

Mutations in *MAPT* give rise to aneuploidy in animal models of tauopathy

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Abstract Tau is a major microtubule-associated protein in brain neurons. Its misfolding and accumulation cause neurodegenerative diseases characterized by brain atrophy and dementia, named tauopathies. Genetic forms are caused by mutations of microtubule-associated protein tau gene (*MAPT*). Tau is expressed also in nonneural tissues such as lymphocytes. Tau has been recently recognized as a multifunctional protein, and in particular, some findings supported a role in genome stability. In fact, peripheral cells of patients affected by frontotemporal dementia carrying different *MAPT* mutations showed structural and numerical chromosome aberrations. The aim of this study was to assess chromosome stability in peripheral cell from two animal models of genetic tauopathy, JNPL3 and PS19 mouse strains expressing the human tau carrying the P301L and P301S mutations, respectively, to confirm the previous data on humans. After demonstrating the presence of mutated tau in spleen, we performed standard cytogenetic analysis of splenic lymphocytes from homozygous and hemizygous JNPL3, hemizygous PS19, and relevant controls. Losses and gains of chromosomes (aneuploidy) were evaluated. We detected a significantly higher level of aneuploidy in JNPL3 and PS19 than in control mice. Moreover, in JNPL3, the aneuploidy was higher in homozygotes than in hemizygotes, demonstrating a gene dose effect, which appeared also to be age independent. Our results show that mutated tau is associated with chromosome

instability. It is conceivable to hypothesize that in genetic tauopathies the aneuploidy may be present also in central nervous system, possibly contributing to neurodegeneration.

Keywords Tau · *MAPT* · Mutation · Tauopathy · Animal models · Cytogenetics · Aneuploidy

Introduction

Tauopathies are neurodegenerative diseases caused by accumulation of filamentous and hyperphosphorylated tau protein in nerve and glial cells of the central nervous system, giving rise to brain atrophy and dementia. Tau belongs to the family of microtubule-associated proteins (MAPs), which regulate microtubule polymerization and dynamics. Tau is the major MAP in brain neurons, but it is also expressed in nonneural tissues such as lymphocytes and fibroblasts [1–3].

Recent findings pointed to new functions of tau besides its traditionally recognized role in neuronal morphogenesis and axonal transport [4]. In particular, a relationship between tau and DNA/chromosomes has emerged. The presence of tau in interphasic nucleoli and in the nucleolar organizer regions of mitotic acrocentric chromosomes has suggested a role in ribosome RNA biogenesis [2, 5]. The in vitro ability of tau to efficiently bind to DNA, preventing it from oxidative damage [6], and the in vivo recruitment of tau into the nucleus and its binding to chromatin during heat stress [7] supported its involvement as DNA-protecting chaperone protein. Very recently, a role of tau in chromosome stability has also been advanced, based on the presence of chromosome aberrations in cells from patients affected by frontotemporal dementia carrying different tau mutations, including the P301L [3, 8], and in cells derived from tau knockout mice [9].

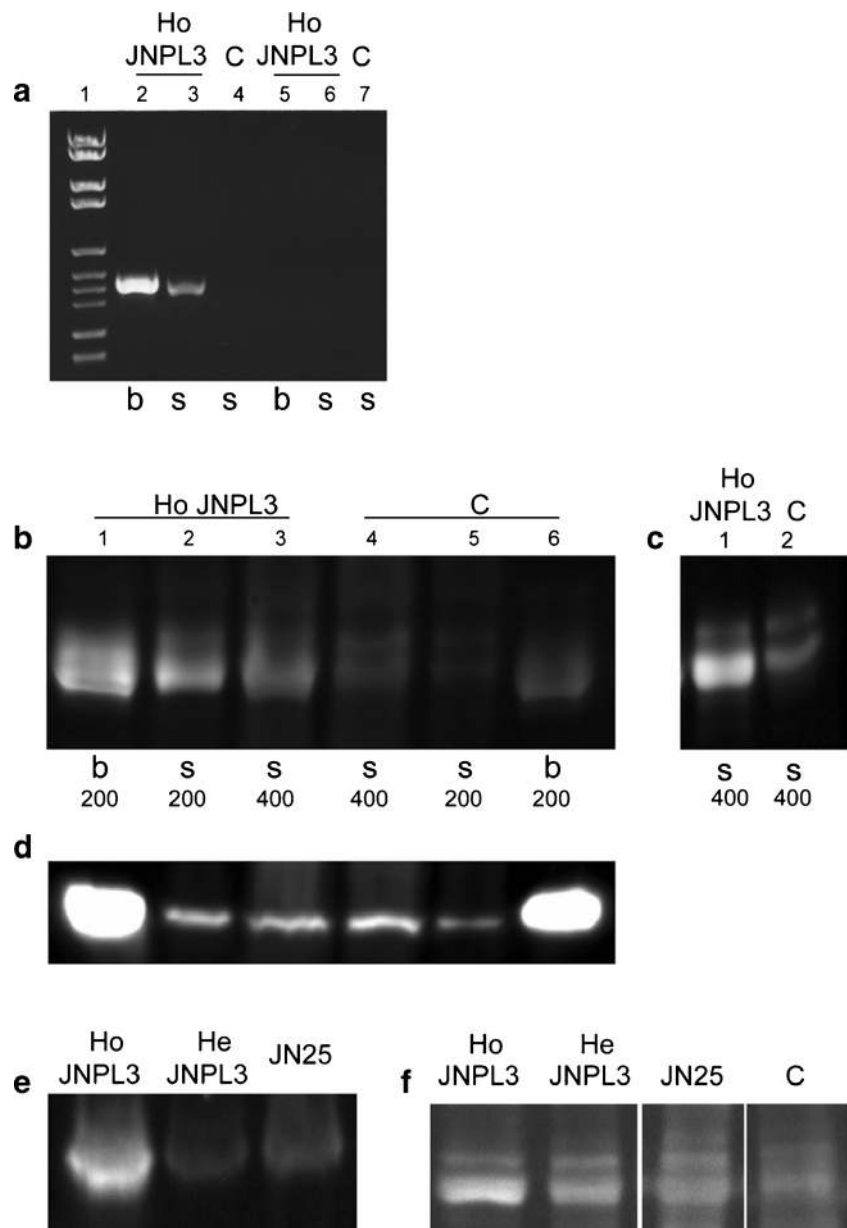
Genetic tauopathies are caused by mutations in the microtubule-associated protein tau gene (*MAPT*). These mutations either reduce the ability of tau to promote the

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Fig. 1 Analysis of expression of tau protein in JNPL3 and control mice. **a** Human *MAPT* mRNA analysis. Agarose gel electrophoresis showing the products of RT-PCR spanning the mutated region. *Lanes 2–4* DNase-treated samples; *lanes 5–7* DNase- and RNase-treated samples. **b, c, e, f** Western blot analysis of tau present in brain, spleen, and isolated lymphocytes. **b** Tissue homogenates. **c** Immunoprecipitated tau. *Numbers at the bottom* indicate micrograms of total proteins loaded. **d** Tubulin revealed by DM1A antibody, in the same samples as in **b**. **e, f** Comparison of expression levels of tau in different strains in spleen (**e**) and lymphocytes (**f**); 200 μ g of total proteins were loaded in each lane. For tau detection, blots were developed with RD4 antibody. *Ho* homozygous, *He* hemizygous, *C* control (B6D2F1), *b* brain, *s* spleen



microtubule polymerization or alter the physiological ratio of different tau isoforms, leading to production of tau deposits and cytoskeleton disruption [10].

Several animal models of genetic tauopathies have been generated since the discovery of the first mutations in *MAPT*. One of the first and most studied mutations in patients affected by tauopathy is the P301L. Transgenic mice expressing the human tau carrying the P301L mutation (JNPL3 strain) have been widely described at clinical, neuropathological, and biochemical level [11–13].

In this study, we first investigated the presence of chromosome aberrations, in particular aneuploidy, in the JNPL3 mouse model, to confirm and extend results from humans [3, 8]. Cytogenetic analysis of lymphocytes from hemizygous and homozygous JNPL3 and control mice showed

chromosome instability linked to the P301L mutation. This instability appeared to be dependent on gene dosage and independent from disease status. We then analyzed an additional mouse model, PS19 expressing the human tau carrying the P301S mutation [14], and confirmed the presence of aneuploidy in lymphocytes. Thus, *MAPT* mutations appear to be associated with chromosome instability.

Materials and methods

Mouse strains

JNPL3 Transgenic mouse strain JNPL3 expresses human tau (isoform 0N4R, containing four microtubule-binding repeats

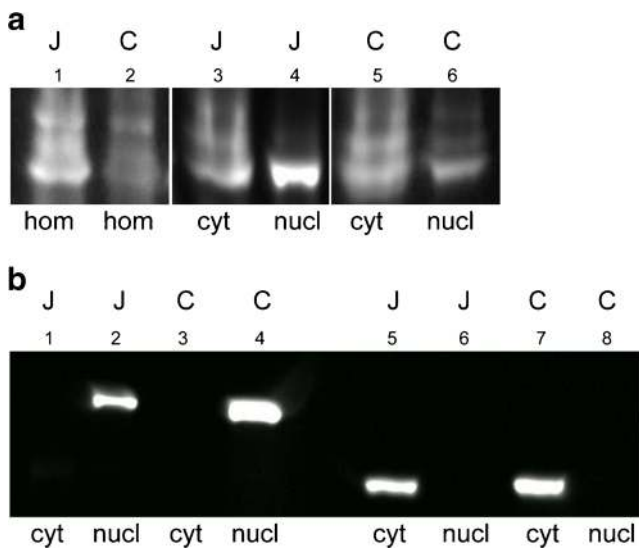


Fig. 2 Presence of nuclear tau in spleen. **a** Western blot analysis of tau present in total tissue homogenates (lanes 1 and 2) and cytosolic (lanes 3 and 5) or nuclear (lanes 4 and 6) fractions from homozygous JNPL3 and control mice. Two hundred micrograms of tissue homogenate were loaded in lanes 1 and 2; 200 μ g of cytosolic fraction were loaded in lanes 3 and 5; nuclear fraction was threefold concentrated, thus corresponding to 600 μ g of cytosolic fraction. Blots were developed with RD4 antibody. **b** The same subcellular fractions probed with anti-lamin B1 (lanes 1–4) or anti-tubulin (lanes 5–8) antibodies, showing lack of cross-contamination. *J* homozygous JNPL3, *C* control, *hom* tissue homogenate, *cyt* cytosol, *nucl* nucleus

and lacking exons 2 and 3) carrying the P301L mutation [11]. Wild-type (wt) non-transgenic strain B6D2F1 was used to maintain hemizygous JNPL3 by breeding.

For cytogenetic analysis, we used both hemizygous and homozygous JNPL3 and, as negative controls, their negative littermates, B6D2F1 and JN25. The latter (gift from J. Lewis) is a transgenic strain expressing the identical (but wt) tau isoform at the same level as JNPL3 strain [11]. JN25 mice were used only as hemizygotes because homozygosity was lethal at about 3 weeks of age for unknown reasons. Homozygous and hemizygous JNPL3 were sacrificed at different ages, corresponding to presymptomatic, early symptomatic, and fully symptomatic stages of the disease; negative controls were sacrificed at matching ages.

JNPL3 mice were assessed weekly for the presence of motor and behavioral dysfunctions. Evaluation of activity level, arousal, posture, gait, and occurrence of involuntary motor movements was performed. In the early symptomatic stage, animals showed a reduction of escape extension. Progression of symptoms included hunched posture, tremors, leg scissoring, decrease of hind limb tone, and loss of weight. In the latest stage, the ability to ambulate was reduced, resulting in paraparesis with lateral recumbency in some animals.

PS19 Transgenic mouse strain PS19 expresses human tau (isoform 1N4R, containing four microtubule-binding repeats and lacking exon 3) carrying the P301S mutation [14]. Wt non-transgenic strain B6C3F1 was used to maintain hemizygous PS19 by breeding. These mice cannot be bred to homozygosity. For cytogenetic analysis, we used hemizygous PS19 and their negative littermates as negative controls. PS19 were sacrificed from 2 to 4 months of age, at presymptomatic stage of the disease; negative controls were sacrificed at matching ages.

Human *MAPT* mRNA expression

Total RNA was isolated from brain and spleen with the QIAamp RNA blood mini kit (Qiagen). We treated RNA with DNase (Ambion) to remove DNA before reverse transcribing it with random primers using cloned AMV First-Strand Synthesis Kit (Invitrogen). A 485-bp fragment comprising the mutated region was obtained by PCR using primers specific to *MAPT* cDNA. PCR fragments were visualized by electrophoresis on agarose gel and then subjected to direct sequencing. In an additional set of samples, we used RNase on DNase-treated RNA samples before reverse transcription, to exclude the amplification product could have derived from traces of undigested original *MAPT* cDNA.

Biochemical analysis of tau protein

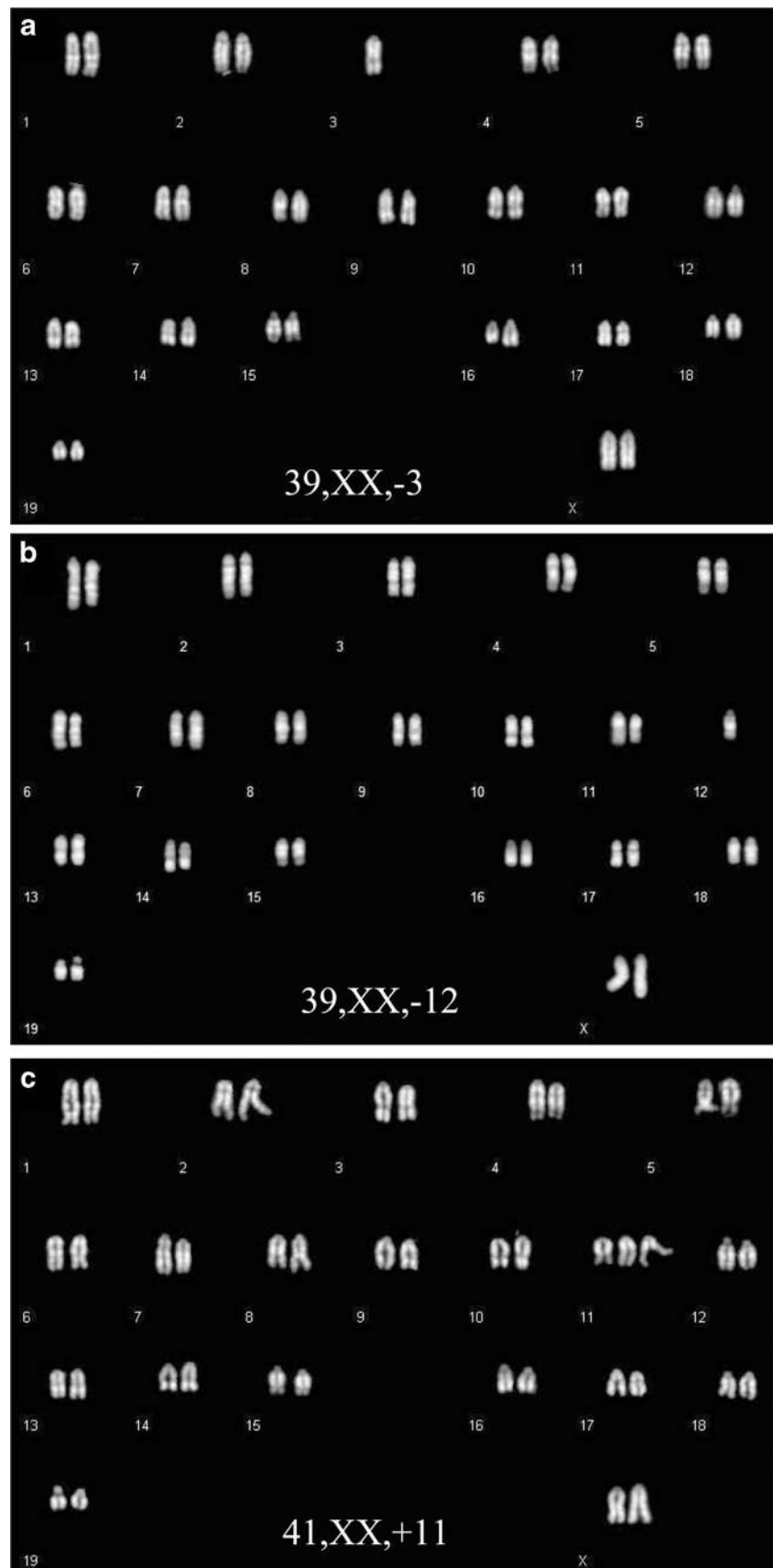
Tissue homogenates Samples of brain or spleen were homogenized in nine volumes of lysis buffer (10 mM Tris-HCl, pH

Table 1 Summary of analyzed data and cytogenetics results

Strain	<i>N</i> animals	<i>N</i> metaphases		% loss (<i>N</i> /total)	% gain (<i>N</i> /total)	Total % (<i>N</i> /total)
		Total	Mean/animal			
Homozygous JNPL3	26	1,549	60	29.8 (462/1,549)	5.2 (81/1,549)	35.0 (543/1,549)
Hemizygous JNPL3	22	1,017	46	20.3 (206/1,017)	1.7 (17/1,017)	22.0 (223/1,017)
JN25	21	961	46	10.3 (99/961)	2.2 (21/961)	12.5 (120/961)
Negative littermates/B6D2F1	22	1,074	49	12.1 (130/1,074)	1.6 (17/1,074)	13.7 (147/1,074)

% loss the percentage of metaphases with chromosome loss, % gain the percentage of metaphases with chromosome gain, total % total percentage of aneuploidy (sum of losses and gains), *N* number

Fig. 3 Examples of aneuploid metaphases. Reconstructed karyotypes showing monosomy of chromosome 3 (**a**) and 12 (**b**) and trisomy of chromosome 11 (**c**)



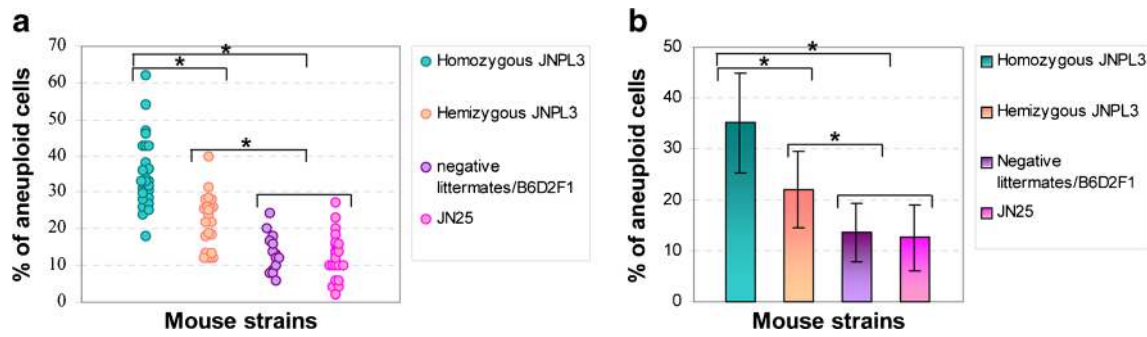


Fig. 4 Total levels of aneuploidy in splenic lymphocytes. **a** Distributions and **b** means and standard deviations of the percentages of aneuploidy (sum of losses and gains). Asterisks indicate significant differences ($p \ll 0.001$; Student's t test)

7.4, 100 mM NaCl, 10 mM EDTA, 0.5 % Nonidet P-40, 0.5 % sodium deoxycholate), briefly sonicated, and clarified by centrifugation at $16,000 \times g$ for 3 min. The total protein content was quantified by BCA assay (Pierce).

Immunoprecipitation Tau protein was concentrated by immunoprecipitation with the phosphorylation-independent polyclonal anti-Tau antibody (Sigma), using the Protein G Mag Sepharose kit (GE Healthcare). Briefly, 400 μg of tissue homogenate was added to 15 μl of antibody after crosslinking to the beads, in 1 ml final volume of PBS buffer. Tau protein was eluted in 50 μl sample buffer.

Subcellular fractionation Subcellular fractionation was performed on tissue homogenate according to de Barreda et al. [15] with minor modifications.

Lymphocyte isolation Blood lymphocytes were isolated by stratifying whole blood on Ficoll-Paque Plus (GE Healthcare). After centrifugation on Ficoll-Paque, lymphocytes were recovered, washed with PBS, homogenized in lysis buffer, and their protein content determined as above.

Western blot analysis Protein extracts were dissolved in sodium dodecyl sulfate (SDS) sample buffer (50 mM Tris-HCl, pH 6.8, 100 mM β -mercaptoethanol, 2 % SDS, 0.1 % bromophenol blue, 10 % glycerol), subjected to 10 % SDS-polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride membranes (Immobilon, Millipore), and probed with the following monoclonal antibodies: four-repeat isoform RD4, recognizing tau isoforms carrying four microtubule-binding repeats (Millipore, 1:1000); DM1A, raised against alpha-tubulin, used as cytoplasmic marker (Sigma, 1:1000); and antibody to lamin B1, used as nuclear marker (Santa Cruz, 1:200). Immunoreactive bands were visualized by enhanced chemiluminescence (GE Healthcare).

Culture of splenic lymphocytes and cytogenetic analysis

Spleens were removed aseptically, placed in RPMI 1640 medium supplemented with 10 % fetal calf serum, homogenized, and filtered through a cell strainer to obtain a free cell suspension. This suspension was cultured in the above

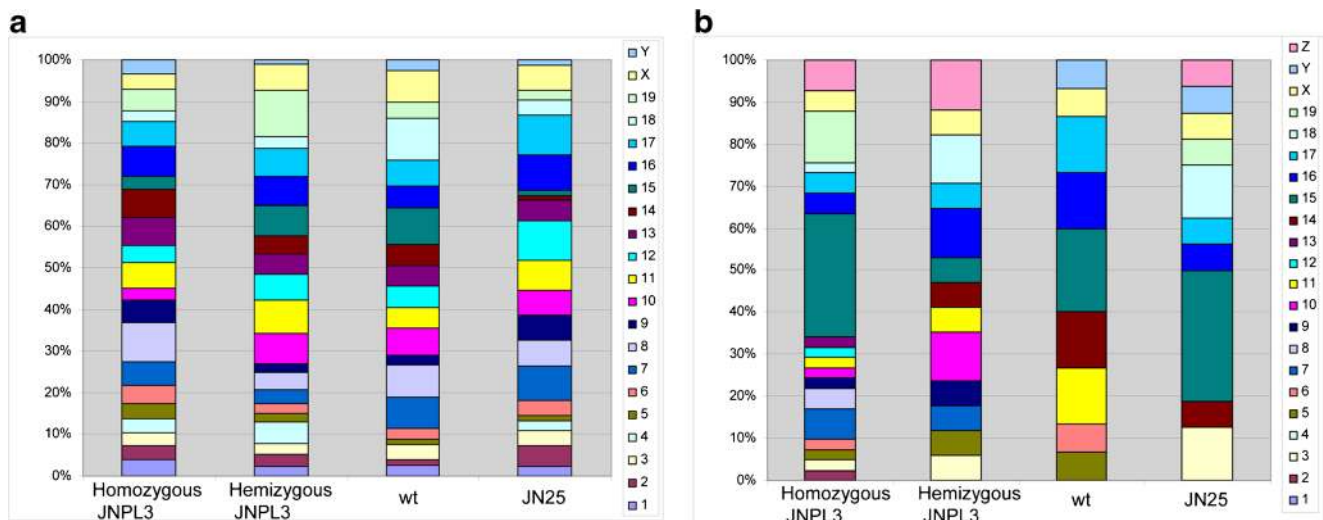


Fig. 5 Distribution of the percentages of loss or gain among all the chromosomes. **a** Loss. **b** Gain. The actual percentages of loss or gain for each genotype are reported in Table 1; in this graph, they are assumed as 100 %. Z marker chromosome

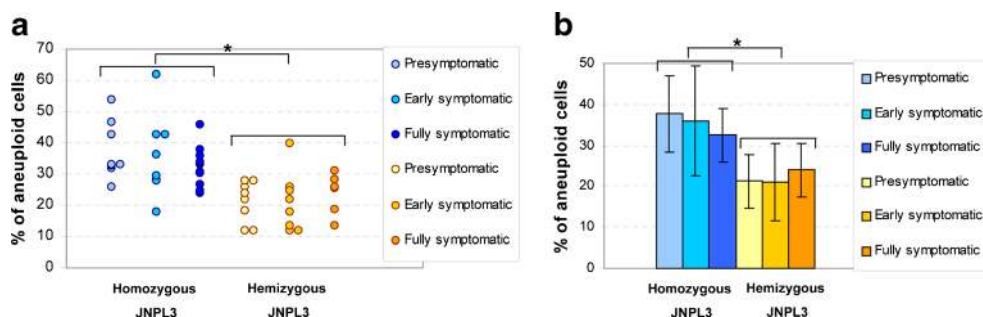


Fig. 6 Levels of aneuploidy in splenic lymphocytes: comparison among different stages of the disease. **a** Distribution and **b** means and standard deviations of the percentages of aneuploidy (sum of losses and gains).

medium added with 5 $\mu\text{g/ml}$ concanavalin A for 42 h and then treated with colcemid for 45 min before cell harvesting. Hypotonic treatment in 75 mM KCl for 20 min at 37 $^{\circ}\text{C}$ was followed by fixation with 3:1 methanol/acetic acid for 30 min on ice. Cells were then dropped onto glass slides and left to dry. Karyotype was analyzed by QFQ banding (Chromowin software, Tesi Imaging) according to the guidelines of Mouse Genome Database [16]. All chromosome spread scores were performed by two independent operators.

Statistical methods

Student's *t* test was performed to compare the results from the different mouse strains.

Results

JNPL3

Molecular and biochemical analysis In JNPL3, the human *MAPT* cDNA carrying the P301L mutation is expressed under

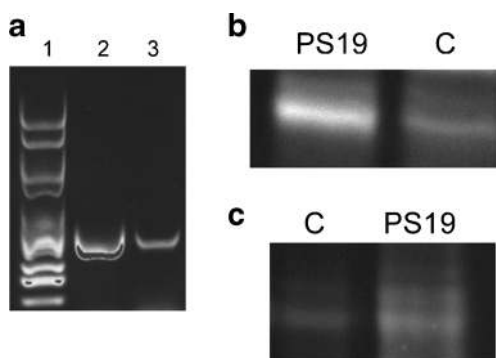


Fig. 7 Analysis of expression of tau protein in PS19 and control mice. **a** Human *MAPT* mRNA analysis. Agarose gel electrophoresis showing the products of RT-PCR spanning the mutated region. Lane 2: brain; lane 3: spleen. **b, c** Western blot analysis of tau present in spleen (**b**) and isolated lymphocytes (**c**); 200 μg of total proteins were loaded in each lane. Blots were developed with RD4 antibody. C control (B6C3F1)

Asterisks indicate significant differences ($p < 0.001$; Student's *t* test). Significant differences are only between homozygotes and hemizygotes, as previously depicted in Fig. 4

the control of the mouse prion protein promoter [11]. This promoter is not tissue restricted. To verify the presence of mutated tau in the spleen, the peripheral tissue we chose for cytogenetic analysis, we analyzed both RNA and protein expression (Fig. 1).

Total RNA was extracted from the brain and spleen of homozygous JNPL3 and from the spleen of B6D2F1 mice. After RT-PCR, amplification products were detected only in the brain and spleen from JNPL3 (Fig. 1a, lanes 2 and 3), while the control mice did not amplify (Fig. 1a, lane 4). By direct sequencing, these PCR fragments showed exclusively the mutated codon CTG corresponding to 301L (not shown). In the set of samples subjected to RNase, no amplification was detected (Fig. 1a, lanes 5–7), confirming that the above-described amplification was derived only from RNA. These results demonstrated the expression of transgenic tau in spleen, apparently at a lower level than in brain (compare lanes 2 and 3).

Endogenous tau was present in the spleen from control mice (Fig. 1b, lanes 4 and 5; Fig. 1c, lane 2). A much higher amount of tau was detected in JNPL3 mice (Fig. 1b, lanes 2 and 3; Fig. 1c, lane 1), clearly due to the additional expression of transgenic human tau. Endogenous and transgenic proteins migrated with similar molecular weight. Immunoprecipitation partially purified and enhanced the amount of tau loaded in the gel (compare Fig. 1b, lanes 3 and 4, to Fig. 1c). RD4 antibody detected only tau isoforms with four repeats, in particular transgenic tau, in addition to endogenous tau isoforms. The anti-tubulin antibody showed the same amount of total proteins loaded in JNPL3 and control samples (Fig. 1d).

The level of tau in spleen was similar for hemizygous JNPL3 and JN25, as previously reported [11] (Fig. 1e). This made JN25 a good control for JNPL3. Further biochemical analysis on purified lymphocytes demonstrated the expression of tau in these cells (Fig. 1f).

Since tau has been recently recognized as a nuclear protein, we verified this finding on subcellular fractions and demonstrated the presence of nuclear tau in the spleen of JNPL3 and control mice (Fig. 2). The amount of nuclear tau is higher in JNPL3, accordingly to results shown in Fig. 1.

Table 2 Summary of analyzed data and cytogenetics results

Strain	N animals	N metaphases		% loss (N/total)	% gain (N/total)	Total % (N/total)
		Total	Mean/animal			
Hemizygous PS19	13	650	50	15.1 (98/650)	2.3 (15/650)	17.4 (113/650)
Negative littermates	10	500	50	5.4 (27/500)	1.0 (5/500)	6.4 (32/500)

% loss the percentage of metaphases with chromosome loss, % gain the percentage of metaphases with chromosome gain, total % total percentage of aneuploidy (sum of losses and gains), N number

Cytogenetic analysis Table 1 summarizes data regarding analyzed animals and results from cytogenetic analysis. Figure 3 shows examples of aneuploid metaphases. The analysis revealed a low percentage of aneuploid cells that was similar ($p=0.60$) in wt (B6D2F1 and negative littermates, mean \pm SD=13.7 \pm 5.7) and JN25 (mean \pm SD=12.5 \pm 6.5) mice. JNPL3 mice showed a significantly higher level of aneuploidy, which was more pronounced in homozygotes (mean \pm SD=35.0 \pm 9.8) than in hemizygotes (mean \pm SD=22 \pm 7.5) (Fig. 4). Chromosome losses prevailed over gains (Table 1) and both of them were evenly distributed among all chromosomes without preference (Fig. 5).

We also divided JNPL3, both homozygous and hemizygous, into three groups based on the clinical stage of disease (presymptomatic, early symptomatic, and fully symptomatic) and compared the respective levels of aneuploidy (Fig. 6). No differences were found among the groups (p values ranged from 0.19 to 0.96), suggesting that aneuploidy is a phenomenon independent from the stage of disease, depending rather on the genetic status (wt or mutated tau). This was also confirmed by the significantly higher level of aneuploidy in homozygous than in hemizygous JNPL3, revealing a gene dosage effect.

PS19

We decided to analyze this additional mouse model of genetic tauopathy in order to determine the presence of chromosome aberrations in association with a different tau mutation.

Molecular and biochemical analysis In PS19, the human *MAPT* cDNA carrying the P301S mutation is expressed under

the control of the same mouse prion protein promoter as for JNPL3 [11, 14, 19]. Thus, we expected tau was expressed in spleen. In fact, RNA analysis showed the expression of the transgene in spleen, although at a lower level than in brain (Fig. 7a). RNase treatment of DNase-treated RNA gives no amplification (not shown). Sequencing of amplified fragments showed exclusively the mutated codon TCG corresponding to 301S (not shown).

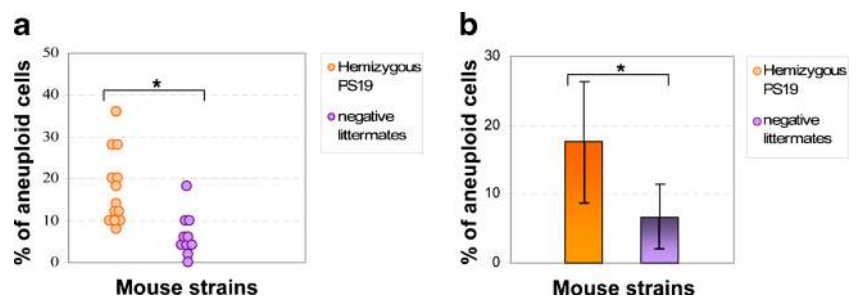
Endogenous tau was present in spleen from control mice and a higher amount of tau was detected in PS19 mice (Fig. 7b), due to the additional expression of transgenic human tau. The same findings were present in isolated lymphocytes (Fig. 7c).

Cytogenetic analysis Results showed a higher percentage of aneuploid cells in PS19 (mean \pm SD=17.4 \pm 8.7) than in controls (mean \pm SD=6.4 \pm 5.2) (Table 2 and Fig. 8). Chromosome losses prevailed over gains (Table 2) and were distributed among all chromosomes (Fig. 9). Losses of four chromosomes (4, 9, 12, 19) were detected only in PS19. Chromosome gains showed no overlap between PS19 and controls, except for marker chromosomes.

Discussion

Tau is well known as a cytoskeletal protein which interacts with microtubules and regulates their formation and dynamics. In the nervous system, tau has a role in neuron morphogenesis and axonal transport and has been characterized as major misfolded protein involved in the pathogenesis of

Fig. 8 Total levels of aneuploidy in splenic lymphocytes. **a** Distributions and **b** means and standard deviations of the percentages of aneuploidy (sum of losses and gains). Asterisk indicates significant difference ($p=0.002$; Student's t test)



Alzheimer's disease and frontotemporal dementia. Presently, tau is regarded as multifunctional protein, with a role in ribosome biogenesis and signal transduction [4], this latter function possibly being connected to amyloid-beta toxicity in Alzheimer's disease [17].

A recent and previously unrecognized function of tau in genome stability has also been suggested. In fact, the protein has the ability to interact with DNA *in vitro*, protecting it from oxidative stress [6, 18], and with chromatin *in vivo* preventing DNA damage caused by heat stress [7]. Tau involvement in chromosome stability has been advanced based on the findings of structural and numerical chromosome aberrations and chromatin and spindle abnormalities in cells from patients affected by frontotemporal dementia carrying different tau mutations [3, 8], as well as by the presence of chromosome aberrations in cells from tau knockout mice [9]. We hypothesize that tau affects genome stability due to (a) its role as a MAP, localized in particular on mitotic spindle where it influences correct dynamics and chromosome segregation, and (b) its presence in the nucleus, associated with chromatin, affecting DNA integrity. As a result, mutations in tau protein can alter its function and lead to genome instability.

To confirm and widen our previous findings in humans, in this study, we used two animal models of tauopathy. We first chose the JNPL3 strain expressing the P301L mutation because of our earlier observation of chromosome aberrations in patients carrying the same mutation [3, 8] and the availability of large amount of clinical and neuropathological data on these mice. After obtaining significant findings about chromosome instability in JNPL3 mice, we performed the same analyses on PS19 mice.

In JNPL3 mice, the transgene is under the control of mouse prion promoter [11], which is not tissue restricted, thus allowing the transgene expression in all tissues. However, the expression is variable and can be dependent on transgene

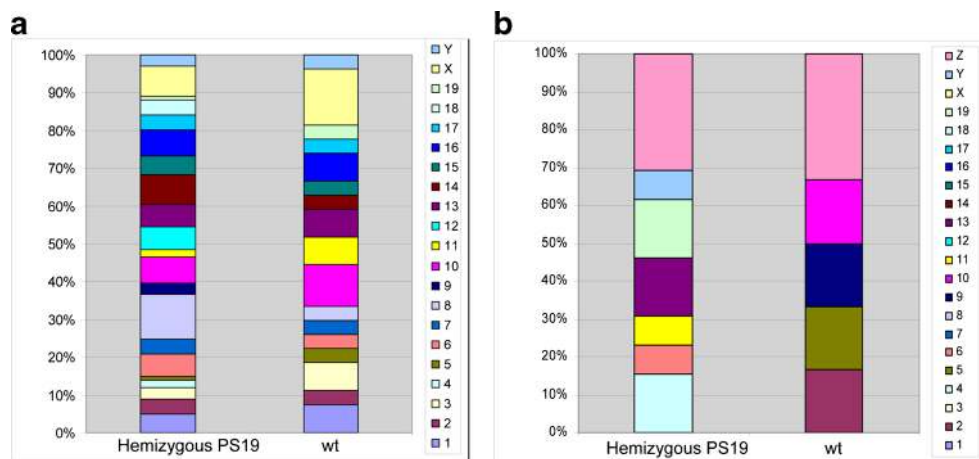
itself [19]. We then assessed the expression of transgenic tau in the peripheral tissue we decided to use for cytogenetic analysis, i.e., the spleen. We used splenic instead of peripheral blood lymphocytes since the former have a greater ability to divide and grow *in vitro*, enabling the cytogenetic analysis of mitotic chromosome spreads, paralleling the previous analysis on human subjects. By analysis of RNA and protein expression, we actually demonstrated the presence of mutated tau in the spleen, although at lower level than in the brain. In addition, we demonstrated the expression of tau in isolated blood lymphocytes. We confirmed the expression of tau in spleen and lymphocytes of PS19, as expected because of the use of the same promoter as in JNPL3.

We detected tau not only in the cytoplasmic, but also in the nuclear fraction of splenic cells. Evidence of both cytoplasmic and nuclear tau was given some years ago in neuroblastoma cell lines [20, 21] and very recently in primary cultures of mouse neurons [7, 15]. In our opinion, nuclear localization of tau is very important for preserving genome stability by physical interaction with chromatin and DNA.

As negative controls for JNPL3, in addition to the B6D2F1 mouse strain and negative littermates originating from breeding JNPL3 and B6D2F1 mice, we also used the JN25 strain because it expresses the identical but wt tau isoform at the same level than JNPL3 mice [11]. This allowed to assign any phenotypes present in JNPL3 mice to the mutation itself rather than to transgene expression levels.

JN25 mice were only described in the original paper from Lewis et al., where it was not specified if they were hemizygotes or homozygotes. After embryo revitalization, we produced hemizygous mice, which did not show any pathological phenotype, but we were not able to grow homozygous mice, since they all died at about 3 weeks of age. Hypotheses that can be considered to explain this lethality are that (a) the site of transgene insertion is very critical for survival and its disruption is not tolerated in homozygous state, or (b) the revitalization process itself has introduced a

Fig. 9 Distribution of the percentages of loss or gain among all the chromosomes. **a** Loss; **b** Gain. The actual percentages of loss or gain for each genotype are reported in Table 2; in this graph, they are assumed as 100 %. Z marker chromosome



defect which is lethal in homozygotes. So, we only used hemizygous JN25 for cytogenetic analysis.

We found significantly higher percentages of aneuploidy in JNPL3 and PS19 mice than in their controls. This result is in agreement with the finding that tau carrying the P301L mutation influences microtubule dynamics, giving rise to overly dynamic microtubules [22]. This may affect proper chromosome segregation, leading to aneuploidy. This effect may also be true for the P301S mutation. A relatively low aneuploidy level was detected in controls of JNPL3 (13.7 % of aneuploid cells in wt and 12.5 % in JN25), in accordance with previous findings of a mean aneuploid level of about 13 % in splenic lymphocytes of non-transgenic mice [9]. In controls of PS19 (B6C3F1) the aneuploidy level was lower (6.6 %), probably depending on the genetic background. Thus, a low aneuploidy level should be considered as a normal, intrinsic phenomenon, consistent with a normal phenotype.

The higher percentages of aneuploid cells in JNPL3 mice (35.0 % and 22.0 % in homozygotes and hemizygotes, respectively) can thus be attributed to the presence of mutated tau. Particularly relevant was the difference between JNPL3 and JN25 mice because it can only be ascribed to the mutation. In addition, aneuploidy was dependent on gene dosage, as homozygotes had a higher level than hemizygotes. Aneuploidy seems to be a precocious effect, as it was present in presymptomatic animals, and no increase in aneuploidy level was detected with age, excluding a progressive accumulation; accordingly, no positive correlation was found between progression of disease and aneuploidy level.

The prevalence of chromosome losses over chromosome gains may only partially be attributed to a technical bias, being present in control mice. However, the significantly higher percentages of losses in JNPL3 and PS19 than in the control mice suggest that mutated tau (as a MAP) causes true chromosome losses. Mutated tau preferably would produce aneuploidy through the mechanism of anaphase lagging, which results in both euploid and monosomic cells (loss only), rather than through the mechanism of non-disjunction, which results in abnormal cells, both monosomic and trisomic.

The higher percentage of aneuploid cells in JNPL3 and PS19 mice did not cause pathological phenotype such as tumor growth. We found no tumor susceptibility of JNPL3 or PS19 with respect to control mice. We think this aneuploidy may not be sufficient per se to induce tumors, necessitating other genetic and/or environmental causative events.

There is of course the possibility that the transgenic mutated *MAPT* has integrated into and disrupted a locus important for correct chromosome segregation. Several genes having a role in spindle assembly checkpoint, centrosome constitution, microtubule dynamics, or chromosome cohesion are involved in chromosome segregation. Disruption of one of them can lead to aneuploidy. We do not exclude this possibility; however, it is highly unlikely that this “unfortunate” integration

should just happen in two different unrelated animal models carrying a *MAPT* mutation and not in a third one (JN25) carrying wt *MAPT*. In addition, it is very interesting and intriguing the fact that tau protein is a microtubule-associated protein, present also on mitotic spindle, and then it may contribute per se to chromosome segregation.

High levels of aneuploidy were recently detected in neurons of transgenic mice carrying an *APP* mutation [9] as well as in the brains from patients affected by Alzheimer’s disease and ataxia telangiectasia [23]. It would be very interesting to investigate whether the same scenario occurs in primary tauopathies and, if so, to search for a contribution of aneuploidy to neurodegeneration.

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Conflict of interest The authors declare that they have no conflict of interest.

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- Our animal experiments conformed to ethical standards (Declaration of Helsinki) and complied with the current laws, and all efforts were made to minimize the suffering of animals.