Linkage Analysis of a Kindred Segregating an Inversion of Chromosome 3

A thesis submitted to

The Faculty of Graduate Studies
University of Manitoba

In partial fulfillment of the requirements for the
Degree of Master of Science

by

Graham M. Casey
Department of Human Genetics
May, 1991

ISBN 0-315-76667-0

LINKAGE ANALYSIS OF A KINDRED SEGREGATING AN INVERSION OF CHROMOSOME 3

BY

GRAHAM M. CASEY

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

Abstract

To contribute to the genetic map of human chromosome 3, data obtained from eight DNA probes, two protein polymorphisms and a cytogenetic polymorphism were analyzed in a linkage study focusing on a kindred in which a pericentric inversion, designated inv(3)(p25q21), was segregating. The segregation of RFLP alleles in both inversion and normal families were analyzed, lod scores calculated and allele frequencies determined for both inversion and normal chromosomes. The data were also analyzed to determine if there was any evidence of an intrachromosomal Schultz-Redfield effect.

Allele frequencies indicated an association of specific alleles at loci located within the inversion segment of the chromosome in addition to specific alleles of PCCB and TF. Both PCCB and TF have been shown to be located at 3q21 outside of the inversion, but the association data were insufficient to determine which locus was closer to the 3q21 breakpoint. Alleles most commonly associated with the inv(3) chromosome were: RAF1*2, D3F15S2E*1, PCCB*C2, and TF*C.

The two point lod scores were inconclusive for some locus pairs on 3q, but when combined with three point segregation analysis an order of 3cen:PCCB:TF:BCHE has been proposed.

The lod scores and recombination fractions from both inversion and normal families for loci located in the 3q21→3qter segment were compared and tested for evidence of heterogeneity. No conclusive evidence of significant deviations was detected and, therefore, no evidence of an intrachromosomal Schultz-Redfield effect could be demonstrated.

Acknowledgements

First and foremost I would like to thank Dr. P. J. McAlpine for the opportunity to study in her laboratory. The experiences in class and the laboratory were both enriching and rewarding.

I would also like to thank the members of my committee, Dr. R. A. Woods and Dr. M. Lewis, for their advice and criticism of this thesis.

Thank you also to Gwendda Coopland for her expert technical assistance and to Margaret Dickson, Anthony Wiens, Don Paetkau for their assistance at the bench.

Special thanks to Linda Komarnicki for her assistance in processing the data through an at times uncooperative computer system, and Helena Lukes for additional lodsums.

Thanks also to the other graduate students, Mike Carpenter, Denise Belsham, Pranati Samaddar, and Shibani Bal, for helpful scientific discussion; and discussions in general that often had nothing to do with science.

I would also like to thank Dr. P. W. Allderdice for her cooperation in providing the blood samples and cytogenetic and *in situ* results as well as her encouragement during this project, and Dr. D. W. Cox and Dr. N. E. Simpson for protein analysis results.

A special note of thanks goes to the members of the A3 kindred. Without the interest in the scientific research projects and their generosity, this research project would not have been possible.

This research project was supported in part by a studentship from the Manitoba Health Research Council, and a grant from the Medical Research Council (P. J. M.).

List of Figures

List of Tables

List of Abbreviations

lod	- log of the odds
θ	- recombination fraction
PCR	- polymerase chain reaction
RFLP	- restriction fragment length polymorphism
VNTR	- variable number of tandem repeats
PIC	- polymorphic information content
cM	- centiMorgan
cDNA	- complementary DNA
3p	- chromosome 3, p arm
3q	- chromosome 3, q arm
3cen	- chromosome 3 centromere
ter	- terminal end of chromosome
inv(3)	- inv(3)(p25q21) [Specific inversion in this study.]
SDS	- sodium dodecyl sulfate
nm	- nanometre
µg	- microgram
µL	- microlitre
TAE	- tris-acetate-EDTA
ng	- nanogram
V	- volt
TE	- tris-EDTA
^{32}P	- radioisotope of phosphorous
dATP	- deoxyadenine triphosphate
dCTP	- deoxycytosine triphosphate
N	- Normal
µci	- microcurie
pg	- picogram
cpm	- counts per minute
dTTP	- deoxythymine triphosphate
dGTP	- deoxyguanine triphosphate
SSPE	- sodium-sodium phosphate EDTA (1X=0.15M NaCl, 0.01M NaH_2PO_4, 0.001M EDTA pH 7.7)
Denhardt's	- 2% Denhardt solution (2% Ficoll, 2% Polyvinylpyrrilidone, 2% Bovine serum albumin fraction V)
ddH_2O	- distilled deionized water

Table of Contents

1.000 Introduction

1.100 The Human Gene Map

The human genome contains an estimated 50,000 to 100,000 genes distributed over 23 pairs of chromosomes. At present, only a relatively small number of these loci have been identified and mapped. However, over the past few years, due to the initiative of the Human Genome Project, considerable progress has been made in developing both physical and, to some extent, genetic maps of the human chromosomes (Watson, 1990). The ultimate goal of the Human Genome Project is to produce the complete base sequence of the human genome, and to interpret the data in terms of both the structure and function of the human organism.

Mapping a large genome requires a means of storing and cataloguing massive amounts of data, as well as distributing information to investigators in a timely manner and a usable format. To deal with this problem the Human Gene Mapping Workshops are currently held annually. The objectives of these meetings include overseeing the collection of data and the generation of genetic and physical maps of human chromosomes, establishment of a common nomenclature used in naming loci as they are identified, and, perhaps most importantly, the dissemination of information in practical and useful forms.

The collection and the sorting of this information can be a difficult task, since data come from numerous investigators working in laboratories around the world. Furthermore, the

data are derived from a variety of sources such as linkage analysis of family data, somatic cell hybrids, in situ hybridization, gene dosage, exclusion mapping and neighbour analysis (McAlpine et al., 1989). Initial progress in developing the chromosomal maps was slow, but the pace has increased dramatically such that in the most recent publication of the human gene map, 1631 mapped gene markers, 113 fragile sites, more than 3,300 anonymous DNA segments and 54 mitochondrial genes have been mapped (McAlpine et al., 1989; Kidd et al., 1989). The more than 5,100 genetic markers already mapped increase the potential for mapping other loci linked to any of the individual markers or to known linkage groups.

1.200 Linkage Analysis

The number of genes present in the human genome is much greater than the number of chromosomes in a cell and therefore the prior probability that two loci will be located on the same chromosome (i.e., syntenic) has been estimated at 1/18.5 (Renwick, 1969). Recombination between homologous chromosomes during meiosis, allows for the exchange of alleles at various loci on the chromosome pairs. The closer two syntenic loci are together, the less likely their respective alleles will be separated by homologous recombination. Such loci are said to be linked, and their alleles will tend to be inherited together (Conneally and Rivas, 1980). This principle forms the basis for genetic linkage, the analysis of which involves

the use of various classes of markers and statistical methods as a tool for genetic mapping.

In order to detect linkage between two genes, at least one parent must be heterozygous at both loci under consideration so that segregation analysis will identify recombination between them i.e., recombinant and non-recombinant gametes from the informative parent. For the double heterozygote, two possible phases exist: alleles on the same homologue are in coupling; alleles on opposing homologues are in repulsion. Phase is important since identification of recombinants is only possible in phase known pedigrees as opposed to pedigrees in which the phase of the informative parent is unknown (Maynard-Smith et al., 1961).

Morton (1955) used this logic to develop his sequential test method for analysis of human linkage data. The sequential test allows for the continuing accumulation of data from successive pedigrees (families); this is important since human families tend to be relatively small and phase information is not always available. The sequential test, by allowing a third possible result to the hypothesis (i.e., results of analysis are inconclusive), means that the process of analysis can be continued until the required levels of significance are reached to prove or disprove the hypothesis. In addition to the sequential test protocol, Morton also incorporated the probability test ratio method (Haldane and Smith, 1947) and the lod score method (Barnard, 1949). The

probability ratio is the ratio of the probability of the data at a given specific value of θ to the probability of the data assuming independent assortment. Use of the logarithm of this ratio (lod score) means that the values are additive and data from many families can be combined readily.

In 1956 Morton applied this protocol to test for linkage between elliptocytosis and the Rh blood locus. After analyzing several pedigrees from the literature, Morton found that in four pedigrees elliptocytosis was closely linked to the RH locus ($z>2$ at $\theta=0.00$). However, in at least three other pedigrees linkage was not found ($z<-2$ at $\theta<0.05$) suggesting that at least two genetic entities were capable of causing the elliptocytosis phenotype. Morton was proved to be correct when independent studies demonstrated that non-RH linked elliptocytosis was due to a mutation at the α-spectrin locus (Ravindranath and Johnson, 1985) and the RH linked form of elliptocytosis was shown to be due to a mutation of the erythrocytic membrane protein 4.1 (McGuire and Agre, 1987). Morton not only demonstrated that the sequential test method was functional for analyzing family linkage data, but also described the first example of genetic heterogeneity in humans (Morton, 1956).

1.300 Linkage Analysis and Genetic Markers

Linkage analysis requires that defined genetic markers be available for study, and that these markers be readily

identifiable and segregate frequently. Markers can be divided into three general categories: (i) cytogenetic markers (ii) protein markers including serologically defined systems and (iii) DNA markers.

Cytogenetic markers are those that can be detected by microscopic examination of chromosomes. Initially, the centromere, or primary constriction and the satellites of human chromosomes 13, 14, 15, 21 and 22 were the only commonly useful cytogenetic markers. However, with the advent of banding techniques in the early 1970s (Casperson et al., 1970; Drets and Shaw, 1971), both the number of markers detectable and the sensitivity of detection increased. The chromosome specific patterns produced by G-, Q- and R- banding allowed the identification of each chromosome as well as relatively small regions within a given chromosome (i.e., individual bands). C- banding allowed the identification of highly repetitive sequences such as those around centromeres and the heterochromatic pericentric sequences frequently found on chromosomes 1, 9, 16 and the Y chromosome. Banding techniques made possible the identification of regions of each chromosome so that the physical breakpoints associated with specific chromosome rearrangements are also detectable. The only limitation in identifying such breakpoints is that the rearrangement must involve a portion of the chromosome large enough to be detected at the limits of resolution of both the banding technique used and the microscope. Application of

this technique can potentially identify the breakpoints of insertions, deletions, translocations and inversions such that they can be used as genetic markers (see for example Fain et al., 1989).

Protein polymorphisms are determined by a broad spectrum of tests or assays to detect, through some biochemical method, heritable variation in proteins (Harris, 1980). Starch or acrylamide gel electrophoresis can be used to detect variation in size or charge of a protein while isoelectric focusing can detect variation across a pH gradient. These variations can be due to amino acid substitutions, insertions or deletions which can potentially be traced back to a change at the DNA level. Quantitative variation in catalytic proteins can be measured through in vitro assays. Provided that the phenotypes of the individuals can be defined and segregation detected with accuracy, these protein polymorphisms can be used as genetic markers in linkage analysis. Blood group markers (i.e., ABO, RH and many others) as well as HLA antigen types have been used extensively in linkage studies and still have an important role. However, in many recent studies these are being replaced by DNA polymorphism markers which are estimated at an order of magnitude larger in terms of the frequency of their variability (Harris, 1980).

DNA polymorphisms are heritable variations in the nucleotide sequence of DNA (Botstein et al., 1980; Harris, 1980). Since restriction endonucleases recognize specific

base sequences, changes in the DNA sequence can be reflected in the presence or absence of a restriction endonuclease recognition site. This variation can be detected by the size of the DNA fragment produced by endonuclease digestion, and can be transmitted from one generation to the next (Grodzicker et al., 1974). These heritable variations in fragments size following restriction endonuclease digestion are referred to as restriction fragment length polymorphisms (RFLP's). DNA variation can also result from small duplications or deletions of short sequences, both of which will alter the size of the fragments following endonuclease digestion or the polymerase chain reaction (PCR) which utilizes sequence specific primers to amplify short sequences of DNA (Litt and Luty, 1989; Weber and May, 1989). Such variation between individuals can be identified by probing with the appropriate DNA sequence, restriction endonuclease digested DNA that has been separated by electrophoresis, and usually blotted onto a supporting membrane.

DNA probes are cloned sequences which can be used to identify different classes of polymorphisms such as RFLP's or mini-satellites (Jeffreys et al., 1985). Mini-satellite probes are sequences which detect a variable number of tandem repeats (VNTR's) consisting of short sequences of DNA repeated in tandem. It has been postulated that these variations arise by unequal exchange or gene conversion (Jeffreys et al., 1988) during mitosis or meiosis, or by slippage of the DNA during

replication (Jeffreys et al., 1985; Jeffreys et al., 1988). The polymorphic nature of the mini-satellite sequences arises from variation in the number of times the core sequence is repeated. Examination of these VNTR's has shown them to be highly polymorphic and they are applied in the new systems of DNA finger printing for forensic and biological relationship studies.

More recently, an abundant class of dinucleotide repeats, which can be identified through the use of PCR, has been described. These dinucleotide repeats, known as microsatellites, consist of (dT-dG)n repeats where n is approximately 10-60 (Litt and Luty, 1989) or (dC-dA)n repeats where n is about 15-30 (Weber and May, 1989). They have been shown to be highly polymorphic and several have been identified within specific genes (Weber and May, 1989). Because these repeat sequences are dispersed throughout the human genome, there is great interest in examining their usefulness for human gene mapping.

RFLP's are more commonly used as genetic markers for linkage studies than protein polymorphisms or VNTR's because it is not necessary for the mutation leading to a change in restriction sites to be located within the gene of interest. In practise the fragments detected by a given probe may be larger or smaller than the gene itself. To be useful for linkage studies, four simple criteria must be met by any given locus exhibiting an RFLP (Conneally and Rivas, 1980).

1. The mode of inheritance of the polymorphic fragments must be clear. In many cases, RFLP phenotypes are inherited as simple Mendelian co-dominant traits, however, in the case of the +/- types, the inheritance is described as dominant/recessive.
2. Phenotypic expression of each genotype must be clear. Phenotypic expression is relatively straightforward in the case of RFLP's as the identification of the phenotypes is accomplished by identifying the restriction fragment patterns.
3. The locus must be reasonably polymorphic. With regard to this, the polymorphic information content (PIC) of a given locus is used as a guide. The PIC is a measure of the informativeness of the offspring of various mating types given the frequency of heterozygotes based on the number of alleles found at a given locus, and the population frequencies of each allele (Botstein et al., 1980). PIC values typically range from about 0.15 to 0.35, with the higher values indicating a greater degree of variability. As indicated above, the VNTR's are more variable than most conventional RFLP's, but the distribution and number of types of VNTR's are not yet well defined. Consequently, the more numerous and more widely distributed RFLP's are often chosen for linkage analysis. This distribution is an important consideration as estimates suggest that if linked markers are 20 cM apart (i.e., an estimated 20

million base pairs) it could be possible to map any new marker in the human genome (Botstein et al., 1980).

4. The phenotypic variability must be detectable (i.e., expressed) in tissues amenable to study. Because the polymorphisms exist in the DNA, there is a ready supply of genomic DNA easily accessible in white blood cells. DNA can be extracted from fresh blood samples and portions of the lymphocytes may also be transformed in vitro, with Epstein-Barr virus, to yield a more or less permanent reserve of genomic DNA on selected individuals.

RFLP's possess all the required characteristics to be used as markers, and so they have come into general use in linkage studies. The ability to accumulate sufficient data (in the form of lod scores) and generate genetic maps depends on whether a given RFLP has a high enough PIC value, or enough informative families exist, to yield cumulative lod scores that meet the criteria for demonstrating or refuting linkage (Morton, 1955). If the cumulative lod scores exceed +3.0 at any given value of θ, linkage is indicated at that recombination fraction (θ). Conversely, cumulative lod scores of -2.0 or less refute linkage at values of θ less than that θ at which the score is $\leq$-2.0. According to the criteria recommended by Morton, scores between -2.0 and 3.0 are inconclusive and data accumulation must continue. These values are the constants A and B, respectively, in the following equations:

$A=(1-\beta)/\alpha$

$B=\beta/(1-\alpha)$

and α and β are the probabilities of type I and type II errors respectively. Morton (1955) suggested the prior probability of linkage in humans could be approximately 0.05 and, therefore, α should be at least 0.02 to ensure the posterior probability of a type I error (the chance of finding linkage where no linkage exists) is less than 0.05. By setting log A=3 and log B=-2, α=0.001 and β=0.01. Rao et al. (1978) determined that less than 2% of studies with total lod scores greater than 3 were type I errors.

More recently it has been suggested that if a priori mapping information exists (i.e., two loci are known to be located on the same chromosome) a lod score of +2.0 is sufficient to indicate linkage (Keats et al., 1987).

1.400 Inversions

Chromosomal rearrangements can be categorized as insertions, deletions, ring chromosomes, translocations, isochromosomes and inversions. Using chromosome banding techniques it is generally possible to define quite clearly any rearrangement under examination provided it is large enough to be detected at the limits of resolution of the microscope and the banding technique used.

This study is concerned with a pericentric inversion of chromosome 3. Inversions occur when two double stranded

breaks occur in the chromosome, the intervening segment rotates 180° and is religated at the breakpoints. If the centromere is located between the two breakpoints, the inversion is referred to as pericentric; if the centromere is not located between the two breakpoints, it is referred to as paracentric. Each of these two general classes of inversions can be further subdivided to define more complex rearrangements, such as tandem, included or overlapping inversions (Schulz-Schaeffer, 1985).

Sturtevant (1926) first identified inversions and showed that they acted as crossover suppressors in Drosophila. Only double crossovers involving two strands within the inversion will result in balanced recombinant chromosomes. Therefore, single (or, in rare instances, triple) crossovers will result in unbalanced gametes leading to gametic/zygotic lethality. In the case of an inversion heterozygote one member of the homologous pair will form a loop during pachytene and spatial separation at the minor loops of the pairing structure may inhibit crossing over in the regions adjacent to the breakpoints. In this way chromosomal inversions act to disrupt the normal patterns of homologous recombination.

Inversions have been shown to have played a major role in the evolution of some species of Drosophila (Dobzhansky and Spassky, 1947). Specific inversions have also been used in the laboratory to study induced mutations, such as the ClB chromosome in Drosophila (Muller, 1928).

In humans, the frequency of pericentric inversions has been estimated to be as high as 1-2% (Kaiser, 1984), however, most data suggest that the frequency is as low as 0.02% (Hook and Hamerton, 1977; Hamerton, 1983). Kaiser (1984) also suggested that there may be preferred breakpoints in some chromosomes as the same cytogenetically - identifiable breakpoints can be found in inversions from different kindreds (Kaiser, 1984). With the methods available for banding chromosomes, identification of the breakpoints of an inversion can be fairly accurate, and these breakpoints can be used as markers in genetic analysis.

1.500 The Inv(3)(p25q21) Chromosome

The Newfoundland kindred used in this study was ascertained through the birth of a child with multiple congenital anomalies, and an abnormal karyotype (Allderdice et al., 1975). Cytogenetic analysis also showed that child carried a duplication-deficient chromosome 3, and that the mother was heterozygous for an inversion designated inv(3)(p25q21). Searches of both family and public records which extend as far back as the early 1800s, indicated that the distribution of the inversion in the present day population probably resulted from a founder effect (Allderdice et al., 1975). Blood samples from selected members of the kindred have been obtained and DNA analyzed for a variety of genetic markers. Linkage studies using families derived from the main kindred are the subject of this study.

1.600 The Genetic Map of Chromosome 3

At Human Gene Mapping 10 (HGM10) the map of chromosome 3 consisted of 59 genes, 3 fragile sites and 114 anonymous DNA segments for a total of 176 markers (Naylor and Bishop, 1989). More than 50% of these markers were new assignments as of the most recent Human Gene Mapping Conference (Naylor and Bishop, 1989).

Selected probes defining several genetic loci previously assigned to chromosome 3 were examined in a linkage study, focusing on the inv(3) kindred, in an effort to improve the genetic map.

1.610 The RAF1 Locus

The viral oncogene v-raf has been shown to induce tumours in epithelial tissues in mice. Using v-raf as a probe, Bonner and coworkers (1984) identified two related sequences in humans: RAF1 the presumed active gene, which they mapped by in situ hybridization to chromosome 3p25; and RAF1P1, a processed pseudogene, located on chromosome 4. Three types of tumours: renal cell carcinoma, small cell lung carcinoma and mixed parotid gland tumours are all associated with specific rearrangements on 3p (Bonner et al., 1984). Since these tumours are in epithelial tissues, the tissue in which v-raf can induce tumour formation, they could involve RAF1 (Bonner et al., 1984).

Subsequently, a cDNA clone and several genomic clones of the human RAF1 gene were isolated and characterized (Bonner et

al., 1986). RFLP's detected with this cDNA probe have been used in the analysis of linkage between RAF1 and Von Hippel Lindau (VHL) disease (Seizinger et al., 1988; Kidd et al., 1989).

1.620 The D3F15S2E Locus (Formerly DNF15S2)

The complex locus DNF15S1 was originally mapped by in situ hybridization to chromosome 1, however somatic cell hybrid studies gave positive results for both chromosome 1 and chromosome 3. Subsequent analysis of the segment on chromosome 1 demonstrated that it represented several copies of a portion of a unique chromosome 3 segment repeated in tandem, (Goode et al., 1986). The unique sequence from chromosome 3 was then designated DNF15S2 (Welch and Carritt, 1987). These, and other studies (Carritt et al., 1986; Goode et al., 1986) demonstrated that the probe for this locus detects RFLP's in human genomic DNA digests. More recently mapping studies with somatic cell hybrids (Naylor et al., 1989) and in situ hybridization studies (Kok et al., 1987) place DNF15S2 at 3p21.

The locus has since been renamed D3F15S2E to reflect its chromosomal location (3) and the fact that it is an expressed sequence (E). Probes detecting this locus in humans hybridize strongly to mouse DNA indicating evolutionary conservation of the sequence. By probing normal lung cDNA libraries, clones were recovered and used to examine small cell lung carcinoma cell lines. The results showed that the locus DNF15S2E was

expressed in both normal cell and tumour cell lines (Naylor et al., 1989). As in the case of RAF1, deletion of some sequences of 3p have been associated with tumour formation. In the case of D3F15S2E, the strongest association is found with small cell lung carcinoma. While this locus itself is not responsible for the disease, it is believed to be very close to the locus that is (Naylor et al., 1989).

1.630 The PCCB Locus

Propionyl-coenzyme A carboxylase (PCC) is a biotin dependent enzyme which acts in the pathway for the degradation of branched chain amino acids as well as fatty acids with an odd number of carbon atoms. Enzyme molecules are dimers of an α and a β polypeptide (Vagelos, 1971). The α polypeptide containing the binding site for the biotin group (Lamhonwah et al., 1986).

Two cDNA PCC clones were isolated, one for the α polypeptide (PCCA) and one for the β polypeptide (PCCB) and through the use of somatic cell hybrids mapped to chromosomes 13 and 3 respectively (Lamhonwah et al., 1986). Subsequent analysis demonstrated that the PCCB gene mapped to 3q13.3→q22 (Kraus et al., 1986). Several RFLP's have been identified as associated with PCCB: a +/- RFLP in EcoR1 digests (Lamhonwah et al., 1986) and two codominant RFLP's in PstI digests (Waye et al., 1988).

1.640 The SST Locus

Somatostatin (SST) is a neuropeptide and hormone composed of 14 amino acid residues. It inhibits the release of pituitary, pancreatic and intestinal hormones, regulates gastrointestinal activity and may be a neurotransmitter (Brazeau et al., 1973; Koerker et al., 1974; Yen et al., 1974).

A cDNA copy and a genomic copy of this gene have been isolated and sequenced (Shen et al., 1982). SST was shown to be located on human chromosome 3 by analysis of the segregation of SST with previously assigned chromosomal markers in rodent-human somatic cell hybrids. Regional localization to 3q21-qter was achieved from the analysis of somatic cell hybrid lines carrying specific translocations involving chromosome 3 (Naylor et al., 1983). The probe for the SST locus has been shown to detect codominant allele RFLP's in EcoRI and in BamHI digests of DNA (Naylor et al., 1983).

1.650 The APOD Locus

Apolipoprotein D (APOD) is a protein component of high density lipoprotein (HDL), accounting for about 5% of the total plasma HDL in normal individuals (McConathy and Alaupovic, 1973; McConathy and Alaupovic, 1976). The mRNA coding for APOD was isolated and a cDNA copy prepared from this. Analysis of the sequence of the cDNA showed that it coded for a protein containing 169 amino acids (Drayna et al.,

1986). The APOD locus has been regionally mapped to human chromosome 3p14.2→qter, and the cDNA probe has been shown to detect a codominant RFLP in MspI digested DNA (Drayna et al., 1987).

1.660 The BCHE Locus

Serum cholinesterase (BCHE; formerly CHE1) is a plasma protein which hydrolyses a number of choline esters such as acetylcholine, butyrylcholine and benzocholine (Harris, 1980). Qualitative and quantitative variation of the activity of this enzyme has been identified as the cause of suxamethonium sensitivity (Bourne et al., 1952; Evans et al., 1952).

BCHE has been cloned (McGuire et al., 1989) the mutations identified and mapped to 3q26 (Allderdice et al., 1991). Using specific exons from the BCHE gene, RFLP's have been identified and correlated to specific phenotypes (McAlpine et al., 1991).

1.670 Protein Polymorphisms

Data from two protein markers transferrin (TF) and alpha-2-HS glycoprotein (AHSG) were also used in this study.

Transferrin which maps to 3q21 (Yang et al., 1984), is the major iron carrier in the body (Starkenstein and Harvalik, 1933) and is a member of a TF-like family of proteins, including lactotransferrin and ovatransferrin, which probably arose from gene duplication events (Metz-Boutique et al., 1981; Williams et al., 1982; Jeltsch et al., 1982;

MacGillivary et al., 1983).

AHSG, which maps to 3q27→qter (Magnuson et al., 1988; Naylor and Bishop, 1989), is a plasma protein synthesized in the liver. It has been shown to be concentrated in bone matrix (Ashton et al., 1976; Triffitt et al., 1976) and its concentration is highest in the bone tissue of the fetus and neonate (Quelch et al., 1984; Dickson and Bagga, 1985). AHSG plasma levels drop in certain cancers (Bradley et al., 1977) and inflammatory diseases (Lebreton et al., 1979). The age and disease related fluctuations in the serum levels of AHSG suggest that it is involved in bone formation and turnover (Quelch et al., 1984; Dickson and Bagga, 1985).

Both of these loci have been shown to be polymorphic by conventional isoelectric focusing (Kuhnl and Spielmann, 1979; Constans et al., 1981) and appropriate histochemical or immunological staining methods.

1.680 Cytogenetic Markers

Cytogenetic markers studied included the inversion breakpoints (inv(3)(p25q21)) (Allderdice et al., 1975) and a centromeric heterochromatic marker (3cen) located on 3q close to the centromere (Figure 1) and within the inversion (Allderdice et al., 1975).

1.700 The Schultz-Redfield Effect

Accumulated data from the experimental organisms Drosophila and rye show that when crossing over is suppressed

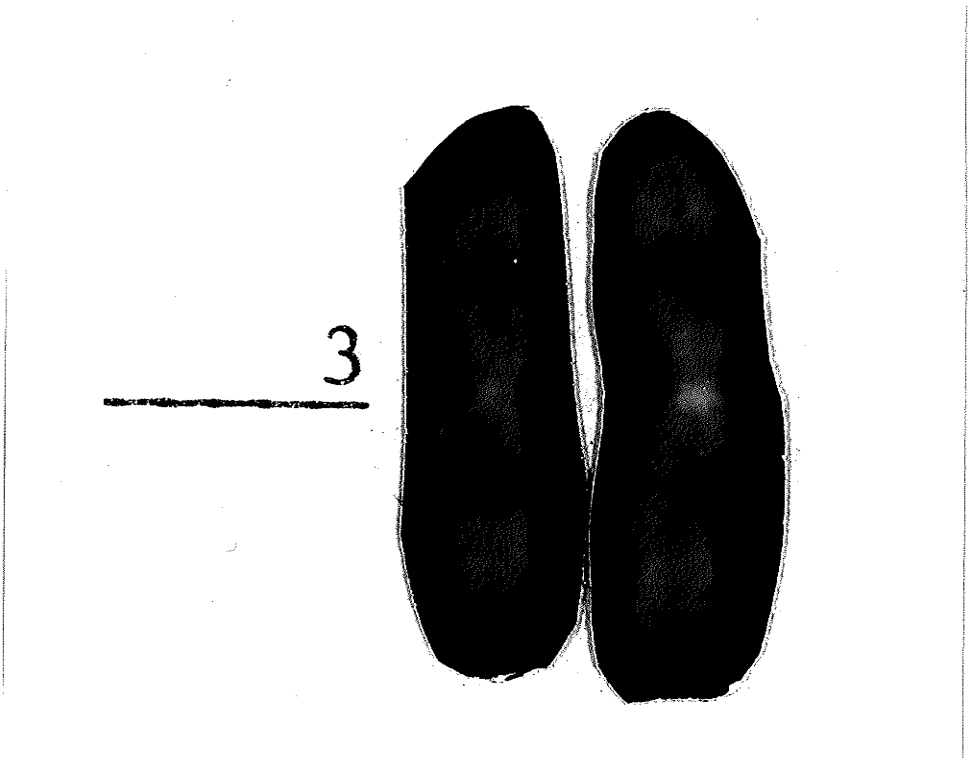

Figure 1. Photograph of normal human chromosome 3 showing the bright staining heterochromatic marker 3cen (right). A normal human chromosome 3 without the 3cen marker is shown for comparison (left).

in one region of the genome, there is an increase in crossing over in other regions (Schultz and Redfield, 1951). This phenomenon, originally observed in Drosophila, is known as the Schultz-Redfield effect and has been shown to be both intra- and interchromosomal.

Since the family groups used in the study are part of a large kindred in which an inversion segregates, segregation of the various markers in families from this kindred maybe expected to demonstrate an intrachromosomal Schultz-Redfield effect. To investigate this possibility, data from cytogenetically normal parents (controls) and the data from families in which one parent is an inversion heterozygote must be partitioned and compared to determine if the data sets indicate an increase in crossing over in the region 3q21→qter.

1.800 Specific Objective of the Current Study

1. To determine RFLP phenotypes for selected markers within nuclear families from the inv(3) kindred and from control families.
2. To examine phenotypes of offspring with normal chromosomes and inversion heterozygotes for the association of specific alleles with chromosome structure.
3. To generate lod scores from the segregation data, and examine the three point segregation to order loci where possible.
4. To examine the recombinational data for evidence of an

intrachromosomal Schultz-Redfield effect.

2.000 Materials and Methods

2.100 Sample Selection

The majority of DNA samples used were from selected families from the large Newfoundland kindred ascertained by Allderdice (1975), obtained through a collaborative study for gene mapping. The families were selected on the basis of one parent carrying the inv(3) (p25q21) chromosome (inversion heterozygote) or on the basis of being cytogenetically normal (control family). Other families studied were cytogenetically normal families within this kindred, as well as a series of families ascertained through a variety of genetic markers made available for use as controls; none of these families were ascertained for chromosome rearrangement. Segregation of a number of polymorphic markers was tested to verify biological relationships.

2.200 Preparation of DNA Samples

DNA was extracted from fresh white blood cell pellets, frozen white blood cell pellets or from lymphoblastoid cell cultures (Steel and Edmond, 1971). All blood samples were obtained from sources describe in section 2.100.

To establish lymphoblastoid cultures, about one million peripheral blood lymphocytes were isolated on a Histopaque 1077 (Sigma) gradient and incubated with 10^6 transforming units of Epstein Barr virus (Showa University Research Institute for Biomedicine, St. Petersburg, Florida) in RPMI

media supplemented with 15% fetal calf serum and cyclosporin (2 µg/mL). Following transformation cultures were grown for two to three weeks, supplemented with fresh media as required. Cultures were split into new flasks to prevent overgrowth and harvested for DNA extraction when approximately eight flasks were available. If required a sub-culture (i.e., 1 flask) of cells were placed in 0.8% DMSO and RPMI media and frozen in liquid nitrogen.

DNA was extracted from frozen or fresh lymphocytes or lymphoblastoid cell lines according to the method of Maniatis et al., (1982) with the following modifications. The cells were suspended in 1.6 mL of 1X suspension buffer (10 mM Tris-HCl pH 8.0, 2 mM EDTA, 10 mM NaCl) and then lysed overnight at 60°C by the addition of 0.4 mL of 5X lysis buffer (1X Suspension buffer, 5% SDS, 50 µg/mL proteinase K). Proteins and insoluble components were removed by two extractions with equal volumes of buffered phenol following which the two phases were separated by centrifugation at 3,000 rpm for 15 minutes at 20°C (IEC PR-6,000). Residual protein and phenol were removed by two extractions with equal volumes of chloroform:isoamyl alcohol (24:1) followed by centrifugation at 3,000 rpm for 15 minutes at 20°C. After the addition of NaCl (final concentration of 0.1 M) the DNA was precipitated by the addition of an equal volume of ice cold isopropanol. The DNA was then pelleted, washed twice with cold 70% ethanol to remove the excess salts and then resuspended in TE-8 (10 mM

Tris-HCl pH 8.0, 1 mM EDTA) on a rotator rack.

Once resuspended in TE-8, the DNA was quantitated spectrophotometrically at 260 nm and the purity estimated by measuring the ratio of absorbance at 260 nm to the absorbance at 280 nm. A ratio of 1.8 indicated pure DNA, while lower values indicated contamination with protein or phenol and higher values indicated contamination with RNA. If the ratio was not 1.8 the sample was re-extracted and re-precipitated. When the samples were purified, resuspended and quantitated, they were stored at 4°C until analyzed.

2.300 Analysis of Samples

2.310 Digestion of DNA by Restriction Endonuclease

Three to five µg of genomic DNA were digested with the appropriate restriction endonuclease (purchased from Boehringer Mannheim, Laval PQ, Promega, Ottawa ON, or Pharmacia, Baie D'Urte PQ) according to the manufacturers specifications. Following incubation (8-16 hours) reactions were terminated by the addition of 0.1 volume of 10X loading buffer (50 mM EDTA, 10% Ficoll, saturated with Orange G crystals). Samples were then incubated at 65°C for 10 minutes after which they were placed on ice for 5 minutes or until loaded into agarose gels for electrophoresis.

2.320 Electrophoresis of Restricted DNA Samples

Agarose gels were prepared from a 0.8% solution of electrophoresis grade agarose in TAE (0.04 M Tris-acetate, 0.001 M EDTA, pH 7.90). The solution was heated to boiling, cooled to approximately 55°C after which ethidium bromide (10 mg/mL) was added to a final concentration of 1 µg/mL. One hundred and fifty mL of the molten agarose was then poured into a mould with a 14.5 cm X 19.5 cm base (or 175 mL for 12.5 cm X 26.0 cm base) containing a plastic comb to form wells and allowed to cool to room temperature. The plastic comb was removed and the gel placed in an electrophoresis tank containing TAE buffer such that the gel was about 2 cm below the surface. Approximately 250 ng of HindIII digested lambda DNA (molecular weight marker II, Boehringer Mannheim, Laval PQ) were added to the first sample well, while the digested genomic DNA samples were added to the remaining wells. Electrophoresis was conducted at room temperature with the gels running for 850 V hours to 1,000 V hours depending on the gel size and the fragment size resolution required. Gels were photographed following electrophoresis.

2.330 Southern Transfer

Following electrophoresis, DNA was transferred to BIOTRANS (TCN Biomedicals, Inc, Irvine CA) GENESCREENPLUS (NEN Research Products, Mississauga ON) or HYBOND-N+ (Amersham, Oakville ON) nylon membranes by the method of Southern (1975),

modified to the manufacturer's specifications (Table I). High molecular weight DNA fragments were nicked by a 10 second exposure to ultraviolet radiation (254 nm) from a transilluminator (Spectroline TR-254) prior to transfer to BIOTRANS and GENESCREENPLUS membranes. This step was replaced with a 10 minute acid depurination step (0.25 M HCl) prior to transferring the DNA to HYBOND-N+ membranes; alternatively transfer to HYBOND-N+ proceeded with no pre-treatment of the DNA. DNA transfer proceeded from 4 to 24 hours, following which the membranes were briefly rinsed in 2X SSC (1X SSC:0.15 M sodium chloride, 0.015 M sodium citrate) and air dried. BIOTRANS filters were baked for 2 hours at 80°C to bind the DNA permanently to the membrane, while the GENESCREENPLUS and HYBOND-N+ membranes were used without further treatment.

2.340 DNA Probes

A total of eight DNA probes were used for the DNA studies (Table II). For nick translation labelling, plasmid vectors with inserts were labelled intact, while plasmids were linearized, or inserts excised and purified prior to labelling by the multiprime labelling reaction (section 2.350).

Table I. Modified Southern Transfer Protocols for BIOTRANS, GENESCREENPLUS and HYBOND-N+ Membranes

TREATMENT	BIOTRANS	GENESCREENPLUS	HYBOND-N+
Denaturation	2 washes in 1.5 M NaCl, 0.5 M NaOH for 20 minutes at 25°C	1 wash in 0.4 N NaOH, 0.6 M NaCl for 30 minutes at 25°C	Not required
Neutralization	3 washes in 0.5 M Tris-HCl (pH 7.0), 3.0 M NaCl for 20 minutes at 25°C	1 wash in 0.5 M Tris-HCl (pH 7.5), 1.5 M NaCl for 30 minutes at 25°C	Not required
Pre-treatment of membrane	Not required	Wet with dd H_2O; soak 15 minutes in 10X SSC.	Not required
Transfer Buffer	20X SSC	10X SSC	0.4 N NaOH

Table II. Human Chromosome 3 Loci Examined by RFLP Analysis

Symbol	Locus Name	Regional Location[a]	Probe[b]	Restriction Endonuclease[b]
RAF1	Murine leukaemia virus (v-raf-1) oncogene homologue 1	3p25	prB2	TaqI
D3F15S2E	DNA segment, numerous copies	3p21	pH3H2	HindIII
PCCB	Propionyl Coenzyme A carboxylase, β polypeptide	3q13.3→q22	pPCC41A2	PstI
BCHE	Butyryl-cholinesterase	3q26→qter	pUC1.7	MspI
SST	Somatostatin	3q28	pgHS7-2.7	EcoRI
APOD	Apolipoprotein D	3q26.2→qter	papoD6	MspI

[a] Regional localization data from McAlpine et al., 1989.

[b] Probe designations and restriction endonucleases as summarized by Kidd et al., 1989.

2.341 Isolation of Probes

Bacteria carrying the appropriate vector containing the probe insert were inoculated into two 500 mL LB broth cultures containing the antibiotic required to complement the resistance conferred by the plasmid. The cultures were grown at 37°C for about 3 hours (to an optical density of 0.4) and chloramphenicol added to a final concentration of 200 µg/mL prior to culturing overnight. The overnight cultures were centrifuged 15 minutes at 4°C and 5,000 rpm (Beckman J2-21; rotor JA-10), washed with 100 mL TE-8 (10 mM Tris-HCl pH 8.0, 1 mM EDTA) and centrifuged 15 minutes at 5,000 rpm and 4°C again. The pellet was resuspended in 12 mL of glucose buffer (50 mM glucose, 25 mM Tris-HCl pH 8.0, 10 mM EDTA) containing 40 mg of lysozyme, and incubated 10 minutes at room temperature. The cells were lysed by the addition of 27.6 mL of 1% SDS in 0.2 N NaOH and placed on ice for 15 minutes. Protein, chromosomal DNA and high molecular weight RNA were precipitated by addition of 14 mL of potassium acetate (3 M potassium, 5 M acetate pH 4.8) followed by chilling on ice for 15 minutes. Following a 15 minute centrifugation at 7,000 rpm and 4°C (Beckman J2-21, rotor JA-10) the supernatant was removed and extracted with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1). The aqueous phase was separated by centrifugation at 6,000 rpm for 5 minutes and 0.6 volumes of cold isopropanol added, following which the solution was chilled overnight at -20°C. The

precipitated DNA was pelleted by centrifugation at 10,000 rpm for 20 minutes at 4°C (Sorvall RC2-B, rotor SS34), dried briefly and resuspended in TE-8. Cesium chloride (1 g/mL of solution) and ethidium bromide (10 mg/mL) were added to the solution and thoroughly mixed. After ultracentrifugation at 45,000 rpm for 36 hours at 20°C (Beckman L8-80 m, rotor Ti 80), the plasmid DNA was removed with a syringe and washed 2-3 times with H_2O-saturated isoamyl alcohol to remove the ethidium bromide. The DNA solution was then diluted with 3 volumes of TE-8. To precipitate the DNA, 2.5 volumes of 95% ethanol were added and the solution then chilled overnight at -30°C. Following pelleting of the DNA by centrifugation at 10,000 rpm for 15 minutes (Sorvall RC2-B, rotor SS34), the DNA was dried, resuspended in TE-8 and the concentration determined spectrophotometrically at 260 nm.

Alternatively, plasmid DNA containing probe sequences was isolated using a pz523 spun column (5 Prime→3 Prime, Inc., West Chester PA) following the protocol supplied by the manufacturer.

2.350 Labelling of Probes

DNA probes were labelled by either a nick translation or multiprime labelling reaction with α^{32}P dATP and α^{32}P dCTP (NEN). The nick translation reaction utilized a commercially available kit (N.5500 Amersham).

Between 150 ng and 250 ng of closed circular plasmid DNA,

unlabelled dTTP and dGTP (final concentration:1 μM) and 4 μL of enzyme solution (2 units DNA polymerase I/μL and 40 pg DNase I/μL) were incubated with 50 μCi each of α^{32}P dATP and α^{32}P dCTP at 15°C for 2 hours. The nick translation reaction was terminated by the addition of 1 volume 2X stop buffer (0.05% bromphenol blue, 10% glycerol, 20 mM EDTA). Unincorporated nucleotides were separated from the labelled plasmid DNA by centrifugation of the solution over a Sephadex G-50 column saturated with TE-8 and 0.1% SDS at 2,500 rpm for 1 second (IEC PR-6,000, rotor 269). Alternatively, labelled DNA was purified on a Nick Column (Pharmacia) according to manufacturers' specification. Approximately 200,000 cpm of HindIII digested, labelled lambda DNA was added to the eluate and both were denatured by heating to 100°C for 10 minutes and cooled on ice for 5 minutes.

Linearized plasmid DNA and insert (probe) DNA were labelled by the multiprime labelling reaction using the Multiprime DNA labelling (RNP.1600z, Amersham). The DNA (about 100 ng for linear plasmid, and 10-35 ng for insert DNA) was denatured for 10 minutes at 100°C following which 2 μL of a solution containing random hexamer primers were added and the solution cooled on ice for 5 minutes. Unlabelled dGTP and dTTP (final concentration:2 μM), 2 units of DNA polymerase I Klenow fragment (supplied with Amersham kit or purchased from Pharmacia) and 50 μCi each of α^{32}P dATP and α^{32}P dCTP were

added and the solution incubated 2 hours at 37°C or overnight at room temperature. Labelled probe DNA was separated from unincorporated nucleotide by purification on a Nick Column (Pharmacia). Subsequently 200,000 cpm of Hind III digested labelled lambda DNA was added to the probe DNA and both denatured at 100°C for 10 minutes and chilled on ice for 5 minutes.

2.360 Hybridization of Probe Sequence

Membranes were prehybridized for 2-4 hours as detailed in Table III. The prehybridization solution was removed and replaced with the appropriate hybridization solution (Table III). The denatured probe was added and the membrane was then sealed in a plastic bag and incubated at 65°C in a shaking water bath overnight.

2.370 Post Hybridization Washing and Autoradiography

Following hybridization, membranes were removed from the bags and placed in plastic containers for washing under the appropriate stringency conditions (Table IV). The post-hybridization washes ranged from 15 minutes to 1 hour in duration depending on the amount of background radiation remaining on the membrane as measured by a Geiger-Muller detector (ion chamber detector). When background radiation levels reached <100 cpm, membranes were rinsed briefly in 2X SSC at room temperature, blotted lightly to remove excess

Table III. Pre-Hybridization and Hybridization Conditions for BIOTRANS, GENESCREENPLUS and HYBOND-N+ Membranes

TREATMENT	BIOTRANS	GENESCREENPLUS	HYBOND-N+
Pre-Hybridization Solution	5X Denhardt's 5X SSPE 0.2% SDS 500 µg/mL denatured salmon sperm DNA	5X Denhardt's 10% Dextran SO_4 0.5% SDS 50 mM Tris-HCl (pH 7.5) 1 M NaCl 500 µg/mL denatured salmon sperm DNA	5X Denhardt's 5X SSPE 0.5% SDS 400 µg/mL denatured salmon sperm DNA
Total Volume	20.0 mL	20.0 mL	25.0 mL
Incubation	65°C	65°C	65°C
Hybridization Solution	5X Denhardt's 5X SSPE 0.02% SDS 10% Dextran SO_4 500 µg/mL denatured salmon sperm DNA	As above	As above
Total Volume	10.0 mL	10.0 mL	12.5 mL

Table IV. Post-Hybridization Washing Protocols for BIOTRANS, GENESCREENPLUS and HYBOND-N+ Membranes

BIOTRANS	GENESCREENPLUS	HYBOND-N+
1. 0.2% SDS, 0.1 mM EDTA 5 mM $NaPO_4$ buffer at room temperature	1. 2X SSC and 0.1% SDS at room temperature	1. 2X SSPE and 0.1% SDS at room temperature
2. 0.2% SDS, 0.1 mM EDTA 5 mM $NaPO_4$ buffer at 40°C - 45°C (if required)	2. 2X SSC and 0.1% SDS at 50°C - 55°C	2. 1X SSPE and 0.1% SDS at 50°C - 55°C
	3. 0.1X SSC and 0.1% SDS at 50°C - 55°C (if required)	3. 0.1X SSPE and 0.1% SDS at 50°C - 55°C (if required)

liquid, and sealed in plastic bags. The filters were then placed in X-ray cassettes with one or two Kodak XAR-5 films between two Cronex intensifying screens and left overnight to 10 days at -70°C. Films were developed according to the manufacturer's protocol.

2.380 Removal of Bound Probe

Hybridized probes were removed by adding approximately 250 mL of 0.1X SSC and 0.1% SDS (at 100°C) to a plastic container with the membranes (BIOTRANS and GENESCREENPLUS) and allowing the solution to cool to room temperature on a shaker table. The probe was removed from HYBOND-N+ membranes by a similar method, except that a 0.5% SDS solution (100°C) was used. The washing procedure was usually repeated twice following which the membranes were sealed in plastic bags and exposed to film for 48 hours to ensure all the previously hybridized probe had been stripped. Membranes could then be re-probed as described in section 2.360.

2.400 Analysis of TF and AHSG Phenotypes and Cytogenetic Markers

Data from protein analysis had already been collected by standard isoelectric focusing and histochemical staining techniques. AHSG and some TF phenotypes were provided by Dr. D. W. Cox (Hospital for Sick Children, Toronto). All cytogenetic analyses including karyotypes, analysis of the centromeric heterochromatic marker and in situ hybridization

were performed by Dr. P. W. Allderdice. Several families were typed by quantitative analysis for BCHE by Dr. N. E. Simpson (Queen's University, Kingston).

Lod scores calculated from AHSG, TF and BCHE segregation reported previously (McAlpine et al., 1987) have been included with the data for these loci for the control families.

2.500 Linkage Analysis

All markers were tested for linkage in pair wise combinations using the lod score method of Morton (1955) performed by the Mark III computer program of Cote (1975). Lod scores were partitioned by sex of the informative parent and according to the presence or absence of the inv(3)(p25q21) chromosome. Maximum values of Z and the corresponding value of θ were determined using the MAP83 computer program (Sherman et al., 1984). Testing various orders of loci and testing the lod scores for heterogeneity was also carried out with the MAP83 program. The χ^2 results for allele association tests were calculated manually.

3.000 Results

3.100 Description of Phenotypes

3.110 RAF1

The pattern of hybridization of the RAF1 probe sequence to TaqI digested human genomic DNA is illustrated in Figure 2. Two bands occur, one at approximately 7.6 kilobases (kb) and a second at approximately 7.0 kb. Individuals were phenotyped on the basis of the 7.6 kb band designated type 1, the 7.0 kb band designated type 2 and the presence of both the 7.6 and 7.0 kb bands as 2-1. The bands detected by this probe are consistent with those detected by the probe pF10-7 (Seizinger et al., 1988).

3.120 D3F15S2E

Probe pH3H2, containing the insert detecting the D3F15S2E RFLP, produces a hybridization pattern with HindIII digested human genomic DNA as shown in Figure 3. Two intense constant bands are present at 8.0 kb and 3.8 kb, while the polymorphic bands are found at approximately 2.3 kb and 2.0 kb. Individuals were phenotyped on the basis of the 2.3 kb band designated type 1, the 2.0 kb band designated type 2 and the presence of both the 2.3 and 2.0 kb bands as 2-1. The RFLP's detected by this probe are consistent with the literature (Carritt et al., 1986).

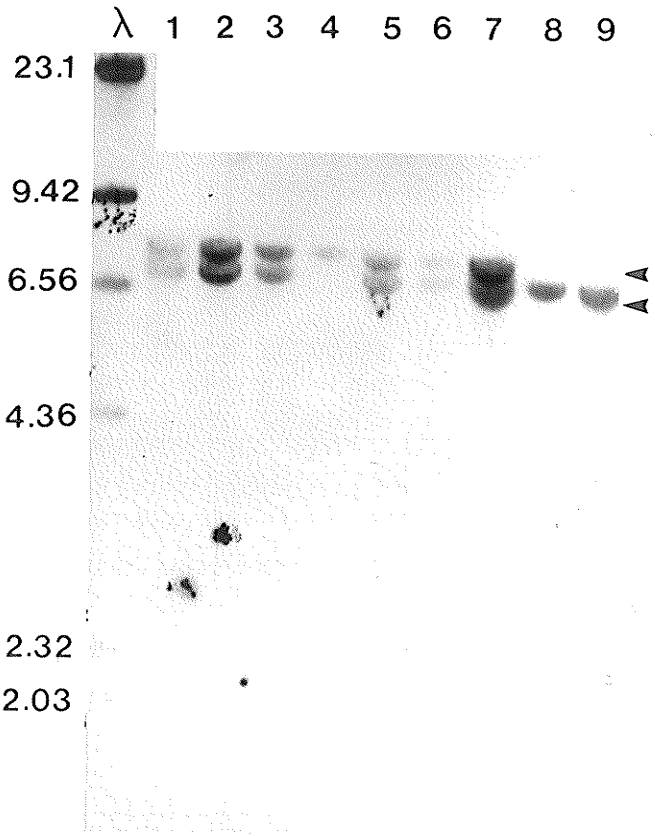

Figure 2. Photograph showing the TaqI RFLP phenotypes for RAF1. Lane 4: RAF1 1. Lanes 8 and 9: RAF1 2. Lanes 1-3, 5-7: RAF1 2-1.

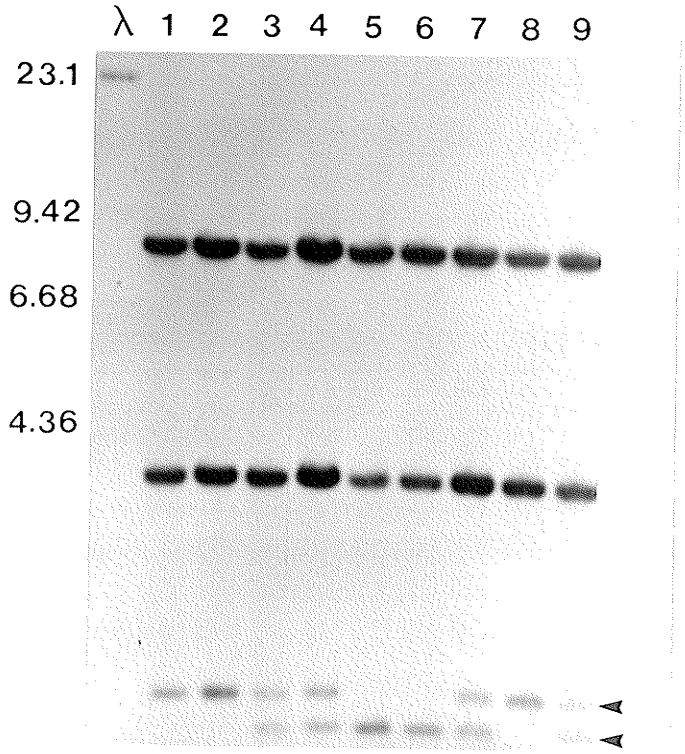

Figure 3. Photograph showing the HindIII RFLP phenotypes for D3F15S2E. Lanes 1, 2 and 8: D3F15S2E 1. Lanes 5 and 6: D3F15S2E 2. Lanes 3, 4, 7 and 9: D3F15S2E 2-1.

3.130 PCCB

The patterns of hybridization of the PCCB probe sequence to PstI digested human genomic DNA are shown in Figure 4. The patterns of hybridization are complex and posed some difficulties in phenotyping as there are two codominant RFLP systems (designated B and C) and several constant bands (Waye et al., 1988). The constant bands appeared at approximately 12.0 kb, 8.1 kb and 4.2 kb; the 3.0 kb constant band was extremely weak or not seen. The first codominant RFLP (B) is defined by bands at approximately 10.2 kb and 7.8 kb (Figure 4). The 7.8 kb band was occasionally difficult to separate from the 8.1 kb constant band, so comparisons of both band position and relative width were used. Phenotypes of individuals were determined based on the presence of only the 10.2 kb band (B1) as type B1, the presence of only the 7.8 kb band (B2) as type B2, and the presence of both the 10.2 kb and 7.8 kb bands as type B2-1.

The second codominant RFLP (C) was defined by bands at approximately 6.2 kb and 1.7 kb (Figure 4). Phenotypes of the individuals were determined based on the presence of only the 6.2 kb band (C1) as type C1, the presence of only the 1.7 kb band (C2) as type C2, and the presence of both the 6.2 kb and 1.7 kb bands as type C2-1. In all cases, attempts were made to determine phenotypes for individuals using both RFLP systems, however segregation analysis and subsequent calculation of lod scores utilized only one or the other RFLP

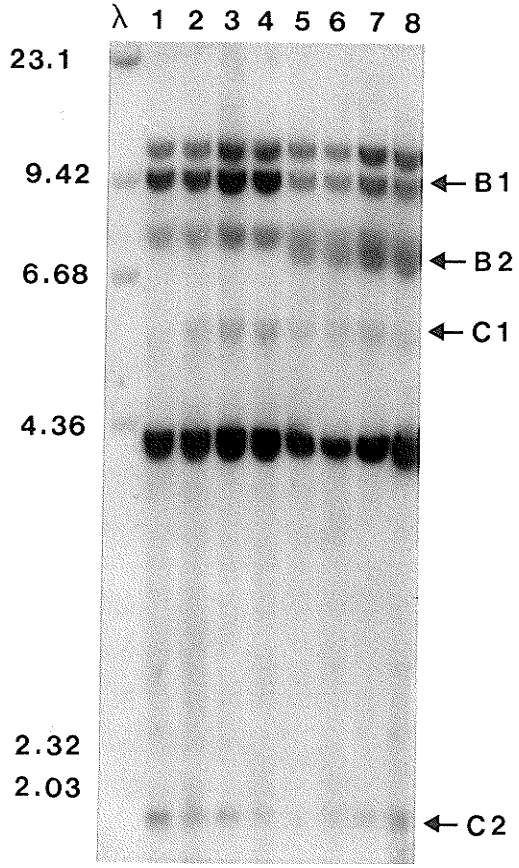

Figure 4. Photograph showing the PstI RFLP B and C phenotypes for PCCB. Lanes 1-4: PCCB B1. Lanes 5-8: PCCB B2-1. Lane 1: PCCB C2. Lanes 2-8: PCCB C2-1.

system.

For the most part, the bands seen were consistent with those described in the literature (Waye et al., 1988). However, the 3.0 kb constant band described in the literature was not seen in the inv(3)(p25q21) kindred and was rarely seen in the other normal control families tested.

The same probe can be used to detect RFLP's in EcoRI digested human genomic DNA (data not shown). The hybridization pattern seen is that of a "plus/minus" RFLP system (Lamhonwah et al., 1986) and, therefore, there can be a problem clearly identifying all heterozygotes. For this reason, the probe was used only on the DNA of a few selected individuals to attempt to identify points of recombination.

3.140 SST

The pattern of hybridization of the probe for the SST sequence to EcoRI digested human DNA is shown in Figure 5. There are no constant bands present and the polymorphic bands found at approximately 12.0 kb and 6.4 kb are consistent with the literature (Naylor et al., 1983). Individuals were phenotyped on the basis of the 12.0 kb band designated type 1, the 6.4 kb band designated type 2, and the presence of both the 12.0 and 6.4 kb bands designated type 2-1.

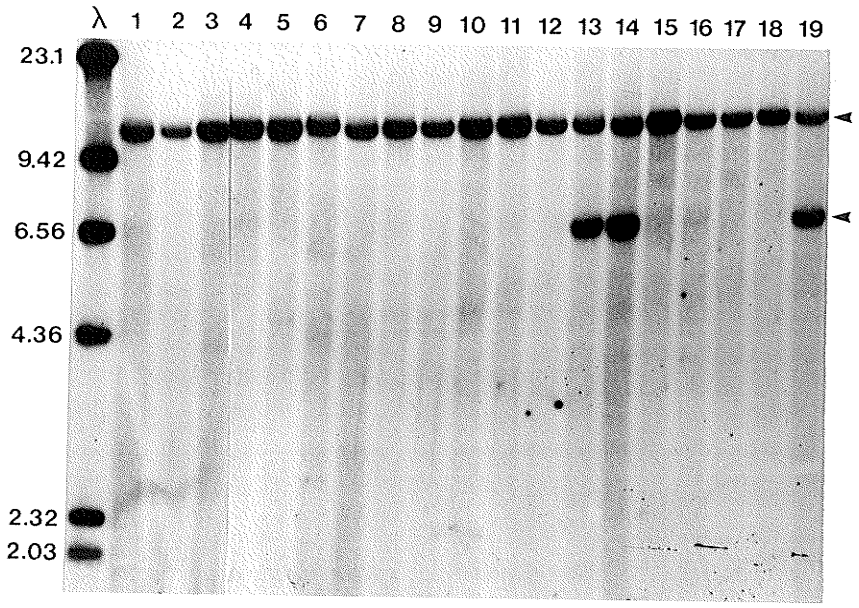

Figure 5. Photograph showing the EcoRI RFLP phenotypes for SST. Lanes 1-12, 15-18: SST 1. Lanes 13, 14 and 19: SST 2-1.

3.150 BCHE

The hybridization pattern of the probe for the BCHE sequence to MspI digested human DNA is shown in Figure 6. There are no constant bands present and the polymorphic bands are seen at approximately 10.5 kb and 4.5 kb. These results are consistent with results of recent studies testing unrelated individuals (McAlpine et al., 1991). Individuals were phenotyped on the basis of the 10.5 kb band designated type 1, the 4.5 kb band designated type 2, and the presence of both the 10.5 and 4.5 kb bands designated type 2-1.

3.160 APOD

The hybridization pattern of the probe sequence for APOD to MspI digested human DNA is shown in Figure 6. Only the 2.9 kb constant band is seen; the 1.3 kb and 0.7 kb constant bands are not visible. The polymorphic bands seen at approximately 2.20 kb and 2.15 kb are consistent with the literature description (Drayna et al., 1987). In some instances, it was difficult to distinguish between the two bands, and it was necessary to compare relative band width as well as size. Individuals were phenotyped on the basis of the 2.20 kb band designated type 1, the 2.15 kb band designated type 2, and the presence of both the 2.20 and 2.15 kb bands designated type 2-1.

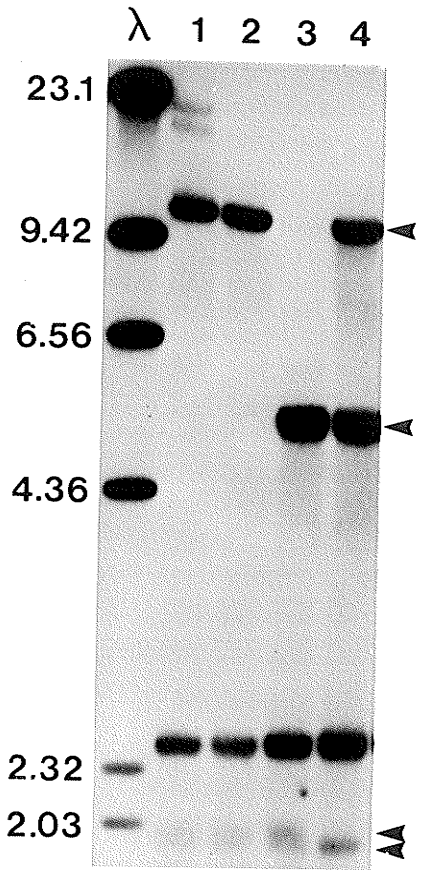

Figure 6. Photograph showing the MspI RFLP phenotypes for BCHE (upper arrows) and APOD (lower arrows). Lanes 1 and 2: BCHE 1 and APOD 2-1. Lane 3: BCHE 2 and APOD 2-1. Lane 4: BCHE 2-1 and APOD 2.

3.200 Allele-Chromosome Associations

The alleles carried on inv(3) and normal chromosomes for selected loci were tabulated in 2X2 or 3X2 contingency tables and chi-square values determined (Table V). The RAF1 locus results show that RAF1*2 was associated with the inv(3) chromosome while the RAF1*1 allele was associated with the normal chromosome (χ^2=74.57, P<0.005, df:1). The D3F15S2E locus also showed evidence of an allele-chromosome association with the inv(3) chromosome usually carrying D3F15S2E*1 while the normal chromosomes tended to carry D3F15S2E*2 (χ^2=58.41, P<0.005, df:1). The PCCB C RFLP showed a clear allele association with a χ^2=10.26 P<0.005 at one degree of freedom.

The results of the 3X2 contingency test for the TF locus indicate that the TF*C allele was associated with the inv(3) chromosomes examined (χ^2=17.18, P<0.005, df:2). The TF*C allele was also commonly found on the normal chromosomes, but the TF*S and TF*Z alleles were found only on normal chromosomes.

None of the more distal loci on 3q showed evidence of a specific allele associated with either normal or inversion chromosomes. The χ^2 for BCHE was 0.244 (P>0.50, df:1), for SST was 3.28 (P>0.05, df:1), for APOD was 2.09 (P>0.10, df:1) and for AHSG was 0.013 (P>0.90, df:1).

Based on these results the inv(3) chromosome tended to carry the RAF1*2, D3F15S2E*1, PCCBC*2 and TF*C alleles.

Table V. Contingency Tables and Chi-Square Analysis of Allele Distribution Relative to the inv(3) Chromosomes and normal Chromosomes

RAF1

	RAF1*1	RAF1*2
inv(3)	2	50
Normal	162	68

χ^2 =74.57, P<0.005 (1 df)

D3F15S2E

	D3F15S2E*1	D3F15S2E*2
inv(3)	41	2
Normal	54	127

χ^2 =58.41, P<0.005 (1 df)

SST

	SST*1	SST*2
inv(3)	45	8
Normal	218	15

χ^2 =3.28, P>0.05 (1 df)

PCCB - C RFLP

	PCCB*C1	PCCB*C2
inv(3)	21	37
Normal	184	128

χ^2 =10.26, P<0.005 (1 df)

BCHE

	BCHE*1	BCHE*2
inv(3)	35	5
Normal	184	38

χ^2 =0.244, P>0.50 (1 df)

Table V. continued

	APOD		AHSG	
	APOD*1	APOD*2	AHSG*1	AHSG*2
inv(3)	5	48	32	15
Normal	42	180	102	53
	χ^2=2.09, P>0.10 (1 df)		χ^2=0.013, P>0.90 (1 df)	

	TF		
	TF*C	TF*S	TF*Z
inv(3)	54	2	0
Normal	162	48	22

χ^2=17.18, P<0.005 (2 df)

Numbers in each cell of the contingency tables indicate the number of alleles scored for each chromosome type.
df: degrees of freedom.

3.300 Linkage Studies

Figure 7 shows a schematic representation of the normal chromosome 3 (left) and the inv(3)(p25q21) chromosome (right); the bands in which the breakpoints of the inversion are located are indicated by arrows. The regional localizations of each of the other markers used in this study are shown on the schematic diagram of the normal chromosome 3 (left).

Tables VI and VII contain lod scores at various values of θ as well as the maximum lod score ($\hat{Z}$) and the corresponding value of θ ($\hat{\theta}$) for each locus pair derived from families segregating the inversion chromosome and chromosomally normal families respectively. Only those lod scores which were formally significant for at least one of the classes of chromosomes are included in the tables. Lod scores obtained for locus pairs known (from the physical map) to be located sufficiently far apart to make detection of useful linkage unlikely as well as the lod scores which were inconclusive for both inv(3) and normal chromosomes are given in Appendices 1 and 2. In all tables of lod scores locus pairs are listed alphabetically. Unless otherwise specified, the results for two point lods involving the inv(3) marker represent the combined lod scores for the 3p25 and 3q21 breakpoints.

3.310 inv(3) Family Lod Scores

Lod scores obtained (Table VI) indicated tight linkage between RAF1 and the inversion breakpoint at 3p25 ($\hat{Z}_m$=3.659,

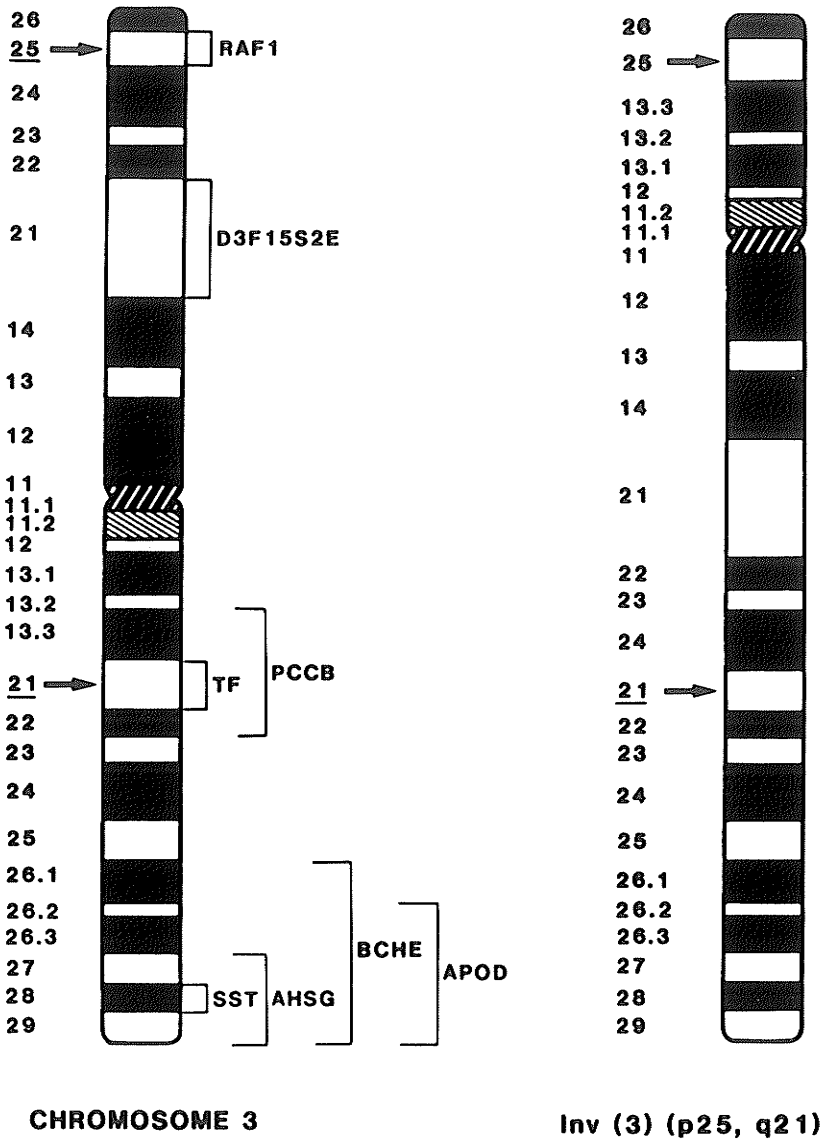

FIGURE 7. SCHEMATIC REPRESENTATION OF THE NORMAL HUMAN CHROMOSOME 3 (LEFT) AND THE inv (3) CHROMOSOME (RIGHT). REGIONAL LOCALIZATIONS FOR SELECTED CHROMOSOME 3 LOCI ARE INDICATED ON THE NORMAL CHROMOSOME. THE INVERSION BREAKPOINTS ARE INDICATED BY ARROWS ON THE NORMAL CHROMOSOME.

Table VI. Lod Scores Derived from Families Segregating for the Inversion Chromosome

Locus Pair	Type	Segregation F	Segregation C	R:NR	Recombination Fraction 0.05	0.10	0.20	0.30	0.40	$\hat{Z}$	$\hat{\theta}$
3cen:D3F15S2E	P	7	24	0:4	4.221	3.768	2.790	1.736	0.699	4.457	0.03
	M	10	35	0:7	6.097	5.639	4.344	2.818	1.212	6.426	0.02
3cen:inv(3)	P	24	89	0:16	16.831	16.449	11.834	7.592	3.097	16.854	0.06
	M	25	88	2:26	15.945	14.931	11.694	7.695	3.375	15.945	0.05
3cen:RAF1	P	7	20	0:3	3.652	3.468	2.738	1.797	0.772	3.667	0.04
	M	8	22	0:7	4.632	4.127	3.073	1.974	0.905	5.117	0.00
3cen:TF	P	6	21	0:1	-0.163	0.645	1.003	0.783	0.329	1.003	0.20
	M	7	21	2:17	2.664	2.864	2.601	1.977	0.456	2.868	0.11
BCHE:TF	P	2	7		-0.907	-0.422	-0.070	0.019	0.013	0.026	0.34
	M	2	4		-0.163	0.067	0.214	0.216	0.140	0.225	0.25
D3F15S2E:inv(3)	P	7	25	0:4	4.427	3.971	3.126	1.871	0.764	4.675	0.03
	M	12	43	0:6	6.736	6.431	5.099	3.348	1.429	6.742	0.06
D3F15S2E:RAF1	P	3	14		2.999	2.671	1.963	1.191	0.421	3.311	0.00
	M	6	16	0:8	2.302	2.277	1.890	1.326	0.680	2.314	0.07
D3F15S2E:TF	P	2	11		2.464	2.206	1.645	1.021	0.372	2.709	0.00
	M	4	11	1:2	1.167	1.257	1.068	0.712	0.289	1.257	0.10
inv(3):RAF1	P	6	23	0:3	3.631	3.428	2.668	1.714	0.698	3.659	0.03
	M	11	28	0:7	5.706	5.061	3.724	2.336	1.016	5.719	0.00
inv(3):TF	P	6	24	0:3	0.673	1.411	1.615	1.220	0.555	1.624	0.18
	M	7	28	1:13	2.084	2.314	2.123	1.604	0.890	2.326	0.12

Table VI. continued

Locus Pair	Type	Segregation F	Segregation C	R:NR	Recombination Fraction 0.05	0.10	0.20	0.30	0.40	$\hat{Z}$	$\hat{\theta}$
PCCB:TF	P	1	5		1.093	0.975	0.720	0.436	0.149	1.204	0.00
	M	2	3	2:1	-1.721	-1.143	0.592	-0.298	-0.115	0.000	0.50

Abbreviations used are: P - paternal; M - maternal; F - number of families scored; C - number of children scored; R:NR - recombinant:non-recombinant phase known score.

θ_m=0.03; $\hat{Z}_f$=5.719, θ_f=0.00), with one definite crossover between the inversion breakpoint and the RAF1 locus being identified among 55 offspring scored. One child carrying the recombinant chromosome, dup q inv(3)(p25q21), was heterozygous for the RAF1 RFLP which allowed the RAF1 locus to be placed between the 3p25 breakpoint and the centromere (compare Figures 7 and 11).

The D3F15S2E locus was shown to be tightly linked to RAF1 ($\hat{Z}_m$=3.311, θ_m=0.00; $\hat{Z}_f$=2.314, θ_f=0.07) as well as to 3cen ($\hat{Z}_m$=4.457, θ_m=0.03; $\hat{Z}_f$=6.426, θ_f=0.02). D3F15S2E:TF yielded positive lods ($\hat{Z}_m$=2.709, θ_m=0.00; $\hat{Z}_f$=1.257, θ_f=0.10) in the inv families. Lods for BCHE:TF were inconclusive ($\hat{Z}_m$=0.026, θ_m=0.34; $\hat{Z}_f$=0.225, θ_f=0.25)

3.320 Normal Families and Control Families

Lod scores from families with normal chromosomes gave inconclusive results for D3F15S2E:RAF1 ($\hat{Z}_m$=0.602, θ_m=0.00; $\hat{Z}_f$=0.301, θ_f=0.00) and 3cen:RAF1 ($\hat{Z}_m$=0.083 θ_m=0.39); but lod scores were relatively small for other RAF1 pairs.

PCCB was found to be tightly linked to TF ($\hat{Z}_m$=5.418, θ_m=0.00; $\hat{Z}_f$=2.321, θ_f=0.06) but showed no evidence of linkage to 3cen ($\hat{Z}_m$=0.000, θ_m=0.50; $\hat{Z}_f$=0.000, θ_f=0.50). TF showed

Table VII. Lod Scores Derived from Families With Normal Chromosomes

Locus Pair	Type	Segregation F	C	R:NR	Recombination Fraction 0.05	0.10	0.20	0.30	0.40	$\hat{Z}$	$\hat{\theta}$
3cen: D3F15S2E	P	8	18	1:3	-2.531	-1.279	-0.294	0.040	0.102	0.104	0.39
	M	2	4		-1.442	-0.888	-0.388	-0.152	-0.036	0.000	0.50
3cen:RAF1	P	9	29	3:5	-3.902	-1.930	-0.428	0.029	0.082	0.083	0.39
3cen:TF	P	9	32	1:3	-10.001	-5.929	-2.367	-0.779	-0.108	0.000	0.50
	M	13	53	1:1	-11.602	-6.641	-2.514	-0.840	-0.170	0.000	0.50
BCHE:TF	P	11	30	0:4	2.448	2.749	2.390	1.581	0.651	2.762	0.11
	M	8	21	1:1	-1.042	-0.200	0.262	0.229	0.077	0.300	0.24
D3F15S2E:RAF1	P	2	4		0.516	0.430	0.268	0.128	0.034	0.602	0.00
	M	1	2		0.258	0.215	0.134	0.064	0.017	0.301	0.00
D3F15S2E:TF	P	3	7	1:1	-1.184	-0.673	-0.552	-0.088	-0.019	0.000	0.50
	M	2	4	1:1	-0.463	-0.229	-0.062	-0.012	-0.001	0.000	0.50
PCCB:TF	P	5	23	0:4	4.651	4.163	3.116	1.972	0.784	5.418	0.00
	M	4	16	0:2	2.279	2.228	1.769	1.121	0.432	2.321	0.06

Abbreviations used are: P - paternal; M - maternal; F - number of families scored; C - number of children scored; R:NR - recombinant:non-recombinant phase known score.

moderate linkage to BCHE ($\hat{Z}_m$=2.762, $\hat{\theta}_m$=0.11; $\hat{Z}_f$=0.300, $\hat{\theta}_f$=0.24).

3.400 Recombinational Diagrams for Chromosome 3

Using the lod scores (Section 3.310 and 3.320, Tables VI and VII, Appendices 1 and 2) and available three point segregation data a recombinational diagram was developed as illustrated in Figures 8a and 8b. Due to the apparent crossover suppression effects of the inversion, separate recombinational diagrams were produced for both the inv(3) chromosome and the normal chromosome 3. In both diagrams, loci are placed in the presumed order and recombinational fractions corresponding to $\hat{Z}$ values which meet the requirements for formally significant lod scores (i.e., ≥ +2) are underlined.

3.410 The Inversion Chromosome

In the inversion chromosome (Figure 8a) 3cen would be expected to be located within the inversion near 3pter. Formally significant lod scores were obtained for 3cen:inv(3) for both male and female segregation results and showed a recombination fraction of 0.06 and 0.05 respectively. The recombination fractions for D3F15S2E:RAF1 and inv(3):RAF1, also corresponding to formally significant lod scores, suggested RAF1 was closer to the inversion breakpoint, which was consistent with physical mapping data.

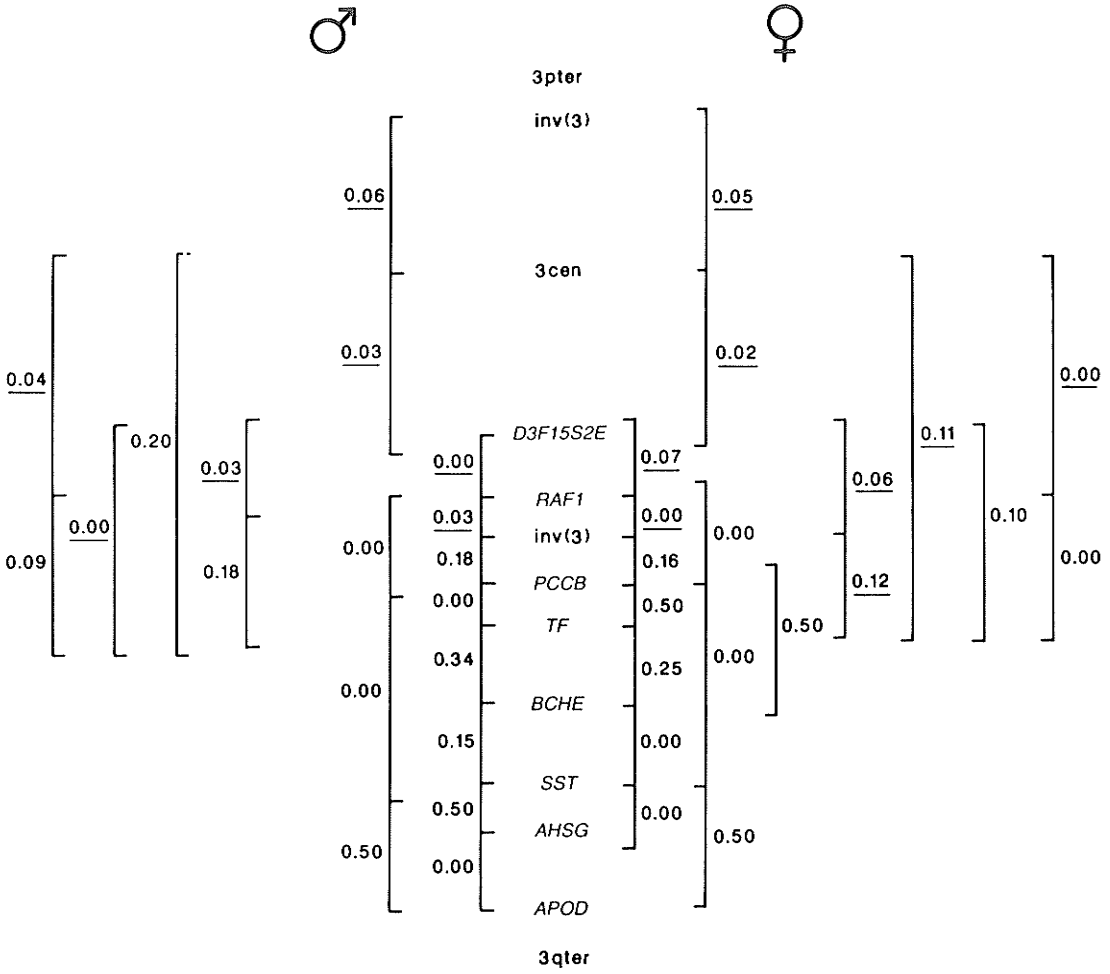

Figure 8a. Schematic representation of the combined results of linkage analysis and three point segregation analysis of inv(3) (p25 q21) chromosomes. Loci are shown in the presumed order. Recombination fractions corresponding to formally significant lod scores are underlined.

PCCB was placed outside the inversion since one family studied had two PCCB:inv(3) recombinant children, both of whom had normal karyotypes (compare Figures 7 and 11). This result was consistent with results of in situ and human-rodent somatic cell hybrid analysis using the PCCB probe (Allderdice et al., 1991). Lod scores for PCCB and the inv(3) breakpoint were inconclusive. However, inv(3) - PCCB - TF three point segregation analysis of a phase known family showed recombination between PCCB and TF and between the 3q21 breakpoint and TF but not between PCCB and 3q21 breakpoint. When the least number of crossovers is assumed, PCCB appears to be located between the 3q21 breakpoint and TF (Figure 9). BCHE and TF are placed on the diagram based on their physical map locations. The distal 3q markers could not be placed on the diagram using either linkage data or three point segregation results and as such the order shown is speculative and given only for the purposes of data presentation.

3.420 The Normal Chromosome 3

Physical mapping data were used to place D3F15S2E and RAF1 as no formally significant lods were obtained (Figure 8b). Both PCCB and TF appeared to segregate independently from 3cen. BCHE appeared moderately linked to TF but segregated independently from PCCB. Three point segregation analysis of a phase unknown family suggested an order of PCCB:TF:BCHE with five sibs informative for all three markers (Figure 10), supporting the results seen in the phase known

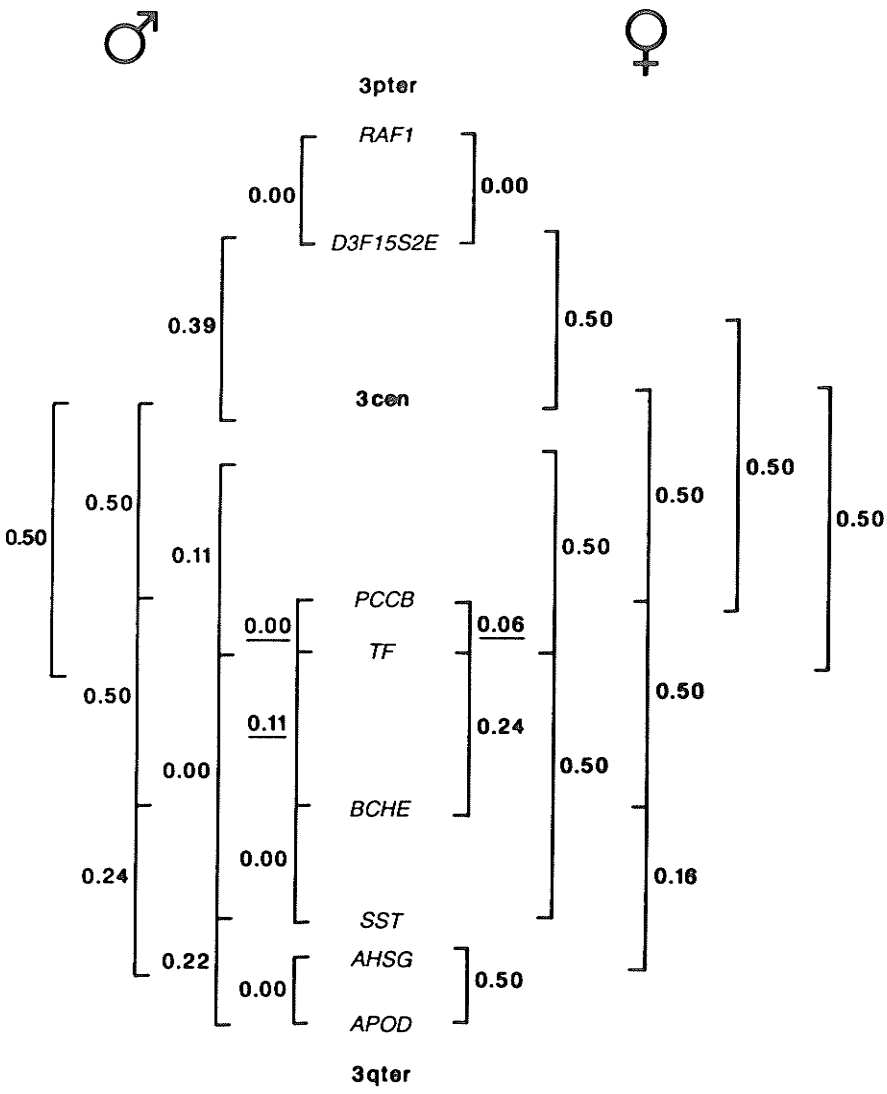

Figure 8b. Schematic representation of the combined results of linkage analysis and three point segregation analysis of normal human chromosome 3's. Loci are shown in the presumed order. Recombination fractions corresponding to formally significant lod scores are underlined.

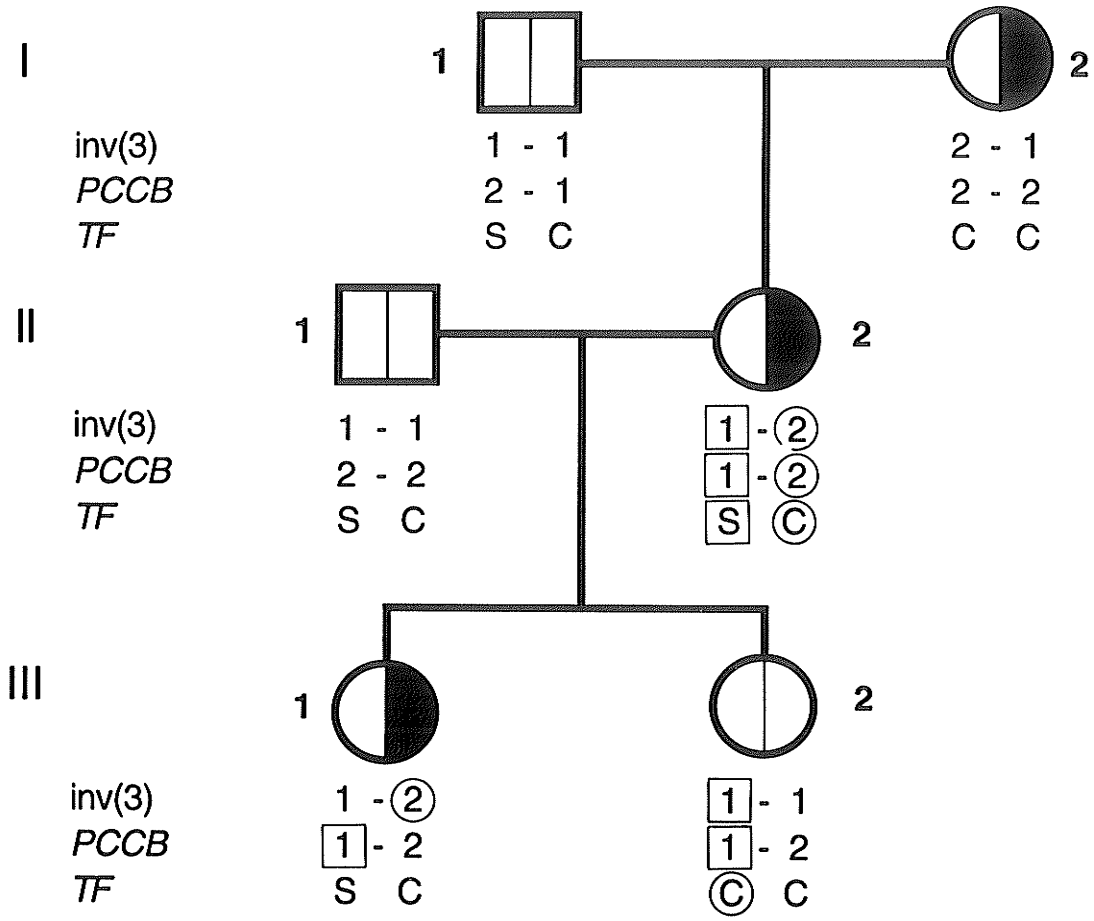

Figure 9. Phase known nuclear family segregating the inversion chromosome, informative for the inv(3), *PCCB* and *TF* markers. ◑ : Inversion heterozygotes; ◫, ⓘ : Normal karyotypes.
Grandpaternal alleles are within squares,
Grandmaternal alleles are within circles.

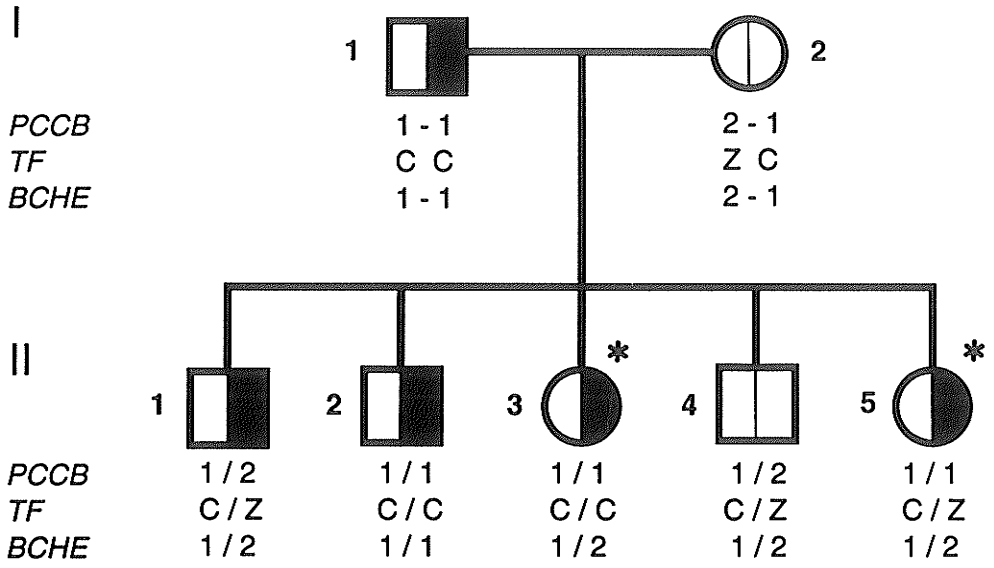

Figure 10. Phase unknown nuclear family with normal chromosomes, informative for the *PCCB, TF* and *BCHE* markers. In Generation II, paternal alleles are shown on left side of slash, maternal alleles are shown on the right. Asterisks indicate sibs of different class. ◨, ◑: Inversion heterozygote. ◫, ⦶: Normal karyotype.

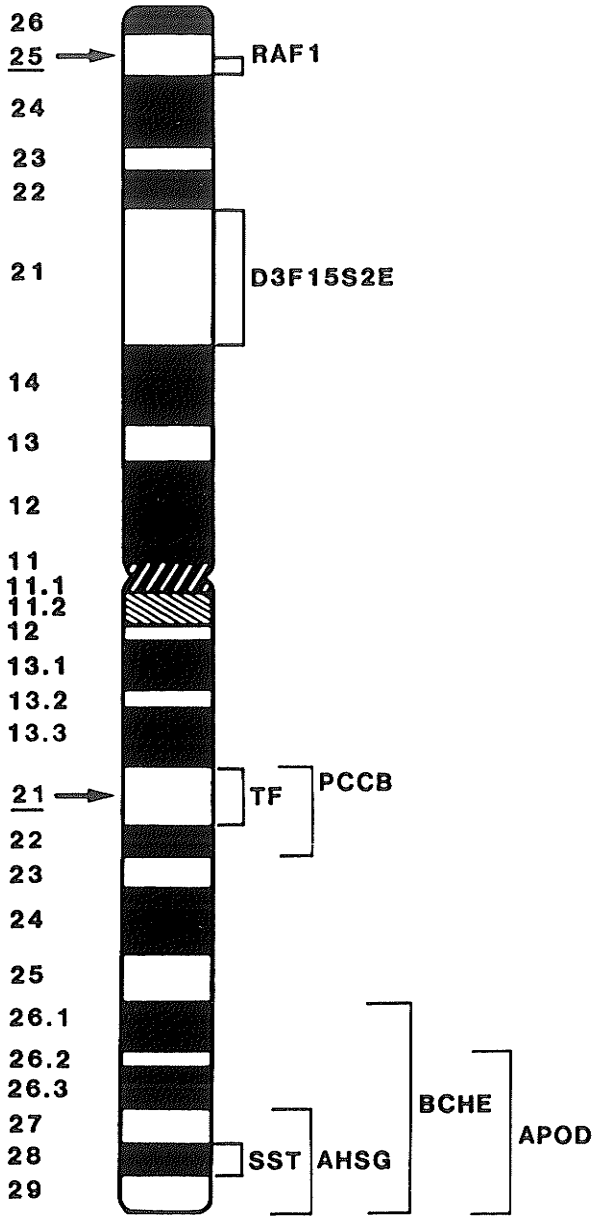

FIGURE 11. SCHEMATIC REPRESENTATIONS OF HUMAN CHROMOSOME 3 SHOWING REVISED REGIONAL LOCALIZATONS FOR RAF1 AND PCCB. (COMPARE WITH FIGURE 7).

inversion family. Because the phase unknown data are not formally conclusive, the order given for PCCB and TF must be regarded as tentative and the changes in the physical map reflect only the linkage and *in situ* hybridization results (Figure 11). As in the case of the inv(3) chromosome diagram, no formally significant lods were observed for the loci on distal 3q and, therefore, the order shown is simply used to represent the data and is speculative at this time.

3.500 Estimates of Heterogeneity in Recombinational Data

Table VIII gives the results of heterogeneity tests of lod scores for both inv(3) and normal chromosomes for the region 3p25→3q21. Results of heterogeneity tests for the region 3q21→qter are discussed separately in section 4.500.

Statistically significant deviations were found between inversion and normal chromosome 3 linkage data for the 3cen:D3F15S2E (male and female) 3cen:RAF1 (male) 3cen:TF (male and female) D3F15S2E:TF (male) PCCB:RAF1 (male) PCCB:TF (female) and RAF1:TF (male) locus pairs. With one exception, at least one marker of each locus pair was known to be located, by physical mapping or segregation analysis, within the inversion. The PCCB:TF locus pair is believed to be located at 3q21, the band containing the 3q breakpoint of the inversion. Although the lod scores for some of these locus pairs are not all formally significant, segregation was reasonably frequent in both the inv(3) and normal chromosome

linkage data (Table VI and Table VII) suggesting that the statistical analysis would be valid and that heterogeneity detected is probably not due to limited data of one chromosome type.

Table VIII. Chi-Square Test for Heterogeneity Between Recombination Fractions in the 3p25→3q21 Region of the inv(3) and Normal Chromosome 3 Results

Locus Pair	Sex	Chi-Square	DF	P	Sig.
3cen:D3F15S2E	M	8.66	1	0.00	**
	F	7.17	1	0.00	**
3cen:RAF1	M	6.63	1	0.00	**
	F	0.00	1	1.00	
3cen:TF	M	3.16	1	0.03	**
	F	8.02	1	0.00	**
D3F15S2E:TF	M	4.67	1	0.01	**
	F	0.93	1	0.27	
PCCB:RAF1	M	3.88	1	0.03	**
	F	0.99	1	0.25	
PCCB:TF	M	0.53	1	1.00	
	F	5.14	1	0.01	**
RAF1:TF	M	5.32	1	0.01	**
	F	0.00	1	1.00	

** Indicates significant deviation ($p<0.05$).

4.000 Discussion

4.100 Variation Detected in RFLP Phenotypes

All of the RFLP phenotypes examined coincided with the hybridization patterns described in the literature with the exception of PCCB, for which the 3.0 kb constant band was not detected in the Newfoundland kindred and rarely seen in chromosomally normal families.

The most likely explanation for this is that the stringencies of the washing protocols varied between the two laboratories. It is quite likely that the washing stringencies were variable as stringencies depend on a number of parameters including type of membrane used to bind the genomic DNA, the specific activity of the probe, the size of the homologous sequence of the probe and its stability during hybridization and the subsequent washing procedures. These factors, either alone or in concert, could conceivably lead to some variation.

4.200 Allele Associations with the inv(3) Chromosome

Suppression of crossing over within the inversion could be indicated by the relatively strong associations of RAF1*2 and D3F15S2E*1 with the inv(3) chromosome (section 3.200). Comparison of the linkage data also indicates an apparent reduction in crossing over within the inversion based on the heterogeneity tests for locus pairs in which RAF1 or D3F15S2E were included. These results suggest that recombination

within the inversion is not common in spite of the relative size of the inversion and the potential for double crossovers. However, given the physical localizations of RAF1, D3F15S2E and 3cen on chromosome 3 (Figure 7) some double recombinational events may not be detected. In order to examine this possibility in more detail additional probes detecting polymorphisms within the inversion could be tested to improve the likelihood of detecting most double crossovers.

Suppression of crossing over due to an inversion in a chromosome could also be evident for loci close to the breakpoints of the inversion but not located inside the inverted segment itself. Therefore, loci on 3q close to the 3q21 breakpoint could also show chromosome specific-allele associations. In the case of TF, the TF*C allele shows a significant association with the inv(3) chromosomes. PCCB, which appears to be closely linked to 3q21, also showed an allele-specific association of PCCB*C2 segregating with the inv(3) chromosome.

The remaining loci on 3q are located at a sufficient physical distance from the breakpoint at 3q21 such that they probably would not be expected to show the effects of the crossover suppression and no significant associations of specific BCHE, AHSG, SST or APOD alleles with the inv(3) chromosomes were detected. From the allele-chromosome associations that were found, the inv(3)(p25q21) chromosome usually carried the RAF1*2, D3F15S2E*1, PCCB*C2 and TF*C

alleles. These results may represent the alleles carried on the founder chromosome as recombination within the inversion does not appear to be very frequent.

4.300 Comparison of the Recombinational Data for the Normal and inv(3) Chromosomes

The physical map of chromosome 3, (refer Figure 7) shows that RAF1 would be expected to be the most distal locus at 3p25 and D3F15S2E more proximal to the centromere at 3p21. Both the inv(3) and normal chromosome 3 recombinational diagrams (refer Figure 8a and 8b respectively) are constructed to reflect these relative positions and the recombinational fractions observed indicate close linkage between them on the inv(3) chromosome.

Tests for heterogeneity between the linkage data for inversion and normal chromosomes indicate possible crossover suppression within the inversion in the RAF1 - D3F15S2E - 3cen segment (Table VI, Table VII and Table VIII). Tight linkage of about 3% recombination in males is observed for 3cen:D3F15S2E on inv(3) chromosomes, while on normal chromosomes linkage is formally excluded at less than 5% recombination in males. Similarly, tight linkage is observed for 3cen:RAF1 on inv(3) chromosomes (4% recombination) while linkage is formally exclude at less than 5% recombination on normal chromosomes. It should be noted that for 3cen:RAF1 lods scores from chromosomally normal individuals come close to formally excluding linkage at less than 10% recombination

(Z=-1.930). The placement of RAF1 near 3q21 on the inversion chromosome versus its typical placement at 3p25 (compare Figures 8a and 8b) is also supported by the linkage data for RAF1:TF (Appendix 1 and 2). Moderate linkage of 9% is suggested on inversion chromosomes, while linkage is formally excluded at less than 10% recombination on normal chromosomes.

The lod scores and segregation data also provide support for a proposed ordering of loci in the region 3q21→qter (Figures 8a and 8b). Two point segregation analysis of the PCCB locus and the 3q21 inversion breakpoint identified two recombinant children, both of which had normal karyotypes, indicating PCCB was located outside the inversion. These results are consistent with data obtained by in situ analysis (Allderdice et al., 1991).

Determining the local order of PCCB and TF relative to the centromere and to BCHE is complicated by the lack of formally significant lod scores and 3 point segregation data. Three point phase known segregation analysis of one inversion family suggested the order inv(3):PCCB:TF with one of two sibs informative for all three loci (Figure 9). Further support was obtained by three point phase unknown segregation analysis of five sibs from a chromosomally normal parent which suggested the order PCCB:TF:BCHE (Figure 10). The consistent order obtained assuming the fewest number of crossovers to explain the observed results is PCCB:TF:BCHE. In toto, these data indicate that both PCCB and TF are located at 3q21 with

PCCB possibly closer to the centromere than TF.

There is reasonably good agreement of the recombination fractions observed in chromosomally normal families with the previously published data for some locus pairs on 3q. The initial data regarding linkage between TF and BCHE indicated a male recombination fraction of 0.11 and a female recombination fraction of 0.19 (Robson et al., 1966). At Human Gene Mapping 7 (HGM 7) Eiberg et al. (1984) reported that TF and BCHE were 24 cM apart while BCHE and AHSG were 10 cM apart with lod scores of Zm=2.21 and Zm=5.02 for these pairs respectively. By Human Gene Mapping 10 (HGM 10) moderate linkage was suggested for TF:BCHE (Z_m=6.51, θ_m=0.18; Z_f=1.51, θ_f=0.27) and for BCHE:AHSG (Z_m=0.40, θ_m=0.34; Z_f=2.21, θ_f=0.18) (Keats et al., 1989). Recombination fractions calculated from chromosomally normal families in this study (refer Table VII and Appendix 2) for BCHE:TF (Z_m=2.764, θ_m=0.11; Z_f=0.300, θ_f=0.24) and for AHSG:BCHE (Z_m=0.852, θ_m=0.24; Z_f=0.217, θ_f=0.16) are consistent with those previously reported. The lods for AHSG:BCHE obtained in this study are not, however, formally significant. The recombination fractions calculated for the same locus pairs from the inv(3) families (refer Appendix 1) are not consistent, probably due to the relative lack of informative families. HGM 10 also indicates linkage between 3cen and TF (Z_m=3.21, θ_m=0.10; Z_f=1.68, θ_f=0.21) (Keats et al., 1989),

however, data from chromosomally normal families in this study (Table VII) suggest independent segregation between these loci with 32 male meioses and 53 female meioses scored.

The recombinational diagram in the region of 3qter contains some hypothetical placements for AHSG, SST and APOD. Lack of significant lod scores for these loci relative to each other and to BCHE made ordering difficult. BCHE has been shown to be proximal to the centromere relative to AHSG (Zelinski et al., 1987, McAlpine et al., 1987) but as no formally significant lod scores were obtained, and no three point segregation data were available, the order used in the diagram must be seen as purely speculative. The increase in recombination in telomeric regions of the chromosomes may explain why no formally significant lod scores were obtained and why the recombinational distances between these loci appear large in some instances.

4.400 Factors Influencing Results of Linkage Analysis

Linkage analysis requires that at least one parent be heterozygous at the loci under study and, unless the phase of the informative parent is known, at least two children available that can be phenotyped. The probability that an individual is heterozygous at a given locus is 2pq and, therefore, the frequency of double heterozygotes is: (2pq)(2p'q'). Depending on the frequency of each of the alleles at the loci under study, the number of heterozygotes detected may be low. This low frequency of heterozygotes

poses a greater problem when searching for triple heterozygotes to determine the order of three loci (Stringham, 1987). In this study lack of heterozygosity at some loci in many parents reduced the number of scorable offspring and consequently made it difficult to obtain lod scores which reached the required levels of significance. Furthermore, phase known information was available for only 23 of 64 segregating parents (including both inversion and normal families), and in some instances the phase information was only partial.

A further complicating factor in the analysis was the presence of untested parents and children. When no phenotype information was available for one parent, segregation information was lost or reduced unless the missing parental phenotype could be inferred from the phenotypes of the tested spouse and available children.

Finally, the fact that the Newfoundland kindred was a relatively isolated group with evidence of inbreeding in the pedigrees, and the inversion segregating in this kindred is believed to be the result of a founder effect, heterozygosity may have further been reduced in this group (Cavalli-Sforza et al., 1971).

In spite of these problems lod scores for a relatively large number of locus pairs were accumulated. The lack of formally significant scores for some locus pairs, particularly around the PCCB locus and SST locus, makes more precise

placement and orientation on the recombinational diagram difficult. This lack of significance will likely be overcome by accumulating additional lod scores for loci in these regions from other sets of families with normal chromosomes.

4.500 The Schultz-Redfield Effect

Comparisons of the recombinational diagrams for the inv(3) and normal chromosome 3 indicate that there may be a reduction in recombinational distances within the inversion. However, it is not clear that there is increased recombination in the 3q21→qter region of the inv(3) chromosome relative to the normal chromosome 3. In some cases the recombinational distances appear to be less on the inv(3) chromosomes while in other cases the reverse is true. This may be a reflection of the relative lack of segregation data, or may indicate that many more families would be required to identify an intrachromosomal Schultz-Redfield effect. There are an estimated average of 2.93 chiasmata per chromosome 3 (male meioses; Hulten, 1974) so the overall increase in recombination might be expected to be small. Therefore, the amount of data collected may be insufficient to identify an intrachromosomal Schultz-Redfield effect.

The maximum values for Z and the corresponding value of θ for loci in 3q21→qter were calculated by the MAP83 computer program (Sherman et al., 1984) after combining the normal and inv(3) lod scores (see Table IX). In calculating these

values, the program first checks for heterogeneity in the data to be combined (by locus pair) and determines whether the p value is significant. If the heterogeneity between the inv(3) lods and the normal lods is significant $\hat{Z}$ and $\hat{\theta}$ are calculated separately. As indicated in Table IX, significant heterogeneity was detected between the lod scores for the PCCB:TF locus pair only. As shown on the recombinational diagrams (Figures 8a and 8b) formally significant lod scores were not obtained in all instances and, therefore, the deviation seen here is likely due to the relatively small amount of data available with only two families segregating for female inversion heterozygotes. No other significant heterogeneity was detected between the lod scores from inv(3) families or those from families with normal chromosomes. Based on this analysis, there is no evidence of a Schultz-Redfield effect in the data presented in this study.

Table IX. Chi-Square Test for Heterogeneity Between Recombinational Fractions in the 3q21-qter Region of inv(3) and Normal Chromosome 3 Results.

Locus Pair	Sex	Chi-Square	DF	P	Sig.
AHSG:BCHE	M	0.00	0	1.00	
	F	0.29	1	0.53	
AHSG:PCCB	M	0.00	0	1.00	
	F	1.23	1	0.20	
AHSG:SST	M	0.00	0	1.00	
	F	0.00	0	1.00	
AHSG:TF	M	0.00	0	1.00	
	F	1.04	1	0.24	
AHSG:APOD	M	-0.14	1	1.00	
	F	0.00	0	1.00	
APOD:BCHE	M	0.00	1	1.00	
	F	0.00	0	1.00	
APOD:PCCB	M	0.00	1	1.00	
	F	1.20	1	0.21	
APOD:SST	M	0.36	1	0.49	
	F	0.00	0	1.00	
APOD:TF	M	0.70	1	0.33	
	F	0.00	1	1.00	
BCHE:PCCB	M	0.00	0	1.00	
	F	0.00	1	0.98	
BCHE:SST	M	0.88	1	0.28	
	F	0.00	0	1.00	
BCHE:TF	M	1.64	1	0.14	
	F	0.04	1	0.81	

Table IX. continued

Locus Pair	Sex	Chi-Square	DF	P	Sig.
PCCB:SST	M	0.00	0	1.00	
	F	1.34	1	0.18	
PCCB:TF	M	-0.58	1	1.00	
	F	5.14	1	0.01	**
SST:TF	M	0.00	0	1.00	
	F	0.00	0	1.00	

** indicates significant deviation ($p < 0.05$).

5.000 Summary

1. Analysis of the segregation data for allele-chromosome associations showed statistically significant associations between each of the RAF1*2, D3F15S2E*1, PCCB*C2 and TF*C alleles and the inversion chromosome. No associations were found for BCHE, AHSG, SST and APOD.
2. Recombination fractions between pairs of loci on normal and inv(3) chromosomes have been estimated. RAF1 has been found to lie within the inversion, close to the 3p25 breakpoint. The order 3cen:PCCB:TF:BCHE has been proposed from three point segregation information. Insufficient segregation information precluded ordering SST, AHSG and APOD.
3. Analysis of segregation and the recombinational data did not show any clear statistical evidence of an intrachromosomal Schultz-Redfield effect.

Appendix 1. Lod Scores Derived from Families Segregating for the Inversion Chromosome

Locus Pair	Type	Segregation F	Segregation C	R:NR	Recombination Fraction 0.05	0.10	0.20	0.30	0.40	$\hat{Z}$	$\hat{\theta}$
3cen:AHSG	P	8	23		-3.018	-1.553	-0.444	-0.092	-0.007	0.000	0.50
	M	7	22		-2.978	-1.517	-0.394	-0.050	0.009	0.014	0.44
3cen:APOD	P	5	19		-3.198	-1.873	-0.746	-0.269	-0.060	0.000	0.50
	M	3	10		-0.371	0.047	0.263	0.211	0.075	0.264	0.21
3cen:BCHE	P	3	10	0:3	-0.348	0.094	0.358	0.351	0.220	0.387	0.25
	M	2	7	2:5	-0.605	-0.122	0.224	0.286	0.201	0.287	0.29
3cen:PCCB	P	3	14		-0.371	0.047	0.263	0.211	0.075	0.264	0.21
	M	6	20	2:2	1.977	1.931	1.496	0.900	0.315	1.979	0.06
3cen:SST	P	5	13		-2.092	-1.095	-0.324	-0.068	-0.006	0.000	0.50
	M	1	8		0.650	0.787	0.730	0.530	0.193	0.804	0.14
AHSG:APOD	P	1	5		1.093	0.975	0.720	0.436	0.149	1.204	0.00
AHSG:BCHE	M	1	2		0.258	0.215	0.134	0.064	0.017	0.301	0.00
AHSG:D3F15S2E	P	1	2		-0.721	-0.444	-0.194	-0.076	-0.018	0.000	0.50
	M	4	12		-1.648	-0.902	-0.314	-0.099	-0.020	0.000	0.50
AHSG:inv(3)	P	11	33		-2.511	-1.039	0.007	0.182	0.077	0.182	0.30
	M	10	40		-1.364	-0.117	0.536	0.438	0.149	0.564	0.23
AHSG:PCCB	M	1	2		0.258	0.215	0.134	0.064	0.017	0.301	0.00
AHSG:RAF1	P	1	2		-0.721	0.444	-0.194	-0.076	-0.018	0.000	0.50
	M	3	7		-1.184	-0.673	-0.254	-0.088	-0.019	0.000	0.50

Appendix 1. continued

Locus Pair	Type	Segregation F	Segregation C	R:NR	Recombination Fraction 0.05	0.10	0.20	0.30	0.40	$\hat{Z}$	$\hat{\theta}$
AHSG:SST	P	2	7		-1.184	-0.672	-0.254	-0.087	-0.018	0.000	0.50
	M	1	2		0.258	0.215	0.133	0.064	0.017	0.301	0.00
AHSG:TF	M	2	5	0:2	-0.163	-0.067	0.214	0.216	0.140	0.225	0.25
APOD:BCHE	P	2	5		-1.442	-0.888	-0.388	-0.152	-0.036	0.000	0.50
APOD:D3F15S2E	P	3	12		-2.349	-1.310	-0.458	-0.133	-0.022	0.000	0.50
	M	2	6		0.093	0.276	0.323	0.222	0.076	0.325	0.18
APOD:inv3	P	6	23		-2.442	-1.198	-0.122	-0.054	0.013	0.018	0.43
	M	4	15		-0.132	0.298	0.473	0.337	0.113	0.473	0.20
APOD:PCCB	P	1	5		-1.442	-0.887	-0.388	-0.151	-0.035	0.000	0.50
	M	2	10		0.093	0.276	0.323	0.222	0.076	0.325	0.18
APOD:RAF1	P	2	10		-1.628	-0.866	-0.264	-0.057	-0.004	0.000	0.50
	M	1	4		0.814	0.720	0.517	0.298	0.094	0.903	0.00
APOD:SST	P	2	5		-1.184	-0.672	-0.254	-0.087	-0.018	0.000	0.50
	M	1	3		-0.721	-0.444	-0.194	-0.076	-0.018	0.000	0.50
APOD:TF	P	2	9		-0.649	-0.207	0.064	0.083	0.031	0.086	0.28
	M	1	2		-0.721	-0.444	-0.194	-0.076	-0.018	0.000	0.50
BCHE:D3F15S2E	P	3	11		0.318	0.522	0.554	0.405	0.195	0.564	0.16
	M	3	6	0:4	1.374	1.235	0.950	0.648	0.333	1.505	0.00
BCHE:inv(3)	P	5	17	0:3	-0.954	-0.028	0.582	0.638	0.422	0.652	0.27
	M	4	11	2:4	-0.368	0.052	0.288	0.268	0.156	0.307	0.24

Appendix 1. continued

Locus Pair	Type	Segregation F	Segregation C	R:NR	Recombination Fraction 0.05	0.10	0.20	0.30	0.40	$\hat{z}$	$\hat{\theta}$
BCHE:PCCB	M	1	3		-0.721	-0.444	-0.194	-0.076	-0.018	0.000	0.50
BCHE:RAF1	P	2	8	0:3	-0.606	-0.121	0.224	0.287	0.203	0.288	0.29
	M	2	5	0:3	1.095	0.980	0.746	0.502	0.254	1.204	0.00
BCHE:SST	P	2	7		0.369	0.529	0.524	0.358	0.130	0.580	0.15
	M	1	2		0.258	0.215	0.134	0.064	0.017	0.301	0.00
D3F15S2E:PCCB	P	2	8		0.372	0.531	0.526	0.360	0.131	0.581	0.15
D3F15S2E:SST	P	3	8		-1.906	-1.117	-0.448	-0.163	0.037	0.051	0.43
inv(3):PCCB	P	4	18		0.166	0.517	0.600	0.413	0.147	0.603	0.18
	M	7	22	1:2	0.496	1.005	1.073	0.710	0.225	1.096	0.16
inv(3):SST	P	4	14		-3.070	-1.753	-0.652	-0.272	-0.040	0.000	0.50
	M	3	10		-0.068	0.342	0.534	0.434	0.174	0.534	0.21
PCCB:RAF1	P	1	5		1.093	0.975	0.720	0.436	0.149	1.204	0.00
	M	1	1	0:1	1.093	0.975	0.720	0.436	0.149	1.204	0.00
PCCB:SST	P	1	3		0.533	0.465	0.318	0.170	0.049	0.602	0.00
	M	1	6		0.533	0.465	0.318	0.170	0.049	0.602	0.00
RAF1:SST	P	1	2		-0.721	-0.444	-0.194	-0.076	-0.018	0.000	0.50

Appendix 1. continued

Locus Pair	Type	Segregation F	Segregation C	R:NR	Recombination Fraction 0.05	0.10	0.20	0.30	0.40	$\hat{Z}$	$\hat{\theta}$
RAF1:TF	P	2	12		1.464	1.508	1.252	0.818	0.309	1.525	0.09
	M	3	5	0:3	1.095	0.981	0.746	0.502	0.254	1.204	0.00

Abbreviations used are: P - paternal; M - maternal; F - number of families scored; C - number of children scored; R:NR - recombinant:non-recombinant phase known score.

Appendix 2 Lod Scores Derived from Families With Normal Chromosomes

Locus Pair	Type	Segregation F	Segregation C	R:NR	Recombination Fraction 0.05	0.10	0.20	0.30	0.40	$\hat{Z}$	$\hat{\theta}$
3cen:AHSG	P	6	21		-6.233	-3.779	-1.611	-0.617	-0.143	0.000	0.50
	M	4	12	1:1	-2.369	-1.346	-0.508	-0.175	-0.037	0.000	0.50
3cen:APOD	P	3	7		-0.045	0.136	0.192	0.126	0.039	0.193	0.19
	M	4	12		-1.370	-0.651	-0.130	0.007	0.012	0.012	0.38
3cen:BCHE	P	3	15		-2.792	-1.498	-0.452	-0.081	0.006	0.015	0.44
	M	5	14		-1.390	-0.687	-0.180	-0.035	-0.003	0.000	0.50
3cen:PCCB	P	5	20	0:3	-5.069	-3.147	-1.428	-0.613	-0.187	0.000	0.50
	M	3	13		-3.348	-2.003	-0.836	-0.313	-0.071	0.000	0.50
3cen:SST	P	2	5		-1.442	-0.888	-0.388	-0.152	-0.036	0.000	0.50
	M	1	3		-0.721	-0.444	-0.194	-0.076	-0.018	0.000	0.50
AHSG:APOD	P	1	2		0.258	0.215	0.134	0.060	0.017	0.301	0.00
	M	2	9		-1.628	-0.865	-0.264	-0.056	-0.004	0.000	0.50
AHSG:BCHE	P	8	25	1:5	-0.882	0.201	0.815	0.737	0.386	0.852	0.24
	M	4	8	1:1	0.053	0.201	0.208	0.116	0.033	0.217	0.16
AHSG:D3F15S2E	M	1	2		-0.721	-0.444	-0.194	-0.076	-0.018	0.000	0.50
AHSG:PCCB	P	3	17		-1.114	-0.437	0.004	0.072	0.030	0.072	0.30
	M	4	13		-2.368	-1.344	-0.508	-0.174	-0.036	0.000	0.50
AHSG:RAF1	P	4	11		0.609	0.706	0.591	0.350	0.110	0.710	0.11
	M	1	3		-0.721	-0.444	-0.194	-0.076	-0.018	0.000	0.50

Appendix 2. continued

Locus Pair	Type	Segregation F	Segregation C	R:NR	Recombination Fraction 0.05	0.10	0.20	0.30	0.40	$\hat{Z}$	$\hat{\theta}$
AHSG:TF	P	17	62	2:6	-5.160	-1.690	0.615	0.857	0.484	0.886	0.29
	M	28	88	12:8	-19.307	-11.223	-5.220	-1.855	-0.654	0.000	0.50
APOD:BCHE	P	4	11		-2.626	-1.560	-0.642	-0.239	-0.054	0.000	0.50
	M	2	7		-1.184	-0.672	-0.254	-0.087	-0.018	0.000	0.50
APOD:D3F15S2E	P	1	2		0.258	0.215	0.134	0.064	0.017	0.301	0.00
	M	1	2		0.258	0.215	0.134	0.064	0.017	0.301	0.00
APOD:PCCB	P	1	3		-0.721	-0.444	-0.194	-0.076	-0.018	0.000	0.50
	M	3	10		-1.905	-1.116	-0.448	-0.163	-0.036	0.000	0.50
APOD:RAF1	P	2	4		0.516	0.430	0.268	0.128	0.034	0.602	0.00
	M	1	4		0.814	0.720	0.517	0.298	0.094	0.903	0.00
APOD:SST	P	3	7		-0.205	-0.014	0.074	0.052	0.016	0.077	0.22
APOD:TF	P	1	2		0.258	0.215	0.134	0.064	0.017	0.301	0.00
	M	2	8		-2.163	-1.331	-0.582	-0.227	-0.053	0.000	0.50
BCHE:D3F15S2E	P	2	5		-1.442	-0.888	-0.388	-0.152	-0.036	0.000	0.50
	M	2	4		0.516	0.430	0.268	0.128	0.034	0.602	0.00
BCHE:PCCB	P	1	3		-0.721	-0.444	-0.194	-0.076	-0.018	0.000	0.50
	M	2	7		-0.926	-0.427	-0.120	-0.023	-0.001	0.000	0.50
BCHE:RAF1	P	2	6		-1.185	-0.673	-0.254	-0.087	-0.019	0.000	0.50
BCHE:SST	P	2	5		0.793	0.680	0.452	0.234	0.066	0.903	0.00

Appendix 2. continued

Locus Pair	Type	Segregation F	Segregation C	R:NR	Recombination Fraction 0.05	0.10	0.20	0.30	0.40	$\hat{Z}$	$\hat{\theta}$
D3F15S2E:PCCB	P	3	6	0:1	-1.463	-0.928	-0.458	-0.234	-0.098	0.000	0.50
	M	2	8	1:1	-0.463	-0.229	-0.060	-0.012	-0.001	0.000	0.50
D3F15S2E:SST	P	2	5		0.783	0.680	0.452	0.234	0.066	0.903	0.00
PCCB:RAF1	P	2	11		-3.348	-2.003	-0.836	-0.314	-0.071	0.000	0.50
	M	1	3		-0.721	-0.444	-0.194	-0.076	-0.018	0.000	0.50
PCCB:SST	P	1	2		-0.721	-0.444	-0.194	-0.096	-0.018	0.000	0.50
	M	2	2		-0.463	-0.229	-0.060	-0.012	-0.001	0.000	0.50
RAF1:SST	P	1	2		0.258	0.215	0.134	0.064	0.017	0.301	0.00
	M	1	6		-1.185	-0.673	-0.254	-0.087	-0.018	0.000	0.50
RAF1:TF	P	4	17		-4.790	-2.891	-1.224	-0.466	-0.107	0.000	0.50
SST:TF	P	1	2		0.258	0.215	0.134	0.064	0.017	0.301	0.00
	M	1	3		-0.721	-0.444	-0.194	-0.076	-0.018	0.000	0.50

Abbreviations used are: P - paternal; M - maternal; F - number of families scored; C - number of children scored; R:NR - recombinant:non-recombinant phase known score.

References

Allderdice, P. W. Browne, N., and Murphy, D. P., (1975) Chromosome 3 duplication q21→qter deletion p25→pter syndrome in children of carriers of a pericentric inversion inv (3) (p25q21). Am J Hum Genet, 27:699-718.

Allderdice, P. W. Galutira, D., Lockridge, O., LaDu, B. N., and McAlpine, P. J. (1991) The cloned butyrylcholinesterase (BCHE) gene maps to a single chromosome site 3q26.2. Genomics (submitted).

Ashton, B. A., Hohling, H. J., Triffitt, J. T. (1976) Plasma proteins present in human cortical bone: enrichment of the α2-HS-glycoprotein. Calcif Tissue Res, 22:27-33.

Barnard, G. A. (1949) Statistical inference. J Roy Stat Soc, B11:115-135.

Bonner, T., O'Brien, S. J., Nash, W. G., and Rapp, U. R. (1984) The Human homologs of the raf (mil) oncogene are located on human chromosomes 3 and 4. Science, 223:71-74.

Bonner, T., Oppermann, H., Seeburg, P., Kerby, S. B., Gunnell, M. A., Young, A. C., and Rapp, U. R. (1986) The complete coding sequence of the human raf oncogene and the corresponding structure of the c-raf-1 gene. Nucleic Acids Res, 14:1009-1015.

Botstein, D., White, R. L., Skolnick, M., and Davis, R. W. (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet, 32:314-331.

Bourne, J. G., Collier, H. O. J., and Somers, G. F. (1952) Succinylcholine (succinoylcholine). Muscle relaxant of short action. Lancet, 1:1225.

Bradley, W. P., Blasco, A. P., Weiss, J. F., Alexander, J. C., Silverman, N. A., Chretien, P. B. (1977) Correlations among serum protein-bound carbohydrates, serum glycoproteins, lymphocyte reactivity, and tumour burden in cancer patients. Cancer, 40:2264-2272.

Brazeau, P., Vale, W., Burgus, R., Ling, N., Butcher, M., Rivier, J., and Guillemin, R. (1973). Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. Science, 179:77-79.

Carritt, B., Welch, H. M., and Parry-Jones, N. J. (1986) Sequences homologous to the human D1S1 locus present on human chromosome 3. Am J Hum Genet, 38:428-436.

Caspersson, T., Zech, L., Johansson, C., et al. (1970) Identification of human chromosomes by DNA-binding flourescent agents. Chromosoma 30:215-227.

Cavalli-Sforza, L. L., and Bodmer, W. F. (1971) The Genetics of Human Populations. W. H. Freeman and Company, San Francisco pp. 341-353.

Conneally, P. M., and Rivas, M. L. Linkage analysis in man in Advances in Human Genetics Vol 10, H. Harris and K. Hirschhorn (eds). Plenum Press, New York; 1980, pp. 209-266.

Constans, J., Viau, M., Jaeger, G., and Polisson, M. J. (1981) Gc, Tf, Hp subtype and α_1-antitrypsin polymorphisms in a pygmy bi-aka sample. Hum Hered 31:129-137.

Cote, G. B. (1975) Centromeric linkage in man. Ph.D. thesis, University of Birmingham, Birmingham, U.K.

Dickson, I. R., Bagga, M. K. (1985) Changes with age in non-collagenous proteins of human bone. Connect Tissue Res, 4:77-85.

Dobzhansky, T., and Spassky, B. (1947) Evolutionary changes in laboratory cultures of D. pseudobscura, Evolution 1:191-216.

Drayna, D., Fielding, C., McLean, J., Baer, B., Castro, G., Chen, E., Comstock, L., Henzel, W., Kohr, W., Rhee, L., Wion, K., and Lawn, R. (1986) Cloning and expression of human apolipoprotein D cDNA. J Biol Chem, 261:16535-16539.

Drayna, D., Scott, J. D., and Lawn, R. (1987) Multiple RFLP's at the human apolipoprotein D (APOD) locus. Nucleic Acids Res, 15:9617.

Drets, M. E., and Shaw, M. W. (1971) Specific banding patterns of human chromosomes. Proc Natl Acad Sci USA, 68:2073-2077.

Eiberg, H., Mohr, J., and Nielsen, L. S. (1984) A2HS: new methods of phenotyping and analysis of linkage relations: Assignment to chromosome 3. Cytogenet Cell Genet 37:461.

Evans, F. T., Gray, P. W. S., Lehmann, H., and Silk, E. (1952) Sensitivity to succinyl-choline in relation to serum cholinesterase. Lancet 1:1229.

Goode, M. E., van Tuinen, P., Ledbetter, D. H., and Daiger, S. P. (1986) The anonymous polymorphic DNA clone D1S1, previously mapped to human chromosome 1p36 by in situ hybridization, is from chromosome 3 and is duplicated on chromosome 1. Am J Hum Genet, 38:437-446.

Grodzicker, T., Williams, J., Sharp, P., Sambrook, J. (1974) Physical mapping of temperature-sensitive mutations of adenoviruses. Cold Spring Harbour Symp Quant Biol 39:439-446.

Haldane, J. B. S., and Smith, C. A. B. (1947) A new estimate of the linkage between the genes for colour blindness and haemophilia in man, Ann Eugen, 14:10-31.

Hamerton, J. L. (1983) Population Cytogenetics: a Perspective, in Paediatric Research A Genetic Approach. M. Adinolfi and F. Giannelli eds. Lippincott.

Harris, H. (1980) The Principles of Human Biochemical Genetics 3rd rev. ed., Elsivier/North Holland Biomedical Press, New York.

Hook, E. B., Hamerton, J. L. (1977) The frequency of chromosome abnormalities detected in consecutive newborn studies - differences between studies - results by sex and by severity of phenotypic involvement, in Population cytogenetics - Studies in humans, Hooke, E. B. and Porter, I. H. eds., Academic Press, New York.

Hulten, M. (1974) Chiasma distribution at diakinesis in the normal human male. Hereditas 76:55-78.

Jeffreys, A. J., Wilson, V., and Thein, S. L. (1985) Hypervariable "minisatellite" regions in human DNA. Nature, 314:67-73.

Jeffreys, A. J., Royle, N. J., Wilson, V., and Wong, Z. (1988) Spontaneous mutation rates to new length alleles at tandem-repetitive hypervariable loci in human DNA. Nature, 332:278-281.

Jeltsch, J. M., and Chambon, P. (1982) The complete nucleotide sequence of the chicken ovotransferrin mRNA. Eur J Biochem, 122:291-295.

Kaiser, P. (1984) Pericentric Inversions: Problems and significance for clinical genetics. Hum Genet, 68:1-47.

Keats, B. J. B., Conneally, P. M., and Lalouel, J. M. (1987) Report of the committee on linkage data and gene order. Cytogenet Cell Genet, 46:339-343.

Keats, B., Ott, J., and Conneally, M. (1989) Report of the committee on linkage and gene order. Cytogenet Cell Genet 51:459-502.

Kidd, K., Bowcock, A. M., Schmidtke, J., Track, R. K., Ricciuti, F., Hutchings, G., Bale, A., Pearson, P., and Willard, H. F. (1989). Report of the DNA committee and catalogs of cloned and mapped genes and DNA polymorphisms. Cyto Genet Cell Genet 51:622-947.

Koerker, D. J., Ruch, W., Chideckel, E., Palmer, J., Goodner, C. J., Ensinck, J., and Gale, C. C. (1974) Somatostatin: Hypothalamic inhibitor of the endocrine pancreas. Science 184:482-484.

Kok, K., Osinga, J., Carritt, B., Davis, M. B., van der Hout, A. H., and others, (1987) Deletion of a DNA sequence at the chromosomal region 3p21 in all major types of lung cancer. Nature, 330:578-581.

Kraus, J. P., Williamson, C. L., Firgaira, F. A., Yang-Feng, T. L., Munke, M., Francke, U., and Rosenberg, L. E. (1986) Cloning and screening with nanogram amounts of immunopurified mRNA's: cDNA cloning and chromosomal mapping of cystathionine β-synthetase and the β-subunit of propionyl - CoA carboxylase. Proc Natl Acad Sci USA, 83:2047-2051.

Kuhnl, P., and Spielmann, W. (1979) A Third common allele in the transferrin system, $\underline{TF}^{c3}$, detected by isoelectric focusing. Hum Genet 50:193-198.

Lamhonwah, A. M., Barankiewicz, T., Willard, H. F., Mahuran, D., Quan, F., Gravel, R. A. (1986) Isolation of cDNA clones coding for the α and β chains of human propionyl - CoA carboxylase: Chromosomal assignments and DNA polymorphisms associated with PCCA and PCCB genes. Proc Natl Acad Sci USA, 83:4864-4868.

Lebreton, J. P., Joisel, F., Raoult, J. P., Lannuzel, B., Rogez, J. P., Humbert, G. (1979) Serum concentration of human α2-HS-glycoprotein during the inflammatory process. J Clin Invest, 64:1118-1129.

Litt, M., and Luty, J. A. (1989) A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. Am J Hum Genet 44:397-401.

MacGillvray, R. T. A., Mendez, E., Shewal, J. G., Sinha, S. K., Lineback-Zins, J., and Brew, K. (1983) The primary structure of human serum transferrin. J Bio Chem, 258:3543-3553.

Magnuson, V. L., McCombo, J. L., Lee, C. C., Yang, F., Bowman, B. H., and McGill, J. R. (1988) Human α2-HS-glycoprotein localized to 3q27→q29 by in situ hybridization. Cytogenet Cell Genet, 47:72-74.

Maniatis, T., Fritsch, E. F., Sambrook, J. (1982) Molecular Cloing A Laboratory Manual. Cold Spring Harbour Laboratory, USA.

Maynard-Smith, S., Penrose, L. S., and Smith, C. A. B. (1961) Mathematical Tables for Research Workers in Human Genetics. J. H. Churchill Ltd., London.

McAlpine, P. J., Allderdice, P. W., Cox, D. W., Simpson, N. E., McEarchran, M., Komarnicki, L. (1987) The ordering of TF:CHE1:AHSG and their orientation distal to 3q21, Cytogenet Cell Genet, 46:659.

McAlpine, P. J., Shows, T. B., Boucheix, C., Stranc, L. C., Berent, T. G., Pakstis, A. J., and Doute, R. C. (1989). Report of the nomenclature committee and the 1989 catalog of mapped genes. Workshop on Human Gene Mapping. Cytogenet Cell Genet, 51:13-66.

McAlpine, P. J., Dixon, M., Allderdice, P. W., Lockridge, O., and La Du, B. N. (1991) The butyrylcholinesterase gene (BCHE) at 3q26.2 shows two RFLP's. Nucl Acid Res, in press.

McConathy, W. J., and Alaupovic, P. (1973) Isolation and partial characterization of Apolipoprotein D: A new protein moiety of the human plasma lipoprotein system. FEBS Letter 37:178-182.

McConathy, W. J., and Alaupovic, P. (1976) Studies on the isolation and partial characterization of Apolipoprotein D and Lipoprotein D of human plasma. Biochemistry 15:515-520.

McGuire, M., and Agre, P. (1987) Three distinct variants of protein 4.1 in caucasian hereditary elliptocytosis. Clin Res 35:428A.

McGuire, M. C., Nogueira, C. P., Bartels, C. F., Lightstone, H., Hajra, A., Van Der Spek, A. F. L., Lockridge, O., and La Du, B. N. (1989) Identification of the structural mutation responsible for dibucaine - resistant (atypical) variant form of serum cholinesterase. Proc Natl Acad Sci USA 86:953-957.

Metz-Boutigue, M. H., Mazurier, J., Jolles, J., Spik, G., Montreuil, J., and Jolles, P. (1981) The present state of the human lactotransferrin sequence. Biochem Biophys Acta, 670:243-254.

Morton, N. E. (1955) Sequential Tests for the Detection of linkage, Am J Hum Genet, 7:277-318.

Morton, N. E. (1956) The Detection and estimation of linkage between the genes for elliptocytosis and the Rh blood type. Am J Hum Genet, 8:80-96.

Muller, H. J. (1928) cited in Cytogenetic Plants, Animals, Humans J. Schulz-Schaeffer Springer Verlag, New York (1985).

Naylor, S. L., Sakaguchi, A., Shen, L., Bell, G., Rutter, W., Shows, T. B. (1983) Polymorphic human somatostatin gene is located on chromosome 3. Proc Natl Acad Sci USA, 80:2686-2689.

Naylor, S. L., and Bishop, D. T. (1989) Report of the committee on the genetic constitution of chromosome 3. Cytogenet Cell Genet, 51:100-120.

Naylor, S. L., Marshall, A., Hansel, C., Martinez, P. F., Holley, B., and Sakaguchi, A. Y. (1989) The DNF15S2 Locus at 3p21 is transcribed in normal lung and small cell lung cancer. Genomics, 4:355-361.

Naylor, S. L., and Bishop, D. T., (1989). Report of the committee on the genetic constitution of chromosome 3. Cytogenet Cell Genet 51:106-120.

Quelch, K. J., Cole, W. G., Melick, R. A. (1984) Noncollagenous proteins in normal and pathological human bone. Calcif Tissue Int, 36:545-549.

Rao, D. C., Keats, B. J. B., Morton, N. E., Yeo, S., and Lew, R. (1978) Variability of human linkage data. Am J Hum Genet, 30:516-529.

Ravindranath, Y., and Johnson, R. M. (1985) Altered spectrin association and membrane fragility without abnormal spectrin heat sensitivity in a case of congenital haemolytic anaemia. Amer J Hemat 20:53-65.

Renwick, J. H. (1969) Progress in mapping human autosomes. British med bulletin. 25:65-73.

Robson, E. B., Sutherland, I., and Harris, H. (1966) Evidence for linkage between the transferrin locus TF and the serum cholinesterase locus E1 in man. Ann Hum Genet, Lond. 29:325-332.

Schultz, J., and Redfield, H. (1951) Interchromosomal Effects on Crossing Over in Drosophila in Cold Spring Harbour Symposia on Quantitative Biology Vol. XVI, Genes and Mutations. The Biological Laboratory, Cold Spring Harbour, L. T. New York.

Schulz-Schaeffer, J. (1985) Cytogenetics Plants Animals Humans Spring-Verlag, New York.

Seizinger, B. R., Rouleau, G. A., Ozelius, L. J., Lane, A. H., Farmer, G. E., and others (1988) Von Hippel-Lindau disease maps to the region of chromosome 3 associated with renal cell carcinoma. Nature, 332:268-269.

Shen, L. P., Pictet, R. L., and Rutter, W. J. (1982) Human somatostatin I: sequence of cDNA. Proc Natl Acad Sci USA, 79:4575-4579.

Sherman, S. L., King, J., Robson, E. B., and Yee, S. (1984) A revised map of chromosome 1 Ann Hum Genet, 48:243-251.

Southern, E. M. (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis, J Mol Biol, 98:503-517.

Starkenstein, E., and Harvalik, Z. (1933) in Iron Metabolism I. Bernat ed. Plenum Press, New York 1983, pp. 78-82.

Steel, C. M., and Edmond, E. (1971) Human lymphoblastoid cell lines. I. Culture methods and examination for Epstein-Barr virus, J Natl Cancer Inst 47:1193-1201.

Stringham, E. G. (1987) Gene mapping studies of chromosome 8 in man. M.Sc. thesis, University of Manitoba.

Sturtevant, A. H. (1926) A crossover reducer in Drosophila melanogaster due to inversion of a section of the third chromosome, Biol. Zbl., 46:697.

Thompson, M. W., and Thompson, J. S. (1986) Linkage and Mapping. in Genetics in Medicine (4^{th} ed.). W. B. Saunders Company, Toronto; pp. 193-209.

Triffitt, J. T., Gebaur, U., Ashton, B. A., Owen, M. E., Reynolds, J. J. (1976) Origin of plasma α2-HS-glycoprotein and its accumulation in bone. Nature, 262:226-227.

Vagelos, P. R. (1971) Regulation of fatty acid biosynthesis. in Current Topics in Cellular Regulation, 4:119-166.

Watson, J. D. (1990) The Human Genome Project: Past, Present, and Future. Science, 248:44-49.

Waye, J. S., Gravel, R. A., and Willard, H. F. (1988) Two PstI RFLP's in the PCCB gene on the long arm of chromosome 3. Nucleic Acids Res, 16(5):2362.

Weber, J. L., and May, P. E. (1989) Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet, 44:388-396.

Welch, H. M., and Carritt, B. (1987) Detailed study of the structure and origin of the DNF15S1 locus. Cytogenet. Cell Genet., 46:714.

Williams, J., Elleman, T. C., Kingston, I. B., Wilkins, A. G., and Kuhn K. A. (1982) The primary structure of hen ovotransferrin. Eur J Biochem, 122:297-303.

Yang, F., Lum, J. B., McGill, J. R., Moore, C. M., Naylor, S. L., van Bragt, P. H., Baldwin, W. D., and Bowman, B. H. (1984) Human transferrin cDNA characterization and chromosomal location. Proc Natl Acad Sci USA, 81:2752-2756.

Yen, S. S. C., Siler, T. M., and DeVane, G. W. (1974) Effect of somatostatin in patients with acromegaly. N Engl J Med, 290:935-938.

Zelinski, T., Kaita, H., Lewis, M., Coghlan, G., and Craig, D. (1987) The sequence of chromosome 3 loci AHSG:TF:CHE1. Hum Hered 37:1-6.