Significant frequency deviation of the class I polymorphism HLA-A10 in schizophrenic patients

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**Key points**

- We compared frequencies of HLA-A and HLA-C antigens in two patient panels with schizophrenia, panel 1 (n=31 for both) and panel 2 (n=60 and n=71, respectively) and a random panel (n=367 and n=390, respectively) and found a lower frequency of HLA-Cw5 in both patient panels, which did not reach significance.
- The increase in frequency of HLA-A10 was nearly identical in both patient panels (p=0.006, two sided, in panel 2, relative risk 3.071). Association of other genes in the HLA-A chromosomal region with schizophrenia will be discussed.

**Patients and random controls**

All patients were white and from southern Germany. The diagnosis of schizophrenia was performed at the Psychiatric Hospital of the University of Munich. Schizophrenia was diagnosed according to the third edition of the "Diagnostic and Statistical Manual of Mental Disorders" (DSM-III-R) in patients in panel 1 and panel 2. All patients were inpatients at the time of diagnosis. Different schizophrenic subtypes had no impact on the observed deviation of frequencies of HLA class I alleles and antigens (not shown).

Patient panel 1 (n=31) was previously collected to study HLA class I antigens and HLA class II alleles. Results of HLA class II alleles have already been published. Patient panel 2 (n=71) was collected later over a three year period and HLA-C polymorphism was investigated by sequence based typing (SBT) for best resolution. Only 60 of these 71 patients could be used for serological typing.

Samples for the random control panels were collected from members of the Institute of Immunology in Munich and from medical students at the University of Munich. All subjects were white. The frequencies of HLA antigens did not deviate from other published German HLA antigen frequencies.

**Isolation of peripheral mononuclear cells (PBMC), RNA, and HLA typing**

Peripheral blood mononuclear cells (PBMC) from healthy random controls and schizophrenic patients were isolated by Ficoll gradient centrifugation.

Serological HLA-A typing was performed according to the NIH method with some modifications to the original technique. Our own monoclonal antibodies, reagents obtained by exchange, and commercial reagents were applied. A total of 28 HLA-A and -C antigens were defined using a battery of 120 reagents. Total RNA was prepared from freshly isolated cells or after CD3 stimulation using the Micro RNA isolation kit (Stratagene, La Jolla, CA, USA). RNA was transcribed into cDNA by RAV-2 reverse transcriptase (Amerham, Braunschweig, Germany) and amplified by PCR. Sequence, length, and localisation of primers were as previously described. Sequencing primers were hybridised to conservative regions of HLA alleles. Amplified PCR products were sequenced after strand separation with streptavidin coated Dynabeads M-280 (Dynal, Hamburg, Germany). Sequencing was performed using fluoroprime or indodicabo-cyanine labelled primers and gels were run on an automated DNA sequencer (ALF Express, Pharmacia, Freiburg, Germany). Both strands of every PCR product were sequenced. Twenty-three different HLA-C alleles were identified in patients and controls (not shown).

**Statistical evaluation**

HLA-A and HLA-Cw antigen frequencies of patients and random controls as well as HLA-Cw* allelic frequencies (not...
RESULTS
Both patient panels and controls were typed for 20 HLA-A antigens and eight HLA-C antigens serologically. The following HLA-C alleles were identified by sequence based typing: HLA-Cw*0102, -Cw*02022, -Cw*0302, -Cw*0303, -Cw*0304, -Cw*0401, -Cw*0501, -Cw*0602, -Cw*0701, -Cw*0702, -Cw*0704, -Cw*0802, -Cw*1202, -Cw*1203, -Cw*1502, -Cw*1504, -Cw*1505, and -Cw*1601. The random control panel is not shown because the results of HLA-C typing of a major part of the control panel has previously been published. As shown there, HLA-Cw5 was encoded only by one allele, HLA-Cw*0501. Nine patients were identified as carrying HLA-Cw*0501 and these patients also expressed HLA-Cw5 serologically. We did not find a significant difference between HLA-SBT typing and serological typing for HLA-Cw5/HLA-Cw*0501. No significant HLA-C allelic frequency deviation was found in patient panel 2 as compared to the random controls (not shown), nor of HLA-Cw4 as previously published. Therefore the results were restricted to serological typings of patient panel 1 and patient panel 2 (table 1A). From 20 serologically tested HLA-A antigens (not shown), only HLA-A10 showed a significant frequency deviation. The results of HLA-A10 in patient panel 1 (p=0.025, corrected, not significant) was replicated in patient panel 2 (p=0.006, p value correction not required, legend table 1).

DISCUSSION
Many microbial factors have been implicated in the pathogenesis of schizophrenia, but so far each microbial factor has been identified in a relatively small subgroup of patients. The heterogeneity of these microbial factors is also reflected by the associations with different HLA loci and their alleles. Polymorphic HLA alleles encode individual smell receptors and influence mating preferences. Polymorphic HLA molecules process, select, and present degraded microbial proteins. The set of inherited HLA alleles determines susceptibility or resistance to particular microbes. We recently analysed immune response genes of the HLA class II region in patient panel 1 with sequence specific oligonucleotide typing and reported an increased frequency of the narcolepsy and multiple sclerosis associated HLA class II allele DQB1*0602 in the schizophrenic patients. A former report of a strongly increased frequency of HLA-Cw4 in Czech schizophrenic patients inspired us to reinvestigate HLA-C polymorphism by sequencing the schizophrenia patient panel 2 (not shown). However, we did not find a significantly increased frequency of any HLA-Cw allele in the patients, rather a decreased frequency of the allele HLA-Cw*0501 (not shown). This allele is the only one to encode the serologically defined antigen HLA-Cw5. Although the results of patient panels 1 and 2 showed a similarly decreased frequency of HLA-Cw5, the decrease in frequency did not reach significance.
improvement observed in three psychiatric patients, suffering by adoptive immunotherapy may have led to the substantial ence neuronal adaptive responses. Restoration of such circuits modulate/disturb the neurotrophic system and thereby influ-
(BDNF).

produce, for example, brain derived neurotrophic factor may fail to protect embryonal brain development or fail to

REFERENCES


