
MOLECULAR DIAGNOSIS OF ADULT NEURODEGENERATIVE DISEASES AND MOVEMENT DISORDERS

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Introduction

Molecular genetics provide a powerful tool in the diagnosis of many neurological diseases. Genetic testing of mutations in disease causing genes has allowed us to define and classify many of the heterogeneous inherited neurodegenerative syndromes.¹⁻⁵ Confirmation of diagnosis allows early institution of genetic counselling, enables genotype-phenotype correlation, helps select specific patients for clinical drug trials, and ultimately provides a better understanding of pathogenesis and long-term clinical outcome of the disease. As molecular testing may have serious implications for a patient and his family, it should be performed only after careful consideration and a genetic counselling process involving doctors, professional counsellors, and the affected patient and his family.⁶

A number of genetic tests for adult neurodegenerative diseases have been introduced in recent years. Some are solely for research purpose, while others are used routinely in clinical practice. In this paper, we highlight our local experience in the scientific, ethical, social and legal issues of molecular testing of certain diseases such as Huntington's disease, and the autosomal dominant and autosomal recessive cerebellar ataxias. Genetic tests for these diseases are carried out as part of routine clinical care of patients in Singapore.⁷⁻¹¹ In addition, we draw attention to other neurodegenerative and movement disorders for which genetic screening or testing is available locally. Our experience gained from such testings could be applied to a wider spectrum of neurodegenerative diseases when more routine tests are developed. The listing of the diseases below is based on the capability and experience of some of our major institutions and not meant to be exhaustive unless a national survey of such genetic testing capability is carried out.

Huntington's Disease and Inherited Ataxias

Huntington's Disease

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder associated with basal ganglia and cerebral cortex atrophy. HD is characterised by involuntary choreiform movements, cognitive impairment, and behavioural abnormalities. It is caused by an unstable expanded CAG repeat within the coding region of the HD gene. Variability in age at onset, tendency of paternal transmission, and sporadic new mutations are some of HD's recognised clinical features.¹²

Purpose of test

A genetic test can confirm the diagnosis with abnormal CAG repeats of 40 to over 100 CAG units. Normal chromosomes have 6 to 26 CAG repeats that are inherited in a Mendelian fashion. Sometimes alleles with 36 to 39 repeats are present in unaffected elderly relatives of sporadic *de novo* cases.

Selection criteria

Genetic testing is recommended for patients with dementia, involuntary movements, and neuro-behavioural disorders, and/or a positive family history of HD. It should also be considered for patients without family history but with clinical features of HD, particularly when there is a history of ancestors' early death, non-paternity, or adoption.

Confirmation of diagnosis

Involves a clinical diagnosis of HD and no genetic test previously for the patient or family member.

Presymptomatic testing

Performed for individuals at risk for HD who request this test for purposes like marriage or childbearing.

Prenatal testing

Can be considered when one parent is known to carry the HD gene and the couple wants to determine the carrier status of the foetus.

Procedure of test

Patients suspected of having HD are usually assessed by a neurologist who has an interest and is familiar with the nature of the test. Genetic counselling by a neurologist regarding the nature and implications of the test is carried out. Patients are usually informed of the test results at their subsequent follow-up between 1 to 3 months.

Information used for research

Diagnosed HD patients will be approached separately regarding participation in a research project if such research is available. Sometimes collective information regarding the prevalence of the disease and the genetic findings are presented in scientific meetings or for planning educational or healthcare programmes. Strict confidentiality is maintained to ensure that no patient can be identified.

Accuracy of results

The test is usually repeated twice in the laboratory to ensure accuracy, and is compared with negative controls.

Some characteristic features

Repeat Sizes Up to 26 Units – Normal.

Repeats of 27 to 35 Units – There have been no confirmed reports of persons with repeats in this range expressing HD. However, descendants of fathers with repeats in this range can inherit an expanded allele in the clinical range.

Repeats of 36 to 39 Units – Some persons may develop HD and others may live into old age without clinical evidence of the disease.

Repeats of 40 Units or Larger – All patients with the range of 40 or more will eventually develop HD. However, some individuals with repeats at the low end of this range are reported to exhibit initial symptoms at ages older than common life expectancy.

There is a strong correlation between the length of the expanded CAG repeat with age at onset of the disease. In asymptomatic persons, however, the repeat size cannot reliably predict age of onset. In a study of 63 HD patients and family members in Singapore, the range of CAG repeats in our population's normal and HD alleles is similar to those reported elsewhere.⁹

Autosomal Dominant Cerebellar Ataxias

Autosomal dominant cerebellar ataxias (ADCAs), frequently referred to as SCAs, are a group of neurodegenerative diseases characterised by cerebellar dysfunction either alone or in combination with other neurological abnormalities. The estimated prevalence of ADCA in Singaporean families is at least 1 : 27,000.⁸

Their clinical classifications (ADCA I: cerebellar syndrome with other neurologic involvement such as pyramidal, extrapyramidal, ophthalmoplegia, dementia; ADCA II: cerebellar syndrome with pigmentary maculopathy; ADCA III: relatively pure cerebellar syndrome) have largely been replaced by a genetic classification since

expansions of coded CAG trinucleotide repeats were demonstrated to cause several dominantly inherited SCAs. At least ten genes have been identified for SCAs 1, 2, 3, 6, 7, 8, 10, 12 and 17, dentatorubral-pallidoluysian atrophy (DRPLA), and ten loci responsible for SCAs 4, 5, 11, 13, 14, 16, 18, 19, 21 and 22 have been mapped. These loci have been numbered based on their order of classification. However, a locus for SCA 9 has yet to be assigned.

In SCAs 1, 2, 3, 6 and 7, the mutation is due to CAG repeat expansions within the coding regions of the gene. SCA 8 is associated with an expansion of a CTG repeat in the 3' untranslated region (UTR) of the *SCA 8* gene that produces antisense mRNA to the *KLHL1* gene on the complementary strand. In SCA 10, the disease-causing expansion occurs in the ATTCT pentanucleotide repeat of intron 9 of *SCA10*, a gene of unknown function widely expressed in the brain. In SCA 12 there is an expanded CAG repeat in the 5' untranslated region (UTR) of *PPP2R2B*, a gene coding for a brain-specific regulatory subunit of the protein phosphatase PP2A. SCA 17 is due to an expanded CAG repeat in TATA box binding protein (*TBP*) gene, which gives rise to an elongated polyglutamine tract in the respective proteins.¹³

Purpose of test

A genetic test can confirm the diagnosis when abnormal trinucleotide or pentanucleotide repeats are above the range of normal chromosomes. The genes are inherited in a Mendelian fashion.

Selection criteria

Selection criteria include: patients with clinical features of SCA such as cerebellar ataxia, pyramidal and extrapyramidal signs and with a family history of ataxia; or a history of ancestors' early death, non-paternity, or adoption in the presence of suspected clinical features described above.

It has to be emphasised that a wide phenotypic overlap amongst the SCAs and inter-familial and intra-familial phenotypic variability exists even for each SCA subtype. Based on the history and ancestry of Singaporeans, we previously demonstrated a founder effect for specific SCA subtypes and the association of ethnicity-specific SCA subtypes. SCA 2 is relatively common amongst the Malay race. Priority testing for SCA 3 and SCA 2 for ethnic Chinese, and SCA 2 in ethnic Malays may be cost effective and relevant locally.⁸ Clinical features that were highly predictive of a positive DNA SCA test in our population included presence of a positive family history, chorea and dystonia, muscle and tongue fasciculations, gaze-evoked nystagmus, and hypertonia.⁷

Some characteristic features

SCA 1 – Hypermetric saccades and hyperreflexia.

SCA 2 – Markedly reduced velocity of saccadic eye movements, areflexia and changes similar to those seen in olivopontocerebellar atrophy on brain imaging. May show pure parkinsonian phenotype.

SCA 3 – Combinations of protruded eyes, muscle fasciculations, spasticity, chorea, gaze-evoked nystagmus, parkinsonism and peripheral neuropathy. May show pure parkinsonian phenotype.

SCA 7 – Macular degeneration.

SCAs 5, 6, 10 and 11 – Relatively pure cerebellar signs.

SCA 8 – Mild sensory neuropathy with frequent late-onset spasticity.

SCA 12 – Head and hand tremors.

SCA 17 – Intellectual deterioration and dysphagia.

DRPLA and SCA 10 – A history of seizures with ataxia.

Confirmation of diagnosis

A clinical diagnosis of SCA, and no genetic test done previously for the patient or family member.

Presymptomatic testing

Performed for those at risk of developing SCA, who request this test be performed for purposes like marriage or childbearing.

Prenatal testing

Can be considered when one parent is known to carry the SCA gene and the couple wants to determine the carrier status of the foetus.

Procedure of test

Patients suspected of having SCA are usually assessed by a neurologist who has an interest and is familiar with the nature of the test. Genetic counselling regarding the nature and implications of the test is carried out by a neurologist. Patients are usually informed of the test results at their subsequent follow-up between 1 to 3 months.

Information used for research

Diagnosed SCA patients will be approached separately regarding participation in a research project if such research is available. Sometimes collective information regarding the prevalence of the disease and the genetic findings are presented in

scientific meetings or for planning educational or healthcare programmes. Strict confidentiality is maintained to ensure that no patient can be identified.

Accuracy of results

The test is usually validated and repeated twice in the laboratory to ensure accuracy, and is compared with negative controls.

General features shared by most SCAs

1. Anticipation, where there is progressive increase of expanded CAG repeats in successive generations. Those with larger CAG repeats display earlier ages of onset with greater disease severity than those with relatively smaller repeats.
2. Appearance of a critical size of repeat for most of the SCAs, above which the disease would manifest.
3. Influences of parental origin on repeat size instability. Paternal transmission of many SCAs (such as SCAs 1, 2, and 3) may result in a severe, rapidly progressive phenotype at a young age.

Exceptions

1. In some SCAs, the disease and normal allele sizes overlap in an intermediate range. Alleles in the intermediate range show reduced penetrance in SCA 2. In SCA 7, the intermediate alleles do not cause disease but can give rise to *de novo* expansion to disease causing size in subsequent generations.
2. Some SCAs (such as SCAs 1 and 2) have non-CAG repeat (CAA, CAT) interruptions. The CAT interruptions introduce histidines into the polyglutamine tract in the protein product, ataxin 1, which may prevent pathogenicity of expanded polyglutamines in SCA 1. The presence of the CAT interruptions on normal alleles is useful for distinguishing normal from diseased alleles for allele sizes of 36 to 44.
3. SCA 8 exhibits instability of repeat with a bias towards expansion in maternal transmission and frequent contraction in paternal transmission. SCAs 1, 2, 3, and 7 may show length changes during intergenerational transmission with a predisposition to expansion in subsequent generations.
4. In SCA 6, the CAG repeat size shows no size instability in parent-to-child transmission, even though anticipation has been reported. SCAs 1, 2, 3, 5, 10 and 14 also show anticipation, whereas SCAs 8, 12 and 13 do not.

Autosomal Recessive Cerebellar Ataxias

This is a heterogeneous group of autosomal recessively inherited disorders that are characterised by progressive ataxia, and whose disease onset frequently occurs at a young age. However, milder variants with later disease onset have been described. The term early-onset cerebellar ataxia is ascribed to those recessive ataxias in which neither gene mutations nor chromosomal loci are known.

The affected gene and causative mutations have been described for Friedreich's ataxia, ataxia telangiectasia, autosomal recessive ataxia with oculomotor apraxia, autosomal recessive spastic ataxia of Charlevoix-Saguenay, abetalipoproteinemia, ataxia with isolated vitamin E deficiency, Refsum's disease, and cerebrotendinous xanthomatosis. However, routine genetic testing is available for Friedreich's ataxia (FRDA). Biochemical tests are available for some of the recessive ataxias.

Friedreich's Ataxia

FRDA, the most frequent recessive ataxia is characterised by onset in adolescence, progressive gait and limb ataxia, dysarthria, lower limb areflexia, loss of proprioception, and cardiomyopathy. Ninety-six percent of FRDA patients are homozygous for a GAA repeat expansion in the first intron of the X25/frataxin gene.¹³

Purpose of test

A genetic test can confirm the diagnosis when abnormal trinucleotide repeats are above the range of normal chromosomes. The genes are inherited in a Mendelian fashion.

Selection criteria

The test is used to confirm diagnosis in patients with the typical phenotype of FRDA. The test is used as a diagnostic screen in patients whose family history is compatible with autosomal recessive inheritance and whose progressive ataxia is otherwise unexplained. Other selection criteria include a history of ancestors' early death, non-paternity, or adoption in the presence of suspected clinical features described above.

Confirmation of diagnosis

A clinical diagnosis of FDRA and no genetic test done previously for the patient or family member.

Presymptomatic testing

Performed for those at risk of developing FDRA and who request this test be performed for purposes like marriage or childbearing.

Prenatal testing

Can be considered when one parent is known to carry the FDRA gene and the couple wants to determine the carrier status of the foetus.

Procedure of test

Patients suspected of having FDRA are usually assessed by a neurologist who has an interest and is familiar with the nature of the test. Genetic counselling by a neurologist

regarding the nature and implications of the test is carried out. Patients are usually informed of the test results at their subsequent follow-up between 1 to 3 months.

Information used for research

Diagnosed FDRA patients will be approached separately regarding participation in a research project if such research is available. Sometimes collective information regarding the prevalence of the disease and the genetic findings are presented in scientific meetings or for planning educational or healthcare programmes. Strict confidentiality is maintained to ensure that no patient can be identified.

The normal repeat length range is from 6 to 36 units, whereas expanded alleles have 90 to 1,300 repeats. Age of onset is inversely correlated with the size of the shorter allele. Heterozygous mutations (GAA expansion) and point mutations in the frataxin gene are less common. Atypical clinical features (e.g. disease onset in adulthood or preservation of muscle reflexes) have been described in those with homozygous mutations (GAA expansions). Finding of a heterozygous GAA expansion in a symptomatic individual suggests the presence of a point mutation on the second allele.

Routine Biochemical Screening of Recessive Ataxias

Ataxia Telangiectasia (AT)

AT is an autosomal recessive disorder characterised by cerebellar ataxia with onset in early childhood, oculocutaneous teleangiectasias, a high incidence of neoplasia, radiosensitivity, and recurrent infections. More than 200 mutations exist in ATM, the gene involved in AT, which encodes a member of the phosphoinositide-3 kinase family involved in cell cycle checkpoint control and DNA repair. The most useful test is determination of serum-foetoprotein, which is elevated in 90% of AT patients.

Abetalipoproteinemia

Abetalipoproteinemia is an autosomal recessive disorder characterised by a gradual onset of ataxia, limb weakness, disturbed sensation, retinal degeneration, and diarrhoea. It is caused by mutations of the gene encoding a subunit of a microsomal triglyceride transfer protein. The diagnosis can be made by lipid electrophoresis showing low serum cholesterol (<70 mg/dl) and nearly absent, very low-density lipoproteins, acanthocytosis in blood smears, and reduced serum vitamin E levels.

Wilson disease

Wilson disease is caused by mutations in the gene for a copper-transporting p-type ATPase called ATP7B, located on chromosome 13q14-q21. The disease is characterised by a combination of neurological (e.g. parkinsonism, chore, dystonia etc), hepatic (cirrhosis, liver failure), or psychiatric dysfunctions (depression, personality changes). More than 170 mutations have been described so far, most being point mutations or

small deletions. Mutational analysis is difficult because of the large size of the gene and the various mutations. A diagnosis in a symptomatic individual can be made based on low serum ceruloplasmin, high urinary copper and/or increased hepatic copper content, Kayser-Fleischer ring, and copper deposits on imaging.

Parkinson's Disease, Dystonia and Alzheimer's Disease

Parkinson's disease

Parkinson's disease (PD) is a progressive neurodegenerative disease characterised by loss of dopaminergic cells in the substantia nigra pars compacta and by the presence of Lewy bodies. The cardinal clinical symptoms and signs of PD are bradykinesia, rigidity, tremor, postural instability and freezing attacks.^{14, 15} Ten gene loci have been identified by linkage analysis on human chromosome 4q21-23 (PARK 1), 6q25-27 (PARK 2), 2p13 (PARK 3), 4p15 (PARK 4), 4p13 (PARK 5), 1p35-p36 (PARK 6), 1p36 (PARK7), 12p11.2-q13.1 (PARK 8), 1p36 (PARK 9), and 1p32 (PARK 10).¹⁵ Genetic susceptibility and gene-environmental interaction in Singaporean PD population and those reported in the literature have not been conclusive.¹⁶⁻²²

Mutations in the alpha-synuclein (PARK 1), Parkin (PARK 2), ubiquitin carboxy-terminal hydrolase (PARK 5), PINK1 (PTEN-induced kinase 1) (PARK 6), DJ1 (PARK 7) and LRRK 2 (leucine-rich repeat kinase 2) (PARK 8) genes have been described.¹⁵ In particular, mutations in the Parkin gene on chromosome 6, first reported in Japanese patients with an autosomal-recessive syndrome of juvenile parkinsonism, is of great significance as mutations in Parkin are much more common than mutations in other genes.²³⁻²⁵ Many different mutations of Parkin have been identified, including exon deletions or duplications, and point mutations.^{24, 25}

Epidemiologic data of Parkin gene mutations in the Singaporean PD population is currently being determined. The Parkin gene is large and more than 100 different types of mutations have been described. It is difficult to distinguish the phenotype between those with and without Parkin mutations. However, genetic testing can be considered in young-onset cases involving levodopa-responsive parkinsonism, particularly in patients less than 20 to 30 years of age and with a family history suggesting a possible recessive inheritance. In Caucasian populations, mutations can be detected in 50% of families with autosomal recessive parkinsonism, and 70% in those with age of onset less than 20 years old. However, we do not recommend genetic testing in the general PD population because the chance of detecting Parkin mutations is low. Furthermore, heterozygous Parkin mutations have been described in healthy controls and exonic Parkin rearrangements (not uncommon) are difficult to detect unless quantitative gene dosage studies are carried out. Due to technical complexity and the lack of clarity regarding the pathogenicity of some Parkin mutations/variants, genetic testing for Parkin mutations should thus preferably be considered in a research setting.

More recently, PINK 1 mutations have been found in young onset and recessive forms of PD. In addition, a common LRRK 2 mutation in exon 41 has been found in a number

of White PD patients with or without family history. More information and research are still needed at this time before we consider genetic testing for these genes in our local clinical setting.

Dystonia

Dystonia is characterised by excessive spasms of both agonist and antagonist muscles resulting in abnormal posturing. Primary dystonia is of idiopathic origin, but a number of disease causing genes or genetic loci have been discovered for a number of dystonia syndromes. The most extensively studied is DYT 1 (Dystonia Musculorum Deformans), which exhibits an autosomal dominant inheritance with reduced (30 to 40%) penetrance. DYT 1 is caused by an underlying GAG in a Torsin A gene on chromosome 9q34. This mutation is present in a number of families of diverse ethnic background.

Most patients develop dystonia before the age of 26 years. One or more limbs are almost always affected and over 95% have an affected arm. The DYT 1 GAG-deletion accounts for 90% of early-onset limb dystonia in the Ashkenazi population, compared to about 50% in the non-Jewish population.

Genetic testing is recommended for patients with early-onset limb dystonia before the age 26 years, and for patients with late-onset dystonia who have a family history of early dystonia onset (<26 years).

Alzheimer's disease

Alzheimer's disease (AD) is the most common neurodegenerative disease in most countries. Disease causing mutations are rare in AD and other inherited dementias, and hence routine genetic testing is not carried out locally. While apolipoprotein E4 allele is an established risk factor for AD, it is not useful for diagnosis or presymptomatic assessment.

General Considerations of Genetic Testing in Adult Neurodegenerative Diseases

There are presently no universally accepted guidelines for genetic testing of adult neurodegenerative diseases, though some guidelines designed to help clinical neurologists have been proposed by a Movement Disorder Society task force¹ and also by a European consortium.^{2, 3} Like other genetic diseases without a definitive cure, there are a number of ethical, social, legal, and psychological issues to consider for such genetic testing. These include informed consent, confirmatory testing, prenatal diagnosis, predictive testing and asymptomatic testing for children, confidentiality, insurability, finances, employment, disability, and marriage. Genetic counselling forms the cornerstone of any genetic testing programme.^{1-3, 6, 13}

Patients should be provided with information regarding the clinical features and course of their disease, the mode of inheritance and penetrance. They should be counselled on

the testing's potential implications for them and their families. Genetic counselling for all asymptomatic family members is equally important. In predictive testing, psychological counselling by trained persons is essential.

The neurologist should ensure that the patient or legal guardian is capable of understanding the process and of making informed choices. Without the written consent of the patient, such tests should not be performed at the request of members of the patients' families or other third parties. Test results should never be disclosed to a third party without written consent from the patient.

Specific Considerations

There is general consensus that testing in at-risk asymptomatic children is not encouraged, particularly when no effective treatment is available.

If the attending neurologist does not have thorough experience with inherited disorders, referral to a colleague with experience in these disorders is suggested.

Unlike some inherited disorders that present at birth or in childhood and greatly decrease lifespan, patients who carry the disease-causing gene in some of the adult neurodegenerative diseases may not develop any symptoms till middle age (such as HD) or even till old age (such as SCA 6). Furthermore, one cannot predict with any certainty when an asymptomatic individual will develop symptoms in an event of abnormal genetic findings. These considerations will pose potential ethical dilemmas for the physician when issues such as termination of a pregnancy need to be discussed. The implications of asymptomatic testing on the employment, insurance, and general life planning on the person tested are grave and needs to be thoroughly examined. Guidelines for presymptomatic diagnosis issued by the International Huntington's Disease Society and the World Federation of Neurology research group for Huntington's disease are useful references.¹² In general, discussion of such issues during the genetic counselling process must be dealt with carefully on an individual basis. There must also be adequate follow-up care for psychological problems. This will require careful management by a combined team of experts including the neurologist, genetic counsellors, psychologists, and social workers. The neurologist who has been taking care of the patient or a neurologist with experience in dealing with the disease should preferably lead the team and determine the individual needs of the patient.

Due to certain cultural beliefs and practices amongst our various ethnic groups, sensitivity and skill is needed to manage patients and their relatives when genetic testing is discussed.^{4, 5} For instance, we have encountered difficulties in getting some at-risk relatives and family members of SCA patients to come forward for an examination. In some instances, patients have falsely given a negative family history. The disease is perceived as a curse to the family due to ancestral misdoings. They would generally try to avoid the truth through denial, or hide their condition from friends and relatives.

Conclusion

The armamentarium of genetic testing and screening of adult neurodegenerative diseases in Singapore will continue to expand. A molecular diagnosis programme should ideally be managed by a team of neurologists, psychologists, and other trained personnel with the necessary experience in managing the diseases. These persons should be committed to providing such services and should have a good knowledge of the ethical, psychological, cultural, and legal issues in our population.

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