Background and Objective
Charcot-Marie-Tooth disease type 2A (CMT2A) is the most common form of autosomal dominant axonal CMT, caused by mitofusin 2 (MFN2) gene mutations. Some patients with MFN2 gene mutations also develop optic neuropathy, but the pathological changes of the optic nerve caused by mutant MFN2 have not been fully investigated. In this study, we detected a heterozygous MFN 2 gene mutation (c.733A>C: p. Ser245Arg) in a family lineage of CMT2A, which presented the abnormality of visual evoked potentials. The objective of this study is to investigate the pathological changes in optic nerves of the transgenic mouse expressing this mutant (S245R) MFN 2 gene in neurons.

Material and Method
1. Generation of a transgenic mouse: The pNSE-human S245R MFN2 plasmid was linearized and injected into the embryos of mice. Founder mice and all transgenic progeny were identified by PCR assay of tail genomic DNA.
2. Expression Analysis: To confirm the expression of human S245R MFN2 in neurons of the generated transgenic mice, we performed western blotting analysis using anti-human-MFN2-specific antibody. Western blotting was performed on extracts from several tissue(human cellobeum, cerebrum and liver of transgenic and non transgenic mice)
3. Morphometric and Mitochondria Analysis: Specimen was subjected to electron microscope (magnification×5000) and 8 random pictures were obtained for each mice . The surface area of the mitochondria was obtained by digital analysis(ImageJ software). The number of mice and mitochondria analyzed was as follows: 11 weeks old MFN2 S245R transgenic mice n=3 mitochondria n=286, 11 weeks old non transgenic mice n=3 mitochondria n= 275, 38 weeks old MFN2 S245R transgenic mice n=3 mitochondria n=407, 38 weeks old non transgenic mice n=3 mitochondria n=360.

Results
We detected a point mutation, c.733A>C (encoded p. S245R), in MFN2 gene of the affected family members . In the next step, we generated a transgenic mouse carrying human S245R MFN2 gene which expresses human MFN2S245R specifically in neurons (Figure 1). A random sampling of the optic nerves were examined from 6 transgenic(11 weeks n=3, 38 weeks n=3) and 6 age matched non-transgenic mice, which showed a significant decrease in the average mitochondria surface area in transgenic mice(Figure 2A, B blue arrow, Figure3). Mitochondria in the state of incomplete fusion were observed only in the transgenic mice(Figure 2 C red arrow)

Discussion
We found that in the optic nerves of the MFN2 S245R transgenic mice the size of mitochondria were significantly smaller than that of non-transgenic mice, and closely adjacent mitochondria which appeared to be in a incomplete state of fusion were observed only in the transgenic mice. Our results suggest that optic nerve impairment in CMT2A could be related with the dysfunction of mitochondrial fusion, which has also been observed in the peripheral nerves of CMT2A patients and mutant MFN2 transgenic mice. Pathogenesis of CMT2A in part has been described as the abnormality in mitochondrial axonal transport and its distribution, our results suggest that abnormality in mitochondrial fusion may also contribute to the disease process.

References