# Microtubule defects in mesenchymal stromal cells distinguish Progressive Supranuclear Palsy patients

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## SUPPLEMENTARY MATERIAL AND METHODS

### Flow cytometry analysis

MSCs from age-matched healthy donors and PSP patients were extensively characterized by flow cytometry at P2. Cells were washed in PBS and incubated in the dark for 20 minutes at RT with the following mouse anti-human antibodies: CD3 FITC (Becton Dickinson - BD, San Jose, CA, USA), CD13 PE (Beckman Coulter - BC, Brea, CA, USA), CD14 PerCP-Cy5.5 (BD), CD34 PE (BD), CD40 FITC (BD), CD45 APC-H7 (BD), CD90 PE-Cy7 (BD), CD73 APC (BD), CD105 PerCP-Cy5.5 (BD), CD146 PE-Cy7 (BD), HLA-DR APC (BD), Platelet-Derived Growth Factor Receptor Beta (PDGFRß) PE (BD) and Alkaline Phosphatase (ALP) FITC (BD). No stained cells were used as negative controls under the same conditions. After staining, the cells were washed once with PBS. At least 10,000 events were acquired with FACS CANTOII flow cytometer (BD) and data were analyzed using the FACSDiva analysis software.

### Genetic analysis of *MAPT* haplotype

*MAPT* exons 1 and 9-13 were sequenced as previously described [1] . Briefly, each PCR was carried out in 50 µl total volume containing 25 ng genomic DNA, 12.5 pmol of each specific primer, 0.6 µM of each dATP, dTTP, dCTP, and dGTP, 1U Taq DNA polymerase (Applied Biosystems, ABI). Fragments were then purified using the ExoSAP-IT® Kit (usb, USA), according to instructions of the manufacturer, and then direct sequence was performed with an ABI PRISM 3130 gene analyzer (ABI).

The tagging *MAPT* H1/H2 SNP rs1800547 [2] was genotyped using TaqMan technology by QuantStudio 12K Flex System (Applied Biosystems, Lifetech, USA) according to the procedure of the manufacturer.

## REFERENCES


1. **Villa C, Ghezzi L, Pietroboni AM, *et al.*** A Novel MAPT Mutation Associated with the Clinical Phenotype of Progressive Nonfluent Aphasia. *J Alzheimer's Dis*. 2011;26:19-26.

2. **Tobin JE, Latourelle JC, Lew MF, *et al.*** Haplotypes and gene expression implicate the MAPT region for Parkinson disease: the GenePD Study. *Neurology*. 2008;71:28-34.

**SUPPLEMENTARY TABLES**

**Table S1: Flow cytometry analysis of expanded MSCs**

| *Surface Antigen* | Mean ± SD (%)[a] | |
|---|---|---|
| | **Ctrl** | **PSP** |
| CD90+ | 99.5 (±0.6) | 99.2 (±0.6) |
| CD105+ | 97.5 (±4) | 98.2 (±1.4) |
| CD73+ | 99.5 (±0.7) | 95.3 (± 5.0) |
| CD13+ | 97.8 (±0.8) | 96.5 (± 3.3) |
| CD146+ | 94.2 (±8.1) | 91.8 (±5.9) |
| PDGFRβ+ | 94.4 (±8.2) | 92.9 (±7.2) |
| ALP+ | 92.5 (±2.1) | 91.3 (±5.1) |
| CD45+ | 1 (±0.3) | 0.2 (±0.1) |
| CD34+ | 0.2 (±0.1) | 0.1 (±0.1) |
| CD14+ | 0.4 (±0.6) | 0.1 (±0.1) |
| CD3+ | 0.0 (±0.0) | 0.3 (±0.3) |
| CD40+ | 0.1 (±0.1) | 0.2 (±0.2) |
| HLA-DR+ | 0.9 (±0.9) | 0.3 (±0.3) |

[a]= percentage of cells positive for surface markers expressed as mean and standard deviation (N=5)

## Table S2: Demographic and clinical characteristics of patients whose cells have been investigated

| MSCs Subgroup | Patient ID | Gender | Onset age (years) | Disease duration [a] (years) | Laterality onset | Exposure [b] | Familiarity [c] | Smoke | PSP-RS | UPDRS-III | Pseudobulbar Palsy (item 3 PSP-RS) | Supranuclear Vertical Palsy (item 4 PSP-RS) | Postural Stability (item 30 UPDRS-III) | Neck Rigidity (item 22 UPDRS-III) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **MSC-A** | PSP#1 | F | 63 | 3 | Bilateral | Neg | AD | Pos | 48 | 32 | 3 | 14 | 2 | 1 |
| | PSP#3 | F | 54 | 3 | Bilateral | Neg | Neg | Pos | 49 | 39 | 1 | 10 | 4 | 1 |
| | PSP#7 | F | 63 | 5 | Left | Neg | Neg | Neg | 58 | 46 | 5 | 12 | 4 | 2 |
| | PSP#10 | F | 63 | 4 | Bilateral | Neg | n.a. | Neg | 47 | 39 | 4 | 15 | 3 | 1 |
| | Median | | 63 | 3.5 | | | | | 48.5 | 39 | 3.5 | 13 | 3.5 | 1 |
| | (range) | | (54-63) | (3-5) | | | | | (47-58) | (32-46) | (1-5) | (10-15) | (2-4) | (1-2) |
| **MSC-B** | PSP#2 | F | 64 | 7 | Bilateral | Neg | n.a. | Neg | 59 | 51 | 4 | 10 | 3 | 4 |
| | PSP#4 | F | 62 | 4 | Bilateral | Neg | AD | Neg | 52 | 49 | 5 | 12 | 3 | 4 |
| | PSP#5 | F | 69 | 6 | Right | Neg | Neg | Neg | 46 | 35 | 3 | 12 | 2 | 2 |
| | PSP#6 | F | 59 | 6 | Bilateral | Neg | PD | Neg | 40 | 39 | 2 | 14 | 3 | 2 |
| | PSP#8 | M | 59 | 3 | Bilateral | Neg | Neg | n.a. | n.a. | 50 | n.a. | n.a. | 3 | 3 |
| | PSP#9 | M | 60 | 4 | Bilateral | Neg | Neg | Pos | 39 | 34 | 3 | 6 | 3 | 1 |
| | Median | | 61 | 5 | | | | | 46 | 44 | 3 | 12 | 3 | 2.5 |
| | (Range) | | (59-69) | (3-7) | | | | | (39-59) | (34-51) | (2-5) | (6-14) | (2-3) | (1-4) |

[a]=at time of BM collection; [b] = Professional exposure to toxic or mutagen substances; [c]= familiarity for neurodegenerative disease; AD = Alzheimer's disease; PD = Parkinson's disease; n.a. = not available; PSP-RS= PSP Rating Scale; UPDRS-III= Unified Parkinson's disease Rating Scale, part III.

**Table S3: *MAPT* haplotype analysis of investigated patients**

| MSCs subgroup | Patient ID | Genotype rs1800547 A/G | Haplotype |
|---|---|---|---|
| MSC-A | PSP#1 | A/A | H1/H1 |
| | PSP#3 | A/G | H1/H2 |
| | PSP#7 | A/A | H1/H1 |
| | PSP#10 | A/A | H1/H1 |
| MSC-B | PSP#2 | A/A | H1/H1 |
| | PSP#4 | A/A | H1/H1 |
| | PSP#5 | A/A | H1/H1 |
| | PSP#6 | n.a. | n.a. |
| | PSP#8 | A/A | H1/H1 |
| | PSP#9 | A/A | H1/H1 |

n.a. = not available

## SUPPLEMENTARY FIGURES AND FIGURE LEGENDS

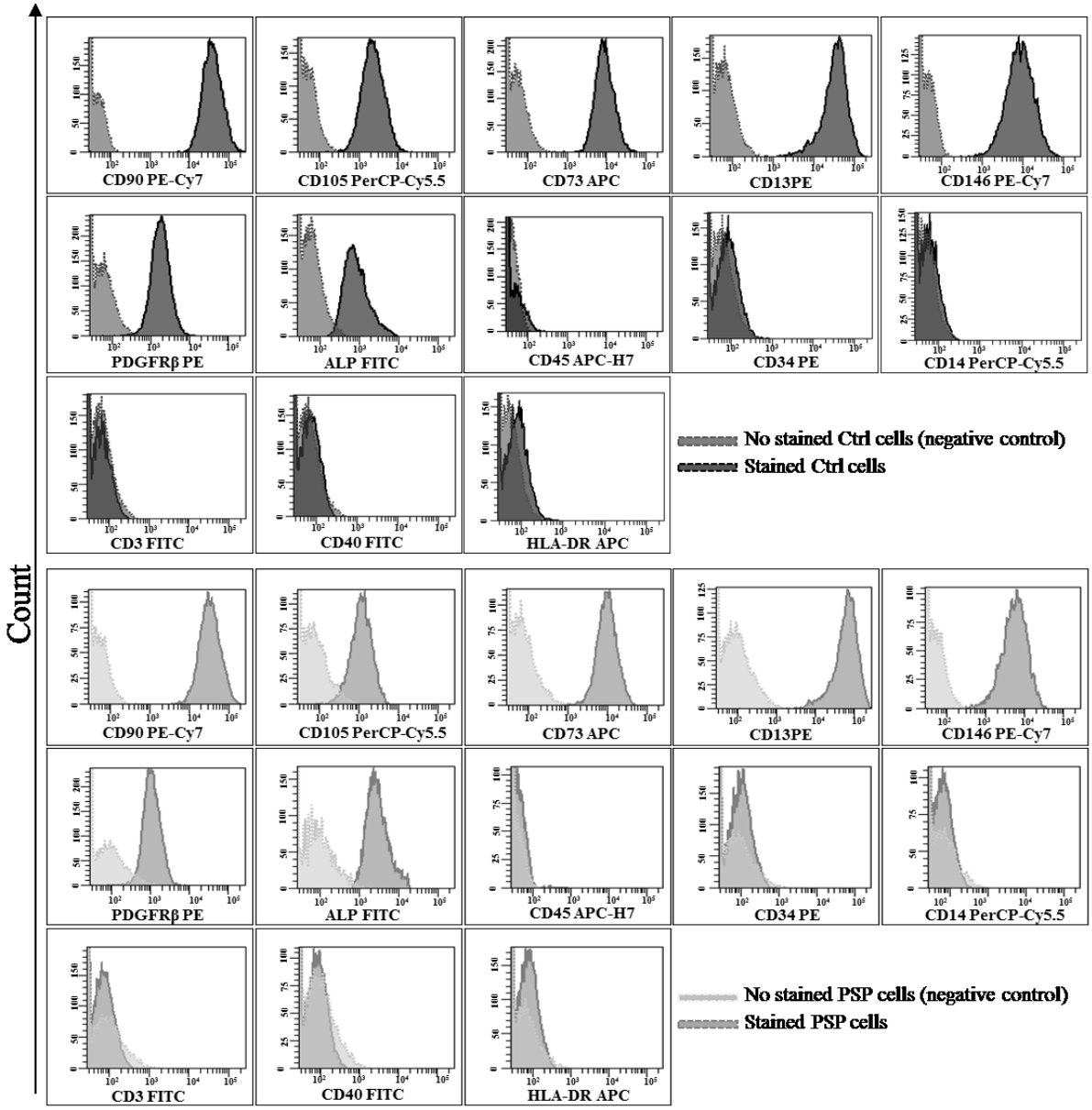


**Fig. S1 Immunophenotypic profile of MSCs from PSP patients and healthy control**

Representative MSC immunophenotypic profile by flow cytometry at passage 2 of healthy controls (Ctrl) and PSP patient (PSP) cells. Fluorescence intensity is displayed on the X-axis and the number of events (Count) in each fluorescence channel is displayed on the Y-axis. Specific histogram of fluorescence for each marker was overlaid with its negative control (no stained cells).

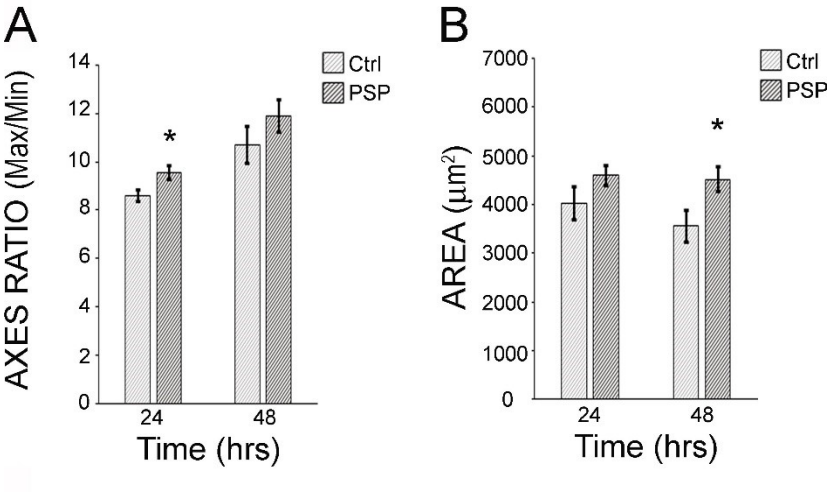


**Fig S2: Morphometric analysis of fibroblast-like subpopulation of MSCs at 24 or 48 hours after seeding**

Morphometric analysis showing ratio between maximum and minimum cellular axes (A) or surface area (B) of MSCs of healthy controls (Ctrl) compared to PSP-affected patients (PSP). *$p<0.05$ according to Student's t-test. All values are expressed as mean ± SEM. Ctrl: controls (N=6), PSP: patients affected by PSP (N=10).

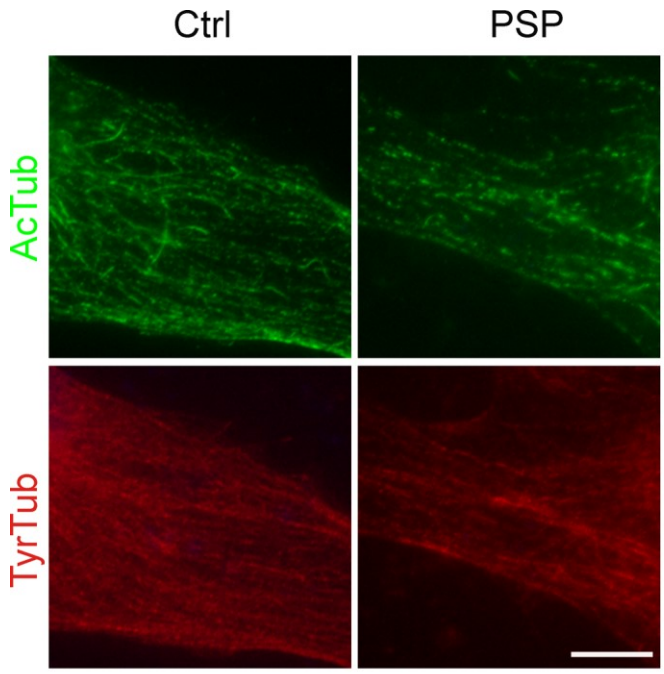


**Fig. S3: Acetylated and tyrosinated microtubules show different staining patterns in MSCs**

High magnification of representative immunofluorescence of MSCs of patients affected by PSP (PSP) or healthy controls (Ctrl) following staining for anti-acetylated (AcTub, green) or anti-tyrosinated (TyrTub, red) tubulin. Anti-acetylated α-tubulin antibodies immunodecorate short segments of MTs, while anti-tyrosinated α-tubulin staining is more homogeneous over the MT network. Scale bar: 10 µm.

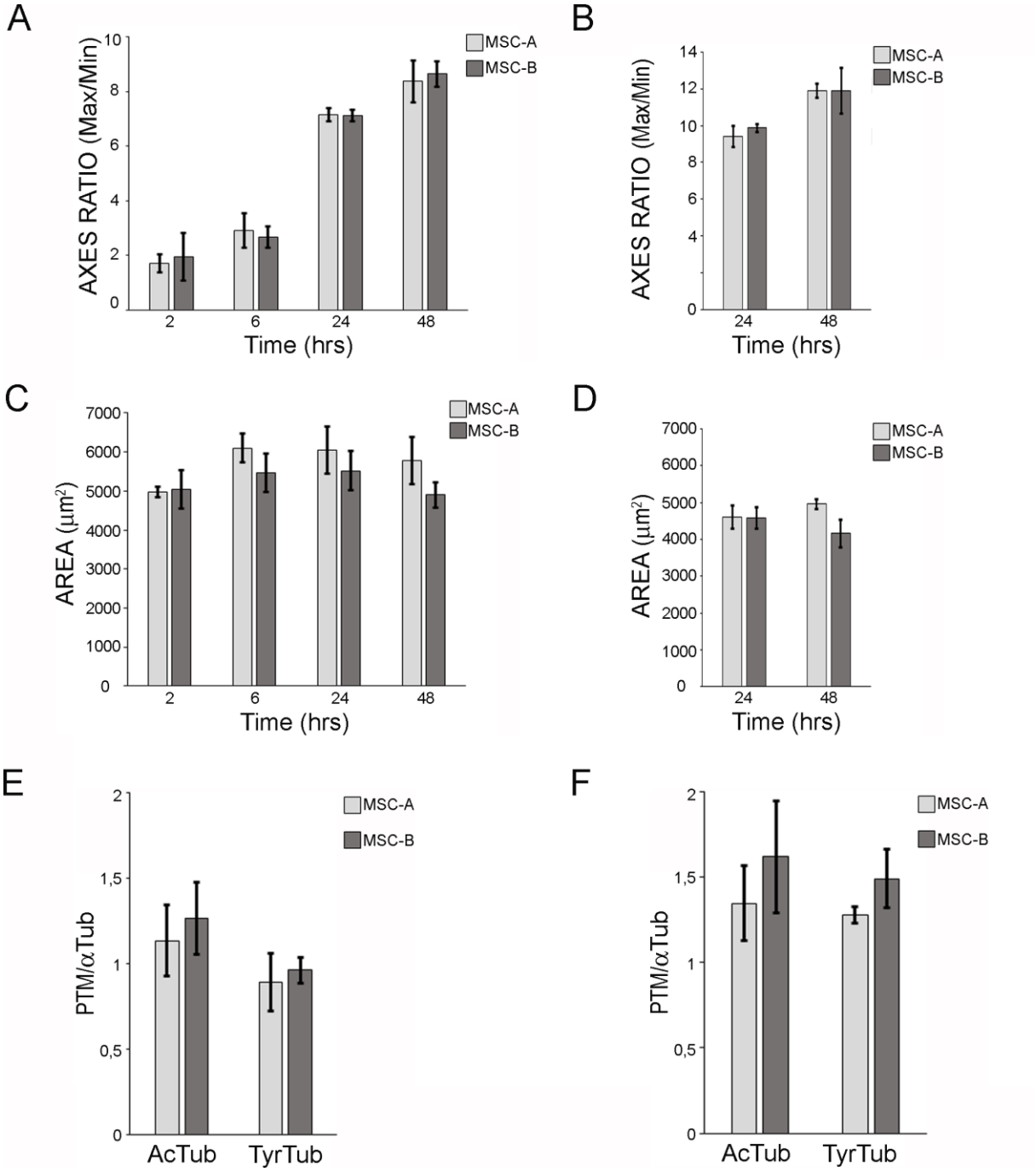


**Fig. S4: Comparative analysis of morphology and α-tubulin PTMs in the two subgroups of MSCs from PSP patients**

Morphometric analysis showing ratio between maximum and minimum cellular axes (A) or surface area (C) of cells from MSC-A and MSC-B subgroups of PSP patients. In (B) and (D) are reported measures restricted to fibroblastic-like cells at 24 and 48 hours (hrs).

Densitometric analysis of the levels of acetylated (AcTub) and tyrosinated (TyrTub) α-tubulin in whole cell extracts of human MSCs of the two subgroups (i.e. MSC-A and MSC-B) at early (P2, E) or later (P5, F) passages in culture. Values of each α-tubulin PTM were normalized on the level of total α-tubulin of the relative sample. Values are expressed as fold change on control level, as in Fig. 4. All values are expressed as mean ± SEM.