

Mutation of *Vps54* causes motor neuron disease and defective spermiogenesis in the wobbler mouse

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Vacuolar-vesicular protein sorting (Vps) factors are involved in vesicular trafficking in eukaryotic cells. We identified the missense mutation L967Q in *Vps54* in the wobbler mouse, an animal model of amyotrophic lateral sclerosis, and also characterized a lethal allele, *Vps54*^{β-geo}. Motoneuron survival and spermiogenesis are severely compromised in the wobbler mouse, indicating that *Vps54* has an essential role in these processes.

The spontaneous, autosomal recessive wobbler (*wr*) mutation of the mouse was discovered almost 50 years ago¹. The mutation is pleiotropic, causing spinal muscular atrophy and defective spermiogenesis^{2,3}. The *wr* mutation was mapped to proximal mouse chromosome 11 (ref. 4) in a region homologous to human chromosome 2p13–14 (ref. 5). To identify the molecular basis of the wobbler phenotype, we refined the genetic localization of the *wr* locus in a large cross between strains C57BL/6J-*wr* and CAST/Ei. Evaluation of >6,000 informative meioses narrowed the candidate interval for *wr* to 0.9 Mb between *D11Hjk30* and *D11Hjk29* (Fig. 1a). We evaluated the six genes in the nonrecombinant interval as candidates for *wr*: *Peli1*, *Vps54*, *Ugp2*, *NM_172792*, *Mdh1* and *NM_145425* (Fig. 1a). We identified the exons and splice variants of each candidate gene by RT-PCR analysis of cDNA from multiple tissues and then screened each exon for mutations by sequencing genomic DNA. In exon 23 of *Vps54*, *wr/wr* genomic DNA contains an A→T transversion in the second position of codon 967 that results in the amino acid substitution L967Q (Fig. 1b,c). The mutation does not seem to affect splicing of the *Vps54* transcript, as all three normal transcripts (Fig. 1b) were detected in wobbler tissues. Leu967 was present in DNA sequences from eight strains of mice (*Mus musculus musculus* strains 129, C57BL/6J, C57BL/10, SWR and SJL; *M. m. castaneus*; *M. m. molossinus*; and *Mus spretus*) and, moreover, is evolutionarily conserved among vertebrates (Fig. 1c).

To confirm that the *Vps54* mutation was responsible for the wobbler phenotype, we carried out transgene-mediated rescue using BAC clone

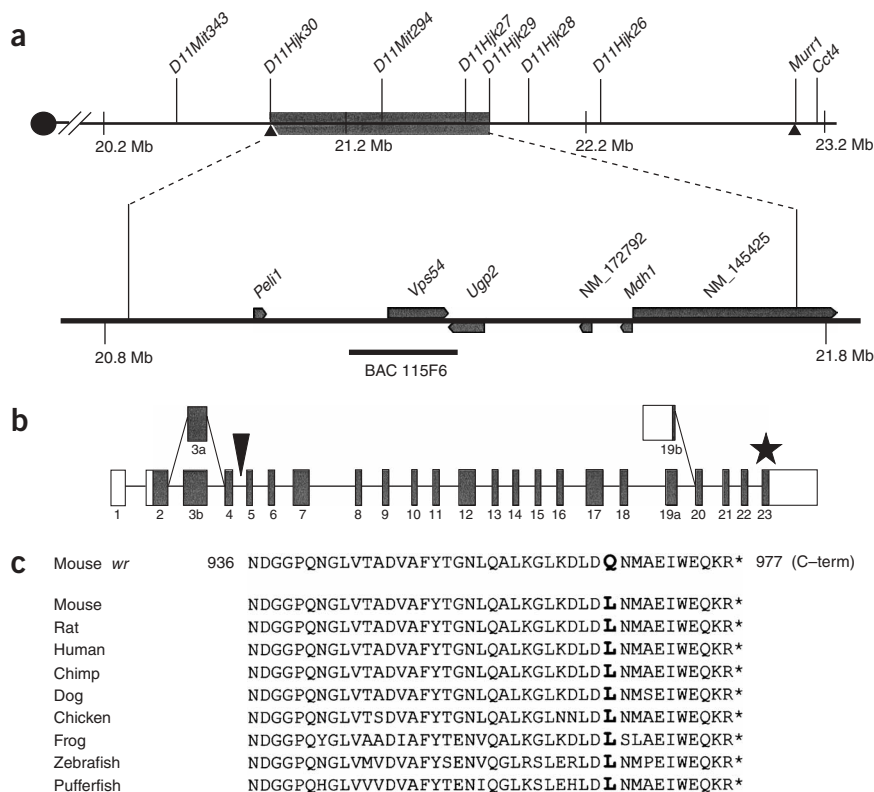
RPC1 24-115F6 from strain C57BL/6J. This BAC contains the complete genomic sequence of *Vps54* plus 63 kb upstream and 18 kb downstream (Fig. 1a). We generated BAC transgenic mice by microinjection of fertilized eggs (Supplementary Methods online). Four independent transgenic lines were successfully established by crossing male founders with C57BL/6J-*wr* females, and we obtained informative mice from all four lines. All (16 of 16) F₂ transgenic wobbler mice (*wr/wr Vps54-tg*) were phenotypically rescued: they had normal mobility on a wire grid and maintained normal body weight and grip strength during the first two months after birth (Fig. 2 and Supplementary Fig. 1 online). Rescue of the neurological phenotype was also indicated by normal histological appearance of motoneurons and astroglia (Supplementary Fig. 1). The fertility of *wr/wr Vps54-tg* males was demonstrated by generation of viable progeny in multiple matings. Spermatozoa from *wr/wr Vps54-tg* mice were normal in motility and appearance under phase contrast microscopy (data not shown). Electron microscopy of testes showed a normal number of flat (wild-type) sperm heads in sections of seminiferous tubules from the transgenic mice (Supplementary Fig. 1). Thus, both neurological and spermatogenic defects of wobbler homozygotes were corrected by the *Vps54* transgene.

To obtain a second mutated allele of *Vps54*, we searched the BayGenomics Clone library and identified a β-geo gene trap insertion in intron 4 of *Vps54* in embryonic stem cell line RRI497 (*Vps54*^{gt(pGT10)2841Ucd} or *Vps54*^{β-geo}; Supplementary Fig. 2 online). The position of the insertion site predicts synthesis of a hybrid protein containing the first 152 amino acids of *Vps54* fused to the full-length β-geo protein. We recovered *Vps54*^{β-geo/+} heterozygous mice, which had a normal phenotype (Fig. 2). X-gal staining of sections from *Vps54*^{β-geo/+} adults showed widespread low-level expression of the *Vps54*-β-geo fusion protein (data not shown). Among more than 80 offspring from mated heterozygotes, we recovered no *Vps54*^{β-geo/β-geo} pups (Fig. 2). Between embryonic day (E) 8.5 and E10.5, *Vps54*^{β-geo/β-geo} embryos were indistinguishable from wild-type embryos. At E11.5, *Vps54*^{β-geo/β-geo} embryos were developmentally retarded. From E12.5 onward, we obtained no homozygous embryos but observed resorption sites with homozygous genotype (Supplementary Fig. 3 online), implying that *Vps54*^{β-geo/β-geo} homozygotes did not survive beyond this embryonic stage. To investigate the cause of lethality, we serially sectioned homozygous E11.5 embryos. The spinal cord was underdeveloped, and dorsal root ganglia were nearly completely absent (Supplementary Fig. 3). In addition, the atrial and ventricular myocardium had severe hypoplasia (Supplementary Fig. 3), suggesting that homozygous embryos died because of cardiovascular malfunction. The prenatal lethality and developmental abnormalities indicate that *Vps54* is essential during development. To prove allelism between *wr* and *Vps54*, we crossed *Vps54*^{β-geo/+} mice

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Figure 1 Positional cloning of the gene underlying the *wr* mutation. **(a)** Genetic and physical map of the *wr* region on proximal mouse chromosome 11. The previously reported nonrecombinant interval⁵ is marked by arrowheads. The refined nonrecombinant region of 894 kb (dotted lines) contains six genes, represented by bars pointing towards their 3' ends. Mb positions are from Ensembl (mouse genome assembly 32). The position of BAC 115F6, used for transgenic rescue, is indicated. **(b)** Exon-intron structure of the *Vps54* gene and position of the *wr* missense mutation in exon 23 (star). Exons on the horizontal line are included in the major splice isoform, encoding a protein of 977 amino acids. The two alternatively spliced exons are shown above. An arrowhead marks the β -geo insertion site in the gene-trap allele. **(c)** An A \rightarrow T transversion at nucleotide 72 of exon 23 in the wobbler mouse changes evolutionarily conserved Leu967 to glutamine.



with *wr*/*+* mice. The compound heterozygotes had the complete wobbler phenotype, including reduced body weight and reduced grip strength (Fig. 2). The severity of the defects was comparable to that of *wr/wr* mice. Noncomplementation of the *wr* and *Vps54* ^{β -geo} mutations indicated allelism, confirming the identification of the gene on the basis of transgenic rescue.

The identification of *Vps54* as the gene underlying *wr* is consistent with the previous finding that the *wr* mutation is cell-autonomous⁶. Although *Vps54* is widely expressed, the *wr* mutation selectively affects motoneuron survival and spermiogenesis, indicating that an intact exon 23 is essential for these processes. In *Caenorhabditis elegans*, the null mutant of the *Vps54* homolog is either infertile or nonviable (allele tm585; Wormbase), and in *Drosophila melanogaster*, null mutants of the *Vps54* homolog scattered (*scat*) have defective sperma-

togenesis⁷. *Saccharomyces cerevisiae* Vps54 forms heterotetrameric complexes with Vps51, Vps52 and Vps53 (ref. 8) to form the Golgi-associated retrograde protein (GARP) complex, which is involved in vesicular trafficking. Mammalian orthologs of yeast Vps52, Vps53 and Vps54 have been identified^{9,10}. The interaction of human Vps52 with Rab6 and the SNARE syntaxin10 (ref. 10) suggests that the mammalian GARP complex has a cellular function similar to that of the *S. cerevisiae* GARP complex.

Mutation in the human gene *VPS33B* is responsible for the kidney syndrome arthrogyrosis, renal dysfunction and cholestasis¹¹ (ARC; OMIM 208085). Among several mouse mutations affecting vesicle transport to lysosome-related compartments, only the mocha mutant has neurological defects¹², but it does not affect motoneurons. The first links between vesicular transport and motoneuron degeneration have been reported for alsin¹³ (ALS2; OMIM 205100) and VABP¹⁴ (ALS8; OMIM 608627). Our investigation of mutant *Vps54* is the first report to our knowledge of a Vps factor causing severe impairment of motoneurons. The precise mechanism by which the missense mutation in *Vps54* causes motoneuron disease is not known. We suggest that *Vps54* might be critical for retrograde vesicular transport and in particular for axonal transport in motoneurons, which is impaired in wobbler mice¹⁵. The phenotype of the wobbler mutant indicates that some of the unmapped hereditary syndromes with neurodegeneration or male infertility might result from mutations affecting *Vps54* or other components of the vesicular transport machinery.

Note: Supplementary information is available on the Nature Genetics website.

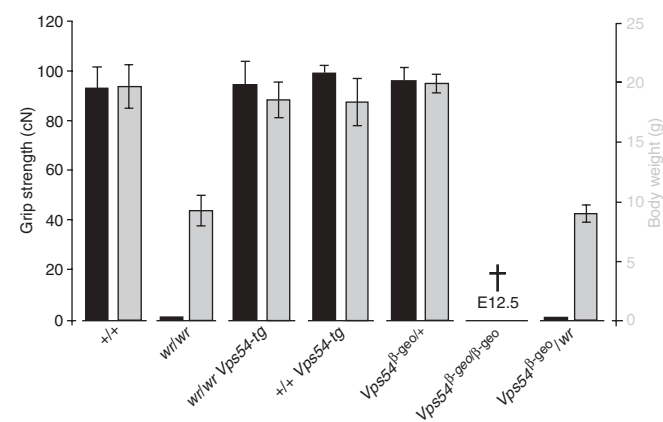


Figure 2 Transgenic rescue of the wobbler phenotype and noncomplementation of a gene-trap allele of *Vps54*. Loss of body weight and grip strength in *wr/wr* mice is rescued by the BAC 115F6 transgene containing a wild-type copy of *Vps54*. Mice of genotypes +/+ *Vps54-tg* and *Vps54* ^{β -geo}+/+ have normal body weight and grip strength 30 d after birth. *Vps54* ^{β -geo}/*wr* mice do not survive beyond E12.5. Compound heterozygotes (*Vps54* ^{β -geo}/*wr*) have reduced body weight and grip strength like *wr/wr* mice. Data shown are mean \pm standard deviation ($n = 10$ in each group).

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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