INTRODUCTION:
Post-infectious autoimmunity has been implicated to play a role in pathogenesis of Tourette's syndrome (TS)
One of most critical aspects for a claim that a disorder results from post-infectious autoimmunity is a demonstration of inflammation at the site of pathology that alters in relation to symptom worsening. Thus far, the evidence came only from imaging studies (2)
This could reflect swelling during immune responses activated by neuronal epitopes resembling GABHS and post-streptococcal autoimmunity may be involved in TS/OCD due to molecular mimicry between streptococcal and neuronal antigens (3).
We selected several inflammatory markers (MCP-1, IL-2 and CD45) and three genes whose expression in post-mortem striatum of TS/OCD patients was most significantly increased (4), and asked whether their expression is enhanced in post-mortem specimen of adult TS patients.

METHODS:
Human brain from four TS cases (age 42 ±8.5 years, 4 males) and four normal control subjects (age 52±10 years, 3 males and 1 female) were obtained from Harvard Brain Bank under sponsorship of Tourette's syndrome association. The characteristics of TS case are presented in Table 1.

Brain specimen were stored in 10% formalin, then rinsed in PBS-0.1%NaN3, cryo-protected in 15% sucrose, and cut coronally into 2.5 cm blocks. The blocks corresponding to basal ganglia region (Palidus, Caudate nucleus and Putamen) were sectioned serially at 24 series of 30 µm sections, which were stored in PBS-0.1%NaN3, at 4°C (5).

Total RNA was extracted from sections of formalin fixed brain tissue using PureLink Total RNA Isolation Kit (Invitrogen, Cat. Number K1560-02) according to manufacturer's instructions. Total RNA was diluted in RNase-free water and stored at -20°C. Two to three µg of total RNA was used for synthesis of cDNA using Sprint RT (reverse transcription) Complete Kit (Clontech, Palo Alto, USA; Cat. Number 639532). Samples were evaluated by quantitative real-time PCR analysis using thermocycler MX3000 (Stratagene).

Quantification is based on number of PCR cycles that are required for the tested cDNA to reach a pre-set threshold of fluorescence emission by SYBR green that is incorporated into dsDNA during the given cycles (Ct). Therefore, the higher number of cycles represents lower expression of a gene of interest. One unit of Ct corresponds to 2-fold change in mRNA expression. The number of cycles was normalized against house keeping gene GAPDH and expressed as ΔCt values.

RESULTS:
Subjects demographics
Mean age for TS group was 42 (± 8.52), and 52 (± 9.95) for NC, and PMI 22 (± 7.11) for TS patients and 18 (± 7.11) for NC. There were no significant differences in age, sex ratio and postmortem interval between patients and controls.

Quantitative Real-Time-PCR
Figure 1. Shows differences in expression levels of mRNA (GAPDH normalized) between TS postmortem basal ganglia and controls in some of inflammation related genes and in previously referred genes: PTPR-N (IA2), PTPR-RI and recoverin. Statistically significant differences between patients and controls were only found in PTP-RN (p=0.047), although levels of expression of PTP-RI and recoverin were higher in patients than controls. Mann Whitney test was used to show significant differences for MCP-1 (p=0.043) and IL-2 (p=0.043) were found. A tendency in higher expression of CD45 was observed although this was not statistically significant.

Figure 2. Relative expression of PTP-RN, PTP-RI, recoverin, CD45, IL-2 and MCP-1. Values are expressed in relative abundance of mRNA amount of each target gene in the patient sample relative to that in controls by using the 2^{ΔCt} calculation. It is shown that PTP-RN expression is 16-fold higher in patients than in controls, MCP-1 was 6.5-fold and IL-2 2.3 -fold, respectively Table 2 shows the mean values of ΔCt for each gene studied in patients and controls.

CONCLUSIONS:
The analysis of post-mortem specimen revealed significantly increased expression of two inflammatory markers, MCP-1 and IL2, in basal ganglia of TS patients. We reproduced a previous observation of increased expression of IA-2 in striatum of TS patients. The increased expression of MCP-1 and IL-2 supports the notion of inflammatory processes occurring in TS basal ganglia. Confirming the elevated expression of IA-2 suggests its significant role in TS pathogenesis. As IA2 and closely related IA-2β (ICA512) are major autoantigens in type 1 diabetes, the increased expression of IA-2 in basal ganglia of TS cases opened a question whether IA-2 could represent also an autoantigen in TS.

REFERENCES:

Figures and tables not included in the natural text representation.