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# Close Associations between Prevalences of Dominantly Inherited Spinocerebellar Ataxias with CAG-Repeat Expansions and Frequencies of Large Normal CAG Alleles in Japanese and Caucasian Populations

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## Summary

**To test the hypothesis that the frequencies of normal alleles (ANs) with a relatively large number of CAG repeats (large ANs) are related to the prevalences of the dominant spinocerebellar ataxias (SCAs)—SCA types 1, 2, 3 (Machado-Joseph disease), 6, and dentatorubral-pallidoluysian atrophy (DRPLA)—we investigated the relative prevalences of these diseases in 202 Japanese and 177 Caucasian families and distributions of the number of CAG repeats of ANs at these disease loci in normal individuals in each population. The relative prevalences of SCA1 and SCA2 were significantly higher in Caucasian pedigrees (15% and 14%, respectively) than in Japanese pedigrees (3% and 5%, respectively), corresponding to the observation that the frequencies of large ANs of SCA1 (alleles >30 repeats) and of SCA2 (alleles >22 repeats) were significantly higher in Caucasians than in Japanese. The relative prevalences of MJD/SCA3, SCA6, and DRPLA were significantly higher in Japanese pedigrees (43%, 11%, and 20%, respectively) than in Caucasian pedigrees (30%, 5%, and 0%, respectively), corresponding to the observation that the frequencies of large ANs of MJD/SCA3 (>27 repeats), SCA6 (>13 repeats), and DRPLA (>17 repeats) were significantly higher in Japanese than in Caucasians. The close correlations of the relative prevalences of the dominant SCAs with the distributions of large ANs strongly support the assumption that large ANs contribute to generation of expanded alleles (AEs) and the relative prevalences of the dominant SCAs.**

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## Introduction

Dominantly inherited spinocerebellar ataxias (SCAs) are a group of heterogeneous neurodegenerative diseases that are characterized by chronic progressive cerebellar ataxia associated with various combinations of other neurological signs (Harding 1982). Although classification of dominant SCAs on the basis of clinical presentation has been quite controversial, because of the overlap in clinical presentations among these SCAs, identification of the causative genes for SCA type 1 (SCA1 [MIM 164400]) (Orr et al. 1993), SCA type 2 (SCA2 [MIM 183090]) (Imbert et al. 1996; Pulst et al. 1996; Sanpei et al. 1996), Machado-Joseph disease/SCA type 3 (MJD/SCA3 [MIM 109150]) (Kawaguchi et al. 1994), SCA type 6 (SCA6 [MIM 183086]) (Zhuchenko et al. 1997), SCA type 7 (SCA7 [MIM 164500]) (David et al. 1997), and dentatorubral-pallidoluysian atrophy (DRPLA [MIM 125370]) (Koide et al. 1994; Nagafuchi et al. 1994) has enabled the classification of dominant SCAs on the basis of molecular diagnosis. In all these disease types, the causative mutations cause expansion of in the number of CAG repeats in the coding region of the corresponding genes. The CAG repeats are generally polymorphic in normal alleles (ANs) of $\leqslant$40 repeats, whereas they usually are >40 repeats in expanded alleles (AEs).

Recent studies suggest that the prevalences of these dominant SCAs are considerably different among different populations (Illarioshkin et al. 1996; Cancel et al. 1997; Geschwind et al. 1997; Lorenzetti et al. 1997). DRPLA and SCA6 seem to be less prevalent in Caucasian populations than in the Japanese population (Silveria et al. 1996; Ikeuchi et al. 1997; Ishikawa et al. 1997; Riess et al. 1997; Stevanin et al. 1997*a*). The molecular basis for the differences in the prevalences of these dominant SCAs, however, is not fully understood.

In Huntington disease (HD), another neurodegenerative disease caused by CAG-repeat expansion (The

Huntington's Disease Collaborative Research Group 1993), new AEs have been demonstrated to arise from ANs with CAG-repeat number that is in the high range for normal individuals but that is lower than the range seen in affected individuals (this category has been termed "intermediate alleles" [IA]) (Goldberg et al. 1993; Myers et al. 1993). Haplotype analyses have demonstrated that the majority of these AIs share the same haplotypes as those of AEs in Caucasian populations (The Huntington's Disease Collaborative Research Group 1993; Squitier et al. 1994), suggesting that these IAs serve as a reservoir for the generation of new AEs and that the frequencies of IAs in various ethnic populations contribute to the variations in the prevalence of HD in the corresponding populations (Squitier et al. 1994).

In regard to dominant SCAs, there is no documentation of the generation of AEs from ANs, making the definition of IAs difficult. Strong linkage disequilibria, however, have been demonstrated in AEs of SCA1 (Wakisaka et al. 1995), SCA2 (Hernandez et al. 1995), MJD/SCA3 (Stevanin et al. 1995;Takiyama et al. 1995; Endo et al. 1996), and DRPLA (Yanagisawa et al. 1996), in particular populations. Furthermore, in French MJD/SCA3 families, a close association has been observed between AEs and a particular haplotype that was also found in all ANs with >33 repeats (Stevanin et al. 1997*b*). In Japanese DRPLA patients, a particular haplotype has been found to be associated with AEs that was also exclusively associated with ANs with >17 CAG repeats (Yanagisawa et al. 1996). These results strongly suggest that AEs of the dominant SCAs are also generated from IAs associated with particular haplotypes and that the prevalences of the dominant SCAs in individual populations correlate with the frequencies of IAs of the corresponding genes. In support of this assumption, ANs with >17 repeats in the DRPLA gene have been described as being overrepresented in the Japanese population versus Caucasian populations (Burke et al. 1994; Deka et al. 1995).

With this background, we determined the relative prevalences of the dominant SCAs in large population-based data sets of Japanese and Caucasian pedigrees, analyzed the distribution of the sizes of ANs of the corresponding genes in both populations, and found that the relative prevalences of the dominant SCAs in Japanese and Caucasian populations are strongly correlated with the frequencies of AN that have a relatively large number of repeats (large ANs) in the causative genes.

## Families, Material, and Methods

### *Pedigrees with Dominant SCAs*

Japanese pedigrees with dominant SCAs who were referred to the Department of Neurology of the Brain Research Institute at Niigata University between April 1993 and March 1997 were included on a consecutive basis, and Caucasian pedigrees with dominant SCAs who were referred to INSERM U289, Hôpital de la Salpêtrière, or Baylor College of Medicine between May 1990 and March 1997 were included on a consecutive basis. Families of northern African, Middle Eastern, and Hispanic origins were not included among the Caucasian pedigrees. These pedigrees were referred to these laboratories for molecular diagnosis of SCAs. Pedigrees were considered to have dominant SCAs when affected individuals with ataxia were observed in at least two generations.

### *Analysis of Number of CAG Repeats*

Genomic DNAs were extracted from peripheral blood leukocytes by a standard procedure. Molecular diagnosis was performed to estimate the number of CAG repeats at five disease loci corresponding to SCA1, SCA2, MJD/SCA3, SCA6, and DRPLA, according to methods described elsewhere (Orr et al. 1993; Kawaguchi et al. 1994; Koide et al. 1994; Sanpei et al. 1996; Ikeuchi et al. 1997). The distributions of the numbers of CAG repeats in ANs at the five loci in unrelated Japanese and Caucasian individuals were determined. For Japanese normal chromosomes, 176 SCA1 loci, 359 SCA2 loci, 275 MJD/SCA3 loci, 327 SCA6 loci, and 307 DRPLA loci were typed, whereas, for Caucasian normal chromosomes, 574 SCA1 loci, 355 SCA2 loci, 641 MJD/SCA3 loci, 303 SCA6, and 156 DRPLA loci were typed.

### *Statistical Analyses*

All statistical analyses were performed by means of SPSS version 3.0. Means, variances, ranges, and skewness were determined for the distributions of ANs at the five loci, in Japanese and Caucasian individuals. Differences, both in the relative prevalences of the dominant SCAs and in the frequencies of the large ANs, between the Japanese pedigrees and the Caucasian pedigrees, were analyzed by means of the $\chi^2$ test with Yates's correction, for each of the dominant SCAs. Differences between the mean sizes of ANs in the two populations were analyzed by means of the Mann-Whitney rank test. The null hypothesis was rejected at $P < .05$.

## Results

### *Relative Prevalences of Dominant SCAs in Japanese and Caucasian Populations*

We identified a total of 202 Japanese and 177 Caucasian families with dominant SCA. The relative prevalences are summarized in figure 1. The relative prevalences of SCA1 and SCA2 were higher in Caucasian

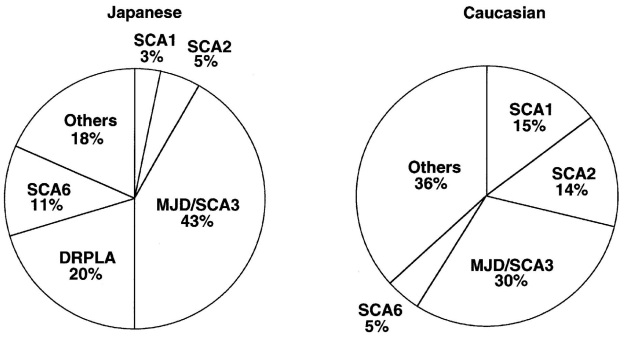


**Figure 1** Prevalences of dominant SCAs in 202 Japanese and 177 Caucasian families (also see the Appendix).

pedigrees (15% and 14%, respectively) than in Japanese pedigrees (3% and 5%, respectively), and the differences were statistically significant (SCA1—$\chi^2 = 13.58$, df = 1, $P = .0002$; SCA2—$\chi^2 = 8.41$, df = 1, $P = .0037$). The relative prevalences of MJD/SCA3, SCA6, and DRPLA were higher in Japanese pedigrees (43%, 11%, and 20%, respectively) than in Caucasian pedigrees (30%, 5%, and 0%, respectively), and the differences were statistically significant (MJD/SCA3—$\chi^2 = 5.05$, df = 1, $P = .024$; SCA6—$\chi^2 = 5.05$, df = 1, $P = .015$; DRPLA—$\chi^2 = 38.21$, df = 1, $P < .0001$).

### *Close Association between Frequencies of Large ANs and Relative Prevalences of Dominant SCAs, in Japanese and Caucasian Populations*

The distributions of the various sizes of ANs are shown in figure 2. The mean sizes of ANs at the SCA1 and SCA2 loci were significantly larger in Caucasians than in Japanese (SCA1, $P < .0001$; SCA2, $P < .0001$) . Mean sizes of ANs at MJD/SCA3, SCA6, and DRPLA loci were larger in Japanese than in Caucasians but were significantly larger only for SCA6 ($P < .0001$), not for MJD/SCA3 ($P = .0757$) and DRPLA ($P = .0795$).

To perform statistical analyses of the differences, between Japanese and Caucasian populations, in the frequencies of ANs larger than the majority of ANs, we defined large ANs as those that correspond to ~5%–10% of the upper tails (see the Appendix). The frequencies of large ANs in SCA1 (ANs >30 repeats) and SCA2 (ANs >22 repeats) were significantly higher in Caucasians than in Japanese (SCA1—$\chi^2 = 22.23$, df = 1, $P < .0001$; SCA2—$\chi^2 = 14.84$, df = 1, $P = .0001$) (Appendix). Cutoff values of 31 or 32 repeats for SCA1 and of 23 or 24 repeats for SCA2 also resulted in significantly higher frequencies of large ANs of SCA1 and SCA2 genes in Caucasian populations than in the Japanese population (see the Appendix). These results were in good accordance with the relatively higher prevalences of SCA1 and SCA2 in Caucasians than in Japanese.

The frequencies of large ANs in MJD/SCA3 (ANs >27 repeats), SCA6 (ANs >13 repeats), and DRPLA (ANs >17 repeats) genes were significantly higher in Japanese than in Caucasians (MJD/SCA3—$\chi^2 = 24.16$, df = 1, $P < .0001$; SCA6—$\chi^2 = 38.64$, df = 1, $P < .0001$; DRPLA—$\chi^2 = 11.80$, df = 1, $P = .0006$). Other cutoff values—28, 29, 30, or 31 repeats for MJD/SCA3; 14 or 15 repeats for SCA6; and 18, 19, 20, or 21 repeats for DRPLA—gave similarly significant differences (see the Appendix). These results are also in accordance with the relatively higher prevalences of MJD/SCA3, SCA6, and DRPLA in Japanese than in Caucasians.

## Discussion

### *Prevalences of Dominant SCAs*

The present study of our large population-based data sets clearly demonstrates marked differences, in the relative prevalences of the dominant SCAs, between Japanese pedigrees and Caucasian pedigrees. As has been estimated elsewhere (Burke et al. 1994; Deka et al. 1995), DRPLA was frequent in the Japanese pedigrees that we studied but was not observed in the Caucasian pedigrees. MJD/SCA3 and SCA6 were also more prevalent in Japanese pedigrees than in Caucasian pedigrees. On the other hand, SCA1 and SCA2 were more prevalent in Caucasian pedigrees than in Japanese pedigrees. MJD/SCA3 was the most prevalent type of dominant SCA in both populations, a finding that is consistent with previous reports of Caucasian populations (Ranum et al. 1995; Schols et al. 1995; Dürr et al. 1996). It should be noted that the causative genes were unknown for ~20%–40% of dominant SCAs (fig. 1), which may include SCA4 (Flanigan et al. 1996) and SCA5 (Ranum et al. 1994). Since the gene for SCA7 has only very recently been identified (David et al. 1997), the analysis of SCA7 could not be performed.

### *Prevalences of Dominant SCAs and Frequencies of Large ANs*

In the present study, we found a close association between the relative prevalences of the dominant SCAs in Japanese and Caucasian pedigrees and the frequencies of large ANs of the corresponding genes. The results suggest that the relative prevalences of these dominant SCAs are determined by the balance between continuous generation of new AEs and loss of AEs that is due to the impaired reproductive fitness of severely affected patients. Recent studies of the frequencies of mutation of ANs, of both the HD gene and the androgen-receptor

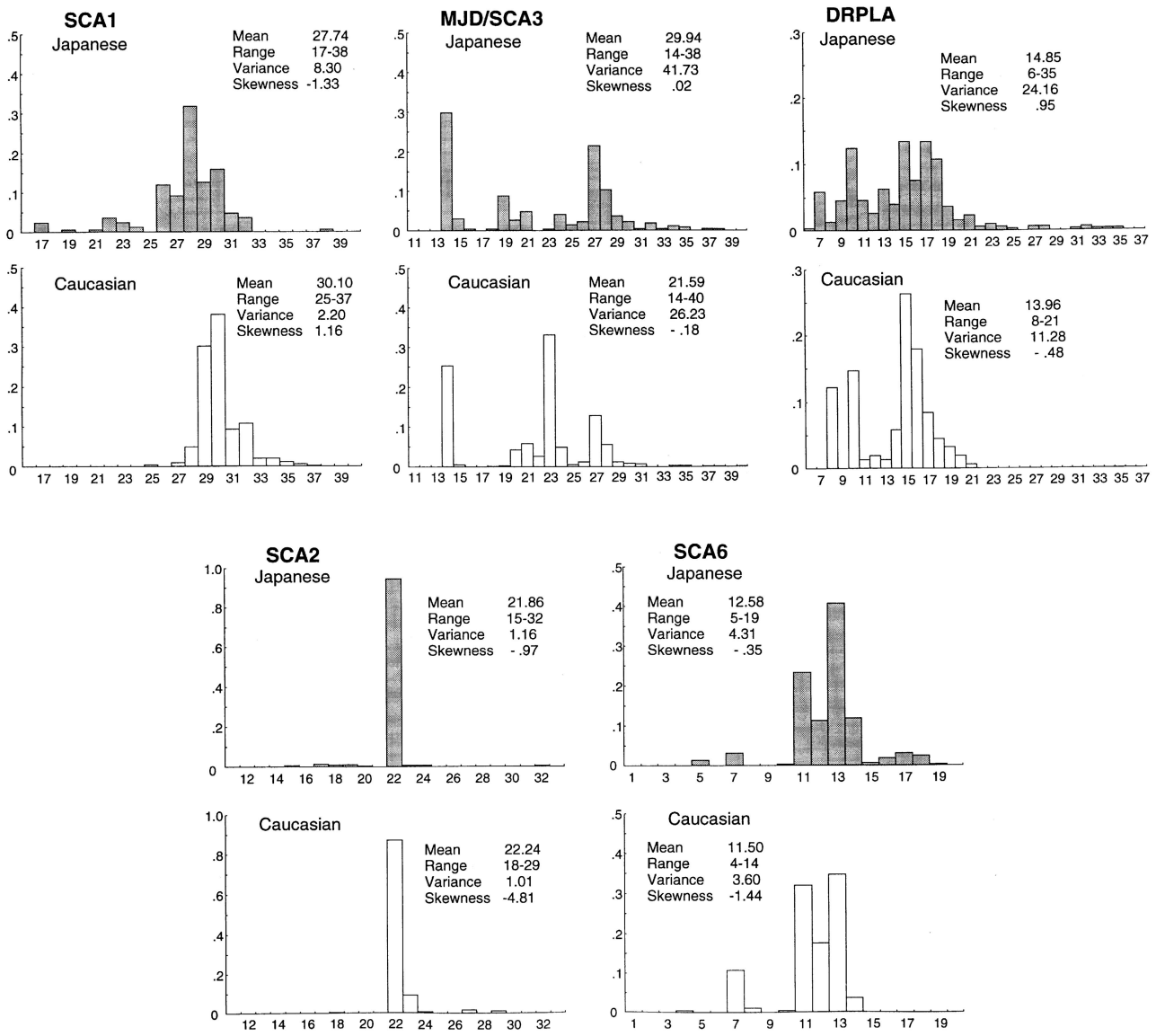


**Figure 2** Distribution of various numbers of CAG repeats in ANs at SCA1, SCA2, MJD/SCA3, SCA6, and DRPLA loci, in Japanese and Caucasian populations. Vertical axes represent allele frequency, and horizontal axes represent number of CAG-repeat units. Distributions of sizes of ANs in Japanese were significantly different than those in Caucasians, as determined by $\chi^2$-fit test, at all the loci (SCA1—$\chi^2$ = 308.79, df = 19, $P < .0001$; SCA2—$\chi^2$ = 51.73, df = 10, $P < .0001$; MJD/SCA3—$\chi^2$ = 207.15, df = 23, $P < .0001$; SCA6—$\chi^2$ = 70.08, df = 13, $P < .0001$; DRPLA—$\chi^2$ = 89.35, df = 26, $P < .0001$). There were no overlaps between the number of CAG repeats in ANs and that in AEs.

gene, by sperm typing, have revealed that the rates of expansion mutations increase depending on the sizes of ANs (Leeflang et al. 1995; Zhang et al. 1995). A large normal-repeat HD allele (30 repeats) showed 9% expansion- and 3% contraction-mutation frequencies (Leeflang et al. 1995). Therefore, some of the large ANs can stochastically undergo expansion mutation to produce the new AEs of the dominant SCAs. Although new mutations arising from CAG repeats within the normal range have not been described in dominant SCAs, the present results strongly support the assumption that new mutations arise from the large ANs. Recent observations that particular haplotypes of large ANs of MJD/SCA3 and DRPLA genes are commonly shared with MJD/SCA3 (Stevanin et al. 1997*b*) and DRPLA (Yanagisawa et al. 1996) patients, respectively, strongly suggest that large ANs with particular haplotypes are particularly prone to further expansion, to the disease range of CAG

repeats; in other words, being at the "large" end of the spectrum is necessary but not sufficient to be a disease-producing IAs. Such an argument is further supported by observations in the HD gene (Goldberg et al. 1995; Chong et al. 1997).

### *Implications of AN Distribution*

It remains unknown why there are differences in the distributions of the sizes of ANs and in the frequencies of large ANs among populations. The differences may simply represent founder effects. However, the distributions of the various numbers of CAG repeats are likely to be in a dynamic state depending on the mutation frequencies of the CAG repeats of the corresponding genes. Interestingly, skewness of the distribution of the sizes of ANs was clearly inverted, in SCA1 and SCA2 loci, between Japanese and Caucasians. It has recently been reported that directional mutational bias at the repeat locus contributes to the skewness of the size distribution (Rubinsztein et al. 1994). Different mutational biases among the populations may represent differences in possible *cis*-elements governing the mutations at these CAG loci, as suggested by the study of HD (Squitier et al. 1994; Goldberg et al. 1995). The existence of such *cis*-elements affecting intergenerational repeat instability has recently been suggested, in the study of androgen-receptor YAC transgenic mice carrying $CAG_{45}$ (La Spada et al. 1998).

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## Appendix

### Comparison of Frequencies, of Large AN Genes of Dominant SCAs, in Japanese and Caucasians

In the following list, the only Japanese/Caucasian frequency difference that is *not* statistically significant is .01/.03—for SCA2 when the number of repeats is >23.

| SCA1 | SCA2 | MJD/SCA3 | SCA6 | DRPLA |
|---|---|---|---|---|
| Frequencies of Large ANs in Japanese/Frequencies of Large ANs in Caucasians, When No. of Repeats for Large AN = | | | | |
| >30 | >22 | >27 | >13 | >17 |
| .09/.26 ($\chi^2$ = 22.23, $P$ < .0001) | .01/.12 ($\chi^2$ = 14.84, $P$ = .0001) | .21/.09 ($\chi^2$ = 24.16, $P$ < .0001) | .20/.04 ($\chi^2$ = 38.64, $P$ < .0001) | .24/.10 ($\chi^2$ = 11.80, $P$ = .0006) |
| >31 | >23 | >28 | >14 | >18 |
| .04/.16 ($\chi^2$ = 16.72, $P$ < .0001) | .01/.03 ($\chi^2$ = 3.65, $P$ = .056) | .11/.04 ($\chi^2$ = 17.60, $P$ < .0001) | .08/.00 ($\chi^2$ = 24.16, $P$ < .0001) | .13/.06 ($\chi^2$ = 5.42, $P$ = .0199) |
| >32 | >24 | >29 | | >19 |
| .01/.04 ($\chi^2$ = 6.88, $P$ = .0087) | .00/.03 ($\chi^2$ = 5.05, $P$ = .0246) | .07/.02 ($\chi^2$ = 10.40, $P$ = .013) | | .10/.03 ($\chi^2$ = 6.88, $P$ = .0087) |
| | | >30 | | >20 |
| | | .05/.01 ($\chi^2$ = 7.03, $P$ = .0080) | | .08/.01 ($\chi^2$ = 9.62, $P$ = .0019) |
| | | >31 | | >21 |
| | | .05/.01 ($\chi^2$ = 10.27, $P$ = .0014) | | .06/.00 ($\chi^2$ = 8.02, $P$ = .0046) |

## Electronic-Database Information

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for SCA1 [MIM 164400], SCA2 [MIM 183090), MJD/SCA3 [MIM 109150], SCA6 [MIM 183086], SCA7 [MIM 164500], and DRPLA [MIM 125370])

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