Genetics and Molecular Biology, 28, 2, 201-204 (2005)
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Research Article

The *paralysé* (*par*) mouse neurological mutation maps to a 9 Mbp (4 cM) interval of mouse chromosome 18

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Abstract

The *Paralysé* mutation is a spontaneous neuromuscular mutation, first observed in 1980 at the Pasteur Institute, which is transmitted by the autosomal recessive *par* allele. Affected homozygote *par/par* mice rarely survive beyond 16 days of age and at the end of their life they are emaciated and completely paralyzed. Several concordant histological and physiological observations indicate that mutant mice might be good models for studying early-onset human motor neuron diseases such as spinal muscular atrophy. Linkage analysis using a set of molecular markers and two F2 crosses indicate that the mutation maps to mouse chromosome 18 in a region spanning 4 cM (or 9 megabase pairs, Mbp) between the microsatellites *D18Mit140 and D18Mit33*. These results positioned the *par* locus in a region homologous to human chromosome 18p11.22 to 18q21.32.

Key words: mouse model, neuromuscular disease, mouse genetic map, *Paralysé* mutation.

Received: April 26, 2004; Accepted: October 14, 2004.

Introduction

The spontaneous neuromuscular mutation *Paralysé* was discovered in mice at the Pasteur Institute in the early 1980's. The disease is transmitted by an autosomal recessive allele (symbol *par*) and the mutation is kept segregating in a moderately inbred mouse strain (PAR/Pas) by repeated brother to sister mating of affected mice (genotypes *+/par?*).

Investigations on the *paralysé* mutation have so far been limited to physiology and histopathology. Henderson *et al.* (1986) reported that extracts of *par/par* mutant muscles exhibited a neuritis-promoting activity 10-fold higher than extracts prepared from control litter-mates. Blondet *et al.* (1989) noted that although the number of muscle fibers and the distribution of acetylcholine receptors in *par/par* mice was normal the diameter of muscle fibers was distributed uni-modally and muscle choline acetyltransferase activity was reduced compared with that of control muscles, indicating that synaptic terminal development might be impaired. Optical and electron microscopy indicated that polyneuronal innervation was retained in the endplates of mutant mice, indicating that the normal process of regression of redundant innervation did not occur. Intramuscular axons failed to become myelinated in mutant animals but sciatic nerve axons were myelinated and had a normal myelin thickness/axon diameter ratio. Blondet *et al.* (1989) concluded that the major pathological feature of the *paralysé* mutant phenotype was an arrest in development of both nerve and muscle during the first week of *ab utero* life.

Morphometric measurements performed by Blondet *et al.* (1989) on lumbar spinal motor neurons of 8 and 14-day old *par/par* and normal control mice indicated that while there was no significant difference in the number of motor neurons between 8-day old *par/par* and control mice there was a significant decrease (30 to 35%) in the number of motor neurons in 14-day old *par/par* mice as compared to control mice and that the motor neurons of the *par/par* mutants were significantly smaller than those of control mice at both 8 and 14 days. These results lead Blondet *et al.* (1989) to hypothesize that the *paralysé* mutation involved atrophy and subsequent death of anterior horn motor neurons and that the rapid progression and the severity of the disease would make *paralysé* mice good models for studying early-onset human motor neuron diseases such as spinal muscular atrophy, these assumptions being confirmed by subsequent work (Houenou *et al.,* 1996, Blondet *et al.,* 1997). A more recent investigation (Pieri *et al.,* 2001) concerning the small heat shock protein Hsp25 and its expres-

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sion levels in the motor neuron of the ventral horn of the spinal cord in *par/par* and control mice indicates that this molecule is expressed at a much lower level in *par/par* mutants as compared normal control mice.

Despite all this research the molecular mechanisms responsible for motor neuron death in *par/par* mutant mice are still unknown, but the technique of positional cloning used in modern mouse genetics offers a relatively straightforward approach by which such mechanisms could be elucidated. The aim of the study reported in this paper was to proceed with the first obligatory step in this direction by attempting to localize the *paralysé* locus within the smallest possible chromosomal segment of the mouse genetic map. This localization would also help in the recognition of the orthologous regions in the human genome and possibly indicate potential candidates for genes acting in the human disease.

Material and Methods

Mice and crosses

Three strains of mice were used for the mapping of the *paralysé* gene: PAR/Pas (symbol P), the sub-strain where the mutation segregates. This strain is moderately inbred and is closely related to, but not identical with, strain C57BL/6 due to some contamination from strains 129/Sv and C3H/Pas that possibly occurred during the first generations; PWK/Pas (symbol K), a highly inbred strain (F75) established from wild *Mus musculus musculus* progenitors trapped in the Czech Republic (Bonhomme and Guénet 1996). This strain is used in our laboratory as a ready-made source of genetic polymorphisms for the purpose of gene cartography; and C57BL/6Cs.18^{SEG} (symbol S), a strain consomic of strain C57BL/6 for a complete chromosome 18 originating from the species *Mus spretus* (strain SEG/Pas). Mice homozygous for the introgressed chromosome 18 are sterile when homozygous $18^{SEG}/18^{SEG}$ but in this experiment, we used heterozygous males and selected the appropriate progenitors by genotyping with microsatellite markers specific for chromosome 18. Maintenance and care of the three strains of mice used in this study were in accordance with the National Institute of Health (NIH) guidelines for the use of laboratory animals.

The genetic localization of the *paralysé* gene was achieved in two successive steps, the first being a cross between C57BL/6-*par*/+ and PWK/Pas mice to rapidly obtain a chromosomal assignment for the *par/par* mutation and the second step a cross between C57BL/6-*par*/+ mice and the consomic strain C57BL/6Cs.18^{SEG} to produce a high resolution map of the critical region. Strain SEG/Pas is very different from both strains C57BL/6 and PWK/Pas and we knew that virtually any molecular markers in the interval would be polymorphic with one or other of these strains. Both crosses were inter-crosses which produced second filial generation (F2) offspring, this type of cross (when possible) having the advantage of doubling the number of meiosis which can be scored for any given sample size. We did not use the normal F2 offspring for the first mapping experiment because their genotype at the *par* locus was ambiguous (+/+ or +/*par*) but all mutant offspring were pooled irrespective of their origin. A piece of tail was clipped from all the mice (24 *par/par* mutants and the 52 +/*par*? non-mutants) and processed to produce good-quality DNA samples. The 24 DNA samples of the mutant offspring were genotyped using 53 single-sequence length polymorphism (SSLP) microsatellite markers which were evenly-distributed over all the 19 autosomic chromosomes.

Once the *par* locus was unambiguously localized on the mouse genetic map, the 52 non- mutant mice were genotyped with specific markers for chromosome 18. Some mice that were found with recombinant haplotypes in the interval known to harbor the par gene were test-bred with +/*par* heterozygous mice to reveal whether they were +/+ or +/*par* at the *par* locus, at least two progenies being bred (producing in all cases more than 10 offspring) to rule out the possibility that mutant mice were not detected because of a chance effect.

Genotyping with SSLP markers

Genomic DNA was extracted and purified by using the phenol/chloroform method. The 53 primer pairs used in this experiment were purchased from Research Genetics (Huntsville, AL, USA) and the polymerase chain reaction (PCR) conditions for amplification of genomic DNA were: 94 °C for 3 min followed by 35 cycles of 94 °C for 40 s, 50 °C for 40 s and 72 °C for 40 s and then 72 °C for 3 min with a final hold at room temperature (21-25 °C). The PCR products were resolved and the by electrophoresis on 3% (w/v) agarose (NuSieve; FCM company, USA), stained with ethidium bromide and analyzed and photographed under UV illumination. The SSLP (microsatellite) polymorphisms were scored using the same electrophoresis technique.

The genotypes of the progeny were scored as P-homozygous when there was only one band characteristic of the marker allele derived from strain PAR/Pas, as K-homozygous when the PWK allele was present, as S-homozygous when the SEG allele was detected or as heterozygous (H) when two bands (*i.e.* both parental alleles) were present. Linkage analysis was performed manually, by looking for divergence from the expected Mendelian proportions. Haplotypes analysis was achieved after ordering and spacing the SSLP markers according to reference database (http://www.informatics.jax.org).

Results and Discussion

At birth, *par/par* homozygotes are indistinguishable from their normal litter-mates (+/*par* or +/+) but from about day 8 growth is impaired and the animals develop progressive generalized muscle wasting (Figure 1). By day

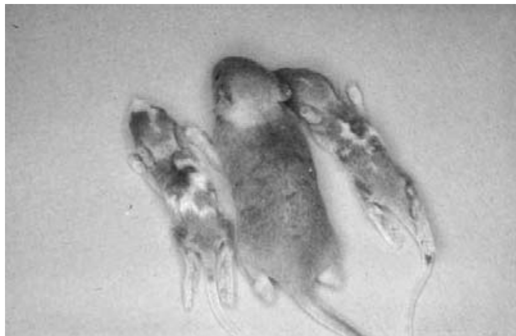

Figure 1 - Normal (center) and *par/par* mutant (left and right) mice. This picture was taken 15 days post-natal.

10 mutant mice exhibit uncoordinated limb-movements and loose the *righting reflex* (*i.e.* they cannot right themselves when placed on their backs) (Blondet *et al.,* 1989). Homozygotes mice rarely survive beyond 16 days of age and at the end of their life are emaciated and completely paralyzed (Duchen *et al.,* 1983). We found, at birth, 24 mutant and 52 normal mice in the merged progenies of the two F2 sets, which means that *par/par* homozygotes accounted for roughly 25% (24/76) of the expected offspring in the population studied and that *par/par* homozygote mortality occurs in a relatively short period of time and only *ab utero*. It also indicates that all homozygotes die and that the other aspects of the genetic background of the mice has no, or very little, influence on the severity of the *paralysé* condition in the phenotype (Duchen *et al*., 1983). Early death is a feature of the mutant pathology which needs to be borne in mind when considering candidate genes for the *par* mutation and should coincide with the onset and transcription pattern of the candidate genes.

Our results concerning the genotyping of the mutant and wild-type mice and our deductions in regard to chromosomal assignment for the *par* locus are presented in Figures 2 and 3.

In our sample of 24 mutant progeny we found a strong deviation from the expected 1:2:1 Mendelian ratio for the SSLP markers (*D18Mit140*, *D18Mit107* and *D18Mit50*), suggesting linkage to Chr 18. We controlled for this by performing complementary genotyping with two other markers (*D18Mit152* and *D18Mit182*) and confirmed the assignment of *par* to chromosome 18. When we considered the haplotypes of affected mice one mouse (indicated by '[' in the last row of Figure 2) allowed us to define the centromeric edge of the critical interval containing the *par* mutation because this mouse was heterozygous (P/K) for the *D18Mit122* marker but homozygous (P/P) for the *D18Mit140* marker, indicating that the edge of the *par* region lies somewhere between *D18Mit122* and *D18Mit140*. In addition to this the haplotypes of three mice (indicated by a 'τ' in the last row of Figure 2) allowed us to define the distal border of the *par*-containing interval as being localized between markers *D18Mit50* and *D18Mit185*.

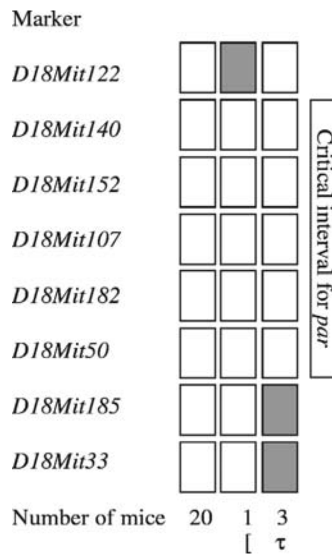

Figure 2 - Schematic representation of the haplotypes of the 24 *par/par* offspring of two merged F2 populations (PARxPWK and PARxSEG). White rectangles indicate the PAR/Pas allele. Gray-filled rectangles are KxP or SxP heterozygotes. The number under each haplotype column indicates the number of animals that share the same haplotype irrespective of the cross. The mouse indicated by a single square bracket in the last row ([) allowed us to define the centromeric border of the *par* interval while the three mice indicated by a 'τ' the last row allowed definition of the distal border.

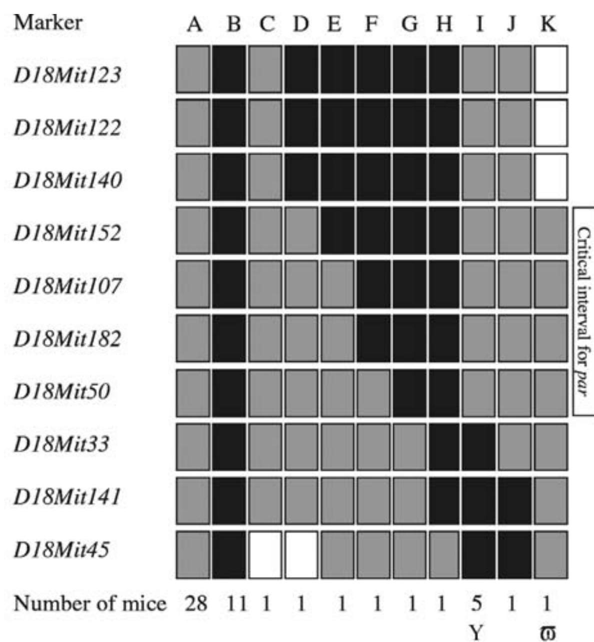

Figure 3 - Schematic representation for the haplotypes of the 52 *+/par*? offspring of two merged F2 populations (PARxPWK and PARxSEG). White rectangles indicate the PAR/Pas allele, gray-filled rectangles are KxP or SxP heterozygotes and solid black rectangles represent K/K or S/S genotypes. Of the 5 mice with a type I haplotype, one (indicated by a 'Y' the last row) was heterozygous (*+/par*) for *par* when test-bred with a *+/par* partner. Unaffected mouse K is indicated by 'ϖ' in the last row.

Analysis of the haplotypes of the 52 unaffected mice confirmed the localization of the centromeric limit of the region harboring the *paralysé* locus because unaffected mouse K (indicated by 'ϖ' in the last row of Figure 3) was found to be homozygous for the P (PAR/Pas) allelic form at loci *D18Mit123*, *D18Mit122* and *D18Mit140* and heterozygous for all other telomeric markers. This group of mice allowed us to refine the location of the distal border of the *par*-containing interval because one of the 5 mice (unaffected an with a I type haplotype, indicated by 'Y' in the last row of Figure 2) was heterozygous for *par* (*+/par*) when test-bred with a *+/par* partner.

Merging all these data indicates that the *par* locus lies within an interval flanked by microsatellite markers *D18Mit140* and *D18Mit33*. On checking the mouse genome sequence database *Ensembl* (http://www.ensembl.org/Mus musculus/) with these entries we found that this segment spans 9 megabase pairs (Mbp) of mouse chromosome 18 in a region which is homologous with rat chromosome 18 and human chromosome 18p11.22 to 18q21.32. We cannot point to any front-line positional candidate gene for the *paralysé* pathology because although the human genome is completely sequenced in the 18p11.22 to 18q21.32 region this region contains some 68 genes, most of which are new. As a functional candidate we might *a priori* retain the orthologous gene responsible for the human mutation ALS3 (Amyotrophic Lateral Sclerosis 3) (MIM # 606640), which maps to human chromosome 18q21 and also results in progressive muscle weakness with motor neuron degeneration (Hand *et al*., 2002). However, in contrast with the *paralysé* mutation, ALS3 is inherited as an autosomal dominant allele. Another possible candidate might be the *Afg3l2* gene, a member of the family of genes encoding ATPase-like proteins, whose human othologue has been found to be involved in hereditary spastic paraparesis (HSP) (Casari G and Rugarli E, 2001).

Considering these mapping data it is important to note that none of the genes encoding Hsp25-like proteins (*Hspb1*, *Hspb7*) map to the critical interval where we found *par* to map, indicating that the decrease in Hsp25 expression levels in the motor neuron of the spinal cord, reported by Pieri and coworkers (Pieri *et al.*, 2001), is not a direct consequence of an alteration in the gene itself but rather is a secondary consequence of the *paralysé* gene mutation. Experiments are in progress to reduce further the critical interval for *paralysé*.

Acknowledgments

This work was supported by the Brazilian agency Fundação de Apoio à Pesquisa do Estado de Minas Gerais (FAPEMIG) and by the Pasteur Institute, France.

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Associate Editor:Emmanuel Dias Neto