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The genes for two neuromuscular diseases of the mouse, 'arrested development of righting response', *adr*, and 'myotonia', *mto*, are allelic

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Summary

Two hitherto unmapped recessive autosomal mutations, 'arrested development of righting response' (*adr*) and 'myotonia' (*mto*, now adr^{mto}), were found to be allelic, so that preliminary linkage data on *adr* and adr^{mto} can now be combined. No linkage was found to the glucose phosphate isomerase (*Gpi*-1) locus on Chr 7 that marks a region of homology between mouse Chr 7 and human Chr 19, and in man is closely linked to the myotonic dystrophy (*DM*) gene. The *adr* gene was expressed on diverse genetic backgrounds including the athymic nude mouse.

1. Introduction

In 1978 Watts, and later Watkins & Watts (1984) described a mouse mutant with abnormal motor behaviour. The mutation showed recessive autosomal inheritance and was termed 'arrested development of righting response', *adr*, because affected mice have difficulties in righting themselves when turned on their backs (phenotype ADR). We have analysed the ADR mutant by contraction measurements (Reininghaus, Füchtbauer, Bertram & Jockusch, 1988) and intracellular electrophysiological recordings (Mehrke, Brinkmeier & Jockusch, 1988) of muscle, and have characterized the disease as a myotonia.

This, in turn, drew our attention to the mutation 'myotonia' (*mto*, now adr^{mto}), described by Heller *et al.* (1982). The two mutations seem to have arisen spontaneously and independently, *mto* in the SWR/J, *adr* in the A2G strain. Although the MTO mouse is more severely affected than the ADR mouse and, at least on its SWR/J background, has a shorter life expectancy, the two mutants share basic physiological features, including the pharmacology of phenotypic curing (Aichele, Paik & Heller, 1985; Reininghaus *et al.* 1988). We therefore suspected that the two mutations might have affected the same gene. Here we show by a complementation test, that 'myotonia', *mto*, and 'arrested development of righting response', *adr*, are indeed allelic. This result has led to the designation *adr* and adr^{mto} for the two alleles (Jockusch & Bertram, 1986).

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2. Mouse strains and methods

(i) *Animals*

The A2G strain carrying the mutation *adr* was obtained in 1982 from Drs D. and R. Watts, Guy's Hospital, London. The SWR/J strain carrying the *mto* mutation was purchased in 1986 and 1987 from Jackson Laboratory, Bar Harbor, Maine. The 'C57BL/6' nude strain was from Bomholt gård, Denmark. As tested by GPI analysis, this strain was not inbred. Progeny were selected for homozygosity with respect to the $Gpi\text{-}1^b$ allele.

(ii) *GPI alloenzyme analysis*

Erythrocytes collected from small samples of heparinized blood were lysed with distilled water and freed from haemoglobin by chloroform treatment (Gracey & Tilley, 1975). The extracts, which were kept at −70 °C for up to two weeks, were electrophoretically separated on 0·8% (w/v) agarose gels in Tris-citrate buffer, pH 7·5, blotted on nitrocellulose and developed for GPI activity (cf. Füchtbauer, Reininghaus & Jockusch, 1988).

3. Results

(i) *Allelism between adr and mto*

Crosses were performed between A2G $adr/+Gpi\text{-}1^a/Gpi\text{-}1^a$ and SWR/J $mto/+Gpi\text{-}1^b/Gpi\text{-}^b$ animals. In nine litters, 23 myotonic animals were obtained among 67 F_1 progeny (Table 1), indicating allelism between *adr* and *mto*.

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Table 1. *F_1 phenotypes of* A2G adr/+Gpi-1^a/Gpi-1^a × SWR/J mto/+Gpi-1^b/Gpi-1^b *and the reciprocal cross*

Mating (no. of litters)	Myotonic	Normal	Total
A2G × SWR/J (8)	20	39	59
SWR/J × A2G (1)	3	5	8
Total (9)	23	44	67
Expected[a] —	17	50	67

[a] allelism of *adr* and *mto* assumed; $0{\cdot}1 > P > 0{\cdot}05$, $F = 1$ (χ^2 test).

The diseased mice were recognized by the typical stiffening response to a sudden challenge like being turned on their backs (Fig. 1). All F_1 animals were heterozygous *Gpi-1^a/Gpi-1^b* as revealed by GPI electrophoresis of erythrocyte lysates (not shown). The degree of the disease was similar to that in ADR mice, and thus milder than the more severe MTO phenotype. The myotonia of affected F_1 hybrids was also demonstrated by after-contractions following repeated stimulation of the anterior tibial muscle (unpublished results of J. Reininghaus; cf. Reininghaus *et al.* 1988).

(ii) *Non-linkage to Gpi-1 (Chr 7) and nu (Chr 11)*

Scoring 95 F_2 individuals of crosses between A2G *Gpi-1^a/Gpi-1^a adr/+* and a nude (NU) mouse strain on a partially inbred C57BL/6 (*Gpi-1^b/Gpi-1^b*) background, no linkage was found between *Gpi-1*, *nu*, and the *adr* gene. There was only one individual (as compared to the expected 6) with the phenotype NU ADR. In further crossings, the genes (cf. Peters, 1988) *Akp-1* (Chr 1), *Pgm-1* (Chr 5), *Es-1* (Chr 8) and *Mod-1* (Chr 9) appeared unlinked to *adr*.

(iii) *Expression of myotonia on different genetic backgrounds*

In F_2 generations obtained after crossing A2G *adr/+* or *adr/adr* mice to the inbred strain C57BL/6 of the house mouse and in backcrosses of A2G × *Mus spretus* hybrids, the numbers of the ADR individuals were as expected, and their symptoms on those highly heterogeneous backgrounds were similar to those in the original A2G strain.

4. Discussion

Recurrent mutations affecting a neuromuscular function are known for the loci *dy* (dystrophia muscularis), *med* (motor endplate disease), *dt* (dystonia musculorum) and for several loci involved in myelin formation (Sidman, Cowen & Eicher, 1979; Peters, 1988). As yet it is unknown whether only a limited number of genes is involved in the control of the highly complex neuromuscular system, whether defects in most other 'neuromuscular' loci are lethal and are therefore not easily detected, or whether the loci with recurrent mutations are highly mutable sites.

Non-linkage of *adr^{mto}* has been shown for markers on 11 autosomes (Heller *et al.* 1982), and we have excluded linkage to genes on chromosomes 1, 5, 8 and 9 (S. Schenk, unpublished results). This leaves six autosomes, 6, 10, 12, 13, 16, 18, and stretches of Chr 2 and 4 for a further search for linkage.

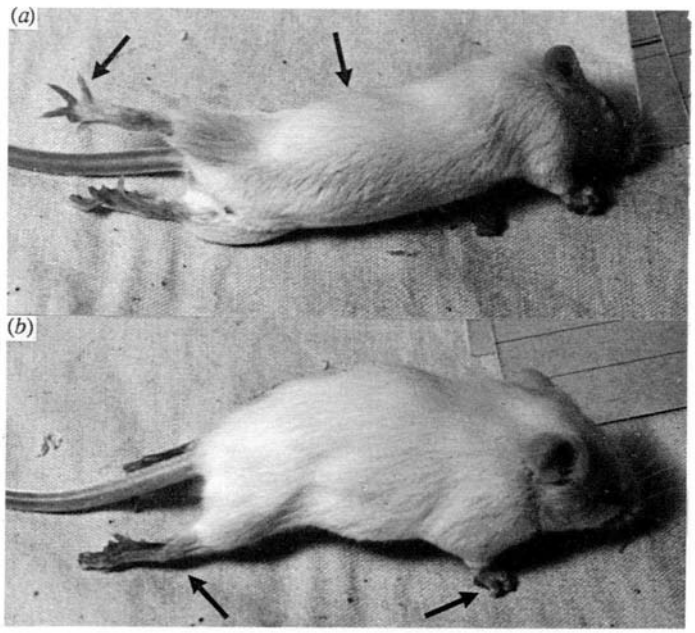

Fig. 1. Myotonic F_1 animal from a cross A2G *adr/+* × SWR/J *mto/+*. The 45-d-old animal has been turned on its back and is showing the typical symptoms of myotonia (arrows) like stiffening of hindlegs (*a*, *b*), stretching of toes, lordotic posture (*a*) and flexing of fingers (*b*).

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The independent assortment of *adr* and *Gpi-1* is compatible with the report of Heller *et al.* (1982), who found no linkage of *mto* (now adr^{mto}) with *c* (albino) and *Hbb* (haemoglobin β-chain) on Chr 7. This non-linkage is of interest because the possibility has been raised that the *adr* gene of the mouse might be homologous to the myotonic dystrophy (*DM*) gene of man (Wieringa *et al.* 1988). The *DM* gene is closely linked to the *GPI* gene on human Chr 19 in a region homologous to the neighbourhood of the *Gpi-1* locus on mouse Chr 7. Thus, if *adr* were homologous to *DM*, a close linkage to *Gpi-1* would be expected. As no linkage was found, the proposed genetic homology of *DM* and *adr* is unlikely. Phenotypically, especially with respect to membrane physiology, the ADR myotonia resembles generalized recessive myotonia (type Becker) of man (Rüdel & Lehmann-Horn, 1985; Mehrke *et al.* 1988), and lacks the pleiotropic symptoms affecting non-muscle organs of *DM* patients.

Because of the nude ADR mouse obtained, an autoimmune etiology of the ADR disease appears unlikely (cf. Jockusch, 1982). This conclusion is supported by the early appearance of the ADR symptoms and by the fact that they are expressed in an autonomous fashion in muscle grafts to a normal host (Füchtbauer *et al.* 1988).

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