

PMP22 plays a dual role in regulating cells growth and peripheral myelin compaction. Charcot-Marie-Tooth 1A neuropathy (CMT1A) and Hereditary Neuropathy with liability to Pressure Palsies (HNPP), are respectively due to a duplication and a deletion of the PMP22 gene, leading to over and underexpression of the protein. It is unknown how the altered PMP22 dosage impairs peripheral myelination. By means of transmission electron microscopy (TEM) morphometry we found that myelin periodicity is significantly increased in CMT1A patients ( $9.79 \pm 0.9$  nm) and compared to normal sural nerves ( $9.1 \pm 0.7$  nm). Conversely, myelin periodicity was slightly reduced in HNPP patients ( $8.9 \pm 0.7$  nm). Interestingly, difference between CMT1A and HNPP patients was highly significant. Moreover, we found an increased myelin periodicity in sciatic nerves of a transgenic rat model of CMT1A ( $8.9 \pm 0.08$ ) and in organotypic dorsal root ganglia cultures ( $10.1 \pm 0.1$  nm) established from this rat line, compared to control animals ( $8.6 \pm 0.1$ ) and cultures ( $9.6 \pm 0.1$  nm).

Since TEM studies suffer considerably from radiation damage, we also used small-angle X-ray scattering (SAXS) with a synchrotron radiation microbeam ( $5 \mu\text{m}$  diameter) for in-situ experiments on single sural nerves of CMT 1A and HNPP patients and of normal controls. The ID13 micro-goniometer was used for these experiments. We were able to recorded myelin patterns from single nerve cells. Fig.1 shows the transversal section through a normal human nerve tissue with several nerve fibres. A single nerve fibre was selected in the center. Selected SAXS-patterns obtained from the myelin lamellae show as expected a strong local orientation. For comparison with the TEM-data, the periodicities were divided by two. Myelin periodicities ranged from 8.4 to 8.9 nm (mean:  $8.7 \pm 0.3$ ) in CMT 1A nerves, from 8.0 to 8.4 nm (mean:  $8.1 \pm 0.3$ ) in normal controls, and from 6.9 to 7.2 nm (mean  $7.1 \pm 0.3$ ) in HNPP.

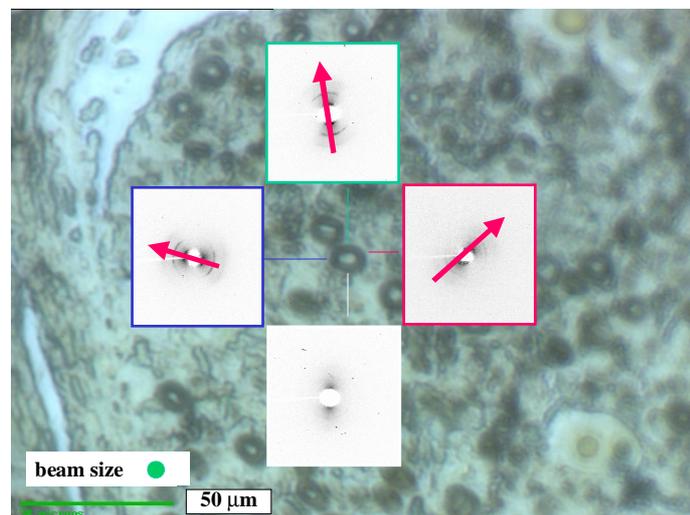


Fig.1 Transversal section through normal human nerve tissue. A green circle shows the size of the beam. The cell selected for SAXS is indicated. Red arrows show the orientation of the myelin reflections.

In conclusion, altered PMP22 levels induce significant changes in myelin lamellae spacing, which may be recorded by appropriate morphometric techniques, and in particular by microbeam SAXS. Differences in term of myelin periodicity between TEM morphometry and x-ray microbeam analysis may be due to the higher resolution and accuracy of the latter technique to measure periodic structures. Having an altered myelin period could lead to demyelination and subsequent remyelination. Therefore, the pathomechanism of both CMT1A and HNPP may be also related to a dysfunction of PMP22 as a structural protein of peripheral myelin.