a millisecond pulsar—may again rival the stability of the best available man-made clocks.

The technical challenge of pushing the precision of pulsar timing as far as possible is alluring to us as experimenters. And it appears likely that considerable further progress can be made. Present observations of PSR 1937+21 are limited not by telescope sensitivity, radiometer noise, or sky background temperature, but rather by systematic measurement errors that can still be reduced. Instrumental limitations of the radio frequency spectrometer are now being worked on. Observations at higher radio frequencies will almost certainly reduce the difficult-to-calibrate variations in propagation time through the interstellar medium, and will become feasible with high sensitivity if a proposed upgrading of the Arecibo telescope is carried out. At present we know of a second millisecond pulsar, PSR 1855+09, for which time-of-arrival measurements at the ~1 μsec level are already possible (*5*). More of these objects will likely be discovered in millisecond pulsar surveys now under way.

The evident need for a better time standard in this experiment is a strong motivation for builders and maintainers of atomic clocks. Steps are now in progress at NBS and at the Bureau International de l'Heure to combine the best clocks in the world in an optimum weighted algorithm to create the "world's best clock" as a reference. Atomic clocks have improved in stability by an order of magnitude every 7 years since their introduction in 1949, and we see no reason for this trend to stop in the near future (*19, 20*). We are also optimistic that more millisecond pulsars will be found, so that timing comparisons can be made among a number of them.

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Molecular Genetics: Applications to the Clinical Neurosciences

JOSEPH B. MARTIN

Application of molecular biology, by means of linkage analysis and DNA probes that demonstrate restriction fragment length polymorphisms (RFLPs), has resulted in the chromosomal localization of the genes responsible for a number of neurological disorders. Characterization of the structure and function of individual genes for these diseases is in an early stage, but information available indicates that the molecular mechanisms underlying phenotypic expression of neurological diseases encompass a wide range of genetic errors ranging from the most minor (a single–base pair mutation) to large chromosomal deletions. Linkage analysis can now be used for genetic counseling in several of these disorders.

UNTIL RECENTLY, THE LIKELIHOOD OF DISCOVERING THE molecular genetic basis of most neurological diseases seemed remote. Now, the application of molecular biology techniques, with DNA probes that reveal restriction fragment length polymorphisms (RFLPs) (*1*) combined with linkage analysis, has resulted in the chromosomal localization of the genes responsible for several of these disorders. In addition, more refined methods of cytogenetics have permitted recognition of chromosomal aberrations (deletions, translocations, or duplications) associated with some of these conditions (*2*). Furthermore, abnormal genes have now been cloned in some of the autosomal recessive lysosomal storage diseases. This review summarizes advances made in selected neurological disorders on which these recent discoveries have had a major impact (Table 1).

Genetic Linkage Analysis

DNA polymorphisms, first demonstrated in the β-globin gene family (*3*), have been used to identify the chromosomal location of the abnormal gene in Huntington's disease (*4*) (chromosome 4), Duchenne dystrophy (*5, 6*) (X chromosome), familial amyloidotic

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polyneuropathy (*7*) (chromosome 18), myotonic dystrophy (*8*) (chromosome 19), familial Alzheimer's disease (*9*) (chromosome 21), von Recklinghausen's neurofibromatosis (*10, 11*) (chromosome 17), bilateral acoustic neurofibromatosis (*12*) (chromosome 22), and autosomal dominant (*13*) (chromosome 11) and X-linked recessive (*14*) manic-depressive illness. These discoveries demonstrate the possibility of locating the gene for any inherited disease, provided that sufficiently large families with the disorder are available.

Two types of DNA variations (single–base pair alterations and repeated sequences) provide the molecular bases of most RFLPs [see (*15*) for review]. Heterozygous RFLPs generated either from random anonymous DNA (function unknown) or from genes in the region can be used to follow the disease in a pedigree. The usefulness of probes for linkage analysis depends on the frequency of the RFLPs generated and the distance of the probe from the gene abnormality. The likelihood of a linkage between the probe and the disease gene is expressed quantitatively as a ratio of the probability of linkage, or logarithm of the odds (LOD score) (*1*). According to conventional criteria, a LOD score greater than 3 defines linkage of a marker to a disease gene (*1, 2*). Extended multigenerational families have been examined to determine the opportunities and limitations of this approach and, in particular, to delineate problems of recombinatorial events between disease genes and linked markers (*1, 2*). Inherent in the methodology are limitations imposed by the analysis of small families, particularly if few living affected individuals are available for study.

Huntington's Disease

Huntington's disease (HD), an autosomal dominant disorder with onset in midlife, is characterized by progressive neurological impairment, behavioral changes, and dementia (*16*). Although chorea is usually the first sign of HD, behavioral changes, most often depression, may occur a decade or more before the movement disorder begins (*17*). Early in the illness, patients often demonstrate personality changes (*16*). The average duration of HD from onset to death is 17 years but may be as long as 30 years. The duration is shorter when the disease begins early in life. Juvenile HD (Westphal variant), for unexplained reasons, is more frequently inherited from the father than from the mother (*16*).

The neuropathology of HD shows the principal lesion to be death of projection neurons in the striatum (*4*). Atrophy also occurs in the cerebral cortex, but the precise neuronal changes accompanying the tissue loss there remain ill-defined (*18*).

Molecular genetics. Molecular genetic linkage studies have localized the HD gene to chromosome 4 (*4*). Initially, RFLPs demonstrated with the DNA probe G8 (locus D4S10) were studied in two large kindreds with HD, one from the United States and the other from Venezuela. The G8 probe, a 17.6-kb segment of single-copy DNA, was shown to be closely linked to the disease gene in both families (*4*). Subsequent studies have revealed additional DNA-sequence polymorphisms at this locus and have confirmed linkage to HD in other pedigrees (*19*). There is as yet no evidence that HD shows nonallelic heterogeneity; no kindred of more than 50 thus far

Table 1. Chromosomal localization and gene abnormalities in selected neurological diseases.

Genetic classification and disease	Chromosome	Gene defect	Comments on genetic heterogeneity	References
Autosomal dominant				
Huntington's disease	4p16	Unknown	None demonstrated in our 50 pedigrees	(*4, 16, 21*)
Myotonic dystrophy	19 centromere	Unknown	None demonstrated	(*8, 33*)
Familial Alzheimer's disease	21q21	Unknown	Not adequately studied	(*9, 45–47*)
Familial amyloidotic polyneuropathy	18q11.2–q12.1	Single–base pair substitution in mRNA for transthyretin	Allelic heterogeneity	(*7, 36*)
Manic-depressive illness	11p, Xp	Unknown	Evidence for nonallelic heterogeneity	(*14, 52, 53*)
Spinocerebellar atrophy	6	Unknown	Unknown	(*65*)
Von Recklinghausen's neurofibromatosis	17	Unknown	None demonstrated in >25 pedigrees	(*10, 11*)
Bilateral acoustic neurofibromatosis	22	Unknown	Unknown	(*12*)
Charcot-Marie-Tooth disease (type 1)	1q2	Unknown	Unknown	(*66*)
X-linked recessive				
Duchenne dystrophy	Xp21	Deletions in 5 to 10%	Multiallelic heterogeneity	(*5, 6, 28, 29*)
Adrenoleukodystrophy	Xq27–q28	Unknown	Unknown	(*68*)
Lesch-Nyhan syndrome	Xq27	HPRT deficiency, many variations	Multiallelic heterogeneity	(*63, 64*)
Pelizaeus-Merzbacher disease	Xq21–q22	Defect in myelin proteolipid protein	Unknown	(*77*)
Autosomal recessive				
Gaucher's disease	1q21	Amino acid substitution in glucocerebrosidase	Allelic heterogeneity	(*60*)
G_{M1} gangliosidosis	3p1	Partially characterized (see text)	Unknown	(*55*)
G_{M2} gangliosidosis				(*55–57*)
Tay-Sachs disease (type 1)	15q22–q25	Mutation in gene encoding α chain of hexosaminidase	Allelic heterogeneity	
Sandhoff disease (type 2)	5q13	Mutation in gene encoding β chain of hexosaminidase	Allelic heterogeneity	
Wilson's disease	13q14.11	Unknown	Unknown	(*67*)
Recessive with germinal chromosomal deletion				
Central neurofibromatosis	22q11–q13	Unknown	Unknown	(*73*)
Retinoblastoma	13q14	Partially characterized	Allelic heterogeneity	(*69, 70*)
Meningioma	22q12.3-qter	Unknown	Unknown	(*74*)

studied has been found with a negative linkage to the G8 marker (*20*). The LOD score for linkage of D4S10 to the HD gene now exceeds 45 (*21*).

Recombinations between the marker locus and the HD gene have been identified, however, that indicate that the G8 probe recognizes a neighboring piece of DNA and not the HD gene itself. It is estimated that the D4S10 locus resides about 2×10^6 to 5×10^6 bp away from the HD gene (*21*). DNA from patients with the Wolf-Hirschhorn syndrome (4p-syndrome), who have deletions on chromosome 4, confirms that the G8 marker and (by inference) the HD gene map to the terminal band on the short arm of chromosome 4 (4p16) (*22*) in a region containing approximately 10 million base pairs of DNA and 50 to 200 genes (*21*). Intense efforts to saturate this region with DNA markers indicate that the D4S10 locus lies proximal to the HD gene. So far, no flanking marker has been identified.

An interesting observation concerning mechanisms of dominance has been made in the Venezuelan pedigree. Individuals recognized by DNA linkage studies likely to be homozygous for HD show identical clinical manifestations compared to those who are heterozygous (*23*). Thus, HD appears to be the first human disorder of genetically documented homozygosity that displays complete phenotypic dominance. Such "complete" dominance of a single gene has been demonstrated in *Drosophila* and is thought to be associated with mutations causing a "gain of function" (*24*), that is, the mutation may be deleterious by expressing a normal cellular function in excess.

Predictive testing. The discovery of a DNA marker linked to the HD gene has raised the possibility of predictive testing for the disorder (*4, 20, 22*). DNA for determining the genotype at the D4S10 locus can be obtained from leukocytes, from cells in amniotic fluid, or from frozen brain tissue of deceased persons. If DNA is available from certain relatives, the inheritance of the HD gene or its normal counterpart can be inferred preclinically or prenatally. Predictive testing entails typing DNA from relatives of the person at risk to determine alleles at the marker locus in a particular family. The DNA of persons at risk is then typed to determine whether they have inherited the particular allele associated with the HD gene in the affected parent. Which family members have to be tested depends on their genetic proximity to the person at risk.

Preclinical testing for HD raises many issues not faced in routine genetic counseling (*4, 25*). The counselor must explain a relatively complicated test procedure in a manner that is understandable not only to the person at risk but also to the members of his or her extended family. Since the test is based on linkage analysis, it can never be 100% predictive; a "positive" or "negative" result can be interpreted only as an altered probability of carrying the gene, rather than a certainty. Determining this probability requires a complicated calculation based on both the genetic relationships of the persons typed and the age of onset of the disorder in gene carriers. Many at-risk persons who wish to be tested will have to be told that they are not testable because the appropriate relatives are not available or are unwilling to cooperate (*25*). Furthermore, even if all necessary samples of DNA can be obtained, making a firm determination is sometimes impossible if the marker alleles do not segregate in an informative fashion (*22*). This difficulty will decrease with time as additional markers are found and will change when the gene itself is cloned. The many ethical issues related to predictive testing for a fatal disease for which there is no treatment have been reviewed elsewhere (*4, 25*). Despite these reservations, presymptomatic and prenatal testing for linkage to the HD gene has begun at Massachusetts General Hospital, Johns Hopkins University, and a few other centers in Canada and Europe.

Duchenne Dystrophy

The application of "reverse genetics," that is, going from a gene to discover the cellular protein for which it encodes (*26*), has been used to advantage in Duchenne muscular dystrophy (DMD), the most common of the childhood dystrophies, which is inherited as an X-linked recessive disorder (*5, 6*). The incidence is approximately 1 in 3500 newborn boys; at least one-third of the cases represent new mutations. The disease is recognized in the early years of life by muscle weakness and muscle fiber necrosis, accompanied by elevation in blood levels of the muscle enzyme creatine phosphokinase. Mental retardation occurs in 30% of DMD-afflicted males (*27*).

The DMD gene has been localized to the middle of the short arm of the X chromosome in band Xp21 by mapping chromosomal deletions associated with the disease in males (*5, 6*), by isolating the junction fragment from a translocation (X;21) in a female with DMD (*28*), and by linkage to cloned anonymous probes containing RFLPs in adjacent DNA sequences (*5, 6*). The development of multiple extragenic and intragenic probes with RFLPs has improved markedly the accuracy of gene diagnosis in female carriers and in prenatal at-risk males (*5, 6*), although the high mutation rate has complicated such analyses. Darras *et al.* (*6*) applied RFLPs from 12 different probes derived from the vicinity of the DMD locus for prenatal and carrier detection in five families with seven at-risk pregnancies. In these studies, pitfalls in accurate diagnosis of the gene defect were due to the large size of the DMD gene, crossover events, and mutations. It is now known, on the basis of RFLP linkage analysis and deletion detection, that the less severe and less common Becker variant (incidence 1 in 30,000, onset in late childhood or early adulthood) is also caused by an abnormality at the same gene locus (*5, 6, 27*).

Physical restriction maps have been generated, by means of pulsed-field gradient gel electrophoresis, of a major fraction of the Xp21 gene locus covering a region containing up to a total of 3×10^6 bp (*29*). The positions of several probes linked to DMD have been assigned to this physical map covering approximately 2% of the X chromosome.

To define precisely the region containing the defective gene in DMD, Monaco and co-workers (*30*) used subtraction hybridization techniques to clone sequences within a large deletion spanning the DMD locus. One cloned DNA segment, pERT87, detected deletions in 5 to 10% of DMD patients. Subclones of pERT87 were used to initiate chromosome walking in genomic phage libraries. A segment of genomic DNA (220 kb, designated the DXS164 locus) was isolated that contained sequences that were deleted or altered in males with DMD. In an international collaborative study, 88 of 1346 (6.5%) of males with DMD or its Becker variant were found to have complete or partial deletions of the DXS164 locus (*30*). The disappointment from these studies arose from the recognition that RFLPs from the DXS164 locus showed recombination rates of 4 to 6% with the DMD gene, which indicated either that the DMD gene locus was extremely large or that the genomic DNA containing it showed recombination at an unusually high rate. Complete cloning of the gene has now confirmed that it encompasses at least 60 exons spanning more than 2000 kb (*31*). To find exons in the DXS164 locus, Monaco *et al.* (*30*) used single-copy genomic clones to hybridize to Southern blots from a variety of mammalian species. One clone was found to hybridize to the DNA of all mammals tested and hybridized to a large transcript of RNA isolated from human fetal skeletal muscle. From this RNA, complementary DNA (cDNA) clones were isolated that cover approximately 10% of this transcript. The cDNA clones map to Xp21 in the region of the DXS164 locus. The function of the RNA and of the large protein that it encodes remains unknown. The most recent evidence indi-

cates that the two known large structural proteins of muscle, titin (2000 kD) and nebulin (600 kD), are not involved in DMD (*31, 32*).

Myotonic Dystrophy

Myotonic dystrophy (MD) is the most common form of adult onset muscular dystrophy (*8*). It is a systemic disorder affecting the lens (cataracts), the heart (conduction defects), the skin and hair (frontal baldness), and, in some families, the brain (mental retardation). It is inherited as an autosomal dominant disorder mapped to chromosome 19 (*33*). No documented cases of new mutation have been found. Several large families have been studied by linkage analysis, first with the use of apolipoprotein C2 (*33*), and, more recently, with anonymous DNA probes with informative RFLPs. Bartlett *et al.* (*8*) examined six large MD families with probes selected from genomic DNA libraries enriched for chromosome 19. One clone, designated LDR152 (D19S19), was shown to be tightly linked to MD with a recombination fraction (theta) of 0.0 and a LOD score of 15.4. No crossover events were found in informative MD families when two different polymorphisms were used, which made the linkage of the probe to the gene locus exceedingly close. New RFLPs developed by chromosome walking should make D19S19 even more informative for carrier detection and prenatal diagnosis. Moreover, isolation of additional DNA probes should lead quickly to physical mapping of the DNA on both sides of the gene locus. The MD locus resides on the q arm of chromosome 19 near the centromere (*8*).

Familial Amyloidotic Polyneuropathy

Neurological abnormalities associated with amyloid deposition take several forms. The genetic basis of one of these, familial amyloidotic polyneuropathy (FAP), has been completely elucidated. FAP is an autosomal dominant disorder first described by Andrade in Portuguese families (*34*). Families of Jewish, Japanese, and Swedish lineage have also been identified (*7*). The disease is characterized by onset in adults of distal extremity sensory loss and weakness. Autonomic nervous system involvement is prominent with postural hypotension, anhidrosis, impotence, and gastrointestinal dysfunction. Symptoms usually begin in the third decade, but in some pedigrees the onset occurs as late as the sixth or seventh decade (*7*). The pathological feature of the disease is the deposition, in an extracellular location, of amyloid fibrils (β-pleated protein polymers) in peripheral nerve, heart, and kidneys, but not brain or other tissues of the central nervous system (*35*).

The disease in most pedigrees examined is caused by a single–base pair mutation on chromosome 18 that encodes for the protein transthyretin (formerly called prealbumin), a tetrameric 56-kD serum carrier protein that transports thyroxine and vitamin A (retinoic acid) (*36*). In Japanese, Portuguese, and Swedish families with FAP there is substitution of methionine for valine at position 30 in transthyretin. A different amino acid substitution of glycine for threonine at position 49 is reported in a Jewish pedigree (*7*), and yet other substitutions have been found in other families (*37*). Family studies of FAP strongly suggest that the mutation in transthyretin is both the genetic and the biochemical basis of the disease.

The gene for transthyretin has now been fully characterized (*36*). The nucleotide sequence of the gene spans 70 kb and consists of four exons and three introns. Consensus TATA and CAAT sequences are found 30 and 101 nucleotides, respectively, upstream from the putative cap site (*38*).

If the mutation in transthyretin is the explanation for the disorder, the question arises as to what mechanism or mechanisms might lead to a delayed onset of the disease until adulthood. Saraiva *et al.* (*7*) examined this question in two Portuguese families, one with typical early-adult onset and the other with late-life onset. Plasma samples were analyzed for protein abnormalities in transthyretin. Leukocyte DNA, subjected to the restriction endonuclease, NsiI, was examined for RFLPs. In both early and late onset cases, and in presymptomatic children, the same quantitative abnormalities were found in transthyretin. Abnormal transthyretin (Met^{30}) circulates in plasma early in life. Therefore, the preclinical phase of the disease cannot be accounted for by developmental repression of the mutant gene, that is, the same genetic mutation was expressed early in life in both early and late onset cases. The factors that determine delayed deposition of the amyloid protein in tissues remain unknown. Conversely, the protective mechanism that delays onset of symptoms in one pedigree and not in another would be important to discern. This disease, present from birth, but manifest clinically only in late life, has potentially major implications for our understanding of the amyloid deposits in the senile plaques of Alzheimer's disease.

Alzheimer's Disease

Alzheimer's disease (AD) is a neurodegenerative disorder of late-life onset characterized clinically by memory loss and other disorders of cognitive function (*39*). Neuropathologic changes, which are prominent in the association areas of cerebral cortex, include intraneuronal neurofibrillary tangles containing paired helical filaments, and senile (neuritic) plaques. The latter contain a central core of extracellular amyloid surrounded by degenerating distended neuronal processes.

Four lines of research have culminated in important new ideas about Alzheimer's disease, resulting in the localization of the gene in familial cases to chromosome 21 and to the cloning of the gene for the amyloid protein. (i) Clinicians recognized that about 10% of patients with typical AD, often with an earlier onset, inherited the disorder in an autosomal dominant pattern; this is so-called familial Alzheimer's disease (FAD) (*40*). (ii) Patients with Down syndrome (formerly called mongolism) who live past the fourth decade invariably manifest the same neuropathologic features as seen in AD, including both neurofibrillary tangles and senile plaques (*41*). Moreover, several careful studies of aging Down syndrome patients give evidence, at least in some patients, of a progressive intellectual deterioration, that is, dementia (*42*). Because Down syndrome is associated in some cases with trisomy 21, it seemed possible that alterations in DNA on chromosome 21 might cause the brain changes associated with AD. (iii) These observations led to the third approach, which was to develop a panel of DNA probes located on chromosome 21 with RFLPs that could be used to study large families with FAD. The results of such an analysis in four families documented genetic linkage to two probes (designated D21S1/D21S11) on the proximal region of the long arm of chromosome 21 (*9*).

(iv) The fourth discovery was the cloning of the gene for the amyloid protein (A_4 or β-amyloid) and demonstration that the gene resides in the same region of chromosome 21 as the DNA markers linked to FAD. The amino acid sequence of cerebral vascular amyloid (the β protein) was first reported by Glenner and colleagues (*43*) and a similar structure confirmed by Masters *et al.* from neuritic plaques (A_4 protein) (*44*). Oligonucleotide probes constructed from its known amino acid structure were used independently in four laboratories to clone the gene (*45–47*). The amyloid gene is

expressed normally in brain but is also present in kidney, thymus, muscle, heart, and liver (*45–47*). Kang and co-workers (*46*) described a cDNA encoding a large precursor of the amyloid protein that they speculated, based on its structure, was a membrane-spanning glycoprotein. Both AD and normal brain contained two transcripts of 3.2 and 3.4 kb, respectively. Tanzi *et al.* (*47*) showed that messenger RNA (mRNA) for the β-amyloid gene is highest in frontal, parietal, and temporal lobe association cortex, regions known to contain abundant numbers of senile plaques in AD. However, an exception was hippocampus where mRNA levels for the amyloid protein were low, but where amyloid plaques are common in AD.

At present, it is unknown whether abnormalities in amyloid gene regulation are the primary pathogenetic mechanism in either familial or sporadic AD. A relation between β-amyloid gene duplication and the occurrence of amyloid deposition in sporadic AD was suggested by the studies of Delabar and associates (*48*), who reported that in three sporadic cases of AD there were three copies of the β-amyloid gene. The gene duplication was also found in Down syndrome without trisomy. However, they did not cite evidence regarding the incidence of this finding in a population of sporadic cases of AD. Current efforts in several laboratories are directed to determining whether the amyloid gene is overexpressed in AD and to define whether the locus of DNA markers linked to FAD on chromosome 21 are coincident with the β-amyloid gene locus. Recently, two separate groups of investigators have shown negative linkage of the β-amyloid gene to FAD, indicating that the two loci both on 21q are separated by more than 15 centimorgans (*49*). Moreover, neither group of investigators found any evidence for gene duplication or overexpression of amyloid protein in FAD (*49*), contrary to the earlier report (*48*).

The origin of the amyloid that deposits in senile plaques in patients with AD remains controversial. Kang *et al.* (*46*) argued for an origin of the amyloid in brain tissues, based on their demonstration that amyloid is derived from a precursor protein with a receptor-like structure. This idea is strengthened by in situ hybridization studies that demonstrate the presence of β-amyloid mRNA within cortical neurons (*50*). On the other hand, the deposition of amyloid in brain vessels and the expression of the message in tissues outside the central nervous system favor the possibility that circulating amyloid may reach the brain through an altered blood-brain barrier. Its deposition in brain tissue could then lead to secondary alterations in neurons. The relation between the factors that cause senile plaque formation and those responsible for neurofibrillary tangles remains unknown.

Although linkage in four families of FAD has been shown, the possibility of genetic nonallelic heterogeneity cannot be excluded until many more families are studied. The use of the markers identified by St George-Hyslop *et al.* (*9*) for presymptomatic recognition of the disease will need to await these additional studies.

Neurofibromatosis

This condition, characterized by multiple tumors originating from Schwann cells, is known to be inherited in an autosomal dominant manner. Two separable phenotypic patterns have emerged, one affecting primarily the peripheral nervous system [von Recklinghausen's neurofibromatosis (VRNF)] and the other the central nervous system, particularly the eighth cranial nerves [bilateral acoustic neurofibromatosis (BANF)]. VRNF is one of the most common autosomal dominant conditions with an estimated prevalence of about one in 3000 (*51*); about half of the cases represent new mutations. In addition to tumors on nerves, patients commonly demonstrate cutaneous pigmentary changes (café-au-lait spots) and iris hamartomas.

Recent linkage studies have identified the gene for VRNF to be the region near the centromere on chromosome 17 (*10, 11*). In 15 kindreds reported from Utah, no evidence of nonallelic heterogeneity was identified. In a confirmatory study reported by Seizinger *et al.* (*11*) an additional 13 families were examined and linkage shown to the locus of the nerve growth factor receptor gene on 17q12 → 17q22. However, crossovers occurred with the VRNF locus, which made it unlikely that the VRNF gene defect is in the nerve growth factor receptor gene itself.

Studies in BANF show the disorder to be linked to chromosome 22 (*12*). This observation has potential major implications for understanding neural crest tumor formation. Linked DNA markers will be particularly valuable in BANF for early identification and treatment of the tumors that occur.

Manic-Depressive Illness

There is growing evidence that individuals in some families may be genetically predisposed to manic-depressive illness. The first clue to the chromosomal location of the gene was derived from studies in the Old Order Amish of Pennsylvania by Gerhard *et al.* (*52*), who provided preliminary evidence for location of the gene to the short arm of chromosome 11. Additional recent evidence from this group has established that two genes known to lie near each other on chromosome 11p are frequently inherited with the affective disorder (*13*). The linkage was established further in this family with cloned genes used as markers adjacent to highly variable regions of DNA. However, two other groups of investigators showed in six other families, three from Iceland (*53*) and three from North America (*54*), that linkage with the same probes to chromosome 11 each gave a negative LOD score, indicating the likelihood of nonallelic genetic heterogeneity for manic-depressive illness. Baron *et al.*, studying pedigrees of patients in Israel, and Mendlewicz *et al.*, examining patients in Belgium, both demonstrated genetic linkage between bipolar affective illness and the X chromosome (*14*). An interesting association between the X linkage and color blindness was noted in these families. These data, taken as a whole, suggest that manic-depressive illness, which in some families takes primarily a bipolar form, and in others is unipolar, is caused by several different disorders. Manic-depressive disorder linked to chromosome 11 is of considerable interest because the marker genes on that chromosome are close to the gene encoding tyrosine hydroxylase, the rate-limiting enzyme for the biosynthesis of the catecholamines. Abnormalities of catecholamine function have long been considered a potential pathogenetic mechanism in manic-depressive illness.

Autosomal Recessive Disorders

G_{M1} and G_{M2} gangliosidosis. Although the enzyme defect is known for many autosomal recessive diseases that affect the central nervous system, the precise molecular genetic basis of the enzyme abnormality has been documented for only a few. G_{M1} gangliosidosis is caused by a deficiency of the lysosomal acid enzyme β-galactosidase (*55*). The main clinical features of the infantile type, also known as generalized gangliosidosis, are early onset of mental retardation, seizures, facial and skeletal dysmorphism, and hepatosplenomegaly. Death usually occurs by 2 years of age. The structural gene for β-galactosidase is on chromosome 3p1 (*56*), but the precise molecular defect in the enzyme is not reported.

Tay-Sachs disease and Sandhoff disease (G_{M2} gangliosidoses) are

lipid storage disorders caused by mutations in the α or β chains, respectively, of the enzyme hexosaminidase (*57*). Two isoenzymes of hexosaminidase composed of different subunits of the α and β chains are known; type A (Hex A) is composed of one α and two β and type B (Hex B) is composed of four α chains. The α chain is encoded on chromosome 15 and the β chain on chromosome 5. Tay-Sachs disease presents clinically during the first year of life with psychomotor retardation, cherry-red spot in the macula region of the eye, and seizures. The child's outward physical appearance is normal, and there is no hepatosplenomegaly. Sandhoff disease has a similar clinical picture with the added feature of lipid-laden foam cells in the bone marrow. The molecular basis of the two diseases has now been partially elucidated. Tay-Sachs disease is caused by a mutation in the α chain, which in families of Ashkenazi Jewish origin is associated with defective α-chain mRNA in fibroblasts (*56*). Comparison of these lineages with French-Canadian pedigrees has defined a different mutation in the latter, which is associated with a 5′ deletion of approximately 5 to 8 kb in the gene encoding for the α chain, whereas Ashkenazi Jewish patients showed an intact gene (*58*). Sandhoff disease is due to defects in the β chain, and a distinction is reported for cases with juvenile versus infantile onset. In 4 of 11 infantile cases, deficiencies in pre-β-chain mRNA were detected and found to be associated with partial gene deletions in the 5′ end of the gene on chromosome 5 (*59*). In juvenile cases, normal or reduced levels of pre-β-chain mRNA were found with no abnormalities in the Hex B gene. These data indicate that each clinical group of Sandhoff disease is comprised of a collection of different Hex B mutations.

Gaucher's disease. Gaucher's disease is another systemic lipid storage disease caused by a deficiency in the lysosomal enzyme glucocerebrosidase (*60*). The disease is classified into three clinical subtypes, 1, 2, and 3. The central nervous system is not involved in type 1. Type 2 is a much rarer form, presenting as a rapidly progressive neuronopathic storage disease; clinical features include cranial nerve and brainstem abnormalities and marked hepatosplenomegaly, with onset usually in the first 6 months of life. In type 3, nervous system degeneration also occurs, but the onset is later and progression more chronic. The molecular basis of the enzymatic defect in type 2 has recently been characterized. Tsuji *et al.* (*60*) discovered a single–base pair change (T to C) on chromosome 1, which led to substitution of proline for leucine in amino acid position 444 of glucocerebrosidase, which resulted in a new cleavage site for the restriction endonuclease NciI. It was speculated that this single–amino acid mutation affects the α-helical structure of the enzyme resulting in inactivation. The new cleavage site provided the opportunity to use restriction fragment length variations generated by NciI digests to detect homozygotes and unaffected heterozygotes in pedigrees of affected families. An unexpected finding was that not all type 2 patients were homozygous for the defect. Not surprising, none of the type 1 patients were homozygous. These findings are of considerable interest in pointing out how minor mutations can cause devastating diseases but leave unanswered how molecular differences can account for the variable phenotypic expression between types 1, 2, and 3 (*61*).

Lesch-Nyhan Disease

Lesch-Nyhan disease is an X-linked recessive disorder caused by a defect in hypoxanthine phosphoribosyltransferase (HPRT), the enzyme that converts hypoxanthine and guanine to inosinic acid and guanylic acid, respectively (*2, 15, 62*). More is known about the genetic basis of this disease than any other neurological disorder. A deficiency of HPRT results in a clinical spectrum of neurological dysfunction: self-mutilation, recurrent vomiting, hypotonia, chorea, and, later, severe pyramidal and extrapyramidal signs. Mental retardation is mild to moderately severe. Despite the major central nervous system manifestations, neuropathologic abnormalities have not been found in autopsied cases.

There is a single active gene encoding for HPRT near the end of the long arm of the X chromosome (Xq27) (*62, 63*). The active HPRT gene is 44 kb in length, contains nine exons, and encodes for a 1.6-kb mRNA. More than one-third of the affected males represent new mutations. Females are rarely affected. Biochemical analyses of HPRT in Lesch-Nyhan syndrome and in gout have revealed four different amino acid substitutions that can be accounted for by single–base pair mutations in the HPRT gene. In 5 of 28 sporadic cases, Southern blot analyses of genomic DNA have revealed rearrangements of DNA resulting in alterations or deletions of sequences of the HPRT gene. However, in approximately 85% of cases, the gene appears normal by Southern and Northern blotting. In these cases, the defect likely is a point mutation, or small DNA deletion or rearrangement, abnormalities demonstrated more often by digestion with ribonuclease (*64*). These studies demonstrate vividly the variety of mutations that can be associated with loss of a single gene function.

Other Diseases

Linkage studies with RFLPs have also shown the chromosomal site for one type of spinocerebellar atrophy (*65*) (chromosome 6) for one form of Charcot-Marie-Tooth disease (*66*) (chromosome 1), Wilson's disease (*67*) (chromosome 13), and for adrenoleukodystrophy (*68*) (X chromosome) (Table 1).

Brain Tumors: Retinoblastoma, Neurofibromatosis, and Meningioma

Molecular genetic studies of neural tumors have revealed changes in DNA, including rearrangements, loss, amplification, and mutation of genomic sequences (*2*). Investigations of two human conditions, retinoblastoma and BANF, suggest strategies that may prove profitable for understanding mechanisms of neural oncogenesis.

Retinoblastoma. Retinoblastoma is a rare malignancy of childhood. Although most cases are sporadic and unilateral, about 15% of cases are inherited and bilateral (*69*). Studies of retinoblastoma have provided evidence of genetic mechanisms involved in tumor formation. Hereditary cases are transmitted as an autosomal dominant trait. The locus for the gene in retinoblastoma is on chromosome 13q14; deletions of DNA in this region are associated with tumor development. Tumors occur when retinoblasts with one defective gene copy in this region (germinal mutation) undergo a second mutational event (somatic mutation or "second hit" of the chromosome carrying the normal allele), resulting in loss of the remaining normal copy of the gene. The defect appears to be the loss of a normal gene that directly or indirectly regulates cell division and growth.

Cavenee and co-workers (*69*) used DNA probes homologous to single-copy DNA sequences spaced along the q arm of chromosome 13 to compare RFLPs of DNA from tumor tissue with DNA derived from lymphocytes in the same patients. Loss of the normal gene that unmasks recessive mutation at the retinoblastoma locus were shown to occur by several different mechanisms, including nondisjunction, reduplication, mitotic recombination, gene conversion, or point mutation. Fung *et al.* (*70*) found that 16 of 40

retinoblastomas probed with a cDNA to the retinoblastoma gene had identifiable structural changes, including in two cases homozygous internal deletions. By "reverse genetics" Friend *et al.* (*71*) identified the normal gene that is mutant in retinoblastoma. Sequential cloning of adjacent DNA by chromosome walking led to isolation of 30 kb, one cloned fragment of which hybridized to RNA derived from human retinal cells. A 4.7-kb RNA transcript was identified, which appears to correspond to the RNA found by Lee *et al.* (*72*) that codes for a protein of 816 amino acids. Discerning the function of this protein, absent in retinoblastoma, will be important for understanding mechanisms of tumor suppression.

Acoustic neurofibromas. Similar strategies have been used to examine another disorder, BANF (*73*). In this condition, bilateral tumors occur on the eighth cranial nerve, leading to deafness. The patients also exhibit a higher susceptibility to a variety of other nervous system tumors including meningiomas, gliomas, and neurofibromas of the spinal cord. Seizinger and colleagues (*73*) examined tissue obtained from these tumors in search of chromosomal deletions that might provide a clue to the site of the genetic abnormality. DNA from patients with bilateral acoustic neuromas was compared with DNA from the patient's own leukocytes. Tumor tissue from these patients consistently demonstrated loss of a region of chromosome 22. These workers have subsequently produced a panel of DNA probes for chromosome 22 and have shown that families with BANF show linkage to chromosome 22 (*12*), whereas families with VRNF, a disorder associated with peripheral neuromas, show negative linkage to chromosome 22 and positive linkage to chromosome 17 (*10, 11*). At this juncture, it appears that the central form of the disease is analogous to retinoblastoma caused by a "second hit" disorder because of loss of a cell growth–controlling gene on chromosome 22. The peripheral form of neurofibromatosis, on the other hand, is a different disorder due to a gene defect on chromosome 17 (*10, 11*). Precise definition of the genetic defects in these conditions remains to be accomplished, but this discovery will have important implications for understanding growth characteristics of several central nervous system tumors.

Meningioma. Two reports, using molecular genetic approaches, have shown a frequent association in meningiomas of DNA deletions on chromosome 22 (*74*). These abnormalities add yet another to the series of tumors in which predisposition may be determined by "recessive oncogenes."

Future Directions

Recent applications of molecular biology, which have been successful in many other genetic diseases, have now yielded important new insights concerning disorders affecting the central nervous system. Characterizations of the structure and function of individual genes causing neurological disorders are in the early stages, but information already available shows that the molecular mechanisms underlying the phenotypic expression of a given disease can encompass a range of genetic errors. Modifying factors that determine variations in the phenotypic expression of a single genetic abnormality as, for example, what factors determine early versus late onset of the illness in different individuals, remain essentially undefined.

What are the implications of these discoveries for neurology and psychiatry in the years ahead? First, linkage analysis with RFLPs can now be applied to many of the other genetic disorders that are at present poorly understood. Second, even before isolation of the genes involved, linkage analysis can be used for genetic counseling in HD, MD, DMD, and in those families with FAD where linkage has been documented. The third issue is more challenging: how to develop improved strategies for moving quickly and effectively from genetic linkage maps to physical maps in order to locate, define, and clone abnormal genes. So far this has proven more difficult than expected, either because genes are large as in DMD or because methods for cloning large DNA segments of 50 to 150 kb are still under development (*75*). The fourth issue will be even more challenging: how to discover the functions of genes that are eventually characterized? After discovery of a protein abnormality, steps taken to identify its normal function will likely be a complex undertaking. One interesting approach, not currently exploited fully, will be to introduce abnormal genes into transgenic animals and to examine for phenotypic expression. Animal models of disease have been of great value in several autosomal recessive diseases, such as those affecting myelin basic protein (shiverer mouse) (*76*), proteolipid protein (jimpy mouse, Pelizaeus-Merzbacher disease) (*77*), and galactocerebroside (twitcher mouse) (*78*). If abnormal genes in FAD or HD can be expressed in animals, the possibility of elucidating disordered function would hold great promise. One interesting model has been the demonstration that transgenic mice bearing the *tat* gene of human T-lymphotropic virus type 1 (HTLV-1) develop multiple peripheral nerve tumors that morphologically resemble those occurring in neurofibromatosis (*79*).

Gene therapy of disorders affecting the central nervous system seems a long way off. Partial correction of the gene defect in the shiverer mouse has been accomplished by transgenic experiments (*76*). Development of viral vectors specific for individual cell types is the subject of current research in many laboratories. At present, one can be optimistic that genes can be introduced into replicating cells with these techniques, as already shown in Gaucher's disease fibroblasts (*80*), but whether site-specific directed gene insertion will be possible into intact postmitotic brain neurons in vivo remains to be shown.

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