

## *Prospects for the Diagnosis of Malignant Hyperthermia Susceptibility Using Molecular Genetic Approaches*

Roy Clifford Levitt, M.D. \*

### CONTENTS

- Introduction
- Diagnosis by Direct Molecular Genetic Techniques
- Prospects for the Diagnosis of Malignant Hyperthermia Susceptibility by Direct Methods
- Diagnosis by Indirect Molecular Genetic Techniques
- Prospects for the Diagnosis of Malignant Hyperthermia Susceptibility by Indirect Methods
- Summary

MALIGNANT hyperthermia susceptibility (MHS) is a clinically heterogeneous, autosomal dominant pharmacogenetic disorder.<sup>1</sup> MHS is generally characterized by accelerated metabolism, hyperthermia, and muscle rigidity.<sup>2,3</sup> All commonly used potent inhalation anesthetics and depolarizing skeletal muscle relaxants are believed to initiate this syndrome in susceptible individuals.<sup>3,4</sup> MHS is widely believed to result from a membrane defect that disrupts calcium metabolism in skeletal muscle. However, the genetic defects in MHS that confer susceptibility to these anesthetic agents and disrupt metabolic regulation remain largely unknown. Efforts to understand the molecular basis of MHS and other Mendelian disorders (those inherited as a single gene) have been greatly aided by recent advances in genetic technology. The clinical application of this technology has provided exciting new opportunities

for the diagnosis of hereditary disorders, malignancies, and numerous other diseases<sup>5-8</sup> (e.g., autoimmune, infectious, immunodeficiency, and cardiovascular). The purpose of this review is to acquaint the reader with selected molecular genetic techniques that will be useful in the non-invasive presymptomatic and prenatal diagnosis of MHS.

### Diagnosis by Direct Molecular Genetic Techniques

The clinical diagnosis of MHS or any Mendelian disorder is easily accomplished once a defective gene or gene product (protein) is identified. Although genes and gene products vary significantly among individuals without producing abnormal phenotypes (nucleotide sequence variation that does not alter the phenotype is referred to as a polymorphism), a true genetic mutation should be unique to affected individuals and should not occur in normal relatives. If a protein is recognized as defective and can be purified, it can be used to isolate and amplify the normal and abnormal genes by propagation in bacterial cells (cloning). The DNA sequence of the normal and abnormal cloned genes can then be examined, providing insight into the structure and function of the defective protein and the pathophysiology of the disorder. Extensive studies on hemoglobinopathies and, more recently, many other heritable disorders have provided considerable information on the nature of mutations that cause such heritable disorders. These and other Mendelian disorders generally arise from either point mutations or gross abnormalities in chromosomal DNA.

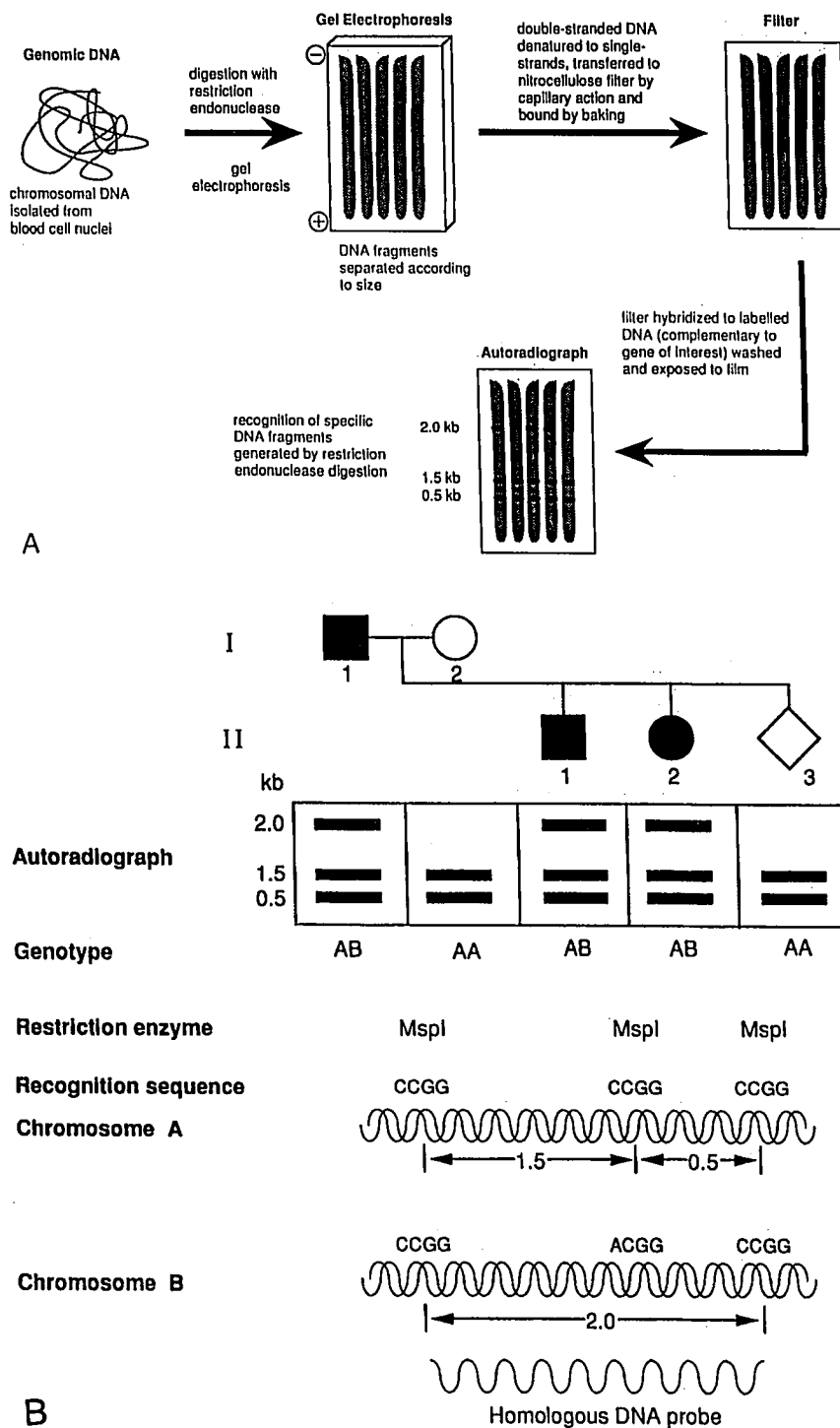
Gross abnormalities are characterized as DNA deletions, insertions, or rearrangements within a critical region of a functioning gene. The consequences of these mutations are readily apparent (e.g., the gene is deleted in part or whole, or the gene is interrupted by an insertion or rearrangement). Point mutations are generally confined to the substitution of a DNA base (nucleotide) or a small deletion or insertion of a few base pairs that significantly affects the function of the gene product. Both types of mutations can now readily be demonstrated by molecular techniques. These methods are generally easy to apply to double-stranded DNA isolated from the nuclei of circu-

\* Associate Professor of Anesthesiology and Critical Care Medicine.

Received from the Department of Anesthesiology and Critical Care Medicine, The Johns Hopkins Medical Institutions, Baltimore, Maryland. Accepted for publication January 29, 1992. Supported by the Foundation for Anesthesia Education and Research, the MDA, and National Institutes of Health grant GM47145-01.

Address reprint requests to Dr. Levitt: The Department of Anesthesiology and Critical Care Medicine, The Johns Hopkins Medical Institutions, Meyer Building, Room 8-134, 600 North Wolfe Street, Baltimore, Maryland 21205.

Key words: Complications; malignant hyperthermia susceptibility. Genetics: allele-specific oligonucleotide analysis; linkage; malignant hyperthermia susceptibility; mapping; molecular; polymerase chain reaction; restriction fragment length polymorphism.



lating white blood cells obtained from a blood sample by simple venipuncture.

Gross abnormalities in gene structure can be detected by restriction fragment length polymorphism (RFLP) analysis<sup>9</sup> (figs. 1A and 1B) and Southern blotting.<sup>10</sup> In RFLP analysis, DNA-modifying enzymes cut double-

stranded DNA at recognition sites dictated by the DNA sequence, producing fragments that are then traditionally separated according to size by electrophoresis. Southern blotting is a method of making DNA single-stranded and transferring and binding the sized DNA fragments to a solid support. The polymorphic fragments produced are

generally detected by labeled DNA probes that recognize this gene by hybridization (complementary single strands of DNA recognize each other and produce double-stranded DNA by hydrogen bonding.) Thus, when a restriction endonuclease is used to cleave chromosomal DNA, a unique pattern of fragments can be detected (fig. 1A).

Nearly all gross rearrangements (mutations) can be recognized by RFLPs because the gene structure is altered near a recognition site for one or more restriction enzymes that cut the double-stranded DNA of interest. For example, insertions may produce a larger DNA fragment than normally expected because the added DNA segment lies within the interval (segment of chromosomal DNA) between two restriction enzyme sites. In a similar way, deletions and rearrangements can significantly alter the pattern of restriction fragments produced by the digestion of genomic (pertaining to the entire complement of chromosomes) DNA. Although gross abnormalities are relatively rare in nature, more than 60% of Duchenne and Becker muscular dystrophies,<sup>11</sup> as well as certain forms of retinoblastoma,<sup>12,13</sup> familial hypercholesterolemia,<sup>14</sup> and numerous other disorders,<sup>6</sup> are caused by this type of mutation.

A small number of point mutations can be demonstrated directly by virtue of an altered DNA sequence within the recognition site for a restriction enzyme (for detailed description, see fig. 1B). The mutation may add or delete a restriction endonuclease site, producing a RFLP that can be detected as described above. Sickle cell anemia<sup>15-18</sup> and hemophilia A<sup>19</sup> are examples of diseases in which the mutation can be recognized directly by RFLPs. Presently, only a limited number of restriction endonucleases are known that collectively can recognize only a small portion of the DNA sequence of any particular gene. Therefore, because most point mutations are likely to lie outside these restriction endonuclease recognition sites, they will not be recognized directly by RFLPs.

Allele-specific oligonucleotides (ASOs)<sup>20,21</sup> can in theory recognize all known nucleotide polymorphisms and point mutations (fig. 2), regardless of whether they occur within a recognition site for a restriction endonuclease or not. ASOs are synthetic oligonucleotides (usually approximately 20 nucleotides or longer) that under optimal hybridization conditions can be forced to recognize only one allele (gene copy) or homologous DNA sequence, and not one that differs by even a single nucleotide. Oligonucleotides can then be synthesized that recognize all known point mutations within a gene. ASOs are particularly useful for the diagnosis of autosomal dominant and recessive disorders in which a small number of point mutations predominantly account for the phenotype (e.g. cystic fibrosis,<sup>22</sup> Marfan syndrome,<sup>23,24</sup> sickle cell anemia<sup>18,21</sup>). ASO technology also is being put to use in

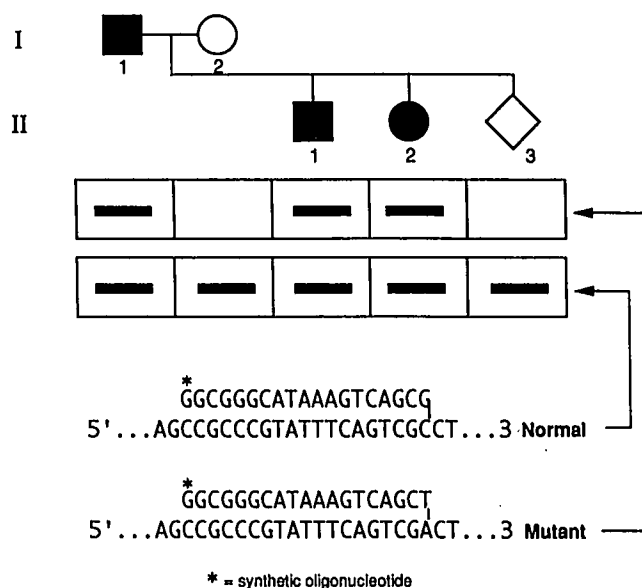


FIG. 2. The use of allele-specific oligonucleotide analysis. Individuals I-1, II-1, and II-2 are affected with a Mendelian disorder (solid symbols). In this example, a C-to-A nucleotide substitution in the DNA sequence of one chromosome causes the disorder. Only those individuals affected with this disorder will demonstrate this abnormal gene by allele-specific oligonucleotide analysis.

other important areas of clinical medicine. HLA class II genotyping by ASO analysis has recently been demonstrated to be an efficient technique for improving the selection of unrelated donors for bone marrow transplantation as compared to standard serologic tests.<sup>25</sup> Unfortunately, ASO analyses can be used only to detect mutations that have been described. Unrecognized mutations, even very close to a known mutation in the same gene, would not be detected by this technique.

A recently described method amplifies a small fragment of genomic DNA directly. This technique is referred to as the polymerase chain reaction (PCR).<sup>26</sup> PCR is a method of selective replication *in vitro* of a small region of chromosome or DNA of interest (usually 2,000 base pairs or fewer). This method is extremely powerful because, without cloning, it can amplify a small region from a single chromosome. For our purpose in describing PCR, the genomic source of DNA that is amplified will be referred to as a template. PCR, like chromosomal replication *in vivo*, depends on a number of components: a template, a polymerase that can synthesize the new nucleotide chain, free nucleotides that can be added to the end of the growing nucleotide chain, and various cofactors ( $Mg^{2+}$ , buffers, etc.). PCR also depends on two oligonucleotide primers (usually 18 nucleotides or longer) that are homologous to the DNA at each end of the region to be synthesized. PCR is a cyclic process with an amplification of the region between the primers by  $2^n$  (where n equals the number

of cycles in the process). During the first cycle, the double-stranded template is denatured (separating the strands) by heating. Oligonucleotide primers are annealed to each complementary template strand by cooling. (There is a large excess of primers so they are much more likely to anneal to the template than to the complementary strand.) These oligonucleotide primers are then extended by the polymerase (adding nucleotides in succession) to synthesize replicas of each strand of the original template. During the second cycle, the products of the first cycle and the original template are denatured, annealed to primers, and replicated by the polymerase. If we start with one double-

stranded template molecule, after the first cycle of PCR we would have two; after the second cycle we would have four; after the third cycle we would have eight; and so on (for a detailed description, see fig. 3).

This method obviates the need to cleave large amounts of genomic DNA with a restriction endonuclease, separate the fragments by electrophoresis, and transfer them from the gel to a solid support to detect a mutation by RFLP analysis (fig. 1A). The small fragment of genomic DNA containing the mutation that alters the recognition site for a restriction endonuclease can be amplified to high concentration by PCR. The RFLP can then be demon-

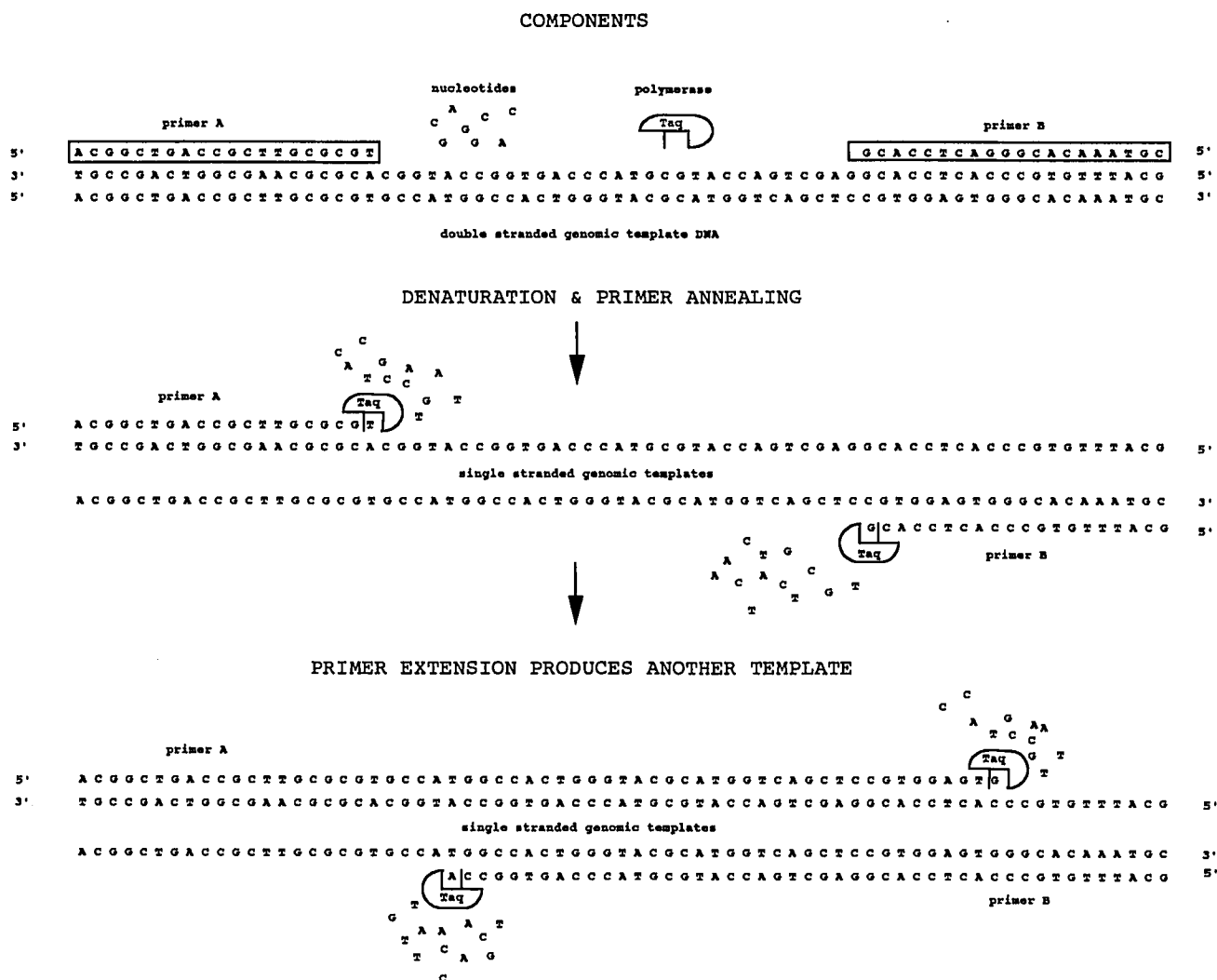


FIG. 3. Amplification of DNA by polymerase chain reaction. The components shown are combined for the polymerase chain reaction. The double-stranded template DNA is denatured (*i.e.*, the strands are separated) using heat, and then the components are cooled, allowing each primer to anneal to a template strand. The polymerase (thermostable) then extends the primers by adding nucleotides complementary to those on the template strand. An excess of primer ensures that the template strands do not reanneal with each other. Primer extension produces another template in each case. When this process is repeated (heat denaturation, primer annealing, primer extension) an amplification of  $2^n$  results (where  $n$  = the number of cycles). During a typical polymerase chain reaction amplification, approximately 2 h is required to complete 30 cycles.

strated directly in this PCR fragment by restriction endonuclease digestion<sup>26</sup> (fig. 1B). The entire process from blood collection to the description of a mutation would take hours with PCR, rather than days. Alternatively, this PCR fragment can be bound directly on a solid support and hybridized with an ASO to demonstrate a mutation that cannot be detected by RFLP analysis.

Further refinements in these methods use a labeled ASO as one of the PCR primers (see fig. 4); the labeled ASO amplifies only the mutant allele of interest and can be detected directly by autoradiography without time-consuming DNA hybridizations with an ASO.<sup>8</sup> For example, in figure 4, if primer A recognizes a specific mutant sequence, then the PCR product would be expected only when this allele was present. This is true because we can force the oligonucleotide primer to recognize only the mutant sequence and not the normal allele. The prenatal diagnosis is now routinely carried out for numerous genetic disorders.<sup>6,8</sup> For example, the sensitivity and specificity of the prenatal and presymptomatic molecular genetic diagnosis of sickle cell anemia is essentially 100%. The specificity of direct molecular genetic techniques re-

mains at 100% in heterogeneous disorders (similar phenotypes produced by different mechanisms or genetic defects); however, the sensitivity is inversely related to the number of unidentified mutations. These methods are limited by the inability to detect unknown genetic defects within the same gene or in genes elsewhere. However, it is likely that ASO with PCR will be the direct method of choice for diagnosing the defects that produce MHS.

### Prospects for the Diagnosis of Malignant Hyperthermia Susceptibility by Direct Methods

Similar to most other genetic disorders, MHS is recognized only by an abnormal phenotype. The MHS phenotype is currently described as a fulminant malignant hyperthermic reaction to triggering anesthetics *in vivo* or an abnormal caffeine-halothane contracture test *in vitro*.<sup>27,28</sup> Little is understood at present as to how the MHS phenotype arises. A number of membrane proteins might alter calcium regulation after exposure to triggering anesthetics, if they were defective. Therefore, as in most genetic disorders, it will be necessary to localize and identify the causative gene(s) for MHS before identifying the defective protein responsible in each case. Studying how the abnormal and normal genes differ will ultimately provide an understanding of the altered proteins function and of the pathophysiology involved.

The first genetic locus (MHS-1) shown to cause MHS was localized on human chromosome 19q13.1 by two laboratories simultaneously. McCarthy *et al.*<sup>29</sup> established linkage by chromosomal homology with a similar disorder in pigs. The linear order of genes on the pig and human chromosomes suggested conservation of chromosomal structure and a possible location for the homologous unmapped gene in the human. MacLennan *et al.*<sup>30</sup> also mapped MHS-1 to chromosome 19q13.1 along with the ryanodine receptor gene (RYR-1) or calcium release channel in skeletal muscle, and, on the basis of linkage, suggested that the MHS phenotype arises from a defect in RYR-1. Central core disease is commonly considered to be invariably associated with MHS. The recent mapping of central core disease to the same subchromosomal region as MHS-1 confirms this chromosomal localization for at least one form of MHS and raises the possibility that central core disease may arise from a mutation in the same gene.<sup>31,32</sup>

A systematic search for the genetic alterations in RYR-1 from both porcine and human MHS individuals has disclosed a single genetic mutation.<sup>33-35</sup> A replacement of cytosine 1843 in the complementary DNA (cDNA, a stable copy of the mRNA) of normal pigs with thymidine in MHS pigs, was the only nucleotide substitution found that resulted in an amino acid substitution.<sup>33-35</sup> The resulting substitution of a cysteine for arginine 615 was cor-

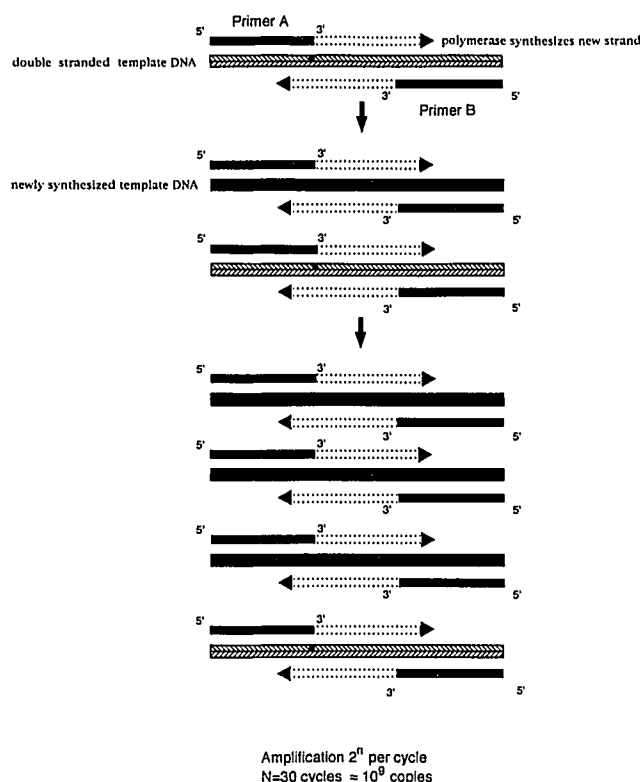


FIG. 4. The use of allele-specific oligonucleotide polymerase chain reaction analysis. The 3' ends of primers A and B are extended (as seen in fig. 3) by a polymerase in each cycle (dotted lines). If primer A is an allele-specific oligonucleotide and recognizes (as in fig. 2) a specific mutant sequence (solid block), it can be used selectively to amplify a mutation and diagnose a disorder directly.

related with, and likely to be causative of, MH in more than 450 swine of six breeds.<sup>33</sup> Therefore, in all strains of pigs that were examined, it appears that a single mutation in RYR-1 may be causative of MHS.<sup>35</sup> The analysis of approximately 70 human families believed to be predisposed to MH has revealed the corresponding substitution in one family, making this potential cause of MHS rare.<sup>34,†</sup> Clearly, in contrast to the pig, a single genetic mutation does not appear to explain MHS in the human. However, this rare ryanodine receptor mutation can be directly demonstrated by a RFLP resulting from the loss of a Rsa I restriction site within a MHS RYR-1 PCR fragment.<sup>34</sup>

The likelihood of genetic heterogeneity (similar phenotypes produced by different mechanisms) in MHS makes the practical application of direct molecular detection somewhat more complicated. Multiple genetic etiologies have been proposed in MHS<sup>1</sup> because of the highly variable clinical expression.<sup>1-4</sup> Genetic heterogeneity is further supported by an association between MHS and numerous other neuromuscular disorders,<sup>36</sup> including Duchenne muscular dystrophy, myotonic dystrophy, myotonia congenita, nonspecific myopathies, and the King-Denborough syndrome. The King-Denborough syndrome is characterized by MHS, myopathy, and severe dysmorphic features.<sup>37</sup> The sporadic occurrence of this disorder suggests that it may arise from a new mutation. Furthermore, the involvement of multiple organ systems suggests either a gross abnormality in the DNA with an effect on multiple genes or a mutation in a single gene with pleiotropic (multiple) effects. Gross chromosomal abnormalities have aided the localization and isolation of mutant genes in a number of disorders. High-resolution cytogenetic studies may prove useful as a diagnostic test in the King-Denborough disorder, if a gross chromosomal abnormality is at fault. The association between Duchenne dystrophy (which maps to the X chromosome) and myotonic dystrophy (which maps to 19q13.2) with MHS is not explained by the genetic mapping data (linkage data) described above. The genetic locus for myotonia congenita has also been excluded from the MHS-1 region of chromosome 19.<sup>38,39</sup> Hence, the mapping data support the notion that there may be a variety of genetic defects that can cause MHS. Recent linkage studies in my laboratory<sup>40</sup> confirm that at least one additional, perhaps common, genetic locus (MHS-2) can produce the MHS phenotype. Others also are finding MHS families that do not map to 19q13.1.<sup>41,‡</sup> MHS-2 has not yet been mapped but has

been excluded from the 19q13.1 linkage group surrounding MHS-1.

Genetic heterogeneity is a common theme in human genetic disease. Elliptocytosis is a hereditary disorder characterized by an abnormal shape of erythrocytes and occasionally mild-to-moderate hemolytic anemia. This phenotype is apparently produced by genetic defects in one of three separate genes.<sup>42-45</sup> Mutations in the  $\alpha$ -spectrin gene on chromosome 1q22-25,<sup>43,44</sup> the  $\beta$ -spectrin gene on 14q32,<sup>45</sup> and the gene for protein 4.1 on 1p36.2-p34<sup>42</sup> all have been shown to produce a similar red cell appearance or phenotype. Similarly, hereditary spherocytosis has been shown to arise either from genetic mutations in the  $\alpha$ - or  $\beta$ -spectrin genes on chromosomes 1q22-q25 and 14q32 respectively.<sup>46,47</sup> Osteogenesis imperfecta is a heritable disorder characterized by brittle bones. Osteogenesis imperfecta type III is characterized by the autosomal dominant inheritance of progressive deformation of bones, dentinogenesis, hearing loss, and very short stature. Osteogenesis imperfecta type III is commonly caused by various point mutations in either the COL1A1 (collagen 1A1) gene on chromosome 17 or the COL1A2 gene on chromosome 7.<sup>48</sup> Separate mutations in these genes also can produce the similar osteogenesis imperfecta type IV phenotype.<sup>48</sup> Ehlers-Danlos syndrome is another heterogeneous group of heritable disorders of connective tissue.<sup>49,50</sup> Ehlers-Danlos syndrome type VII is characterized by extreme joint hypermobility and dislocations, skin hyperextensibility, easy bruisability, and abnormal scarring.<sup>50</sup> This disorder appears to be caused by a deficiency in processing of the N-propeptide of type I procollagen.<sup>49</sup> Genetic mutations in three separate genes (COL1A1, COL1A2, and procollagen N-proteinase) have been associated with this phenotype.<sup>49</sup>

Only speculation can be offered as to how a genetic defect(s) in each of a number of genes could produce the MHS phenotype by altering the regulation of myoplasmic calcium. However, both the clinical characterization and genetic mapping data clearly support genetic heterogeneity in this disorder. It is likely, therefore, as in numerous other genetic disorders, that a number of common mutations in different genes produce the MHS phenotype.

The rapid noninvasive presymptomatic diagnosis of MHS can be carried out, like any routine preoperative screening test, using the direct molecular methods discussed above, once the genetic mutations that cause this disorder have been characterized. The more genetic defects there are that can produce the MHS phenotype, the more difficult and time-consuming it will be to identify each of them. For these reasons, indirect molecular genetic techniques are likely to be required to identify the abnormal gene in many cases and to follow its inheritance to establish the clinical diagnosis of MHS.

† MacLennan DH: Personal communication.

‡ MacLennan DH, Deufel T: Personal communication.

# Diagnosis by Indirect Molecular Genetic Techniques

Chromosomes are organized in a linear fashion. Therefore, the genetic locus (physical location) of the defective gene that produces MHS will be inherited with closely surrounding genetic markers. Polymorphisms in and around a gene (which have no known effect on the individual's phenotype) occur an average of about once in every 500 nucleotides.<sup>51-55</sup> At present, the indirect diagnosis of a heritable disorder is dependent on the use of these polymorphisms as genetic markers. These polymorphisms usually are recognized by the molecular techniques described above (RFLP, ASO, and PCR analyses). Genetic markers are used to demarcate the interval of chromosome surrounding a mutation, which allows the clinician to follow its inheritance (figs. 5 and 6). Therefore, knowledge of the exact mutation is unnecessary for diagnosis of the disorder. As an example, in figure 5, markers 1-4 would be useful to evaluate the inheritance of the putative MHS locus. Markers 2 and 3 are in close proximity to the MHS locus and are least likely to be separated by homologous recombination from this gene. Hence, the inheritance of markers 2 and 3 would indicate that, most likely, the subchromosomal segment between them, including the MHS locus, has also been inherited. Figure 6 illustrates how polymorphic DNA markers can be associated with a phenotype and used as a noninvasive method of diagnosis (linkage analysis). Individuals I-1, II-2, II-4, II-5, II-7, and III-1 are MHS, and individuals I-2, II-1,

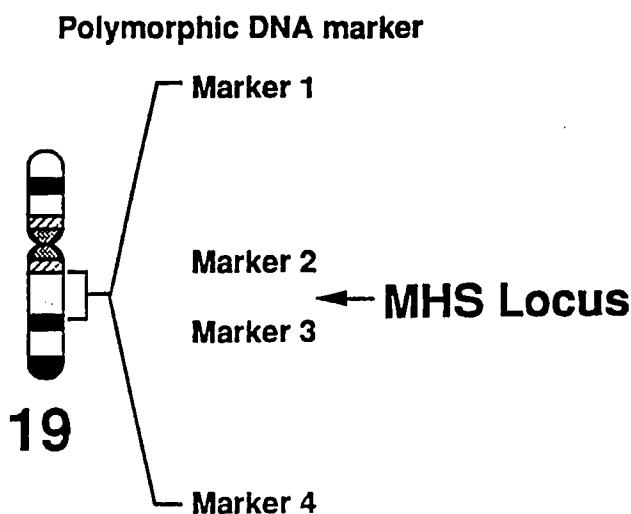


FIG. 5. The use of a genetic map of DNA markers in the indirect diagnosis of malignant hyperthermia susceptibility (MHS). A map of genetic markers can be used to define the subchromosomal interval containing the gene for MHS. Genetic distance is defined by the frequency of recombination (crossing over and exchange of maternal and paternal chromosomal material at meiosis) between markers. Markers closest to MHS (i.e., 2 and 3) are least likely to undergo homologous recombination.

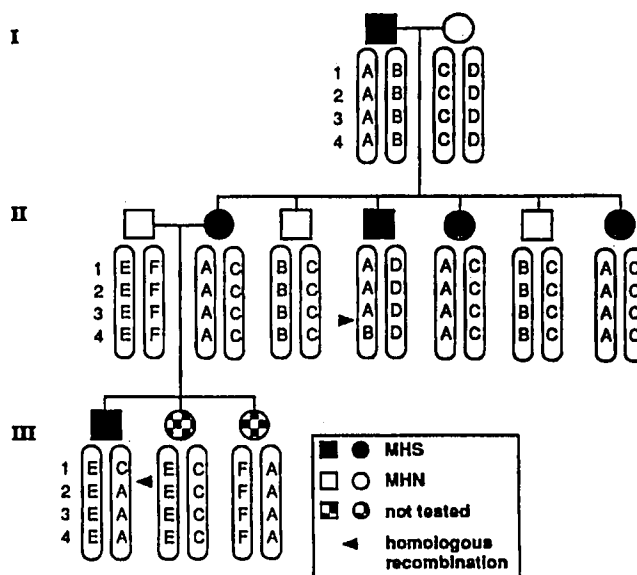


FIG. 6. Indirect diagnostic techniques (linkage analysis). The relationship between the phenotype and genotype defines which chromosome (haplotype) carries the gene for malignant hyperthermia susceptibility (MHS). Individuals at risk, III-2 and III-3, can be evaluated to determine if they have inherited the defective gene. The inheritance of an A allele for markers 1-4 would indicate that most likely the subchromosomal interval between markers 2 and 3, including the mutant gene, has been inherited in those individuals at risk in this family. MHN = not susceptible to malignant hyperthermia.

II-3, and II-6 are normal. These data can be used to establish a close association between markers 2A and 3A and the MHS phenotype in this family and to provide a likelihood estimate with confidence limits as to whether individuals III-2 and III-3 inherited the mutant gene. In this example, individual III-2 is likely to be normal, and III-3 is likely to be MHS. The same analysis can be used for other individuals at risk within this family. Recombination occurs in individual II-4 between markers 3 and 4 and in III-1 between markers 1 and 2, illustrating that these markers would be less useful to predict the inheritance of MHS. This approach is limited because the data from this type of indirect analysis cannot be generalized to other families. In addition, to demonstrate an association between the MHS phenotype and a particular set of chromosomal markers, the family must be of sufficient size, and the phenotype must be known for a sufficient number of family members. The sensitivity of indirect techniques is directly dependent on the frequency of recombination exhibited between a disorder and surrounding DNA markers. The closer that markers can be found to the disorder (the lower the rate of recombination), the higher the sensitivity of the test.

This physical association can also be used in research to identify a previously unknown location for a defective

gene, by demonstrating that the phenotype produced by the gene is consistently inherited along with a particular set of DNA markers (they cosegregate at meiosis because they are located in close proximity on the same chromosome and are not readily separated by homologous recombination). These mapping studies are laborious and require data from sufficient numbers of affected families to generate likelihood estimates for the inheritance (linkage) of the disease phenotype with a set of DNA markers.<sup>56</sup> In addition, the results of these mapping studies are critically dependent on the validity of the phenotype. However, once these linkage relationships are firmly established, they provide a sensitive, noninvasive technique for the presymptomatic diagnosis of MHS. The surrounding markers can be used to identify the chromosome (haplotype) and region on the chromosome that carries the defective gene in a particular family. Ultimately, the map locations generated in this fashion may also suggest gene candidates that may harbor the genetic mutation(s) that cause MHS. Studying the DNA sequence of these candidate genes may ultimately disclose a mutation, which can be used as a direct, highly accurate, and specific method of diagnosis in the future.

#### Prospects for the Diagnosis of Malignant Hyperthermia Susceptibility by Indirect Methods

The clinical diagnosis of MHS can now be attempted by indirect molecular methods. However, a diagnosis can be offered only to those individuals whose family members clearly demonstrate an association between the MHS phenotype and DNA markers closely spaced around MHS-1 on 19q13.1. The closer the DNA markers are located to the MHS-1 locus, the less likely they will be separated from the chromosomal interval carrying the disease gene by homologous recombination. Therefore, the precision of the diagnosis will depend on the reliability of the phenotype established in the other family members and the proximity of the DNA markers used that flank the disease gene. Furthermore, the lack of an association between DNA markers on 19q13.1 and the MHS phenotype cannot exclude the diagnosis because the disorder may be produced by a mutation elsewhere in the genome. Mapping MHS-2 (and potentially other MHS loci) will eventually provide indirect molecular methods of clinical diagnosis in individuals whose disorder does not originate from a mutation at the MHS-1 locus.

#### Summary

MHS is a heterogeneous pharmacogenetic disorder in the human that is likely to be caused by one of a variety of genetic defects, in one of a number of genes. Direct molecular methods will provide a rapid, efficient, non-

invasive, and low-cost screening test once the causative genetic mutations have been identified. However, until this objective is met, indirect molecular genetic methods can be used to demonstrate the inheritance of an abnormal gene in certain family members at risk. This requires localizing the gene that produces the abnormal phenotype to a subchromosomal segment by linkage analysis and showing the coinheritance of MHS and DNA markers in a number of family members. Indirect molecular genetic methods are likely to be particularly useful in the diagnostic evaluation of children too small to be biopsied in families where others have been biopsied or their phenotypes are known. It appears likely that molecular genetic methods will not eliminate the usefulness of the muscle biopsy and caffeine-halothane contracture test in the near future. Rather, these diagnostic tests will complement one another and significantly improve our understanding of the complexity of this disorder.

Many thanks to Dr. Mark C. Rogers for his enthusiastic support and Ms. Kelly Battaglia for her secretarial assistance.

#### References

1. McPherson E, Taylor CA: The genetics of malignant hyperthermia: Evidence for heterogeneity. *Am J Med Genet* 11:273-285, 1982
2. Gronert G: Malignant hyperthermia. *ANESTHESIOLOGY* 53:395-423, 1980
3. Britt BA, Kalow W: Malignant hyperthermia: A statistical review. *Can Anaesth Soc J* 17:293-315, 1970
4. Britt BA: Dantrolene: An update, Malignant Hyperthermia. Boston, Martinus-Nijhoff, 1987, pp 325-367
5. Lowe JB: Clinical applications of gene probes in human genetic disease, malignancy, and infectious disease. *Clin Chim Acta* 157: 1-32, 1986
6. Antonarakis SE: Diagnosis of genetic disorders at the DNA level. *N Engl J Med* 320:153-163, 1989
7. Buffone GJ, Shearer WT, Finegold MJ: New methodologies: Their role in pediatric pathology. *Pediatr Clin North Am* 36:227-256, 1989
8. Caskey CT: Disease diagnosis by recombinant DNA methods. *Science* 236:1223-1229, 1987
9. Nathans D, Smith HO: Restriction endonucleases in the analysis and restructuring of DNA molecules. *Annu Rev Biochem* 44: 273-293, 1975
10. Southern EM: Gel electrophoresis of restriction fragments. *Methods Enzymol* 68:152-176, 1979
11. Bakker E, Bonten EJ, Veenema H, den Dunnen JT, Grootscholten PM, van Ommen GJB, Pearson PL: Prenatal diagnosis of Duchenne Muscular Dystrophy: A three year experience in a rapidly evolving field. *J Inherited Metab Dis* 12(suppl 1):174-190, 1989
12. Bookstein R, Lee EY, To H, Young LJ, Sery TW, Hayes RC, Friedmann T, Lee WH: Human retinoblastoma susceptibility gene: Genomic organization and analysis of heterozygous intragenic deletion mutants. *Proc Natl Acad Sci USA* 85:2210-2214, 1988

13. Friend SH, Horowitz JM, Gerber MR, Wang XF, Bogenmann E, Li FP, Weinberg RA: Deletions of a DNA sequence in retinoblastomas and mesenchymal tumors: Organization of the sequence and its encoded protein. *Proc Natl Acad Sci USA* 84: 9059-9063, 1987
14. Lehrman MA, Russell DW, Goldstein JL, Brown MS: Exon-Alu recombination deletes 5 kilobases from the low density lipoprotein receptor gene, producing a null phenotype in familial hypercholesterolemia. *Proc Natl Acad Sci USA* 83:3679-3683, 1986
15. Geever RF, Wilson LB, Nallaseth FS, Milner PF, Bittner M, Wilson JT: Direct identification of sickle cell anemia by blot hybridization. *Proc Natl Acad Sci USA* 78:5081-5085, 1981
16. Chang JC, Kan YW: A sensitive new prenatal test for sickle cell anemia. *N Engl J Med* 307:30-32, 1982
17. Orkin SH, Little PFR, Kazazian HH Jr, Boehm CD: Improved detection of the sickle mutation by DNA analysis: Application to prenatal diagnosis. *N Engl J Med* 307:32-36, 1982
18. Conner BJ, Reyes AA, Moric C, Itakura K, Teplitz RL, Wallace RB: Detection of sickle cell  $\beta$ -globin allele by hybridization with synthetic oligonucleotides. *Proc Natl Acad Sci USA* 80: 278-282, 1983
19. Youssoufian H, Kazazian HH Jr, Phillips DG, Aronis S, Tsiftis G, Brown VA, Antonarakis SE: Recurrent mutations in hemophilia A give evidence for Cpg mutation hotspots. *Nature* 324:380-382, 1986
20. Wallace RB, Schold M, Johnson MJ, Dembek P, Itakura K: Oligonucleotide directed mutagenesis of the human  $\beta$ -globin gene: General method for producing specific point mutations in cloned DNA. *Nucleic Acids Res* 9:3647-3656, 1981
21. Studencki AB, Wallace RB: Allele-specific hybridization using oligonucleotide probes of very high specific activity: Discrimination of the human beta A- and beta S-globin genes. *DNA* 3:7-15, 1984
22. Shrimpton AE, McIntosh I, Brock DJ: The incidence of different cystic fibrosis mutations in the Scottish population. *J Med Genet* 28:317-321, 1991
23. Dietz HC, Cutting GR, Pyeritz RE, Maslen CL, Sakai LY, Corson GM, Puffenberger EG, Hamosh A, Nanthakumar EF and Currstin SM: Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene. *Nature* 353:337-339, 1991
24. Lee B, Godfrey M, Vitale E, Hori H, Mattei MG, Sarfarazi M, Tspouras P, Ramirez F, Hollister DW: Linkage of Marfan syndrome and a phenotypically related disorder to two different fibrillin genes. *Nature* 353:330-334, 1991
25. Tiercy J-M, Morel C, Freidel AC, Zwahlen F, Gebuhrer L, Bétuel H, Jeannet M, and Mach B: Selection of unrelated donors for bone marrow transplantation is improved by HLA class II genotyping with oligonucleotide hybridization. *Proc Natl Acad Sci USA* 88:7121-7125, 1991
26. Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA, Arnheim N: Enzymatic amplification of  $\beta$ -globin genomic sequences and restriction site analysis for diagnosis of sickle cell anaemia. *Science* 230:1350-1354, 1985
27. Larach MG. Standardization of caffeine halothane muscle contracture test. *Anesth Analg* 69:511-515, 1989
28. Fletcher JE, Rosenberg H: Laboratory methods for malignant hyperthermia diagnosis, *Experimental Malignant Hyperthermia*. Edited by Williams CH. New York, Springer-Verlag, 1988, pp 121-140
29. McCarthy TV, Sandra Healy JM, Heffron JJA, Lehane M, Deufel T, Lehmann-Horn F, Farrall M, Johnson K: Localization of malignant hyperthermia susceptibility locus to human chromosome 19q12-13.2. *Nature* 343:562-564, 1990
30. MacLennan DH, Duff C, Zorzato F, Fujii J, Phillips M, Korneluk RG, Frodis W, Britt BA, Worton RG: Ryanodine receptor gene is a candidate for predisposition to malignant hyperthermia. *Nature* 343:559-561, 1990
31. Kausch K, Lehmann-Horn F, Janka M, Wieringa B, Grimm T, Müller CR: Evidence for linkage of the central core disease locus to the proximal long arm of human chromosome 19. *Genomics* 10:765-769, 1991
32. Haan ER, Freemantle CJ, McCure JA, Friend KL, Mulley JC: Assignment of the gene for central core disease to chromosome 19. *Hum Genet* 2:187-190, 1990
33. Otsu K, Khanna VK, Archibald AL, MacLennan DH: Cosegregation of porcine malignant hyperthermia and a probable causal mutation in the skeletal muscle ryanodine receptor gene in backcross families. *Genomics* 11:744-750, 1991
34. Gillard EF, Otsu K, Fujii J, Khanna VK, deLeon S, Derdemezi J, Britt BA, Duff CL, Worton RG, MacLennan DH: A substitution of cysteine for arginine 614 in the ryanodine receptor is potentially causative of human malignant hyperthermia. *Genomics* 11:751-755, 1991
35. Fujii J, Otsu K, Zorzato F, deLeon S, Khanna VK, Weiler JE, O'Brien PJ, MacLennan DH: Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. *Science* 253:448-451, 1991
36. Heiman-Patterson T, Rosenberg H, Fletcher JE, Tahmouh AJ: Halothane-caffeine contracture testing in neuromuscular diseases. *Muscle Nerve* 11:453-457, 1988
37. King JO, Denborough MA: Anesthetic induced malignant hyperthermia in children. *J Pediatr* 83:37-40, 1973
38. Koch M, Harley H, Sarfarazi M, Bender K, Wienker T, Zoll B, Harper PS: Myotonia congenita (Thomsen's disease) excluded from the region of the myotonic dystrophy locus on chromosome 19. *Hum Genet* 82:163-166, 1988
39. Bender K, Senff H, Steiert A, Lagodny H, Wienker TF, Koch M: Linkage studies of myotonia congenita and paramyotonia congenita. *Clinic Genet* 36:92-99, 1989
40. Levitt RC, Nouri N, Jedlicka AE, McKusick VA, Marks A, Shutack JG, Fletcher JE, Rosenberg H, Meyers DA: Evidence for genetic heterogeneity in malignant hyperthermia susceptibility. *Genomics* 11:543-547, 1991
41. MacKenzie AE, Allen G, Lahey D, Crossan ML, Nolan K, Mettle G, Worton RG, MacLennan DH, Korneluk R: A comparison of the caffeine halothane muscle contracture test with the molecular genetic diagnosis of malignant hyperthermia. *ANESTHESIOLOGY* 75:4-8, 1991
42. Conboy J, Mohandas N, Tchernia G, Kan YW: Molecular basis of hereditary elliptocytosis due to protein 4.1 deficiency. *N Engl J Med* 315:680-685, 1986
43. Lawler J, Liu S-C, Palek J, Prchal J: A molecular defect in spectrin with a subset of patients with hereditary elliptocytosis: Alterations in the alpha-subunit domain involved in spectrin self-association. *J Clin Invest* 73:1688-1695, 1984
44. Lecomte M-C, Dhermy D, Solis C, Ester A, Feo C, Gautero H, Bournier O, Boivin P: A new abnormal variant of spectrin in black patients with hereditary elliptocytosis. *Blood* 65:1208-1217, 1985
45. Ohanian V, Evans JP, Gratzer WB: A case of elliptocytosis associated with a truncated spectrin chain. *Br J Haematol* 61:31-39, 1985
46. Goodman SR, Shiffer KA, Casoric LA, Eyster ME: Identification of the molecular defect in the erythrocyte membrane skeleton

- of some kindreds with hereditary spherocytosis. *Blood* 6:772-784, 1982
47. Palek J: Hereditary elliptocytosis, spherocytosis and related disorders: Consequences of a deficiency or a mutation of membrane skeletal proteins. *Blood Rev* 1:147-168, 1987
  48. Byers PH: Brittle bones—fragile molecules: Disorders of collagen gene structure and expression. *Trends Genet* 6:292-300, 1990.
  49. Vasan NS, Kuivaniemi H, Vogel BE, Minor RR, Wootton JAM, Tromp G, Weksberg R, Prockop DJ: A mutation in Pro $\alpha$ 2(1) gene (COL1A2) for type 1 procollagen in Ehlers-Danlos syndrome type VII: Evidence suggesting that skipping of exon 6 in RNA splicing may be a common cause of the phenotype. *Am J Hum Genet* 48:305-317, 1991
  50. Byers PH: Inherited disorders of collagen biosynthesis: Ehlers-Danlos syndrome, the Marfan syndrome, and osteogenesis imperfecta, *Clinical Medicine*. Edited by Spittel JA Jr. Philadelphia, Harper & Row, pp 1-41, 1983
  51. Orkin SH, Kazazian HH Jr, Antonarakis SE, Goff SC, Boehm CD, Sexton JP, Waber PG, Giardina PJV: Linkage of  $\beta$ -thalassemia mutations and  $\beta$ -globin gene polymorphisms with DNA polymorphisms in the  $\beta$ -globin gene cluster. *Nature* 296:627-631, 1982
  52. Jeffreys AJ: DNA sequence variants in the G gamma-, A gamma-, and  $\delta$ - and  $\beta$ -globin genes of man. *Cell* 18:1-10, 1979
  53. Chakravarti A, Buetow KH, Antonarakis SE, Waber PG, Boehm CD, Kazazian HH Jr: Nonuniform recombination within the human  $\beta$ -globin gene cluster. *Am J Hum Genet* 36:1239-1258, 1984
  54. Chakravarti A, Elbein SC, Permutt MA: Evidence for increased recombination near the human insulin gene: Implication for disease association studies. *Proc Natl Acad Sci USA* 83:1045-1049, 1986
  55. Antonarakis SE, Boehm CD, Giardina PJV, Kazazian HH Jr.: Nonrandom association of polymorphic restriction sites in the  $\beta$ -globin gene cluster. *Proc Natl Acad Sci USA* 79:1237-1241, 1982
  56. Morton NE: Sequential tests for the detection of linkage. *Am J Hum Genet* 7:277-318, 1955