HLA-DRB1*04:01 has a Neuroprotective Role in a Mouse Model of Parkinson's Disease

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BACKGROUND

Parkinson's Disease (PD) is the second most common age-related neurodegenerative disease in North America. The pathological hallmarks of PD include death of dopaminergic neurons in the substantia nigra, mitochondrial dysfunction, neuroinflammation, and accumulation of abnormal α-synuclein (α-SYN) aggregates termed Lewy bodies. Current treatment for PD relies on high dose L-DOPA replacement to improve motor function, but there is a lack of treatment for the underlying cause of PD. A recent study sequencing the HLA alleles from 1,597 PD patients and 1,606 healthy controls found a strong genetic association between shared epitope containing *HLA-DRB1*04:01* allele and PD risk. The shared epitope (SE) is a five amino-acid sequence (QKRAA) on the HLA-DRB chain that was previously identified as a risk factor for more severe rheumatoid arthritis (RA). The SE is not involved in canonical antigen presentation of the MHCII complex, but instead increases the innate immune response by binding other cell-membrane proteins on the immune cell surface including calreticulin. In the central nervous system (CNS), *HLA-DRB1* expression is restricted to the resident immune microglial cell population which were previously implicated in PD to phagocytize and destroy abnormal α-SYN aggregates. To better understand the role of the *HLA-DRB1*04:01* allele in PD, here we have used an *in vivo* mouse model of α-SYN in aggregation to show that the *04:01* allele caused increased microglial activation, neurocognitive changes in behavioral tasks, and protected against α-SYN spread.

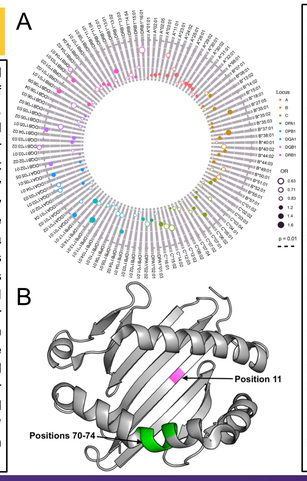


Figure 1: A specific amino acid motif of HLA-DRB1 mediates risk of Parkinson's Disease
A) Results of a next-generation sequencing of 11 *HLA* alleles from 1,597 PD patients and 1,606 healthy controls. Findings revealed a risk association between *HLA* allele carriers and developing PD (Hollenbach et al., *PNAS.* 2019). From this study, the *HLA-DRB1*04:01* allele was found to have a strong protective effect against PD (P_{adj} < 0.02; OR, 0.78). Further examination of the *HLA-DRB1*04:01* amino acid sequence revealed that the protective effects of the allele were mediated by the SE and a valine residue at position 11 (P = 0.001; OR, .76). The protective effect was additionally increased by a positive history of smoking (P = 10^{-4}; OR, .51). *HLA-DRB1*04:02* does not contain the SE and does not show a protective effect. *B)* The shared epitope is seen in green and consists of the residues Q/R-K/R-R-A-A at positions 70-74. Position 11 is shown in pink, and the presence or absence of V at this position plays a significant role in the allele's protective role in the case of Parkinson's Disease.
Adapted from Hollenbach et al., PNAS. 2019.

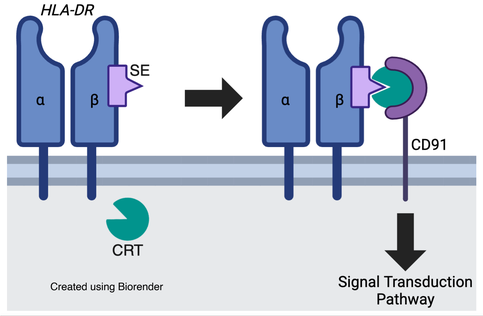


Figure 2: SE binding of Calreticulin activates a signal transduction pathway
Binding of the SE ligand by cell surface calreticulin (CRT) has been shown to activate a signal transduction pathway leading to increased intracellular calcium, activation of peptidylarginine deiminase, and higher levels of protein citrullination (Van Drongelen et al., *J. Immun.* 2020). The *HLA* molecules then present the citrullinated proteins to helper T cells, leading to production of Anti-citrullinated protein antibodies by B cells which have been more commonly found in SE (+) RA patients. This results in increased osteoclast activation and bone destruction.

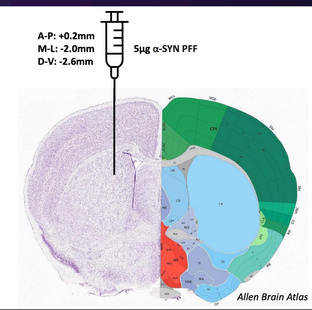
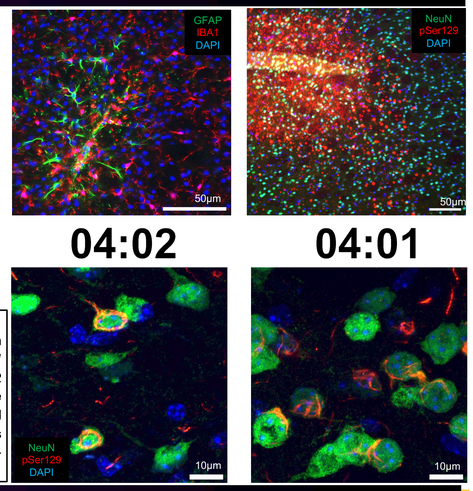


Figure 3: Surgical injection of α-SYN PFFs into striatum
Transgenic mice were utilized, which expressed human *HLA-DR* molecules on their cell surfaces. These molecules contained either the SE (+) *HLA-DRB1*04:01* allele (04:01) or the SE (-) *HLA-DRB1*04:02* allele (04:02). *Top:* 04:01 and 04:02 mice were injected with 5μg α-SYN PFFs or 2.5μl sterile PBS into the caudate putamen using stereotactic surgery. After sacrifice, brains were perfused and fixed for cryosection and immunohistochemistry. *Top Right:* The injection site was confirmed, and the α-SYN was visualized at the injection site. *Bottom Right:* Intra-neuronal inclusions of α-SYN (pSer129) was found in both genotypes.



RESULTS & DISCUSSION

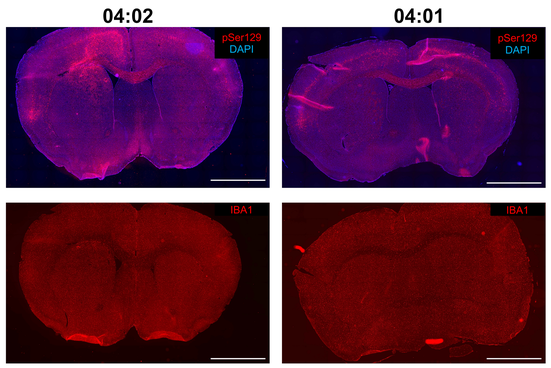
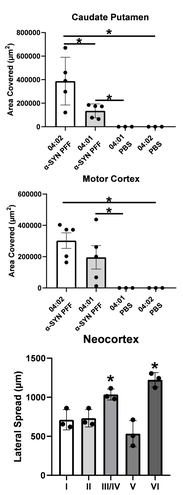
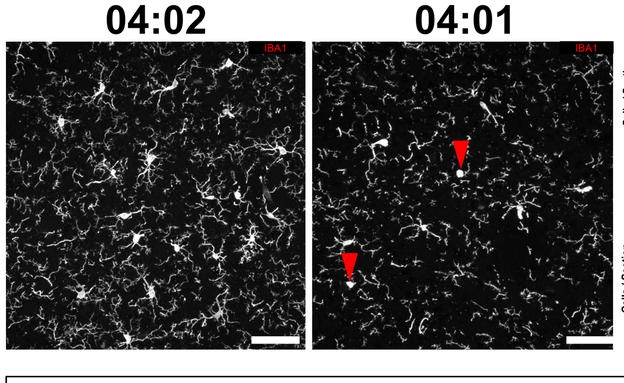


Figure 4: HLA-DRB1*04:01 Protects against α-SYN spreading in vivo
Two months postinjection with α-SYN PFFs, whole brain sections of the mice were stained for pSer129 to quantify pathological, hyperphosphorylated α-SYN fibrils. The area covered by α-SYN fibrils was significantly higher in 04:02 mice than in 04:01 mice, with the difference being strongest in the caudate putamen. No pSer129 staining was detected in PBS-injected mice. Scale bars are 0.5 mm. Mann-Whitney U-test, * P<0.05.





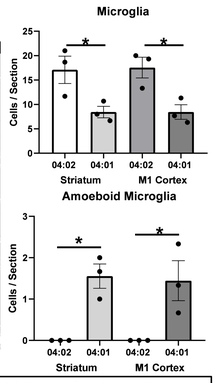


Figure 5: HLA-DRB1*04:01 mice have fewer total microglia, but more reactive microglia in the striatum and motor cortex
Serial sections around the injection site of the brains from the α-SYN injected mice were stained for microglia. 3 visual fields per striatum and cortex were sampled per mouse. The total number of microglia in each visual field was counted and averaged, with 04:01 mice having significantly less microglia per section. Of these microglia, the morphological phenotype was also considered, with some cells being grouped as inflammatory, amoeboid-like microglia. The 04:01 mice contained significantly more amoeboid-like microglia, with none being observed in the 04:02 mice. Mann-Whitney U-test, * P<0.05. Scale bars are 50μm.

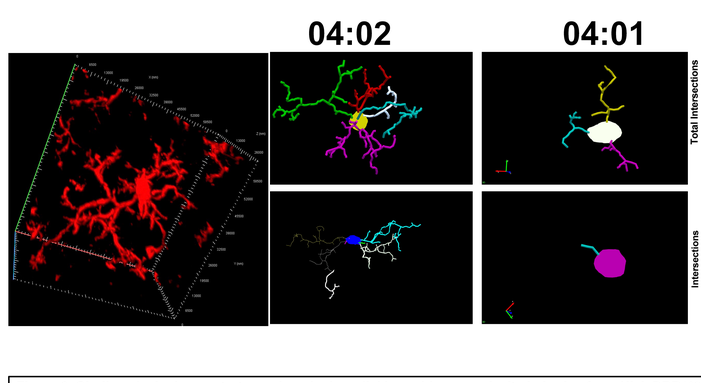


Figure 6: Sholl analysis demonstrates reduced microglial branching complexity in brains from HLA-DRB1*04:01 mice
Using the sections of brains from the mice that had been stained for microglia, further analysis was done to look at branching. For each mouse, 10 microglia were randomly selected with 5 coming from the cortex and 5 coming from the striatum. These microglia were traced using Neurolucida® and evaluated using Sholl analysis. The microglia from 04:01 mice showed significantly fewer total intersections and significantly shorter branches. Using Sholl analysis to measure the number of intersections at different radius sizes, the microglia from 04:01 brains had significantly less complex branching patterns, and fewer branches that made it past 20-30μm. Mann-Whitney U-test, * P <0.05.

Mouse Behavior Analysis

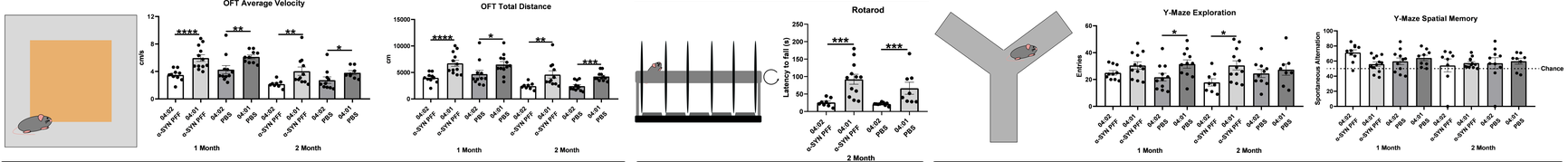


Figure 7: HLA-DRB1*04:01 mice have increased locomotor activity during open field test (OFT)
For the open field test, the 04:01/α-SYN PFFs, 04:01/PBS, 04:02/α-SYN PFFs, and 04:02/PBS mice were evaluated for the total distance that they traveled and their average velocity in an open arena *(Top Left)*. At one month post injection, the 04:01 mice, regardless of treatment, had significantly higher average velocity and significantly longer total distance traveled. At 2 months post injection, the trend continued with 04:01 displaying significantly more locomotor activity than 04:02 mice. * P < 0.05.

Figure 8: HLA-DRB1*04:01 have improved motor coordination on rotarod task
The latency to fall observed for mice with the 04:01 allele was significantly increased as compared to the 04:02 mice, who tended to fall much sooner. Mann-Whitney U-test, * P <0.05.

Figure 9: HLA-DRB1*04:01 mice have increased exploration during Y-maze task
For the Y-maze test, the 04:01/α-SYN PFFs, 04:01/PBS, 04:02/α-SYN PFFs, and 04:02/PBS mice were evaluated for the total arm entries and spatial memory performance. 04:01 mice had a tendency to explore more arms during the Y-maze task, but this was a small effect size and was not consistently significant between groups. When observing the spontaneous alternation of mice, there was no significant difference between groups (P > 0.05) . Mann-Whitney U-test, * P < 0.05.

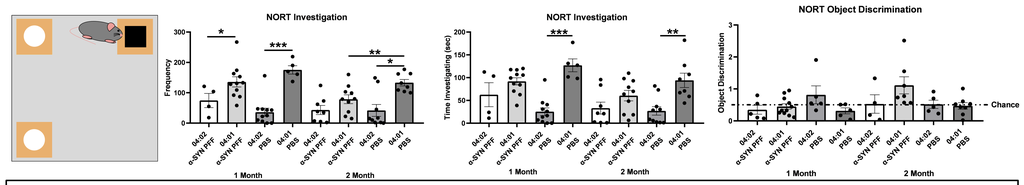


Figure 10: HLA-DRB1*04:01 mice spent significantly longer exploring objects during the novel object recognition task (NORT)
For the novel object recognition test, the 04:01/α-SYN PFFs, 04:01/PBS, 04:02/α-SYN PFFs, and 04:02/PBS mice were evaluated for the frequency of investigations, the total time spent investigating objects, and their object discrimination. Investigation was defined as the nose of the mouse being within a defined proximity to the object. Object discrimination was calculated as (*time with novel object / time with non-novel objects*) A trend was observed of 04:01 mice investigating more, which was significant in most cases. For the object recognition aspect of this test, no significant trends were observed and the results in all groups of mice were not significantly different than that which would be expected to occur by chance. Mann-Whitney U-test, * P < 0.05.

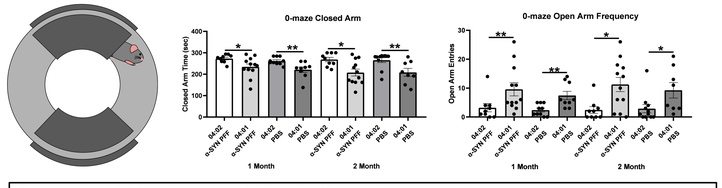
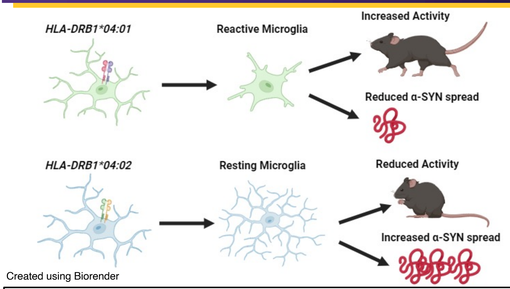


Figure 11: HLA-DRB1*04:01 mice exhibited significantly less anxious behaviors during 0-maze task
For the 0-maze task, the 04:01/α-SYN PFFs, 04:01/PBS, 04:02/α-SYN PFFs, and 04:02/PBS mice were evaluated for their time spent in the closed arm of the maze and their frequency for entering the open arms of the maze. The 04:01 mice spent significantly less time in the closed arm and had significantly more open arm entries compared to the 04:02 group, regardless of treatment type. Mann-Whitney U-test, * P < 0.05.

SUMMARY & FUTURE DIRECTIONS



Future Directions
Future efforts will begin with the investigation of how the 04:02 genotype influences the peripheral immune response to α-SYN by using Luminex-panel to analyze the cytokine panel and flow-cytometry to immunophenotype splenocytes of these mice. We also plan to characterize dopaminergic neuron loss and investigate the molecular mechanisms underlying the observed microglia phenotype. Further work is needed to understand the neurological mechanisms behind these changes.

Summary
The 04:01 mice had significantly less spread of α-SYN in the brain, providing an *in vivo* animal model to support the genetic association between *HLA* alleles and PD protection. The 04:01 mice had fewer total microglia yet higher levels of deramification leading to increased prevalence of amoeboid microglia, indicating higher levels of microglia activation, even in PBS injected animals (*not shown*). The behavior of the mice was found to be significantly different between 04:01 and 04:02 mice in a variety of tasks. Interestingly, this trend was still observed among the control PBS mice that had not been exposed to α-SYN. These findings suggest a direct effect of *HLA* allele variants with microglial activation and behavioral traits.

ACKNOWLEDGEMENTS & REFERENCES

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