Molecular Genetics in Neurology

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Genetically determined diseases with neurological manifestations constitute a big public health problem in many communities including Saudi Arabia. As a result of advances in molecular genetics, the bases of several neurological diseases have now been deciphered. A whole series of genes of neurologic interest have been cloned and sequenced. The analysis of human DNA using recombinant technology is fast becoming an integral part of the diagnosis of number of neurological disorders. It is expected that new methods of mapping the human genome will provide us with a valuable approach in elucidating molecular pathology of these conditions and preventing them. This review presents recent advances in molecular neurogenetics and molecular techniques used for gene analysis as a prelude to possible gene therapy.

The genetic information which is stored in deoxyribonucleic acid (DNA) is normally packed into 23 pairs of chromosomes. Because the chromosomes exist in homologous pairs, so do the genes and as a result of a mutation, individual genes may exist in different forms or alleles. Nowadays, there is instinctive and universal concern about having abnormal offspring and it is estimated that sometime in their reproductive life, many women commence a pregnancy with chromosomal abnormality.1 However, most such pregnancies end in spontaneous abortion and, until recently, many infants born with serious abnormalities died, often undiagnosed, in early childhood. DNA recombinant technology allows the identification of many inherited diseases in fetal life, and chorionic villus sampling in the first trimester of pregnancy has increased the possibility of an early diagnosis. Several direct and indirect methods which will be described below have been used to carry out detailed molecular analyses.

Over the last decade, remarkable advances in the field of molecular neurogenetics have transformed the prospect of using linked genetic markers to predict the transmission of disease-causing genes. Linkage relationships have been described in recent years for several autosomal dominant diseases of the nervous system. Chromosomal assignments have been made for several autosomally inherited neurological diseases.2 Amongst the many loci assigned to chromosome X, several are implicated in neuromuscular diseases, e.g. in the case of Duchenne muscular dystrophy, not only the locus has been precisely mapped on the X chromosome but the gene has also been isolated and its product (dystrophin) defined. Other loci, such as that of Emery-Dreifuss muscular dystrophy, have been aspired to chromosomal bands. Because of their mode of inheritance, mutations occurring on the
X chromosome often result in clinical syndromes whose genetic basis can be relatively easily established by family studies. Accordingly, more disease-causing loci are known to be located on chromosome X than on any autosome and progress toward establishing a physical map is most advanced in the case of the X chromosome.3

In Saudi Arabia, there is an abundance of neurological disorders. Information on the pattern and frequency of neurological diseases is, however, relatively scanty and limited mainly to a survey report4 and few hospital-based studies.5,6 The molecular bases of neurological diseases are almost unknown in Saudis and there is a great potential for advances in the area of molecular neurogenetics. This is because the kingdom not only has the genetic material but is also due to the young population and the high rate of consanguinity,4 large pedigrees are available to investigate linkage relationships and for molecular studies on neurological disorders.

In this concise review, we present the molecular pathogenesis of neurological diseases and a description of DNA recombinant techniques applied for the localization, cloning and identification of genes which mutate to cause or predispose to neurological disorders. It is expected that the information provided here may help in the prevention of inherited neurological disorders and their complications in the Saudi community by definitive diagnosis, carrier detection and genetic counselling, and ultimately for gene therapy when available.

The Gene

The gene is a piece of DNA at a locus on a chromosome and it encodes for a specific protein or several related proteins. The analysis of functional genes have shown that their coding sequences (exons) are joined by sequences of unknown functions known as introns or intervening sequences (IVS) (Fig. 1). The number and size of introns, which are often considerably longer than exons, varies from gene to gene. The DNA sequences extending on either side of a specific locus or gene are called flanking regions. The introns, the 5' and 3' flanking regions and exons are transcribed into nuclear mRNA precursors. At the 5' end of the gene, there is a specific triplet (ATG) (initiation codon) and at the 3' end, there is a termination (stop) codon (TAA, TAG or TGA) controlling protein synthesis. Genes also have sequences of varying lengths at both ends (5' and 3') which determine the structure of non-translated regions of mRNA. Most genes have blocks of sequences in their 5' flanking regions. The first one is about 20–30 base pairs (bp) upstream from the RNA initiation or CAP site called the ATA box; the second, called the CCAAT box, is located about 70–90 bp upstream from the beginning of the gene (Fig. 1).

**Molecular Techniques of Gene Analysis**

Recombinant DNA technology is currently playing an important role in neurosciences.7 During the last decade a large number of neurogenetic diseases have been characterized at the molecular level or mapped by polymorphic markers thus providing us the opportunity to make a firm diagnosis. This rapid expansion of knowledge about the molecular pathogenesis of inherited neurological diseases in which restriction enzymes and molecular probes have played an important role has been the direct result of technical advances of revolutionary magnitude. The availability of a number of powerful techniques which have included gene mapping or restriction endonuclease analysis (Southern blotting technique), restriction fragment length polymorphism (RFLP) analysis, gene cloning, dinucleotide (CA)n repeat polymorphisms (direct DNA sequencing analysis), reverse genetic techniques etc. coupled with the polymerase chain reaction (PCR) have proved to be techniques of potential application in both pre- and postnatal detection of carriers in a number of neurological disorders.

Amongst the above molecular techniques, the dinucleotide (CA)n repeat polymorphism (DNA sequencing), reverse genetics and partial DNA sequencing have found extensive application in most world laboratories and need some discussion here.

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**Figure 1.** A schematic diagram of a gene.
DNA sequencing

Two methods are available for DNA sequencing namely the Maxam & Gilbert method and the chain terminator or dideoxy procedure (Sanger method), the latter considered to be the more powerful technique. It is favoured for large scale DNA sequence determination and is mostly used in conjunction with cloning the DNA to be sequenced in the M13 mp series of single-stranded vectors or Blue script vectors. It is known that repeat elements of the form (dC-dA)n.(dG-dT)n referred to as (CA)n repeats are present in DNA and the human telomere (the stretches of DNA that cap chromosomes) consist of hundreds of these repeating units. In 1989, Weber et al. demonstrated that specific human (CA)n repeats are polymorphic in length among individuals and therefore represent potential genetic markers. The function of these (CA)n repeats is unknown but it has been suggested that they may act as hot spots for recombination or participate in gene regulation. To identify these (CA)n repeats PCR amplified DNA is required, which is then incubated with one or more radiolabelled deoxynucleoside-triphosphates (e.g. dATP, dCTP, dGTP and dTTP) in four separate incubation mixes; and the primer being annealed either side of the (CA)n repeat to be studied. After a suitable incubation period the DNA in each mixture is denatured, electrophoresed side by side and the radioactive bands of single-stranded DNA are detected by autoradiography. The sequences (e.g. T, C, G, A) can be then read directly from radioautograph (Fig. 2). The use of (CA)n repeat analysis offers several advantages over standard RFLP analysis. It is more informative due to its highly polymorphic nature, thus facilitating linkage studies; in contrast RFLPs are less polymorphic showing only two or three alleles (Fig. 3). Use of the PCR to detect DNA polymorphisms offers improved sensitivity and speed as only a small volume of DNA (0.05 ng) is needed and several markers can be run at once in a multiplex reaction.

Reverse genetics

The availability of markers distributed throughout the whole human genome has given birth to the concept of reverse genetics which consists of first isolating the disease gene, then working backwards to identify the metabolic defect. This approach has led to the localization of the gene responsible for Huntington’s chorea on the short arm of chromosome 4 and the identification of dystrophin, an abnormal gene product in Duchenne muscular dystrophy.

Partial DNA sequencing

Recently, a new approach for the rapid characterization of expressed genes by partial DNA sequencing to generate ‘expressed sequence tags’ (EST) has been described. ESTs have been applied in the discovery of new genes, mapping of the human genome and identification of coding regions in genomic sequences and were used in the sequence identification of 2375 human brain genes which probably represents as many as 5% of the genes in human genome.

The details of the procedures used in DNA recombinant technology are beyond the scope of this review but can be found in any of the available reviews of molecular biology. However, mastery of these DNA recombinant techniques requires familiarity with the basic concept of these procedures and their choice and usefulness in investigation of a genetic disorder of particular interest.
All individuals have Ban1 cleavage sites creating a 2.6 kb DNA fragment from chromosome 19.

Individual A
2.6kb
2.6kb

Individual B
2.6kb

Individual C
Base change creating a new Ban1 cleavage site
Base change creating a new Ban1 cleavage site

Cleavage of DNA with Ban1
2.6kb
2.6kb
1.2kb
1.4kb
1.2kb
1.4kb

DNA hybridized against marker apolipoprotein C2 (APO2)
This probe hybridizes to the 1.2 kb fragment of the DNA and therefore also the 2.6 kb fragment

2.6kb
2.6kb
1.2kb
1.4kb
1.2kb
1.4kb

Autoradiograph
2.6kb
1.2kb
1.2
1.2
2.2

Figure 3. Schematic representation of RFLP linkage analysis in a patient with myotonic dystrophy.

Classification of Genetic Disorders

The genetic disorders can be classified into three main groups. First, there are single gene defects which can be traced through families and are clearly defined as dominant, recessive or X-linked. The approximate prevalence of these disorders in Western populations are 9/1000, 2/1000 and 1–2/1000 births respectively. Second, there are chromosomal abnormalities (3/1000 births) some of which can be related to specific clinical syndromes. The third group is comprised of congenital malformations (15–20/1000 births), at least some of which seem to have a strong genetic component.

With advances in DNA recombinant technology, it is becoming increasingly possible to detect healthy carriers of many single gene defects. The diagnosis of an individual with a dominant or X-linked condition can lead to the identification of many members of the family who are at risk. By contrast, for common recessively inherited disorders, the relatively large numbers of carriers and the rarity of a family history of a disorder makes population screening the only realistic way to identify them. At present, in the absence of a family history, accurate methods for carrier detection are available for many neurodegenerative disorders.

Single gene defects

Inherited neurological disorders due to single gene
Table 1

<table>
<thead>
<tr>
<th>Disorder</th>
<th>McKusick (MIM) No.</th>
<th>Chromosomal mapping</th>
<th>Frequency/1000 births</th>
<th>Biochemical defect</th>
<th>Clinical manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Charcot-Marie-Tooth disease (HMSN lb)</td>
<td>118220</td>
<td>1q2</td>
<td>—</td>
<td>U</td>
<td>Hyporeflexia, distal weakness, pes-calls, nerve hypertrophy, mild/moderate sensory loss, very slow motor NCV</td>
</tr>
<tr>
<td>Huntington’s chorea</td>
<td>143100</td>
<td>4pter-p16.3</td>
<td>0.7</td>
<td>U</td>
<td>Involuntary movement and dementia from middle life</td>
</tr>
<tr>
<td>FSH muscular dystrophy</td>
<td>182970</td>
<td>4</td>
<td>0.05</td>
<td>U</td>
<td>Weakness and/or atrophy in the muscles of the face and shoulder girdle</td>
</tr>
<tr>
<td>Spinal muscular atrophy</td>
<td>182980</td>
<td>5q11.2–13.3</td>
<td>0.04</td>
<td>U</td>
<td>Symmetrical weakness and atrophy of limb muscles, Degeneration of anterior horn cells</td>
</tr>
<tr>
<td>Juvenile myoclonic epilepsy</td>
<td>159700</td>
<td>6</td>
<td>—</td>
<td>U</td>
<td>Juvenile onset of grand mal and myoclonic seizures</td>
</tr>
<tr>
<td>Tuberous sclerosis</td>
<td>191100</td>
<td>9q34</td>
<td>0.01</td>
<td>U</td>
<td>Mental retardation, epilepsy, skin changes</td>
</tr>
<tr>
<td>Charcot-Marie-Tooth disease (HMSN 1a)</td>
<td>118210</td>
<td>1pter-p11.2–P12</td>
<td>0.05–0.4</td>
<td>U</td>
<td>Similar to HMSN I but often less pronounced, no nerve hypertrophy normal or mild slowing of motor NCV</td>
</tr>
<tr>
<td>Neurofibromatosis (peripheral)</td>
<td>162200</td>
<td>17q11.2</td>
<td>0.3</td>
<td>U</td>
<td>Multiple cafe-au-lait spots, multiple cutaneous neurofibromata</td>
</tr>
<tr>
<td>Periodic paralysis (hyperkalaemic)</td>
<td>170500</td>
<td>17</td>
<td>—</td>
<td>U</td>
<td>Intermittent attacks of weakness</td>
</tr>
<tr>
<td>Familial amyloid neuropathy</td>
<td>104800</td>
<td>18</td>
<td>—</td>
<td>Transthyretin</td>
<td>Progressive sensory autonomic neuropathy, amyloid infiltration in nerves</td>
</tr>
<tr>
<td>Myotonic muscular dystrophy</td>
<td>160900</td>
<td>19cen–q18.2</td>
<td>0.2</td>
<td>U</td>
<td>Myotonia with atrophy and several degenerative changes in other organs</td>
</tr>
<tr>
<td>Familial Alzheimer’s disease late onset</td>
<td>104310</td>
<td>19</td>
<td>—</td>
<td>U</td>
<td>Profound memory loss; impaired judgement, confusion, and even dual disintegration of cognitive functions</td>
</tr>
<tr>
<td>early onset</td>
<td>104300</td>
<td>21q11.2–22.2</td>
<td>—</td>
<td>U</td>
<td>Neurofibromas, meningiomas, gliomas, schwannomas</td>
</tr>
<tr>
<td>Neurofibromatosis (central) type 2</td>
<td>162210</td>
<td>22q</td>
<td>0.01</td>
<td>U</td>
<td></td>
</tr>
</tbody>
</table>


Defects are caused by mutant genes and usually exhibit obvious and characteristic pedigree patterns. A mutation may be present on only one or on both of a pair of autosomes or on the X chromosome. Tables 1–3 describe the incidence and chromosomal assignments for several inherited neurological diseases with single gene defects.

**Autosomal dominant**

These disorders (Table 1) are usually manifest in all carriers of a single abnormal gene. Some of these dominant conditions occur at a relatively high frequency presumably because they have little effect on reproduction fitness. Dominant genetic disorders which become manifest late in life usually have a strong family history and the
Table 2
Incidence and regional mapping for some autosomal recessive inherited neurological disorders

<table>
<thead>
<tr>
<th>Disorder</th>
<th>McKusick (MIM) No.</th>
<th>Chromosomal mapping</th>
<th>Frequency/1000 births</th>
<th>Biochemical defect</th>
<th>Clinical manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gaucher disease</td>
<td>230800</td>
<td>1q 21</td>
<td>—</td>
<td>Acid β-glucosidase</td>
<td>Anaemia, loss of intellectual achievements followed by progressive spasticity</td>
</tr>
<tr>
<td>Gangliosidosis (GM1)</td>
<td>230600</td>
<td>3p 21</td>
<td>—</td>
<td>β-galactosidase-1</td>
<td>Severe cerebral degenerative disease and poor psychomotor development</td>
</tr>
<tr>
<td>Gangliosidosis type 2 (GM2)</td>
<td>268800</td>
<td>5q 13</td>
<td>—</td>
<td>Hexosaminidase A and B (β chain)</td>
<td>Mental retardation blindness</td>
</tr>
<tr>
<td>Spinal muscular atrophy</td>
<td>313200</td>
<td>5q 12–14</td>
<td>—</td>
<td>U</td>
<td>Progressive neurogenic muscle atrophy</td>
</tr>
<tr>
<td>Friedreich’s ataxia</td>
<td>13660</td>
<td>9q 13</td>
<td>0.1–0.2</td>
<td>U</td>
<td>Ataxia and loss of reflexes</td>
</tr>
<tr>
<td>McArdle’s disease</td>
<td>232600</td>
<td>11q 13</td>
<td>—</td>
<td>Phosphorylase</td>
<td>Exercise intolerance with myalgia, stiffness of exercising muscles</td>
</tr>
<tr>
<td>Wilson’s disease</td>
<td>277900</td>
<td>13q 14.11</td>
<td>0.003</td>
<td>U</td>
<td>Basal ganglia and hepatic failure</td>
</tr>
<tr>
<td>Tay-Sachs’s disease type 1</td>
<td>272800</td>
<td>15q 23–24</td>
<td>0.1–0.14</td>
<td>Hexosaminidase A (chain)</td>
<td>Mental retardation, blindness</td>
</tr>
<tr>
<td>Metachromatic leukodystrophy</td>
<td>2501000</td>
<td>22q 13.31-qter</td>
<td>—</td>
<td>Arylsulphatase A</td>
<td>Infantile or childhood progressive weakness, spasticity, ataxia mental deterioration</td>
</tr>
</tbody>
</table>


outstanding example is Huntington’s chorea in which the average age of onset is 40 years. Although this disease is disabling and ultimately fatal, the majority of affected persons can unfortunately produce a family of ordinary size before the disease appears in them.

**Autosomal recessive**

Genes determining autosomal recessively inherited disorders (Table 2) are very common but the incidence of affected individuals at birth depends upon the frequency with which carriers of a given defect mate.

Due to this relatively rare event, even a low birth-rate of affected individuals (homozygotes) indicates a sizeable reservoir of heterozygotes in the population. Consanguineous marriages are the most important risk factor for producing individuals with these disorders; that is probably why they are common in Saudi Arabia.

**X-linked**

Classically X-linked disorders show a typical family history of asymptomatic female carriers giving birth to affected males. However, there may be no family history if the disorder is transmitted only in the female line for several generations.

Over 200 genes on the X chromosome are known, including that responsible for the commonest kind of childhood muscular dystrophy. It should be noted that X-linked disorders can further be divided into recessive (i.e. Duchenne muscular dystrophy), and dominant (e.g. fragile X-syndrome). X-linked recessive traits typically occur in males only, whilst rare X-linked dominants are approximately twice as common in females as in males, probably due to an error in the genetic information. A list of several X-linked inherited neurological disorders which have been regionally mapped is given in Table 3.

**Chromosomal abnormalities**

The defect is not due to a single mistake in the genetic blueprint but to a developmental anomaly causing an excess or deficiency of whole chromosomes or chromosome segments which upsets the normal balance of the genome. For example the presence of a specific extra chromosome 21, produces a characteristic disorder, namely Down’s Syndrome, even though all the genes on the extra chromosome may be quite normal. Chromosomal abnormalities are among the
Table 3
Incidence and regional mapping for X-linked inherited neurological disorders

<table>
<thead>
<tr>
<th>Disorder</th>
<th>McKusick (MIM) No.</th>
<th>Chromosomal mapping</th>
<th>Frequency/1000 births</th>
<th>Biochemical defect</th>
<th>Clinical manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duchenne/Becker muscular dystrophy</td>
<td>310200</td>
<td>Xp21.2</td>
<td>0.2</td>
<td>Dystrophin</td>
<td>Progressive muscular weakness</td>
</tr>
<tr>
<td>Charcot-Marie-Tooth X-linked HMSN</td>
<td>302800</td>
<td>Xq13–21</td>
<td>—</td>
<td>U</td>
<td>Similar to HMSN I; females mildly affected</td>
</tr>
<tr>
<td>Ataxia sideroblastic anaemia</td>
<td>301790</td>
<td>Xq 13</td>
<td>—</td>
<td>Iron accumulation</td>
<td>Microcytic, hypochromic anaemia and abnormal iron deposit in bone marrow</td>
</tr>
<tr>
<td>Fabry’s disease</td>
<td>301500</td>
<td>Xq22</td>
<td>0.03</td>
<td>α-Galactosidase</td>
<td>Episodic limb and abdominal pain, cutaneous angiokeratoma, hypertension, renal failure, corneal dystrophy</td>
</tr>
<tr>
<td>Lesch–Nyhan disease</td>
<td>308950</td>
<td>Xq26.2–27.2</td>
<td>—</td>
<td>HGPRT</td>
<td>Psychomotor retardation</td>
</tr>
<tr>
<td>Hunter disease</td>
<td>309900</td>
<td>Xq26–27</td>
<td>0.002</td>
<td>SIS</td>
<td>Abnormal face dwarfism, hepatosplenomegaly, respiratory infections</td>
</tr>
<tr>
<td>Fragile X syndrome</td>
<td>309550</td>
<td>Xq27.3</td>
<td>0.4</td>
<td>U</td>
<td>Mental retardation</td>
</tr>
<tr>
<td>Emery–Dreifuss muscular dystrophy</td>
<td>310300</td>
<td>Xq28</td>
<td>—</td>
<td>U</td>
<td>Humeroperoneal weakness</td>
</tr>
</tbody>
</table>

U: Unknown; HGPRT: Hypoxanthineguaninephosphoribosyltransferase; SIS: sulphate iduronidase sulphatase.

best defined causes of fetal loss or congenital disease and the frequency of chromosomal abnormalities at birth is approximately 7/1000 births.

Multifactorial inheritance

In these conditions there is no one major error in genetic information but rather a combination of small variations that together can produce a serious defect. Environmental factors may also be involved. Some of the congenital abnormalities seem to have a strong genetic component, e.g. Arnold-Chiari malformation, myelomeningocele, encephalocoele. Since 20% of affected infants have multiple malformations, although the total incidence of malformations is 29/1000 births, the actual incidence of affected infants is about 15/1000 births.

Molecular Pathogenesis of Neurological Diseases

Myotonic muscular dystrophy

Myotonic dystrophy (DM) is among the most common forms of muscular dystrophy with a prevalence rate of approximately 1/10 000. It is inherited as an autosomal dominant disorder with variable expression, age-dependent penetrance and a very low rate of mutation. A maternally transmitted congenital form also exists, resulting in mental retardation and muscle hypotonia. As yet, no molecular lesion in the gene has been reported to be associated with DM, and no biochemical defect has been defined. Myotonic dystrophy was one of the first genetic diseases to be linked to a specific chromosome being located at 19q 13.2–13.3. This was discovered initially using the Lutheran blood group (LU) and secretor (Fucosyl transferase 2 FUT2) trait markers and then the protein marker, complement component 3(C3). Tight linkage to the DM gene has been demonstrated with the markers apolipoprotein CII (APOC2) and creatine kinase muscle type (CKMM), both of which are proximal to the DM gene. The most likely order of these markers is cen-ApoC2-CKKM-DM.

Duchenne/Becker muscular dystrophy

Duchenne muscular dystrophy (DMD) is a severe X-linked recessive myopathy with an incidence of 1/3500 male births and is allelic with Becker muscular dystrophy (BMD), a clinically similar but less severe form of myopathy affecting 1/30000 males. DMD results from the deficiency of a large protein known as dystrophin, while in case of BMD, the dystrophin is present, but is abnormal.
with regards to either the amount or molecular structure or both. At the genetic level, both disorders result from mutations of the gene encoding dystrophin and as such the identification of a specific dystrophin-gene mutation in a patient permits precise clinical diagnosis. It is known, that this gene, which when defective, results in DMD or BMD consists of a minimum of 65 exons (coding sequences) spread over approximately 2.4 million bp. Intragenic deletions appear to be the most common gene defects leading to DMD and BMD. Deletion of exon 3–7 produces no consistent clinical pattern, while either deletions of the first exon or large in-frame deletions (30 or more exons) have been associated with DMD. After exclusion of (a) deletions of exon 3–7 and (b) large in-frame deletions, the correlation between deletion and clinical severity was predicted in 96% cases. As such, forming a prognosis based on the extent of the deletion has already reached a relatively high degree of accuracy. Recent data on clinical/molecular correlations have now further shown that there is a strong correlation between specific gene mutations and expected dystrophin abnormality.

The dystrophin gene is so large that screening patients for deletion mutations can be laborious, and a recent study has simplified analysis using the PCR reaction to exons selected from the 70 exon gene; the 18 exon PCR test was capable of detecting 98% of DMD/BMD gene deletions. Direct mutation identification was made possible in the 2.3 x 10^6 bp dystrophin gene in 65–70% of the cases of D/BMD using techniques of multiplex exon amplification through PCR and southern-based RFLP analysis. In the remainder of cases without an identifiable mutation, however, carrier detection and prenatal diagnosis depended solely on DNA linkage studies. To date, linkage analysis has been performed using diallelic RFLP markers which may be limited by small family size, inadequate family member cooperation and deceased DMD males.

**Emery-Dreifuss muscular dystrophy**

Emery-Dreifuss muscular dystrophy (EDMD) is an X-linked disorder which can be clearly distinguished by genetic linkage from both DMD and BMD, which are allelic and map to Xp21. Linkage analysis has assigned EDMD to the terminal region of the long arm of human X chromosome. Several recombinations between EDMD and three proximal Xq28 markers suggest that the EDMD gene is located in distal Xq28. Multipoint linkage analysis indicates that the odds are 2000:1 that the EDMD gene lies distal to marker DXS305. These data substantially refine the ability to perform accurate carrier detection, prenatal diagnosis and the presymptomatic diagnosis of individuals at-risk for EDMD by linkage analysis.

**Spinal muscular atrophies**

Spinal muscular atrophies (SMA) are a hereditary group of neurodegenerative disorders which selectively affect the alpha-motoneuron. These have been divided into SMA type I (Werdnig-Hoffman disease); SMA type II (chronic childhood SMA, intermediate SMA) and SMA type III (Kugelberg-Welander syndrome, mild SMA). Childhood-onset SMA is recessively inherited, whereas the adult-onset disease (coming on at 17–55 years) is transmitted in either a recessive or dominant pattern. An X-linked spinal muscular atrophy (Kennedy's syndrome) is also known. The incidence of infantile SMA is 1/20000, with a carrier frequency of 1/60–1/80. Chronic SMA accounts for a similar number of patients with a similar carrier frequency. In Saudi Arabia, because of the high rate of consanguinity (55%), the disease incidence of SMA type I is at least 40-fold greater than in Western populations at 1.93/100 live births.

The gene responsible for SMA has been precisely mapped on chromosome region 5q 11.2–13.3 and the evidence is consistent with linkage between acute SMA and chromosome 5q markers. Linkage analysis on many families with chronic SMA (type II) and mild SMA (type III) were also mapped by linkage with DMA marker D5S39 on chromosome 5 (5q 11.2–13.3). Thus three forms of SMA, which have been differentiated clinically, are most probably due to different mutations at a single locus on chromosome 5 and the prenatal diagnosis of SMA type I now appears to be possible.

**Neurofibromatosis**

Neurofibromatosis has two clinically distinct forms; von Recklinghausen neurofibromatosis (NF-1) and bilateral acoustic neurofibromatosis (NF-2). Although NF-1 is more common (incidence 1/3000), the neurological morbidity of NF-2 (1/100 000) can be much more devastating. Molecular genetics has altered concepts of neurofibromatosis. The gene for generalized von Recklinghausen disease (NF-1), has been assigned to chromosome 17, whereas the gene for the syndrome of bilateral acoustic neuroma (NF-2)
has been linked to chromosome 22. Rapid progress has been made in refining the genetic map of the NF-1 region and the chromosomal location of the NF-1 has been mapped genetically to the 17q11.2 region.\textsuperscript{19} It has been shown that the primary defect in NF-2 is linked to DNA markers on chromosome 22, and it is suggested that it involves inactivation of a tumour suppressor gene.

**Fragile X syndrome**

The fragile X syndrome [fra (X)] is the most common cause of familial mental retardation and is characterized by a fragile site at the end of the long arm of the X chromosome at position Xq27.3. Recent studies\textsuperscript{20} have discovered the site of the fragile X mutation which is linked to abnormal cytosine methylation and instability of a 550 bp DNA segment detected by probes StB12.3 and StaA22 in a BamI digest. This knowledge can be helpful in the prenatal diagnosis of the syndrome, as well as for carrier detection.

**Friedreich's ataxia**

Friedreich's ataxia (FA) is an autosomal recessive degenerative disease of the nervous system of unknown biochemical defect. It is characterized by selective loss of large myelinated fibres in the dorsal roots and by degeneration of the spinocerebellar tract. The frequency of the disease is estimated at 1/25 000, births. In 1990, Hanauer et al.\textsuperscript{21} demonstrated that the gene for FA was localized on chromosome 9 (9q13–q21) and was in close linkage with the two chromosome 9 markers D9S5 and D9S15. A linkage disequilibrium between FA and D9S15 was recently detected in several French and Italian families which suggests that the disease gene may be at an even shorter distance from this locus marker, which therefore represents a very good starting point for cloning attempts.

**Alzheimer's disease**

Alzheimer's disease (AD) is by far the most common cause of dementia. The age of onset of AD is variable and has been reported to occur from the fourth to the ninth decade and has been arbitrarily divided into early onset (EOAD) and late onset (LOAD), the most common clinical form. An age of 55–60 years has been set as the cut-off age between the two diseases.

To date, neither the aetiology nor the pathogenesis of AD is clearly understood, although a genetic component has been implicated in about 10% of cases. In 1987, St George Hyslop et al.\textsuperscript{22} showed that the genetic defect in familial Alzheimer's disease (FAD) was located on chromosome 21 (21q11.2–21q22.2)—the same chromosome of which there is an extra copy in Down's syndrome. Meanwhile, a result from another group\textsuperscript{23} indicated that gene coding for \( \beta \)-amyloid protein which accumulates in walls of blood vessels and neuronal tissue of both AD and aged Down's brain, also mapped to chromosome 21. A cDNA for the \( \beta \)-amyloid protein suggested that it was derived from a larger protein in a variety of tissues. It was also shown that the production of mRNA for \( \beta \)-amyloid protein was increased in fetal Down's syndrome brain which suggested that the extra dose of chromosome 21 and the gene for \( \beta \)-protein might lead to the abnormal deposition in the brains of these individuals. Critical issues which remain to be solved are: which form of amyloid \( \beta \)-protein precursor (APP) gives rise to \( \beta \)-amyloid deposits and from what cells it derived?

Genetic linkages were demonstrated between AD and two DNA marker D21S1/D21S11 and D21S16 on chromosome 21 in 30 pedigrees with EOAD.\textsuperscript{22} Other investigators, employing both a mixture of EOAD and LOAD families and only LOAD families respectively have been unable to confirm linkage to the same chromosome 21 markers (D21S16, D21S13, D21S11, D2I1S1/S11). These studies indicate that familial AD may be genetically heterogenous.

**Charcot-Marie-Tooth neuropathy**

Charcot-Marie-Tooth disease (CMT) or hereditary motor sensory neuropathy (HMSN) is an autosomal dominant disorder genetically localized on chromosome 1 in a few families and on chromosome 17 in other families. The most common types are HMSN I (hypertrophic type) and HMSN II (axonal type) which were differentiated on the basis of nerve conduction velocity (NCV) and nerve biopsy findings. Earlier studies in a small number of families suggested the localization of the HMSN locus on chromosome 1 because of linkage to the Duffy blood group. However, subsequent studies showed linkage of the HMSN locus to chromosome 1 markers (HMSN Ia) in some cases and no linkage in several other cases (HMSN Ia). Recently, it has been demonstrated\textsuperscript{24} that type Ia is linked to the DNA markers D17S58 and D17S71, located in the pericentromeric region of chromosome 17 (17p11.2–p12).
Tay-Sachs diseases

The sphingolipidoses are a group of lysosomal storage disorders in which the stored substances are sphingolipids. Gangliosides are a form of sphingolipid in which the basic structure is a ceramide linked to a polysaccharide chain. Tay-Sachs disease (TSD), also known as type 1 GM2 gangliosidosis or infantile amaurotic idiocy is a lysosomal storage disease in which the stored substance is a ganglioside. It is a recessively inherited autosomal disease caused by the deficiency of the enzyme hexosaminidase A (Hex A). The disease is rare in the general population, but mutant alleles encoding deficient-synthesis of a subunit of this enzyme Hex A, is highly prevalent in Ashkenazi Jews and French-Canadians of no known Jewish ancestry. Several cases of type 2 GM2 gangliosidosis (Sandhoff’s disease) have been reported from Saudi Arabia.\(^{25}\)

Two point mutations, a splicing defect at 5’ doorsite of intron 12, and a four base insertion within exon 11, account for almost all Ashkenazi Jewish patients. Another defect in which 7.6 kb of DNA including the first exon on this gene for the Hex A α-subunit was deleted was found in two French-Canadian TSD patients. Recently a new point mutation within exon 5 of the β Hex A subunit gene (guanine 509→adenine; arginine 170→glutamine) has been identified as being responsible for the clinical and enzymological phenotype of a Japanese TSD infant.\(^{26}\)

Huntington’s disease

Huntington’s disease (HD), is rare in Saudi Arabia;\(^{4}\) however, the gene for HD is widespread with a variable heterozygous frequency of 1/5000–1/15 000 in different populations. The gene causing HD has been localized to a region close to the telomere of chromosome 4. Huntington’s disease was one of the first disorders in which close linkage of an abnormal gene to a RFLP was shown. This discovery resulted in the establishment of predictive testing programmes for preclinical detection of heterozygotes in many parts of the world. Since the localization of the gene for HD, to the distal short arm of chromosome 4 by linkage to the DNA marker D4S10 (Probe G8),\(^{27}\) a number of strategies have been used in order to obtain more closely linked markers. Most of these probes have been placed on a physical map of the region. This map consists of three large regions of DNA: region 1, the most proximal, contains the locus D4S10; region 2, the central region contains D4S95 and D4S98, and region 3, adjacent to the telomere, includes D4S90 (Probe D5) and D4S141 (Probe 2R3). The order of the markers has now been independently confirmed and the majority of crossover data from family studies have shown that the HD gene is localized to the terminal portion of the short arm of human chromosome 4 (4p16.3).\(^{28}\) However, the difficulty of identifying the 4p16-sited Huntington’s chorea gene is, that no probes are available to ‘clamp’ it at the extremity of short arm.

Tuberous sclerosis (TS)

Tuberous sclerosis (TS) results from an autosomal dominant gene which exhibits variable expression and reduced penetrance with an estimated prevalence of 1/15 000 live births. The gene for TS has been provisionally mapped to chromosome 9 by family linkage studies showing the absence of recombination with the ABO blood group, the red cell enzyme polymorphism for adenylate kinase (AK) and a RFLP at the Abelson oncogene locus (ABL), all these markers being localized to 9q34.\(^{29}\) However, there is now increasing evidence for genetic heterogeneity in TS based upon subsequent data by others who have reported recombination between TS and ABO. Recombination between TS and ABL has also been observed recently and this raises the question of genetic heterogeneity\(^{30}\) suggesting the existence of more than one locus at which mutation can lead to TS.

Conclusion

In this review an attempt has been made to present in detail the molecular lesions associated with neurological diseases. It is hoped that the information available in this review will be helpful in generating considerable interest among Saudi neurologists and scientists to conduct research in molecular neurogenetics which eventually can be helpful in reducing the mortality and morbidity associated with these diseases in Saudi Arabia.

References


