Mutations of PAX3 unlikely in Waardenburg syndrome type 2

Sir — Waardenburg syndrome has been divided with regard to dystopia canthorum (DC), into types 1 (presence) and 2 (absence) of the sign[1]; this can be confidently assessed with index W (ref. 2). Lack of penetrance for DC in WS1 occurs in no more than 1% of cases. Most clinically 'nonpenetrant' cases are those with nonapparent dystopia canthorum (NAD)[2] (1.86<W<2.07) who appear to be 'normal'. Family WS.15 in Tassabehji *et al.*[3], purported to be an example of WS2, is instead a typical case of WS1 (I-2, Fig. 4, left; W=2.23). I-2 has a nonpenetrant son (II-2, Fig. 4, right; W=1.83) very similar to the mother in family 7 of Waardenburg's original paper[4], the first and almost single published case of nonpenetrance for DC in WS1 (if stringent biometic criteria are applied)[2,5]. Individual III-2 (W=2.05), grandson of I-23, is a case of NAD according to definition[2].

It is generally and clinically important to establish that *PAX3* mutations (besides those in alveolar rhabdomyosarcoma[6]) are associated only with WS1 and not WS2, as seems to be the case. The type 1 (WS1) family supposedly not showing linkage to *ALPP* was, however, not excluded from the 2q35–q37 region[7]. On the other hand, at least two out of three WS2 families (none biometrically documented: without stated W index values) did not show linkage to *ALPP*, and furthermore, were heterogeneous (P=0.006) with respect to WS1 (ref. 7).

Thus, *PAX3* does not seem to be mutated in Waardenburg syndrome type 2, according to the available biometric[3] and linkage data[7].

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In Reply — The main point of our paper was to show that loss-of-function mutations in *PAX3* could cause Waardenburg syndrome (WS). The small family to which Arias refers included one affected person without dystopia (W=1.83), one with mild dystopia (W=2.21) and one with W=2.05, an intermediate figure below the diagnostic threshold (W=2.07) of the Waardenburg Consortium. One could argue about diagnostic labels and whether they apply to individuals or to families; as an individual our proband was type 2; the family as a whole was neither typical type 1 or type 2. Any classification based on presence or absence of one of the many manifestations of this variable disease is bound to be fallible. However, molecular analysis has in general supported the distinction, first made by Arias himself, between type 1 and type 2 WS. So far, no family with unambiguous type 2 WS has been shown to have a mutation in *PAX3*, although there are now data showing that in some families unambiguous type 1 WS does not map to the location of *PAX3*.

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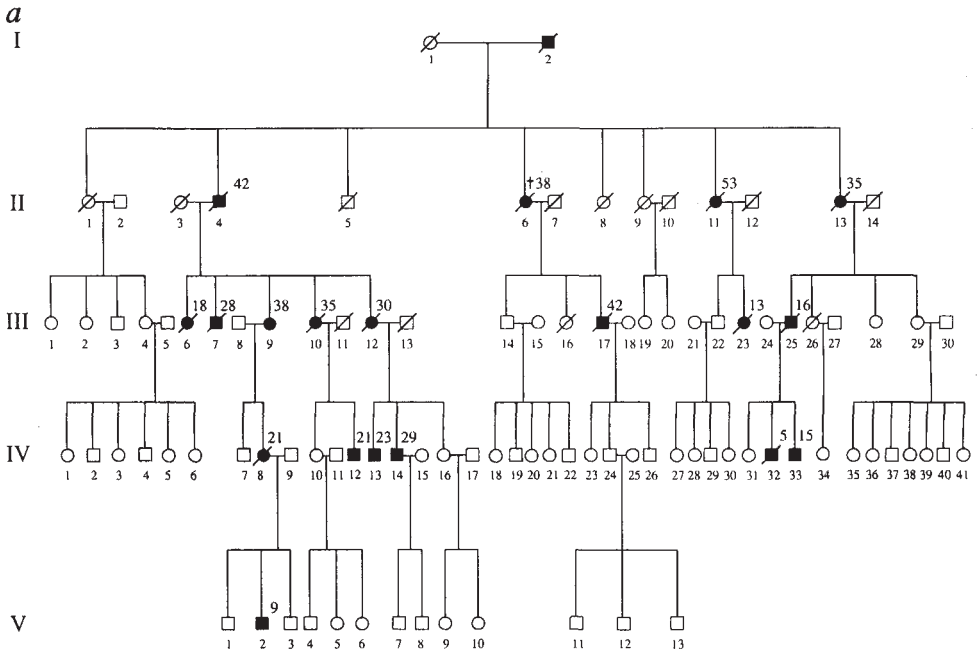
Anticipation in spinocerebellar ataxia type 2

Spinocerebellar ataxias (SCAs) are characterized by a progressive degeneration of specific neurons of the cerebellar cortex, deep cerebellar nuclei, brain stem and spinal cord. Affected individuals suffer from severe ataxia, dysarthria and variable degrees of motor disturbance and neuropathy. The gene for one subtype of the dominantly inherited ataxias (SCA1) maps to chromosome 6P (refs 1,2), and a second subtype (SCA2) was recently mapped to chromosome 12q between *D12S58* and *PLA2*, in a large pedigree of Cuban descent[3]. We have now identified a second pedigree with linkage to 12q and established closer flanking markers for *SCA2*. In contrast to the Cuban pedigree this pedigree reveals a remarkable degree of anticipation of disease onset. Given the recent identification[2] of an expanded CAG triplet repeat in SCA1, this finding suggests a similar mechanism may underlie at least some cases of SCA2 as well.

The FS pedigree is of Southern Italian descent and segregates SCA in five generations (Fig. 1*a*). All affected individuals show marked appendicular and gait ataxia as well as slow saccadic eye movements[4]. DNA was available from 60 individuals including 7 affecteds. Mean age of onset in 19 affecteds was 26.9 ± 12.5. We first excluded linkage to *SCA1* based on the observation of multiple obligate recombinants between the disease phenotype and *D6S89* and *D6S109*, which are known to flank the SCA1 locus[2]. Multipoint analysis excluded the mutation in this family from a 15 cM region including the region between *D6S89* and *D6S109*. To determine whether this family has



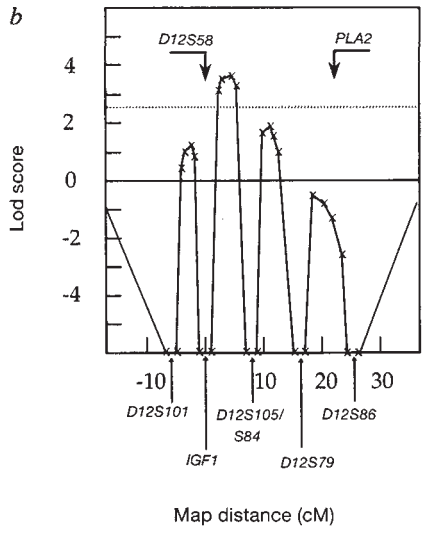


Fig. 1 Linkage of SCA2 to chromosome 12q. *a*, Pedigree structure of the FS family. The age of onset is shown at the upper right corner of affected individuals. Data on age of onset and severity of clinical symptoms have been collected for the past two decades. †, age of onset unknown, 38 is the age of death. *b*, Multipoint analysis of SCA2 versus chromosome 12 markers. The dotted line (Z_{max}-1) intersects the lod score curve at the limits of the 1-lod confidence interval.

a mutation in the SCA2 locus, we analysed linkage to *D12S101*, *D12S58*, *IGF1*, *D12S78*, *D12S105/S84*, *D12S79*, *PLA2*, *D12S86* and *D12S76* (refs 5,6). The highest pairwise lod score was observed for *IGF1* at $\theta_{max} = 0.058$ with $Z_{max} = 3.1$ using age-dependent penetrance classes.

To establish closer flanking markers we ordered the markers in the region with odds >1,000:1 using CRI-MAP[7] (Nechiporuk *et. al*, manuscript in preparation) prior to multilocus linkage analysis. We assigned *SCA2* to an 8.9 cM region, between the flanking markers *IGF1* and *D12S105/S84*, with a maximum lod score of 3.6 (Fig. 1*b*).

Age of onset analysis

Age of onset in the SCAs is extremely variable, and it has been difficult to analyze pedigrees for the presence of genomic imprinting or anticipation. Bias may be introduced into the analysis due to improved disease detection, increased severity of symptoms in index patients, or the failure to account for late disease onset in asymptomatic at-risk individuals. The last of these biases can be corrected if DNA markers flanking the disease gene are used to identify gene carriers that may develop symptoms at a later time point [8]. Among 19 affected members of the FS family we identified 15 parent-child pairs. In 14 pairs, onset of the disease in the offspring occurred earlier than in the parent by 14.4 ± 7.9 years, strongly suggesting the presence of anticipation at the SCA2 locus. We compared this age of onset distribution with the null hypothesis that half of the offspring have an earlier and half a later onset of disease than the parent by χ^2 'goodness of fit' analysis. The null hypothesis was rejected at a highly significant level ($\chi^2 = 11.26$, $p<0.001$).

Failure to account for late-onset of the disease in asymptomatic gene carriers may potentially bias this analysis. Therefore, we used DNA markers flanking *SCA2* to identify likely gene carriers among asymptomatic individuals under age 40. *IGF1* and *D12S105/S84* were informative in all cases. Of 9 asymptomatic at risk individuals, 7 were found to be at <5% risk of carrying the *SCA2* mutation and in one individual flanking markers were recombinant. Only one asymptomatic individual was predicted to carry the *SCA2* mutation with a risk >95%. This person, however, is still 10 years younger than the age of onset in the parent. Even when this person was included in the analysis (assuming that the disease will begin later than in the parent) evidence for anticipation remained highly significant ($\chi^2 = 9.0$, $p<0.002$). We also examined the effect of parental gene origin on anticipation. The mean difference in the age of onset in 8 father-child pairs (10.7 ± 7.1) was not significantly different from 7 mother-child pairs (11.7 ± 16.0) ($t = 0.58$, $p>0.1$).

Unlike the FS pedigree, it is possible that anticipation is not very marked in other SCA2 pedigrees. Recent analysis of repeat expansion in myotonic dystrophy (DM) and HD has suggested that specific chromosome haplotypes predispose to repeat expansion[9]. Also, a threshold effect was observed and repeat

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expansion in DM was much more likely when the repeat number exceeded 60–70 in the parent. Perhaps anticipation is so marked in the FS pedigree because the mutation occurred on a haplotype not commonly found in other SCA2 pedigrees. Alternatively, the DNA repeat number may be higher than in other SCA2 pedigrees, and therefore less stable.

In any event, the anticipation in our SCA2 family, coupled with the elucidation of expansion mutations in SCA1, leave little doubt that more trinucleotide repeat disorders will be uncovered soon.

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