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Classification of free nucleic acids in cerebrospinal fluid of psychiatric patients

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Cerebrospinal fluid (CSF) filtration as experimental therapy proved to be effective in the past for treatment-resistant psychosis and depression in Borna Disease virus- and Epstein-Barr virus (EBV-) seropositive patients. To determine a possible role of free circulating DNA, viral or bacterial infections in the aetiology of psychotic disorders, a method for quantification and classification of free nucleic acids from the CSF should be established. The detection of genomic DNA using the housekeeping gene ALAS-1 and the relative quantification of DNA can be addressed by semi-quantitative PCR.

In order to isolate nucleic acids from blood plasma and CSF three different commercial kits were tested: QIAamp® Virus Kit QIAGEN UltraSens®, GF-1 Viral Nucleic Acid Extraction Kit® from Vivantis and the High Pure Viral Nucleic Acid Large Volume Kit from Roche Applied Science® to select the best performance for analysis. These kits were used for isolation of viral nucleic acids to nucleic acids and small (fragmented DNA, viral DNA/RNA) from 1 ml of cerebrospinal fluid and 1 ml of EDTA-anticoagulated plasma. In comparison, the kit from Roche Applied Science® was the best because it allows the largest sample volumes to work with. The amount of 20 ng/ml DNA were isolated from the CSF of psychiatric patient sample, which was 12.5 times higher than the DNA concentration determined in EDTA-plasma of the same patient, and 16 times less than detected in a plasma sample of a sepsis patient. For the direct detection of potentially circulating cell-free DNA (cfDNA) cfDNA, PCR was performed. To detect genomic DNA, primers were selected which amplified the house keeping gene ALAS-1. To detect viral DNA, the Epstein Barr virus (EBV) gene EBNA-2 was amplified and to detect mitochondrial DNA (mtDNA), we applied short-tandem repeat (STR)-profiling of genomic and mtDNA by using a highly specific PCR in combination with the use of the computer-controlled analyzer ABI PRISMTM 310 Genetic Analyzer. This approach significantly improved the quantification sensitivity of the PCR products.

Finally 16S RNA specific primers were designed to identify DNA of bacterial origin. So far we obtained positive results for the identification of genomic and mitochondrial DNA in 2/2 samples of cerebrospinal fluid. The concentration of genomic DNA in cerebrospinal fluid was similar to the free genomic DNA detected in the corresponding plasma samples. In one patient, EBNA2 could be amplified from plasma but not from CSF. The identification of bacterial DNA was not successful in all samples tested. The present results support the hypothesis that cfDNA of genomic origin and/or mtDNA may represent danger associated molecular pattern (DAMP) molecules and can induce an inflammatory response in patients with psychiatric disorders. Secondly, the method established here is feasible to isolate and further characterize nucleic acids from 1 ml of cerebropinal fluid or plasma. In the future, we intend to address the identification of EBV in CSF and plasma by a multiplexed PCR detecting the EBV-specific genes coding for EBNA1, EBNA2, EBNA3c, BNRF1, LMP1, BMLF1 and BZLF1. Using functional assays, the effect of cfDNA and/or mtDNA to mount an inflammatory immune response will be addressed by whole blood stimulation assays.

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