nor the wt allele affected the Expanded Disability Status Scale score or the age at onset. Our results indicate no association between the chemokine receptor V Δ32 allele and MS.

For the investigation of pathomechanism of PD two different genes were analysed, vitamin D receptor which encodes a transcription factor that influences calcium homeostasis and immunoregulation, and the kynurenine-3-monooxigenase, which is the key enzyme of the kynurenine pathway.

In the vitamin D receptor study 100 PD patients and 109 healthy controls from the Hungarian population were genotyped for four polymorphic sites (BsmI, ApaI, FokI and TaqI). Our results demonstrate an association between the FokI C allele and PD, since the frequency of the C allele was significantly higher in PD patients than in controls, suggesting that this polymorphism may have a role in the development of PD in Hungarian patients.

There is substantial evidence that the kynurenine pathway plays a role in the normal physiology of the brain and it is involved in the pathology of neurodegenerative disorders. 105 unrelated, clinically definitive PD patients and 131 healthy controls were enrolled to investigate the possible effects of the different alleles of kynurenine-3-monooxigenase. None of the four investigated SNPs proved to be associated with the disease or with the age at onset. The investigated SNPs presumably do not appear to influence the gene function and probably do not contain binding sites for regulatory proteins. This was the first study to assess the genetic background behind the biochemical alterations of the kynurenine pathway in PD, directing the attention to this previously unexamined field.

Evidences indicate that there are aberrations in vitamin D endocrine system in ALS too. Our aim was to investigate SNPs from vitamin D receptor gene in 75 sporadic ALS patients and in 97 healthy controls. One of the four investigated SNPs was associated with the disease, but none of the alleles of the four examined SNPs influenced the age at disease onset. The ApaI A allele was more frequent in the ALS group compared to the control group, so it may be a risk factor for getting the disease.

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Identification of novel genes involved in the virulence of *Candida parapsilosis* during the generation of deletion library

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Recently, the increase in the prevalence of fungal diseases has focused attention on understanding the interactions between the pathogens and the host. Despite the growth of sequence information, a large number of fungal genes are uncharacterized and the function of genes are based solely on sequence homology. To characterize gene function in fungi such as the opportunistic pathogen *Candida parapsilosis*, gene knockout methods can be applied. In our previous work we have identified several fungal genes using RNA-Seq data that were overexpressed during host-pathogen interactions. To investigate their functions the creation of a knock out library was prepared. We have adapted a gene knock out strategy from the work of Noble and Johnson (2005). Fusion PCR method was applied to generate gene specific deletion constructions in order to disrupt genes from the genome of *C. parapsilosis* CLIB leu-/his- auxotrophic strain. Primarily we generated the flanking PCR products for the upstream and downstream regions for each of the genes, and the HIS1 and LEU2 marker PCR products. We used HIS1 marker from plasmid vector pSN52, and LEU2 from plasmid vector pSN40. Transformation of *C. parapsilosis* cells was performed chemically, using polyethylene glycol. For each of the identifications we used colony PCR to confirm the total deletion of the genes. All of the mutants were barcoded using a 20 bp tag in order to be able to identify them during later *in vivo* infections. All of the null mutant strains were tested under different conditions such as growth abilities on certain temperatures and medias, survival in the presence of cell wall, osmotic and oxidative stressors, and also pseudohyphae formation. Resistance to antifungal drugs such as fluconazole and caspofungin was also examined. We found null mutants that showed differences in appearance such as increased pseudohyphae formation and resistance to cell wall stressors (CPAR2 200390), regressed growth on different temperatures (CPAR2 303700) and alkali-phobic phenotype (CPAR2 100540). Difference in the virulence of these null mutant strains was also found when using infection models. CPAR2 200390 null mutants were found to be killed less efficiently by murine macrophages *in vitro*. In contrast, null mutants of CPAR2 303700 were killed similarly, however phagocytosed less than wild type cells by macrophages. Furthermore, more murine macrophages were found to phagocytose CPAR2 100540 deletion mutants, however the killing efficiency was lower comparing to the reference strain. In the future using this method we will be able to identify key regulatory factors that may play a role in the virulence of *C. parapsilosis* during host-pathogen interactions.

References

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