The Hereditary Ataxias

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Abstract. Efforts to classify the hereditary ataxias by their clinical and neuropathological phenotypes are troubled by excessive heterogeneity. Linkage analysis opened the door to a new approach with the methods of molecular biology. The classic form of autosomal recessive ataxia, Friedreich's ataxia (FA), is now known to be due to an intronic expansion of a guanine-adenine-adenine (GAA)-trinucleotide repeat. The autosomal dominant ataxias such as olivopontocerebellar atrophy (OPCA), familial cortical cerebellar atrophy (FCCA), and Machado-Joseph disease (MJD) have been renamed the spinocerebellar ataxias (SCA). Specific gene loci are indicated as SCA-1, SCA-2, SCA-3, SCA-4, SCA-5, SCA-6, and SCA-7. In 3 of them (SCA-1, SCA-2, SCA-3, SCA-6, and SCA-7), expanded cytosine-adenine-guanine (CAG)-trinucleotide repeats and their abnormal gene products cause the ataxic condition. The most common underlying loci for olivopontocerebellar atrophy (OPCA) are SCA-1 and SCA-2, although other genotypes may be added in the future. A major recent advance was the identification of the gene for SCA-3 and MJD, and the high prevalence of this form of autosomal dominant ataxia in FA and the SCA with expanded CAG-trinucleotide repeats, clinical and neuropathological severity are inversely correlated with the lengths of the repeats. Anticipation in the dominant ataxias can now be explained by lengthening of the repeats in successive generations. Progress is being made in the understanding of the pathogenesis of FA and SCA as the absent or mutated gene products are studied by immunocytochemistry in human and transgenic murine brain tissue. In FA, frataxin is diminished or absent, and an excess of mitochondrial iron may cause the illness of the nervous system and the heart. In SCA-3, abnormal ataxin-3 is aggregated in neuronal nuclei, and in SCA-6, a mutated \( \alpha \)-calcium channel protein is the likely cause of abnormal calcium channel function in Purkinje cells and in the death of these neurons.

Key Words: Ataxias; Frataxin; Friedreich's ataxia; Genotype; Neuropathology; Olivopontocerebellar atrophy; Phenotype.

INTRODUCTION

In 1907, Holmes (1) proposed that only the study of the neuropathology of the hereditary ataxias would provide a firm classification of a group of disorders which on clinical grounds alone were very heterogeneous. His expectations were not met because gross and microscopic examination of brain, spinal cord, dorsal root ganglia, and peripheral nerves showed equal if not greater heterogeneity. Nevertheless, descriptive neuropathology accompanied innumerable clinical reports on hereditary ataxia. The mode of transmission offered a relatively simple method of classification and remains useful today for office or bedside diagnosis. Knowledge of Mendelian genetics permits the clinician to assess the risk to offspring, which is 50% for the dominant and 25% for the recessive forms. The risk of carrier status in the recessive forms can also be determined (50%). However, the biological reason for anticipation (earlier disease onset in successive generations) and the existence of forms frustes remained unexplained until the era of expanded trinucleotide repeats (2). Efforts at clinical and neuropathological classification continued for many years, and some publications were cited extensively (3–7). The discovery of trinucleotide repeats has catalyzed a rapid growth in hereditary ataxia research, and the number of publications dealing with SCA or FA has risen from 57 in 1994 to 147 in 1997.

Ataxia occurs as a part of many other familial disorders of the nervous system, but this review is restricted to those hereditary forms which have been given the current designation of FA and SCA. Neuropathologists are called upon to view their gross and microscopic observations as a phenotype and to understand the pathogenesis of hereditary ataxia in terms of genotype and gene product.

THE NEUROPATHOLOGY OF THE "CLASSIC" HEREDITARY ATAXIAS

After Friedreich's reports in the period from 1863–1877 (8–11), little effort was made to distinguish other types of hereditary ataxia until 1891, when Menzel (12) described what is now recognized as autosomal dominant olivopontocerebellar atrophy (OPCA). He emphasized the differences between his case and Friedreich's patients, but did not recognize the mode of inheritance. In a remarkable paper without illustrations, Marie (13) made a systematic comparison of the clinical features, inheritance, and anatomic pathology of FA and other forms of hereditary ataxia. He concluded that "cerebellar heredoataxia" and FA were different entities. The term "Marie's ataxia" was used for decades to make the distinction between the recessive (FA) and autosomal dominant forms. However, not all dominant cases revealed OPCA. In many patients, the basis pontis remained normal, although the cerebellar cortex and inferior olivary nuclei were severely affected. Greenfield (14) considered "cerebello-olivary" atrophy a distinct entity and attributed its first description to Holmes (15). His designation
Fig. 1. Friedreich's ataxia. A and B, gross specimen; A, anterior aspect of the thoracic spinal cord; B, dorsal aspect of the thoracic spinal cord. Thinning of the spinal cord and the dorsal roots (arrows) is apparent. The transverse diameter of the spinal cord is less than 1 cm. C-G, microscopic appearance. C, cerebellar cortex (upper vermis). Number and dendritic arborizations of Purkinje cells are normal (40-μm-thick vibratome section; immunocytochemical stain for nonphosphorylated neurofilament protein). D, dentate nucleus. Only a few neurons remain, and there is moderate astrogliosis (Cresyl Violet); E, thoracic spinal cord. The myelin stain reveals pallor of the dorsal columns, the dorsal spinocerebellar tracts, and the lateral corticospinal tracts. The arrows show the position of the dorsal nuclei of Clarke (see also F) (Luxol Fast Blue-periodic acid-Schiff); F, thoracic spinal cord. The immunocytochemical stain for the synaptic protein SNAP-25 shows depletion of afferent connections to the nerve cells of this nucleus (arrow); G, lumbar dorsal root ganglion. Neuronal loss is accompanied by proliferation of satellite cells and residual nodules of Nageotte (Cresyl Violet). Markers: A and B, 1 cm; C and D, 100 μm; E, 1 mm; F, 200 μm; G, 100 μm.

of "Holmes type of ataxia" did not remain without challenge because the condition was recessive or at least not clearly dominant, and the affected males had hypogonadism (6, 15). For the purposes of this review, the neutral term "familial cortical cerebellar atrophy" (FCCA) has been selected to avoid historical inaccuracies. It appeared that most, if not all, forms of autosomal dominant ataxia shared severe Purkinje cell atrophy, which contrasted with the preservation of the cerebellar cortex in FA. However, the cerebellar cortex was intact also in a seemingly new autosomal dominant disease now known as Machado-Joseph disease (MJD) (16). Its occurrence in the Azores, in communities of Portuguese immigrants in the United States, and in Japan (17) could be attributed to migration and Portuguese circumnavigation of the globe. However, the disorder no longer has ethnic restrictions, and the defective gene is the same as in SCA-3.

In this review, FA, OPCA, FCCA, and MJD/SCA-3 are considered to have sufficiently characteristic neuropathological phenotypes to warrant their inclusion as "classic" forms. Their principal gross and microscopic abnormalities that are likely to cause ataxia are illustrated.

Friedreich's ataxia (Fig. 1A–G) typically affects the spinal cord. Transverse and anteroposterior diameters of the thoracic spinal cord are reduced, and the dorsal roots
are greatly thinned (Fig. 1A, B). Transverse slices of the fixed spinal cord show a peculiar gray and gelatinous appearance of the dorsal columns and the anterolateral fasciculi, corresponding to the gracile and cuneate fasciculi, and the lateral corticospinal and dorsal spinocerebellar tracts. Myelin stains readily reveal the spinal lesion (Fig. 1E). There is neuronal loss in the dorsal nuclei of Clarke that can readily be seen on cell stains. Figure 1F illustrates the loss of synaptic terminals in this nucleus. The peripheral nervous system in FA has been studied less extensively but is clearly, and quite severely, affected (18). Figure 1G illustrates the lesion in a lumbar dorsal root ganglion. Neuronal loss is accompanied by proliferation of satellite cells and an abundance of residual nodules of Nageotte. The spinal lesion readily explains the grotesque ataxia of the affected patients, which reminded early observers of tabes dorsalis (12). The severe lesion of the dentate nucleus was underappreciated until 1957 (19; review in 20). Neuronal loss in the dentate nucleus (Fig. 1D) accounts for atrophy of the superior cerebellar peduncles. The cerebellar cortex is generally normal (Fig. 1C).

Severe atrophy of the basis pontis and the cerebellum characterize OPCA (Fig. 2A, B). The pontine tegmentum remains of normal thickness, although tegmental lesions likely account for the supranuclear ophthalmoplegia in some patients with OPCA (21–22). The cortical cerebellar atrophy tends to be severe (Fig. 2D) and causes commensurate retrograde atrophy of the inferior olivary nuclei (Fig. 2G). As in other cerebellar system atrophies, the noduloloculoclar lobe and the cerebellar tonsils reveal preservation of Purkinje cells. Neurons and dendrites of the dentate nucleus are often better preserved despite severe loss of afferents from the cerebellar cortex (Fig. 2E). Degeneration of the basal pontine gray matter is generally very severe (Fig. 2F) and accounts for the atrophy of the middle cerebellar peduncles (Fig. 2B). At spinal levels, long fiber tract degeneration often affects the dorsal columns, but neuronal loss in the dorsal nuclei of Clarke is variable.

The anatomical finding of OPCA does not necessarily imply heredity. A similar constellation of abnormalities occurs in multisystem atrophy where oligodendrogial cytoplasmic inclusions point to nonheredity. However, similar inclusions occur occasionally in OPCA of SCA-1 (23).

Figure 3 shows an example of FCCA, subsequently identified as SCA-6. Cerebellar cortical atrophy (Fig. 3B), commensurate loss of neurons in the inferior olivary nuclei (Fig. 3D), and relative preservation of the dentate nucleus (Fig. 3C) are similar to OPCA. However, the neurons of the basis pontis are not affected. It is often a matter of judgment whether at least some atrophy of pontine neurons exists. Minor numerical reduction in nerve cells may cast a case of FCCA into the category of OPCA, and it is preferable to view lesions of the basis pontis as a spectrum from mild to severe.

Figure 4 illustrates MJD/SCA-3. The overall size of the cerebellum is reduced (Fig. 4A), but microscopy reveals an intact cerebellar cortex (Fig. 4B). The dentate nucleus is severely affected, and surviving neurons show "grumose" degeneration (Fig. 4C). Silver stains show that grumose degeneration is closely related to dendrites (Fig. 4D). The inferior olivary nuclei are intact, reflecting the preservation of the cerebellar cortex (Fig. 4E). At spinal levels, the dorsal columns show degeneration (Fig. 4F), and the lesion in the dorsal nuclei of Clarke is as severe as in FA (Fig. 4F, G). Anterior horn cell loss is fairly typical for MJD/SCA-3, while neuronal loss in dorsal root ganglia is variable (16, 24).

The combined lesions of the dorsal nuclei of Clarke and dentate nuclei in MJD/SCA-3 strongly resemble those in FA. Surprisingly, little attention has been paid to this similarity in efforts to explain the pathogenesis of the disorders.

THE MOLECULAR GENETICS OF THE HEREDITARY ATAXIAS

In 1977, Jackson et al (25) established by linkage analysis that the gene of one form of OPCA (now known to be SCA-1) was located on the short arm of the sixth chromosome. The investigation extended a more limited earlier study (26) that utilized the highly polymorphic human leukocyte antigens (HLA) and the segregation of HLA-haplotypes. Logarithmic odds (lod) scores were over 3.0, the accepted standard for linkage as contrasted with nonlinkage of genetic markers. Subsequent investigations with HLA and other protein and nucleic acid markers showed that not all cases of OPCA (and other dominant ataxias) were linked to loci on the sixth chromosome (27). Comparable techniques established the gene loci of 6 additional dominant ataxias. Current designations are SCA-1 through SCA-7 (reviews in 28 and 29). Machado-Joseph disease and SCA-3 have the same gene locus and are now generally given as MJD/SCA-3 (30–31). The chromosomal locations (including arms) are: 6p (SCA-1); 12q (SCA-2); 14q (MJD/SCA-3); 16q (SCA-4); 11 centromeric (SCA-5); 19p (SCA-6); 3p (SCA-7). At this time, 5 of these 7 forms of dominant ataxia are known to be caused by expanded CAG-trinucleotide repeats. Expanded and unstable CAG repeats were first shown for SCA-1 by Orr et al (2) and provided the first glimpse of the pathogenesis at the molecular level. The occurrence of the expanded CAG repeats in the coding region of the gene implied that a protein with an abnormally long polyglutamine stretch was being biosynthesized. The pursuit of a point mutation or a gene deletion was replaced by a search for the role
Fig. 2. Olivopontocerebellar atrophy (SCA-2). A–C, gross specimens. A, mesial aspect. The anteroposterior diameter of the pons is greatly reduced, and the cerebellum is small and atrophic. The fourth ventricle is dilated. B, transverse slice through the middle pons. The anteroposterior diameter of the basis pontis in this case of SCA-2 (B) is greatly reduced in comparison with a normal specimen (C). The transverse fibers of the basis pontis in SCA-2 are thin and indistinct. The root of the trigeminal nerve is prominent due to atrophy of the middle cerebellar peduncle. Pontine tegmentum and superior cerebellar peduncle appear normal (B). D–G, microscopic appearance. D, cerebellar cortex (vermis). The overall height of the molecular layer is reduced, and there is loss of Purkinje cells. "Torpedoes" are present in the granular layer (arrow; 40-μm-thick vibratome section; immunocytochemical stain for nonphosphorylated neurofilament protein); E, dentate nucleus. Nerve cells are moderately reduced in number, and the neuropil is gliotic (Cresyl Violet); F, basis pontis. Only a few crippled neurons remain, and their dendrites are abnormally smooth (40-μm-thick vibratome section; immunocytochemical stain for nonphosphorylated neurofilament protein); G, inferior olivary nucleus. Subtotal neuronal loss and small-cell astrogliosis are present (Cresyl violet). Markers: A, 1 cm; B, C, 5 mm; D–G, 100 μm.
of an abnormally long protein, and diseases with expanded CAG repeats are thought to be caused by a putative toxic "gain of function." The discovery of expanded CAG repeats in Huntington disease (32) greatly stimulated research efforts to find similar abnormalities in other dominant disorders of the central nervous system. The currently known SCA with expanded CAG repeats, the actual or predicted gene products, normal and expanded repeat sizes, and pertinent references are listed in Table 1. For SCA-1, SCA-2, SCA-3, SCA-6, and SCA-7, an inverse relationship has been demonstrated between the age of onset and the length of the CAG repeats. Longer repeats cause early onset of ataxia, but a correlation with the neuropathological phenotype is much more complex (see below). Zoghbi (37) has recently summarized the clinical manifestations of mutations in the human α1A calcium channel, which include SCA-6, familial hemiplegic migraine, and episodic ataxia type 2. Though SCA-6 is caused by a modest expansion of CAG repeats, the concept of "gain of function" may not apply to calcium channel properties.

Patients with autosomal dominant ataxia are generally heterozygous and have one normal and one abnormal allele. However, homozygosity has been described for SCA-3 (38-40) and SCA-6 (41) and generally causes greater severity, though not in a predictable manner. Paternal transmission of SCA-1 is more likely to cause greater lengths of CAG repeats (42-43) because the length of the repeats is greater in sperm than in other cells, such as lymphocytes. It is likely that the longer repeats in offspring of SCA-2-affected fathers are due to a similar mechanism.

Table 2 lists some epidemiological data on dominant SCA with established expanded CAG repeats. The distribution is likely to change rapidly as more families are studied by molecular techniques. Epidemiological data on SCA-7 have not been published, though the phenotype (dominant ataxia and pigmentary retinopathy) should permit preselection before molecular diagnosis. Perhaps the most impressive result of the compilation in Table 2 is that an estimated 36% of the cases do not belong to any of the known SCA-genotypes.

The chromosomal location of the FA gene is 9q13-21.1 (44). Expanded GAA repeats were recently reported by 2 groups of investigators (45, 46), and the gene product was named "frataxin" (45). Instability of the GAA repeats was seen in parent-offspring transmission, and especially in consanguineous marriages where the length of the allele in the heterozygous parents was the same (45). The abnormal expansion is located in an intron of the gene and causes loss of a normal gene product. A correlation has been shown between clinical features (e.g. age of onset) and length the GAA repeats (47-48). Generally, "loss of function" in FA implies lack of frataxin.

Fig. 3. Familial cortical cerebellar atrophy (SCA-6). A, gross specimen. Atrophy of the cerebellum causes moderate dilation of the fourth ventricle, but the basis pontis is normal. B-D, microscopic appearance. B, cerebellar cortex. Purkinje cells are reduced in number, have small somata and greatly simplified dendrites (40-μm-thick vibratome section; immunocytochemical stain for nonphosphorylated neurofilament protein); C, dentate nucleus. Nerve cells are present in approximately normal number (Cresyl Violet); D, inferior olivary nucleus. Depletion of neurons is commensurate with cortical cerebellar atrophy (Cresyl Violet). Markers: A, 1 cm; B-D, 100 μm.
and requires homozygosity. Compound heterozygosity (expanded GAA repeats in one allele and a point mutation in the second allele) occurs occasionally (45). In light of the molecular data, clinical concepts of FA must be revised. Previously considered a disease of infantile or juvenile onset, FA, as defined by abnormally long GAA repeats, can actually begin in middle or even late adult life (47–48), and patients can have offspring. It is obvious that all children are gene carriers.

In some patients with typical FA, linkage to the 9th chromosome could not be demonstrated. Some of these patients were found to have profound vitamin E deficiency due to a mutation of the alpha-tocopherol transfer protein (chromosome 8q) (49, 50).

The standard methods for the genotyping of SCA and FA are the polymerase chain reaction (PCR), denaturing and non-denaturing gel electrophoresis, and deoxyribonucleic acid (DNA) sequencing. Epidemiological studies
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TABLE 1
Hereditary Ataxias with Expanded and Unstable CAG Trinucleotide Repeats

<table>
<thead>
<tr>
<th>SCA-type</th>
<th>Chromosome and arm</th>
<th>Gene product (protein)</th>
<th>Length of normal allele (in CAG)</th>
<th>Length of expanded allele (in CAG)</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>6p</td>
<td>ataxin-1</td>
<td>19–38</td>
<td>40–81</td>
<td>27</td>
</tr>
<tr>
<td>2</td>
<td>12q</td>
<td>ataxin-2</td>
<td>22–23</td>
<td>36–52</td>
<td>33</td>
</tr>
<tr>
<td>3</td>
<td>14q</td>
<td>ataxin-3</td>
<td>13–40</td>
<td>68–82</td>
<td>27, 34</td>
</tr>
<tr>
<td>6</td>
<td>19p</td>
<td>αv-calciuim channel</td>
<td>4–16</td>
<td>21–27</td>
<td>35</td>
</tr>
<tr>
<td>7</td>
<td>3p</td>
<td>ataxin-7</td>
<td>7–17</td>
<td>38–130</td>
<td>36</td>
</tr>
</tbody>
</table>

TABLE 2
Distribution of Dominant Spinocerebellar Ataxias by Country (In Percent of Cases)

<table>
<thead>
<tr>
<th>Country</th>
<th>Type of SCA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>USA</td>
<td>6</td>
</tr>
<tr>
<td>Japan</td>
<td>3</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>22</td>
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<tr>
<td>Germany</td>
<td>9</td>
</tr>
<tr>
<td>Russia</td>
<td>41</td>
</tr>
<tr>
<td>South Africa</td>
<td>40</td>
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</tbody>
</table>

Summary of data presented at the International Symposium on Inherited Ataxias in Montreal, May 1997 (compiled by Dr Bronya J. B. Keats). Epidemiological information on SCA-7 is not yet available. In India and Cuba, all reported cases have been identified as SCA-2. Most cases in Portugal are MJ/SCA-3.

require only relatively simple technology because the proper selection of the oligodeoxynucleotide primers and cycling parameters for PCR can achieve high sensitivity and specificity. Examples of PCR amplification of genomic DNA in SCA and FA are illustrated below.

THE CORRELATION OF NEUROPATHOLOGICAL PHENOTYPE, GENOTYPE, AND GENE PRODUCT

Clinical and neuropathological phenotypes in FA are caused by diminution or complete absence of frataxin. Exploration of the normal function of this 210-amino acid polypeptide was greatly aided by its partial sequence homology with a protein of Saccharomyces cerevisiae, Yfh1p (45). Disruption of the responsible gene in yeast caused a major deficit in oxidative phosphorylation, suggesting that frataxin is essential for normal mitochondrial function. Furthermore, complementation of a yeast mutant with a frataxin sequence allowed the mutant to overcome a growth deficit in low iron medium (51). A mitochondrial location of frataxin was rapidly confirmed for cells and tissues of mammalian origin (52–54). The evidence supports the concept that mitochondrial frataxin is encoded by the nuclear genome, and that its function is related to iron homeostasis. Frataxin messenger ribonucleic acid (mRNA) was detected in many tissues outside the central nervous system (CNS) of embryonic and neonatal mice (52). However, none of these observations explained the selective vulnerability of the spinal cord, the dorsal root ganglia, and the dentate nucleus. Mitochondrial dysfunction in FA had been suspected for many years because of abnormal oxidation of pyruvate. Recently, it was strongly supported by the study of endomyocardial biopsies of 2 patients with FA (55). Homogenates of these samples showed abnormal activity ratios for the respiratory complexes I, II, and III, and remarkably low activity of aconitase. All these abnormalities were attributed to frataxin deficiency and its consequent mitochondrial iron excess. The stated mitochondrial proteins contain Fe–S clusters, which are susceptible to disruption by iron-catalyzed formation of toxic oxygen radicals (55). Cardiomyopathy is a common companion of FA and may guide further FA research toward possible therapy with iron chelators. Figure 5 shows the typical gross appearance of a “Friedreich heart,” and also illustrates the microscopic abnormalities and accumulation of iron-reactive granules in the hypertrophic myocardial fibers (56). No information is currently available on iron in the CNS lesions of FA, although the high level of the metal in the normal human dentate nucleus may have special importance.

An effort to correlate the complex phenotype of the spinal cord lesion in FA with the length of the GAA repeat has been made in Figure 6. Early onset and death from FA and its complications are thought to be the result of long repeats (= low or absent frataxin). Late onset and long survival may reflect substantial residual frataxin levels. However, in complex hereditary system disorders, it is often uncertain how “severity” can be defined in neuropathological terms and how it can be measured. The cross-sectional area of the spinal cord in FA is a tentative approach and supports the causative relationship between the length of the GAA repeats and the severity of the lesion (Fig. 6).

Normal and abnormal ataxin-1 genes have been well characterized. They are expressed in neural and non-neural tissues (57). Since most SCA-1 patients are heterozygotes, immunoblots of tissue cultures and tissue extracts reveal 2 proteins with similar immunoreactivity.
Immunocytochemistry showed ataxin-1 reactivity in cellular nuclei and in the cytoplasm of Purkinje cells (58). This distribution is difficult to reconcile with the observed composite neuropathological lesion, but a nuclear accumulation of the abnormal protein is true also for other familial diseases with expanded CAG-trinucleotide repeats: Huntington disease (huntingtin) (59) and SCA-3 (ataxin-3) (60). The cytoplasmic distribution of ataxin-1 (58) and ataxin-3 (60) may represent a normal phenomenon, while aggregation inside nuclei may be the important injurious event (61).

Hereditary ataxia is not restricted to the human CNS. Canine and murine mutants with hereditary ataxia are known, but their study has contributed relatively little to the understanding of human hereditary ataxia. In contrast, transgenic mice carrying expanded CAG-trinucleotide repeats have shown some promising results. When targeted to Purkinje cells, a transgene carrying the Pcp2 promoter and expanded human SCA-1 CAG repeats caused several abnormalities, including ectopic Purkinje cells and lack of arborization as shown by immunocytochemistry with anti-calbindin (62). Similarly, transgenic mice carrying expanded CAG repeats of MJD/SCA-3 targeted to Purkinje cells showed poor cerebellar development, selective deficiency of Purkinje cells, and poor dendritic expansion (63). The described transgenic models do not faithfully reproduce the human neuropathology of SCA-1 or MJD/SCA-3, but reveal that expanded CAG repeats have a profound effect on neuronal development and survival.

The extent of the neuropathological lesion is clearly affected by the inherited length of CAG repeats. Figure 7 shows a comparison of father and son who had OPCA due to SCA-2. The lengthening of the abnormal CAG repeats from 41 in the father to 58 in the son caused a much earlier onset and shorter survival. In addition to meiotic instability, causing longer repeats in offspring, mitotic instability, mosaicism, and the effect of the normal allele may also contribute to the phenotype. To date, this phenomenon has been shown for fibroblast and lymphocyte genomic DNA of patients with FA (48). In brain regions of patients with SCA-1 or MJD/SCA-3, the length of the abnormal CAG repeats is stable except for the cerebellar cortex, which reveals smaller CAG tracts in both diseases (64, 65).
In SCA-6, a mutated calcium channel protein is thought to alter Purkinje cell function and survival (35). Neuropathological experience with SCA-6 is still limited (66, 67). Retrospective genotype identification was also made in one family whose neuropathological data had been published (68). The descriptions are in agreement that SCA-6 causes “pure” cerebello-olivary atrophy. Even modest lengthening of the abnormal repeat can cause a more serious clinical and neuropathological phenotype. The patient with SCA-6 whose brain is shown in Figure 3 had a mild form ataxia which began at 40 years of age. He died at 69 from a myocardial infarction. His surviving daughter has the onset of a rapidly progressive ataxic disorder at age 28 years. Her CAG expansion in DNA from white blood cells (25 repeats) is greater than that in her father (22 repeats in DNA extracted from brain tissue).

It is an academic exercise to examine archival cases of hereditary ataxia in which genotypes were not determined. Can they be assigned retrospectively to FA or SCA by studying their gross and microscopic features? Most neuropathologists will have little trouble with the neuropathological phenotype of FA. The tissue changes in the FA-like autosomal recessive ataxia due to hereditary vitamin E deficiency differ substantially from the much more common chromosome 9-linked FA (69). If dominant transmission is known, the constellation of preserved cerebellar cortex and inferior olivary nuclei, neuronal loss and grumose degeneration in the dentate nucleus, and destruction of the substantia nigra and dorsal nuclei of Clarke should permit the diagnosis of MJD/SCA-3. Occasionally, specimens from members of successive generations are available for a comparison. The first full, “unwitting” neuropathological description of MJD/SCA-3 was given by Becker et al (70). The gene locus was not known at that time, but the molecular diagnosis of MJD/SCA-3 has since been confirmed by analysis of lymphocytes in other members of the same family.

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agarose and ethidium bromide stain). S, DNA standards in base pairs. N, amplification of genomic DNA from a person with a normal FA genotype; A1-A3, PCR reaction product corresponding to the spinal cord sections A1-A3. The ages at onset and death, and the length of the GAA trinucleotide repeats were 9 and 28 years, 330/440 repeats for patient 1; 15 and 56 years, 671 repeats (both alleles) for patient 2; and 32 and 87 years, 170 and 104 repeats for patient 3. There is an inverse correlation between the length of the GAA repeats and the cross-sectional area of the spinal cord in FA. Template DNA for PCR in N, A1, and A3 was extracted from frozen brain tissue. The brain of A2 had been fixed, and template DNA was obtained several years before death from white blood cells (courtesy of Dr Bronya J.B. Keats). The oligodeoxynucleotide primers for DNA amplification were Bam and 2500 F (45). Markers, 1 mm.
Fig. 7. Correlation of the severity of OPCA (SCA-2) and length of CAG-trinucleotide repeats. A1 and A2 show the gross pathology of OPCA in father (A1) and son (A2), respectively. The gross appearance is very similar, although the respective ages of onset and death were very different (A1: 20 and 42 years; A2: 7 and 18 years). B, electrophoresis of PCR reaction products (8% polyacrylamide and ethidium bromide stain). S, DNA standards in base pairs; N, PCR with template DNA from a patient with a normal SCA-2 genotype; A1 and A2, father and son, respectively, with expanded CAG-repeats (A1 = 22/41; A2 = 22, 58). The right-pointing arrows indicate the normal alleles; the left-pointing arrows indicate the expanded alleles. The longer CAG repeats in the son (A2) caused comparable atrophy in brain stem and cerebellum in a much shorter duration of illness.
and brain DNA of a patient who died in 1995. The sections of the 2 original cases were still available at the University of Göttingen, Germany, and could be compared with more recent material. The specimens reported by Becker et al (70) showed moderate Purkinje cell loss, preserved inferior olivary nuclei, and severe neuronal loss in the dorsal nuclei of Clarke. Surprisingly, the dentate nuclei were preserved, raising doubt about the neuropathological diagnosis of MJD/SCA-3. In the recent autopsy case, the dentate nucleus was severely affected. It is unclear why this difference in phenotype occurred. The lengths of the CAG repeats in the 2 patients reported by Becker et al (70) are currently unknown, while the recent patient had 78 repeats. His onset was 6 years earlier than in the members of the previous generation. The observations imply that the lesion of the dentate nucleus may be a primary target of the disease process, and that the severity of the lesion is related to the length of the MJD/SCA-3 CAG repeats.

Retrospective phenotype-genotype correlation is even less secure with OPCA because its gene loci may include SCA-1, SCA-2, and perhaps other SCA. Pontine atrophy is generally more severe in SCA-2 than in SCA-1. The tegmental lesion underlying slow saccades in SCA-2 may be subtle and escape detection. Also, similar oculomotor abnormalities occur in other genotypes causing OPCA, making the tegmental lesion somewhat nonspecific. In a systematic study of many brain and spinal cord regions, Robitaille et al (71) compared findings in 11 SCA-1 patients with observations in SCA-2 and SCA-3/MJD. They concluded that the SCA-1 phenotype was quite unique.

Most cases of OPCA are sporadic. The association with striatonigral degeneration, the spinal cord lesions of the Shy-Drager syndrome, and ubiquitinated cytoplasmic inclusions in glial cells generally allow assignment to a nonhereditary multisystem disorder (see exception in reference 23).

The diagnosis of SCA-7 can probably be made if the history of pigmentary retinopathy or a specimen of the retina are available (72). Pure cerebellar cortical lesions have several different genotypes, and a retrospective classification may have to rely on molecular techniques. In some cases, DNA can be extracted from paraffin-embedded or stored fixed tissues for PCR amplification of DNA with specific primers. Although the literature contains several optimistic reports on this technique (e.g. ref. 73), DNA recovery is generally poor, and the procedure remains prone to spurious signals and failed amplification.

OUTLOOK

The seemingly intractable heterogeneity of the hereditary ataxias has given way to a more manageable, although still rather complex variety of disorders. In several types of spinocerebellar ataxia, anticipation and disease severity can be explained by abnormally long, variably reduced, or absent gene products. Onset of FA in late adult life and parental imprinting have logical explanations. The pace of discovery is rapid, and neuropathologists will be able to contribute to the understanding of the genotype-phenotype correlation in this multidisciplinary effort.

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REFERENCES


Genomic DNA was extracted from frozen brain and used as template for amplification of the SCA-2 gene by PCR with primers UH10 and UH 13 (74). Lymphocyte DNA was obtained when these patients were alive, and normal and expanded repeats were sized by Mr Diego Lorenzetti (Houston, Tex). Marker, 1 cm.

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