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The prevalence of antinuclear antibodies in patients with schizophrenia spectrum disorders: results from a large cohort study

Hans C van Mierlo[1], Lot de Witte[1], Ronald HWM Derksen[2], Henny G Otten[3] and the GROUP investigators[4]

BACKGROUND: An increased prevalence of autoantibodies has been found in patients with schizophrenia, suggesting a role for autoimmunity in schizophrenia pathogenesis.
METHODS: We examined the presence of antinuclear antibodies (ANAs), with further determination of specific antibodies, in 368 patients with a schizophrenia spectrum disorder and 283 healthy controls.
RESULTS: No significant difference in prevalence of ANAs between patients (8%) and controls (11%) was found.
CONCLUSION: We did not find an association between ANAs and schizophrenia spectrum disorders. We discuss potential reasons for the discrepancy with some previous studies, such as inclusion of patients using chlorpromazine, which can induce ANAs.

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INTRODUCTION

Several lines of evidence suggest that the immune system is involved in the etiopathology of schizophrenia. This theory is supported by the findings of an increased prevalence of autoimmune diseases among patients with schizophrenia[1] and a higher frequency of several autoantibodies in the blood of patients with schizophrenia, as shown by a recent systematic review,[2] including antinuclear antibodies (ANAs). The presence of ANAs is used to support the clinical diagnosis of various autoimmune disorders and can be seen as a marker of autoimmunity.[3]

Ezeoke *et al.*[2] concluded that ANAs are significantly more prevalent in patients with schizophrenia as compared with controls (22.2 vs. 6.7%). The authors, however, mention that there is a marked heterogeneity and inconsistency among included studies and that additional studies are needed that control for potential confounding factors, such as clinical status, age, genetic background, and medication.

ANAs are autoantibodies that react with intracellular components[4] and can be further divided into antibodies directed against double-stranded DNA or specific proteins such as SSA.[5,6] Examples of autoimmune diseases with a high prevalence of ANAs (up to 93%) are systemic lupus erythematosus, systemic sclerosis, Sjögren's syndrome, mixed connective tissue disease, inflammatory myopathies, and some autoimmune hepatic disorders.[7–9] However, ANAs are also frequently found in the general population with an estimated prevalence ranging from 1 to 20%. The wide range in reported prevalence is caused, among others, by differences in assays used, cutoff values used for a positive result and characteristics of the studied population. The prevalence of ANAs is higher in females than in males and increases with age.[10]

The aim of this study was to examine the prevalence of ANAs among patients with a schizophrenia spectrum disorder as compared with healthy controls and to subsequently analyze the presence of specific antibodies in participants testing positive for ANAs. Previous studies in patients with schizophrenia spectrum disorders often consisted of small cohorts; the present study is one of the largest case–control studies to date—it includes the most important confounding factors and does not include patients on chlorpromazine medication that can induce ANAs.

MATERIALS AND METHODS

Data collection

The data from this study were collected as part of the ongoing multicenter longitudinal Genetic Risk and Outcome of Psychosis (GROUP) study, started in The Netherlands in 2006.[11] This study was approved by the human ethics committees of the University Medical Centers of Utrecht, Amsterdam, Maastricht and Groningen. All included patients and healthy volunteers provided written informed consent before participating.

This study consists of 368 patients and 283 healthy controls whose plasma samples were available for analysis. Plasma samples were collected during a follow-up visit in 2009.

Population

Patients were recruited from mental health centers throughout the Netherlands in 2006. Healthy controls were recruited via mailings to random addresses in the catchment region. Inclusion criteria for patients were: fluent in Dutch and diagnosis of a schizophrenia spectrum disorder at follow-up according to the Comprehensive Assessment of Symptoms and History (CASH)[12] or Schedules for Clinical Assessment in Neuropsychiatry (SCAN)[13] interview. Eligible healthy controls had to meet the following criteria: fluent in Dutch, no history of a lifetime psychotic disorder or lithium use and no first- or second-degree family member with a lifetime psychotic disorder. All subjects with a psychosis due to a general medical condition were excluded from this study. Data on current or previous comorbid physical disorders were collected through questionnaires.

[1]Department of Psychiatry, Brain Center Rudolf Magnus, University Medical Center Utrecht, Utrecht, The Netherlands; [2]Department of Rheumatology and Clinical Immunology, University Medical Center Utrecht, Utrecht, The Netherlands and [3]Laboratory of Translational Immunology, University Medical Center Utrecht, Utrecht, The Netherlands. Correspondence: HC van Mierlo (h.vanmierlo@umcutrecht.nl)
[4]The members of Genetic Risk and Outcome of Psychosis (GROUP) Investigators are listed before references.
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Plasma analysis

Blood was drawn by venous puncture and after centrifugation plasma samples were stored in −80 °C until further analysis.

Plasma samples from all participants were tested for ANAs by indirect immunofluorescence using a commercially available assay, according to the manufacturer's protocol (Euroimmun, Lübeck, Germany). In short, plasma samples were diluted 1:100 and incubated with HEp-20-10 cells and primate liver substrates. After washing, attached antibodies were stained using a fluorescein-labeled antibody against human IgG. Nuclear staining was evaluated by two independent raters unaware of subject status and rated negative (absent or weak staining) or positive (moderate or strong staining). Subsequently, ANA-positive samples were examined for specific antibodies using a commercially available line blot assay according to the manufacturer's protocol (Profile 3 line blot, Euroimmun, Lübeck, Germany). In brief, plasma samples were diluted 1:101 and incubated on a membrane strip containing 14 antigen extracts (histones, nucleosomes, double-stranded DNA, PCNA, centromere protein B, PM/Scl, Scl-70, SSB, SSA, Sm, nRNP, mitochondrial M2, ribosomal-P and Jo-1). Subsequently, the strips were stained using an alkaline phosphatase-labeled anti-human IgG antibody, which was visualized by an NBT/BCIP substrate solution. Assayses were interpreted using the scanning software (Euroimmun, Lübeck, Germany) provided by the manufacturer.

Statistical analysis

Differences in baseline characteristics between the two diagnostic groups were examined using X^2 and Mann–Whitney *U*-tests when appropriate. To assess whether the prevalence of ANAs was significantly different between the group of patients and the control subjects X^2-tests were used. A multiple logistic regression model was used to examine the influence of diagnostic group, gender, age, and ethnicity on ANA status.

RESULTS

Table 1 shows an overview of the characteristics of both patient and control subjects. In total, 29 patients (8%) and 32 controls (11%) tested positive for ANAs. This difference was not significant: $X^2 = 2.212$, $P = 0.137$. The ANA assay was strongly positive in 6/29 (21%) patients and 7/32 (22%) control subjects.

Age, ethnicity, and gender have previously been shown to be associated with ANAs. In our study, significantly more patients compared with control subjects were male (76% of the patients versus 42% of the control subjects). When we analyzed both gender groups separately, 21 out of 281 male patients (7%) and 11 out of 118 male control subjects (9%) tested positive for ANAs ($X^2 = 0.385$, $P = 0.535$), whereas in the group of females 8 out of 87 patients (9%) and 21 out of 165 controls (13%) tested positive for ANAs ($X^2 = 0.698$, $P = 0.404$).

Using multiple logistic regression analysis, we did not find a significant influence of age, gender, or ethnicity on ANA status (data not shown).

To assess the potential influence of antipsychotics on the ANA status, we examined the prevalence of ANAs in the group of patients currently using antipsychotics ($N = 257$) as compared with the group of patients currently not using antipsychotics ($N = 37$). The prevalence of ANAs was 6% and 8%, respectively (no significant difference: $X^2 = 0.190$, $P = 0.663$). Furthermore, we found no significant difference in the prevalence of ANAs between patients currently using first ($N = 24$) and second generation ($N = 183$) antipsychotic agents ($X^2 = 0.206$, $P = 0.650$).

All 61 samples that tested positive for ANAs (29 originating from patients and 32 from control subjects), were tested for specificity in a line blot assay. In total, eight samples had a positive line blot result against the following antigens: histone, pCNA, SSA, and a mitochondrial antigen. Positive results were equally distributed among patients and control subjects (Table 1).

DISCUSSION

Previous studies have not shown an unambiguous relation between ANAs and schizophrenia spectrum disorders (Table 2). Recognized reasons for contradictory results are differences in the techniques used to detect ANAs, differences in definition of positive results, variation and shortcomings in studied populations and whether or not control subjects were included.[2]

We found a similar prevalence of ANAs in patients with a schizophrenia spectrum disorder and control subjects, using indirect immunofluorescence on HEp-2 cells to detect ANAs, which is the gold standard for their detection.[3] Many published studies consisted of small cohorts and did not include a control group. An overview of previous case–control studies is provided in Table 2. Furthermore, most studies did not correct for gender and age.[2] The present study does not suffer from these shortcomings.

Another important reason for the discrepant results in studies on ANA frequencies can be the use of medication. Various earlier studies have included a large proportion of patients using chlorpromazine[14–18] or did not describe medication use. Chlorpromazine is known to induce ANAs.[19,20] None of the patients included in our study were on chlorpromazine as this drug is not available in the Netherlands since 2008;[21] unfortunately, we cannot exclude that some of the included patients might have used chlorpromazine before 2008.

Table 1. Characteristics of patients and controls

	Patients (N = *368*)[a]	*Controls* (N = *283*)	*Group comparison test*
Gender M/F (%males)	281/87 (76%)	118/165 (42%)	$P < 0.01$
Mean age (SD) in years	30.5 (±7.0)	34.5 (±10.5)	$P < 0.01$
Range	18–55	18–55	
Ethnicity Caucasian yes/no/NA	310/50/8	263/16/4	$P < 0.01$
Reported comorbid autoimmune disorder yes/no/NA	3/187/178	9/177/97	$P = 0.072$
ANAs positive/negative	29 (8%) / 339 (92%)	32 (11%) / 251 (89%)	$P = 0.137$
Specific ANAs			
Anti-histones	2	1	
Anti-PCNA	1	1	
Anti-SSA	1	1	
Anti-mitochondrial M2	0	1	

Abbreviations: ANA, antinuclear antibody; F, female; M, male; NA, not applicable.
[a]Diagnosis: 295 schizophrenia, 58 schizoaffective disorder, 15 schizophreniform disorder. Mean duration of illness 7.3 years (±4.3), range 2–43 years. Using antipsychotics y/n/NA: 257/37/74. Clozapine: 58, olanzapine: 54, risperidone: 29, aripiprazol: 27, quetiapine: 15, haloperidol: 12, flupentixol: 4, zuclopenthixol: 4, bromperidol: 2, pimozide: 2, multiple antipsychotics: 41 and not specified antipsychotics: 9.

Table 2. Overview of case–control studies on the prevalence of ANAs in patients with schizophrenia spectrum disorders

Reference	*Patients*	*ANA positive/%*	*Controls*	*ANA positive/%*	*ANA test*
Gottfries and Gottfries[22]	250 patients with schizophrenia	56/22.4%	77	7/9.1%	IF
Zarrabi *et al.*[18]	74 patients with schizophrenia	29/39.2%	15	0/0%	IF
Villemain *et al*[23]	16 patients with schizophrenia	5/31.3%	10	2/20%	IF on mouse liver
Canoso *et al.*[15]	184 males with chronic psychosis	45/24.5%	35	0/0%	IF on HEp-2 cells
Yannitsi *et al.*[17]	179 patients with schizophrenia	86/48.0%	150	10/6.7%	IF on HEp-2 cells
Ganguli *et al.*[24]	225 patients with schizophrenia or schizoaffective disorder	30/13.3%	327	20/6.1%	IF on HEp-2 cells
Sirota *et al.*[25]	108 patients with schizophrenia	42/38.9%	210	8/3.8%	ELISA
Spivak *et al.*[26]	85 patients with schizophrenia	18/21.2%	37	2/5.4%	IF on HEp-2 cells
Zorrilla *et al.*[27]	56 patients with schizophrenia	7/12.5%	84	18/21.4%	IF on HEp-2 cells
Laske *et al.*[28]	34 patients with schizophrenia	2/5.9%	50	0/0%	IF on rat liver
Sidhom *et al.*[29]	60 patients with schizophrenia or schizoaffective disorder	7/11.6%	41	5/12.2%	IF on rat tissue

Abbreviation: ANA, antinuclear antibody; IF, immunofluorescence.

ANA positivity can be caused by antibodies with a wide variety of fine specificities. Only a part of these antibodies are well described and have clinically relevant associations with specific autoimmune disorders.[3] A common way to detect fine specificity of ANAs is by line blot techniques. The method we used can detect reactivity against 14 antigens. The frequency of a positive result with line blot in the ANA-positive samples was low and no clinically relevant results were found. Most importantly, frequencies of positive results were similar for patients and control subjects.

In conclusion, our study shows that the prevalence of ANAs, with determination of specific antibodies, is similar in patients with a schizophrenia spectrum disorder and healthy controls. Our study suggests that previous findings on autoantibodies in schizophrenia should be interpreted with care, paying attention to potential confounders, and that further validation in large cohorts is needed before conclusions can be drawn.

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CONTRIBUTIONS

All authors contributed to and have approved the final manuscript. LdW and RS Kahn designed and supervised the study. HCvM analyzed the data, interpreted the results, and wrote the first draft of the manuscript. RHWMD and HGO contributed to the interpretation of the results and to the completion of the manuscript. GROUP Investigators designed the GROUP project and revised the manuscript.

COMPETING INTERESTS

The authors declare no conflict of interest.

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GENETIC RISK AND OUTCOME OF PSYCHOSIS (GROUP) INVESTIGATORS

Richard Bruggeman[1], Wiepke Cahn[2], Lieuwe de Haan[3], René S. Kahn[2], Carin J. Meijer[3], Inez Myin-Germeys[4], Jim van Os [4], Durk Wiersma[1]
[1]University Medical Center Groningen, Department of Psychiatry, University of Groningen, The Netherlands; [2]University Medical Center Utrecht, Department of Psychiatry, Brain Center Rudolf Magnus, The Netherlands; [3]Academic Medical Center University of Amsterdam, Department of Psychiatry, Amsterdam, The Netherlands; [4]Maastricht University Medical Center, South Limburg Mental Health Research and Teaching Network, EURON, Maastricht, The Netherlands

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