Analysis of spinocerebellar ataxia types 1, 2, 3, and 6, dentatorubral-pallidoluysian atrophy, and Friedreich's ataxia genes in spinocerebellar ataxia patients in the UK

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Abstract

Accurate clinical diagnosis of the spinocerebellar ataxias (SCAs) can be difficult because of overlap in phenotype with other disorders and variation in clinical manifestations. Six SCA loci have been mapped and four disease causing genes identified, in addition to the causative gene for Friedreich's ataxia (FA). All of the identified mutations are expansions of trinucleotide repeat tracts. The SCA2 and SCA6 genes were published recently. The extent of the normal CAG size ranges at these loci and the relative frequencies of the known causes of SCA in the UK are not known. This study first investigated the normal size ranges of the SCA2 and SCA6 loci by genotyping control populations of West African and South African subjects, since African populations generally show the greatest allelic diversity. We found one allele larger than the previously determined normal range for SCA2, and our results at the SCA6 locus agreed with the previously reported normal range. The second component of the study assessed the relative frequencies of the SCA1, 2, 3, and 6, DRPLA, and FA trinucleotide repeat mutations in 146 patients presenting with SCA-like symptoms referred to genetic diagnostic laboratories in the UK. We detected mutations in 14% of patients referred with a diagnosis of autosomal dominant SCA, and in 15% of patients referred with spinocerebellar ataxia where we did not have sufficient family history data available to allow categorisation as familial or sporadic cases. Friedreich's ataxia accounted for 3% of the latter category of cases in our sample, but the most common causes of SCA were SCA2 and SCA6.

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The hereditary ataxias are a group of neurodegenerative disorders characterised by varying degrees of brain stem and cerebellar pathology or dysfunction. Clinical features usually present in the third or fourth decade and can include dysarthria, dementia, pyramidal signs,

and oculomotor and spinal cord dysfunction, in addition to cerebellar manifestations.^{1 2} The dominantly inherited spinocerebellar ataxias (SCAs) are a phenotypically heterogeneous group of disorders which can be difficult to diagnose. In the past two decades at least six SCA loci have been genetically mapped (SCA1-5, 7) and four disease causing genes have been identified (SCA1, 2, 3, and 6).³⁻⁹ Friedreich's ataxia (FA) has a phenotype which can overlap with the autosomal dominant SCAs, but differs in that it is inherited in a recessive manner and usually presents at an earlier age. It is a progressive ataxia with onset usually before the age of 25, which can be accompanied by absence of lower limb reflexes, dysarthria, limb weakness, skeletal deformities, and sometimes cardiomyopathy and diabetes.^{2 10 11} However, a significant proportion of patients with the FA mutation have a later age of onset and incomplete presentation (for example, intact reflexes), suggesting that this disease has a broader clinical presentation.^{10 11}

SCA1, 2, 3, and 6 are associated with abnormal expansions of CAG repeats in the coding regions of their respective genes.³⁻⁹ Thus, they share the same mutation as spinobulbar muscular atrophy (SBMA), Huntington's disease (HD), and dentatorubral-pallidoluysian atrophy (DRPLA).¹² In each of these diseases the tracts of CAG repeats are translated into glutamines. In Friedreich's ataxia, more than 95% of disease alleles are associated with expansion of a GAA trinucleotide repeat tract in the first intron of the causative gene.¹³ This is a large expansion (200 to >900 repeats) which correlates inversely with age of onset of the disease.^{10 11}

The wide variation in clinical manifestations of the SCAs and the overlap in phenotype with other inherited disorders such as DRPLA, FA, and familial spastic paraplegia can confound accurate clinical diagnosis. Molecular characterisation vastly improves upon the use of clinical and histopathological material alone to classify this heterogeneous group of disorders. Identification of a causative mutation can provide a diagnosis in cases who present with minor signs and symptoms, which can manifest years before appearance of the usual symptoms at disease onset,¹ and allows for the options of

predictive testing of asymptomatic at risk subjects and prenatal diagnosis.

Before routine diagnostic testing can be offered, it is critical to know the normal and disease size ranges for these trinucleotide repeat tracts. These size ranges need to be determined using a variety of control populations in order to account for the global variation in allele frequencies among populations.¹⁴ We and others have looked at normal and disease ranges in HD, Machado-Joseph disease (MJD), and myotonic dystrophy (DM) in various populations and have found that there can indeed be variation in allele size ranges among them.¹⁵⁻¹⁸

The normal repeat size ranges in the recently identified SCA2 and SCA6 genes were established using Japanese and white populations for SCA2⁶⁻⁸ and a small white sample in the case of SCA6,⁹ which may not reflect the true genetic diversity in the total human population. Furthermore, while virtually all of the other diseases associated with CAG expansions have more than 36 repeats in their disease ranges,

SCA6 seems to be exceptional in that it has a disease range of only 21-27 CAG repeats,⁹ similar in length to many normal chromosomes in related disorders.¹² To address these issues, we have looked at the allele frequencies of SCA2 and SCA6 in people from Nigeria and South Africa, since African populations are thought to have a high level of allelic diversity at most loci.¹⁴

In view of the recent identification of SCA2 and SCA6 genes and the lack of knowledge of the relative frequencies of disease causing mutations in the UK, we have determined the relative frequencies of SCA1, 2, 3, and 6, DRPLA, and FA in a sample of 146 subjects referred for diagnostic testing for ataxia to molecular genetics laboratories in England and Northern Ireland. Knowledge of the relative frequencies of these diseases in the UK will allow realistic expectations of the proportion of patients for whom clinicians can expect positive results.

Methods

SUBJECTS

Control populations comprised 50 West Africans (mainly from Nigeria) and 100 South African Bantu speaking subjects collected in Cape Town and Johannesburg. All samples were anonymised and apparently unrelated. Although we are not aware that any controls had neurological difficulties, these were not systematically excluded by clinical examination.

The clinical sample comprised 146 patients presenting with SCA-like symptoms referred to genetics diagnostic laboratories in Sheffield (32 males, 34 females, mean age 47 (SD 18), range 2-76 years), Salisbury (nine males, 10 females, mean age 46 (SD 16), range 6-72 years), Manchester (18 males, 18 females, mean age 44 (SD 18), range 3-75 years), Belfast (three males, three females, mean age 42 (SD 11), range 22-49 years) and Cambridge (12 males, seven females, mean age 46 (SD 17), range 28-78 years). This sample included all patients referred to these laboratories with SCA-like symptoms and included cases where a positive result had previously been obtained for SCA1, SCA3, or DRPLA. Twenty-two of these were diagnosed as having autosomal dominant SCA and family histories were complete. The family history details on the laboratory referral forms of 124 cases were not sufficient to allow categorisation as truly sporadic or familial cases and two patients were referred with a diagnosis of familial SCA, but with incomplete family histories. Neurologists referred 98% of cases from Sheffield, 79% of cases in Salisbury, 67% of cases from Belfast, 52% of cases from Manchester, and 47% of cases from Cambridge. The other referring clinicians comprised mainly geneticists but included a GP, paediatricians, and psychiatrists. There was no physician who referred the bulk of patients within any one centre.

PCR ANALYSIS

The SCA2 CAG repeat expansion was analysed using conditions and primers SCA2-A

Figure 1 Distributions of numbers of CAG repeats at the SCA2 (A) and SCA6 (B) loci in two African populations (solid bars) and normal UK chromosomes ($n=235$) (hatched bars). The normal UK chromosomes include data from SCA patients analysed in Cambridge. The distributions indicate the percentage of chromosomes with a given CAG repeat length.

Table 1 Distribution of mutations at known disease loci in patients presenting with SCA-like symptoms in the UK

Locus	Isolated cases (no family history)	Autosomal dominant SCA	Total
SCA1	1 Salisbury	1 Sheffield	2
SCA2	5 Belfast (2) Cambridge (3)	1 Sheffield	6
SCA3	1 Cambridge	0	1
SCA6	6 Salisbury (2) Manchester (3) Cambridge (1)	1 Sheffield	7
DRPLA	2 Salisbury	0	2
Friedreich's ataxia	4 Sheffield	0	4
Total cases	124	22	146

and SCA2-B⁷ and the SCA6 repeats were amplified using primers S-5-F1 and S-5-R1.⁹ Primers Rep1 and Rep2 were used to amplify the SCA1 repeats⁴ and SCA3 repeats were amplified using primers MJD25 and MJD52.⁵ DRPLA expansions were analysed using primers CTG-B37-F and CTG-B37-R¹⁹ and Friedreich's ataxia genotypes were determined using GAA-104F and GAA-629R.¹⁰ Five percent DMSO was added to the reaction mixtures for SCA2 and SCA6. PCR products of the SCA and DRPLA reactions were sized on 6% denaturing polyacrylamide gels compared to a sequence ladder (pGEM 3Z(+)) (Promega)). Homozygotes were amplified twice and all gels were subjected to long exposures to detect any expanded alleles which might have been faint on the autoradiographs.

Results

To confirm the recently established size ranges of normal alleles in SCA2 and SCA6, we genotyped two populations of Africans, one composed mainly of Nigerians and the other from South Africa. We looked at 272 chromosomes at the SCA2 locus and found one allele larger than the previously determined normal range of 15-29 CAGs,⁶⁻⁸ but below the putative disease size (fig 1A). In the case of SCA6, we examined 294 chromosomes from the same two African populations and found that all alleles fell into the previously published normal size range for the CAG repeat tract⁹ (fig 1B).

Our clinical sample of 146 cases ascertained from patients referred to molecular genetics laboratories in the UK with SCA-like symptoms was analysed for SCA1, 2, 3, and 6, DRPLA, and FA mutations. Three of 22 cases referred with the diagnosis of autosomal dominant SCA (14%) had detectable mutations in addition to 19 of 124 of patients referred with spinocerebellar ataxia where we did not have sufficient family history data available to allow categorisation as familial or sporadic cases (15%). The most common SCAs detected in this sample were SCA2 (six cases) and SCA6 (seven cases) (table 1). All expanded mutant alleles fell within previously published disease size ranges. The two cases of DRPLA were not obviously related. One of the cases with DRPLA was from a family which has been described previously.²⁰

Discussion

Identification of causative mutations for SCA1, 2, 3, and 6 allows clinicians to diagnose people

definitively by genotyping. Before determining whether patients have these mutations, we first investigated the upper limits of the CAG repeat sizes in normal subjects for SCA2 and SCA6. Since allele frequencies can differ between populations, it is important to test as many populations as possible when establishing normal and disease ranges of CAG repeat tracts. This study tested two African populations, since they are thought to have great allelic diversity.¹⁴ The presence of an intermediate allele at the SCA2 locus from one Nigerian with 33 repeats could merely indicate a more diverse distribution of non-disease alleles, or it could indicate a disease allele in a patient who has not yet developed the disease. Although we found no alleles outside the putative normal and disease size ranges for SCA6, further studies will be needed to determine the pathological significance of allele sizes that fall between normal and disease ranges. Ideally, the non-pathogenic nature of these long alleles should be confirmed by showing their presence in elderly asymptomatic subjects.

This is the first report of screening for the presence of SCA1, 2, 3, 6, DRPLA, and FA in a large group of patients in the UK referred with clinical features of spinocerebellar ataxia. Fifteen percent of 146 patients had a mutation at one of the six loci tested. The most common causes of SCA in our sample were SCA6 (5%) and SCA2 (4%). Interestingly, 3% (4/124) of isolated cases in our sample were caused by homozygous mutation at the FA locus. Three of these patients presented at age <25 years, while the fourth presented at 60 years of age. This confirms the data of Durr *et al*¹¹ and Filla *et al*,¹⁰ who both found homozygous GAA expansions in the FA gene in subjects with incomplete clinical features of FA presenting after the age of 25. Although we did not screen for point mutations in this gene, our estimate of the frequency of this disease is not likely to be seriously confounded since GAA expansions are found on >95% of disease chromosomes.^{10 11 13}

There was some variation in the frequencies and proportions of the various mutations in patients from different centres, which may have been a function of the diagnostic criteria used by referring clinicians or of regional variations in the distribution of these mutations. However, it is likely that the statistical variation owing to the small sample sizes plays a major role in determining this variance.

We found only one case with SCA3 compared to 15 cases with SCA1, 2, and 6. This contrasts with the data of Silveira *et al*²¹ who found that SCA3 accounted for 17% of SCA cases, and Ranum *et al*²² who diagnosed SCA3 in 21% of their SCA patients. More recent studies have suggested that SCA3 may comprise 23-34% of SCA cases, SCA2 comprises 13-15% of cases, and SCA1 comprises 6-14% of cases.^{23 24} It is important to note that all of these published studies used populations which were ethnically diverse and that the frequencies of the different SCA mutations may vary in different populations, as is the case with HD and DRPLA.^{25 26} All of our patients who had

identifiable mutations are thought to be English (surnames compatible with being English/European), except for a South African of European origin (SCA1) and a Hong Kong Chinese patient (SCA1).

It is necessary to point out that this study was retrospective with regards to the patients who were referred. Thus, the diagnostic criteria which prompted referral to molecular genetics laboratories were not formalised and may vary from clinician to clinician. The most likely result of this ascertainment procedure is contamination of the patient sample with cases who are more likely to have non-SCA diagnoses. This may affect estimates of the absolute proportions of cases of SCA with specific diagnoses. However, this is unlikely to affect the relative proportions of the different identifiable mutations, that is, the proportion of SCA2 cases versus SCA1 cases.

If the referral criteria of these patients are typical, then clinicians can currently expect molecular diagnoses in about 15% of their SCA referrals. SCA2 and 6 were the most prevalent in our clinical sample, which emphasises the importance of these recent discoveries.

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