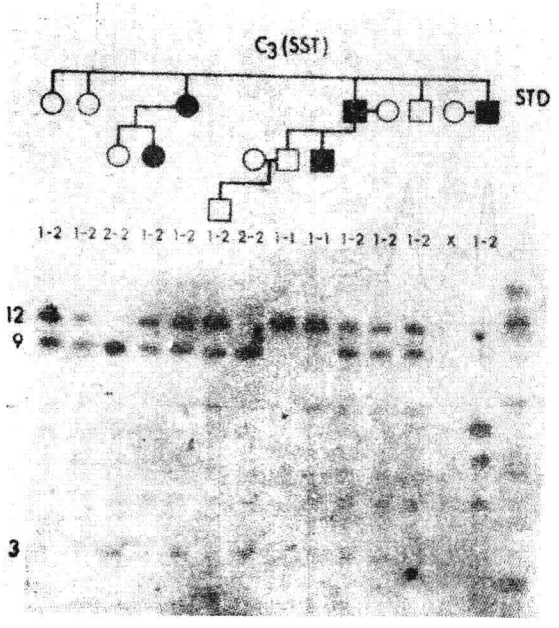

Fig 2.—Southern blot of a DM family using C3 as a probe. DNA samples from each of the illustrated individuals in this DM family have been isolated and digested with the restriction enzyme SST. The DNA has been subjected to electrophoresis on an agarose gel and the gel was blotted using special filter paper that binds the DNA fragments to the paper. The paper is then exposed to a solution containing the radiolabeled unique sequence DNA that codes for the protein complement component 3 (C3). Autoradiography of the blot that was exposed to the probe shows that this probe detects a 12 kb piece of DNA that contains a polymorphic or variable site yielding fragments of 9 kb and 3 kb. Heterozygotes demonstrate the 12 kb fragments from one chromosome 19 and 9 kb and 3 kb fragments from the other chromosome 19. In the extended family, all of whom have been examined, the "polymorphism" for DM is associated with the inheritance of 12 kb fragments. In this part of the family, each of the affected top generation has one chromosome with the 12 kb pattern. In the third and fourth lanes, the affected individual has the 12 kb pattern from one chromosome, the nonaffected does not. In the eighth and ninth lanes there are two individuals, both with each chromosome having the 12 kb polymorphism, yet one is affected and the other cannot be diagnosed. The apparently normal individual represents either a recombination event or a very late age-of-onset affected with no clinical signs. These possibilities are accommodated in the computer programs used for linkage analyses. The frequency of recombination is related to the linkage distance (see Fig 1). The closer the polymorphism is to the disease locus, the less the likelihood for recombination events to occur. The object is to find unique sequences that detect DNA polymorphisms so close to the disease locus that recombination events are extremely rare, so that diagnostic accuracy will be high.

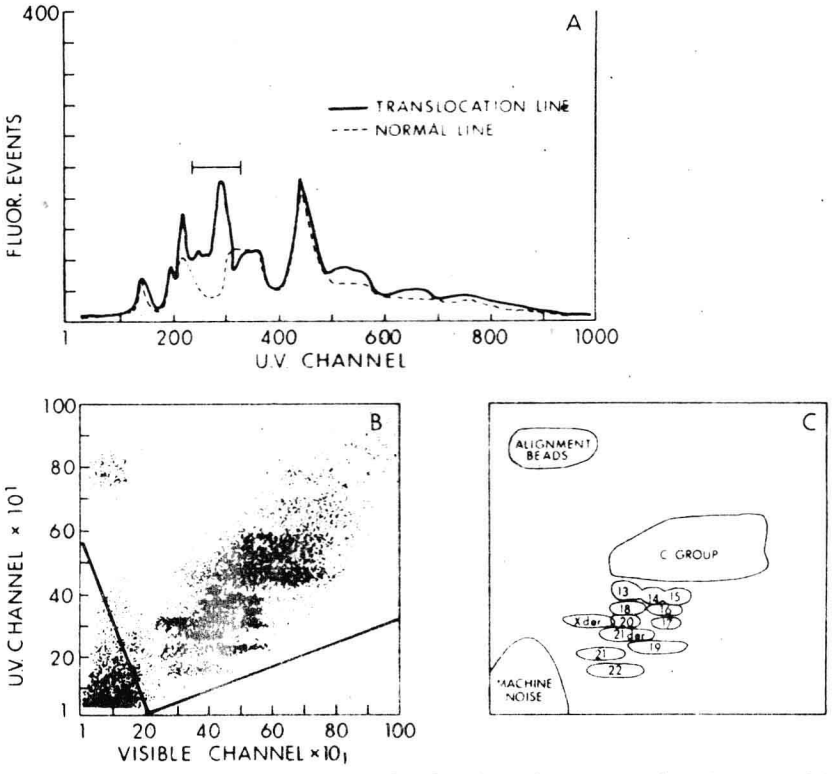

Fig 3.—Flow sorting of chromosome 19. Metaphase chromosomes have been carefully separated and dyes using two different agents, each detected by a different wavelength of fluorescence. The flow microfluorimeter can be tuned to respond to the degree of fluorescence (size) for each chromophore and to select and collect fractions. Chromosome 19 can be detected and collected quite readily because of its exceptional fluorescent dyeing characteristics due to the G-C/A-T ratio. Chromosome 19 can thus be obtained with > 90% purity for the construction of chromosome 19 libraries that can be screened for unique chromosome 19 sequences. In the illustration, chromosomes from a female DMD patient with a balanced X/21 translocation have been sorted. The relative positions of identified chromosomes from this particular sucrose gradient chromosome fraction is indicated. The derived X and derived 21 chromosomes have also been collected for DMD experiments.

locus.[25, 28] It is currently being tested to see if it flanks DM on the other side of one of the other tested probes. Several additional DNA segments are located on the short arm of chromosome 19 and are currently being tested for linkage to DM. As these are being tested, several additional chromosome 19 sequences are being identified, regionally localized, and tested for detection of RFLPs. Thus, it is reasonable to expect that before this chapter is published, additional closely linked and flanking markers will have been identified.

From a clinical point of view, it is important to understand the role that flanking markers (markers on opposite sides of the DM locus on the chromosome) will play in diagnosis and prenatal counseling. In fact, it is highly probable that a panel of useful flanking markers will be available before a very tightly linked marker is identified. Each may not be tightly linked to DM, but together, they may be very useful for accurate diagnosis. For instance, C3, the first available polymorphism, has been studied in several laboratories and is not tightly linked to DM.[24, 25] It is now calculated to be approximately 12cM from the DM locus. Should pBAM47, LDR152, cos 2–21, or one of the other probes (see Table 1) be a measurable distance *on the other side of the DM locus*, the two probes might be used together as flanking markers. Figure 4 illustrates the concept as it could be clinically applied in informative matings. A panel of RFLPs or haplotypes on each side of the DM locus would allow more matings to be informative and increase the general usefulness of this approach.

In Figure 4, a hypothetical locus called F85 is flanking DM on the opposite side of the chromosome from C3. If F85 is calculated from family studies to be 5cM from DM and 17cM from C3 and if both are informative in the mating of interest, then > 99% accuracy could be achieved in predicting the genotype of the individual or pregnancy at risk. The numbers involved in this hypothetical situation are of interest: a single crossover would occur approximately 17% of the time so that the accuracy would revert back to that of the marker that was still linked, a double crossover would occur 0.6% of the time and yield erroneous information; correct prediction would be available from more than 82% of the matings with 99.4% accuracy. In this short distance, the probability of a double crossover is, for practical purposes, also reduced due to the phenomenon of interference. Interference is due to the fact that a crossover in a given region reduces the chance of a second crossover in an adjoining region. Interference is thought to be complete in a region of 10cM or less. It should be clear that as markers become available which are closer and closer to DM, the proportion of single crossovers between them will be reduced and the proportion of correct predictions increased. When markers within 1cM are available, each marker will be 99% accurate and applicable in 99% of the informative matings for that marker.

TABLE 1.—CHROMOSOME 19 RFLPS TESTED FOR LINKAGE TO DM

PROBE NAME*	INSERT SIZE*(bp)	RFLP ENZYME	REGION CH19	LINKAGE TO DM	SOURCE OF PROBE
pBAM47 (D19S5)	2600	Msp-1, Bgl-II	19p	IP	Ross et al.[27]
pBAM34 (D19S6)	1200	Taq-1	19q	no	Ross et al.[27]
LB1-82	1650	Msp-1, Hind III	19p	IP	Litt†
LB2-21	590	Msp-1	19p	IP	Litt†
C3	1390	Sst, Hind III	19p	~ 12cM	Fey et al.[23]
ApoCII	440	Bgl-1, Taq-1	19q	~ 2–4 cm	Myklebost et al.[47]
1J2	1000	EcoRI	19q	> 20cM	Brook et al.†

*Yale Gene Mapping Library designation.
†Unpublished data.

It is important that the term "informative" be understood as it is applied to DM and other genetic diseases. Since DM is inherited as an autosomal dominant trait, the heterozygote is the informative individual. For a marker RFLP to be informative in a particular mating, the affected individual should be a double heterozygote, that is, *heterozygous for the marker and the disease*. In the best case, the nonaffected mate should be homozygous for the marker. The phase of the markers should also be known; that is, which type of the polymorphism for the marker is traveling with the DM trait on the same chromosome 19 in the affected individual. This information is only available from typing the previous generations and other members of the pedigree. It is important for the clinician to realize two essential points: (1) studies of large families are necessary to determine phase; and (2) a particular RFLP may be uninformative in a particular mating and thus useless for diagnostic or counseling purposes in that mating. Assuming that DM is due to a mutation involving the same locus in multiple families, widespread applicability will be achieved when multiple haplotypes of RFLPs or multiple RFLPs are available.

From the above information, it is clear that current linkage strategy involves randomly isolated pieces of DNA that are tested for proximity to the DM locus on chromosome 19. Nothing is learned about the pathogenesis of the disease from any of these studies. On the other hand, these studies will narrow down, flank, and eventually delineate the genetic locus responsible for DM. At that time, the product of that locus can be studied and the molecular pathogenesis of the disease described. Rational, specific therapeutic strategies can then be applied.

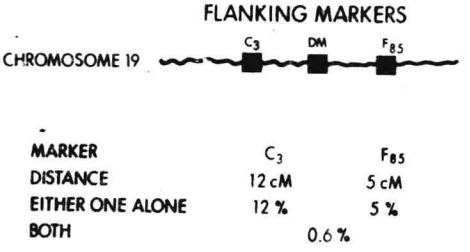

Fig 4.—Hypothetical flanking markers for DM. This diagram illustrates two DNa polymorphic markers, C3 and F85, that are located on chromosome 19 on either side of the DM locus. If either are used alone, the approximate accuracy is indicated by the frequency of recombination events. C3 is an actual linkage marker located approximately 12cM away from DM so that approximately 88% accuracy would be available from its use alone. F85 is a hypothetical marker located on the other side of DM, but only 5cM away allowing 95% accuracy with its use alone. Together they provide > 99% diagnostic accuracy in information matings when no recombinations have occurred (see text).