Prevalence and Determinants of Agonistic Autoantibodies Against α1-Adrenergic Receptors in Patients Screened Positive for Dementia: Results from the Population-Based DelpHi-Study

Jochen René Thyriana,*, Johannes Hertela,b, Lara N. Schulzeb, Marcus Dörrc,d, Harald Prüssede,f, Petra Hempelg, Marion Bimmlerg, Rudolf Kunzeh, Hans Jürgen Grabea,b, Stefan Teipelij and Wolfgang Hoffmannak

aGerman Center for Neurodegenerative Diseases (DZNE), Site Rostock/Greifswald, Greifswald, Germany
bDepartment of Psychiatry and Psychotherapy, University Medicine Greifswald, Greifswald, Germany
cGerman Center for Cardiovascular Research (DZHK), Partner Site Greifswald, Germany
dDepartment of Internal Medicine B, University Medicine Greifswald, Greifswald, Germany
eGeriatric Center for Neurodegenerative Diseases (DZNE), Site Berlin, Germany
fDepartment of Neurology, Charité – Universitätsmedizin Berlin, Germany
gERDE-AAK-Diagnostik GmbH Berlin, Germany
hScience Office, Berlin, Germany
iGerman Center for Neurodegenerative Diseases (DZNE), Site Rostock/Greifswald, Rostock, Germany
jDepartment of Psychosomatic Medicine, University Medicine Rostock, Rostock, Germany
kInstitute for Community Medicine, University Medicine Greifswald, Greifswald, Germany

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Abstract

Background: There is a need to assess promising biomarkers for diagnosis and treatment response in real-life settings. Despite the important role of vascular risk factors, cardiovascular biomarkers have played a minor role in dementia research. Agonistic autoantibodies (agAAB) directed against G-protein-coupled receptors (GPCR) are discussed as modulators of pathology and clinical manifestation.

Objective: 1) Describe prevalence of agAAB directed against GPCR, especially agABB against α1-adrenergic receptors (α1-AR-agAAB) and agABB directed against β2-adrenergic receptors (β2-AR-agAAB) and 2) identify factors associated with agAAB in people with dementia during routine care.

Methods: Blood samples and data from 95 subjects who screened positive for dementia from a primary care cohort, analyzed using an enzyme-linked immunosorbent assay (ELISA) for detecting agAAB. Sociodemographic and clinical data were assessed, and medical records checked.

*Correspondence to: Jochen René Thyrian, German Center for Neurodegenerative Diseases (DZNE), Ellernholzstr. 1-2, 17489 Greifswald, Germany. Tel.: +49 3834 87 7592; E-mail: rene.thyrian@dzne.de.
Results: In 40 (42%) samples, agAAB was detected, with \( n = 29 \) (31%) representing \( \alpha_1\)-AR-agAAB and \( n = 21 \) (22%) \( \beta_2\)-AR-agAAB. There was no association between the presence of any antibody and a formal diagnosis of dementia. However, patients with coronary heart disease were more likely (OR = 4.23) to have \( \alpha_1\)-AR-agAAB than those without coronary heart disease. There were no associations between agAAB and age, sex, education, or cognitive impairment.

Discussion: For the first time, we show that autoantibodies have a significant prevalence in people with dementia in a routine care setting. Previous findings were restricted to highly selective samples. We replicated the association between \( \alpha_1\)-AR-agAAB in patients with coronary heart diseases but were not able to find other factors associated with the presence of agAAB.

Keywords: Antibodies, biomarker, immunoabsorption, prevalence, primary care

INTRODUCTION

According to the World Health Organization (WHO), dementia is a public health priority with 47.5 million people living with dementia and 7.7 million new cases diagnosed every year [1]. The global estimation of the dementia prevalence ranges from 5% to 7% for those aged \( \geq 60 \) years [2]. In Germany, approximately 1.5 million people are affected [3]. Dementia is a major cause of disability and dependency among older people worldwide. It is a complex condition that has physical, psychological, social, and economic impacts on caregivers, families, and society [1]. Dementia is linked to multiple pathologies, such as Alzheimer’s disease (AD), vascular dementia, and frontotemporal dementia. Currently, there is no curative treatment available and treatment guidelines focus on the treatment of symptoms with the aim to delaying onset or the course of the disease [4].

A biomarker is defined as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention”. In dementia research, biomarkers provide for the selection of people at risk for primary or secondary prevention trials [5]. They can be divided into three groups: 1) diagnostic markers, used to enrich, select, and stratify individuals at risk of dementia; 2) endpoint biomarkers, used as outcome measures to monitor the rate of disease progression and detect treatment effects; and 3) markers of target engagement, used to directly target the pathophysiology of dementia [5].

One of the most common causes of dementia is AD, often accompanied by cerebrovascular comorbidity. There are different pathologies of cognitive decline, but recent epidemiological, clinical, and neuropathological data indicate considerable overlap between cerebrovascular disease and AD [6]. This agrees with epidemiological findings indicating that lifestyle factors are independently increasing the risk for both cardiovascular diseases and developing dementia [7–9]. Despite the important role of cerebrovascular comorbidity in AD, cardiovascular biomarkers have played a minor role in dementia research to date. One pilot study delivered promising results when targeting agonistic autoantibodies (agAAB) directed against G-protein-coupled receptors (GPCR) [10]. In this study, however, the sample size was very small and selective, and only eight patients out of 31 who were positive for autoantibodies underwent immunoabsorption [10].

agAAB directed against GPCR are discussed as modulators of the pathologies and outcomes of several cardio- and cerebrovascular diseases, such as dilated cardiomyopathy [11, 12] and dementia [13, 14]. The pathogenic effect of agAAB has been demonstrated in rat models for the \( \beta_1\)-adrenergic receptor autoantibodies involved in dilated cardiomyopathy [15] and for the \( \alpha_1\)-adrenergic receptor in the context of cerebrovascular impairment [13].

Little is known about the prevalence of these antibodies in subjects with dementia or their associations with demographic factors. The studies to date were conducted in selective samples. In a clinical sample of 169 patients with mild to moderate AD, 54% were positive for agAAB; 47% of patients and/or those with vascular dementia were harboring agAAB acting against \( \alpha_1\)-AR. Of these patients, 71% were positive for both \( \alpha_1\)-AR-agAAB and \( \beta_2\)-AR-agAAB. Only five patients showed agAAB acting solely against the \( \beta_2\)-AR [10]. The prevalence in people with dementia in primary care is still unknown, and recent evidence suggests that biomarker accuracy might differ between clinical and primary care samples [16, 17]. However, this information would be important to identify selection criteria and estimate the proportion of people eligible for treatment (i.e., immunoabsorption therapy) in the population of people with dementia. This is in line with a roadmap of a phase model from preclinical studies through...
trials in expert center settings for the evaluation of biomarkers in primary care [18, 19].

The objectives of this article are 1) to describe the prevalence of agAAB directed against GPCR, especially agABB directed against α1-adrenergic receptors (α1-AR-agAAB) and agABB directed against β2-adrenergic receptors (β2-AR-agAAB) and 2) to identify factors associated with the presence of agAAB.

MATERIALS AND METHODS

These analyses are based on blood samples and the baseline data of the DelpHi-trial (Dementia: life- and person-centered help in Mecklenburg-Western Pomerania). DelpHi was a pragmatic, GP-based, cluster-randomized intervention study with two arms, an intervention group and a care as usual group. The intervention delivered was “Dementia Care Management (DCM)”. DCM is a complex intervention that aims to provide “optimal care” by integrating multiprofessional and multimodal strategies for improving patient- and caregiver-related outcomes. DCM individualizes and optimizes dementia treatment and care within the framework of the established health care and social service system. It was developed according to current guidelines targeted at the individual participant level and delivered at participants’ homes by 6 nurses with dementia-specific training. Nurses were supported by a computer-based intervention-management system to improve systematic identification of patients’ and caregivers’ unmet needs and the subsequent recommendation of interventions to address these needs. The study protocol was approved by the Ethical Committee of the Chamber of Physicians of Mecklenburg-Western Pomerania, Germany (registry number BB 20/11). The study is described in more detail elsewhere [20–22].

Sample

Patients were systematically screened for dementia by their GP during routine visits, when they were 70 years or older and lived at home. Patients meeting the eligibility criteria were screened for dementia in participating GP practices using the DemTect. This personal interview-based instrument is widely used for dementia screening in general practices in Germany [23]. Patients who met the inclusion criteria for DelpHi-MV (DemTect <9) were informed about the study by their GPs, were invited to participate and asked to provide written informed consent. When the patient was unable to give a written informed consent, his or her legal representative was asked to sign the consent form on his or her behalf (as approved by the Ethical Committee of the Chamber of Physicians of Mecklenburg-Western Pomerania, registry number BB 20/11). The study physicians received allowances for performing the screening test (10€ per patient) and study enrollment (100€ per patient). The participants were randomly assigned to an intervention and a control group. Identical, standardized, computer-assisted face-to-face interviews with all participants were conducted at the participants’ homes by specially trained nurses over an average of three separate visits 1) immediately after study inclusion (baseline) and 2) 12 months later (follow-up). To minimize participant burden, the assessment sessions were restricted to one hour. All participating GPs provided information from the patients’ records. Participants in the intervention group (n = 348) were asked for blood samples in the course of and after the intervention.

Blood samples

By the first of September 2016, n = 115 participants (33.05%) had given blood samples, 15 (4.3%) were in the waiting list for blood sampling, in 7 (2.01%) blood sampling was not possible, and n = 8 (2.30%) were not asked yet. A total of 121 (34.78%) did not give written informed consent and the rest (n = 82; 23.56%) dropped out of the intervention study before blood samples could be obtained. The time for blood analyses (8/2015) was determined by available funding and thus had to be conducted while the study was still running.

Blood samples were obtained according to standard operating procedures in the participants homes. In total, 29 ml were obtained per participant (8.5 ml for serum analysis, 8 ml for plasma analytics, 10 ml EDTA, 2.5 ml for blood RNA). Samples were processed immediately and stored in aliquots and put into cryoboxes on dry ice for transportation by car to the study center (max. 8 × 0.5 ml serum, 8 × 0.5 ml lithium-heparin-plasma, 8 × 0.5 ml EDTA-plasma and 2 × 1 ml cell suspension). At the study center they were stored in a freezer (−80°C). For this analysis, 100 aliquots were available.

Data assessed

For the present analysis, we analyzed variables concerning age, sex, living situation (alone/not alone), cognitive status, functional status, level
of impairment, comorbidities, formal diagnosis of dementia, and pharmacological therapy.

Cognitive status was assessed using the German version of the Mini-Mental Status Examination (MMSE). The MMSE provides a total score as well as a categorization that indicates “no indication or mild” (score, 20–30), “moderate” (score, 10–19) and “severe cognitive impairment” (score, 0–9). The functional status was assessed using the Bayer Activities of Daily Living Scale (B-ADL), which yields a mean score of 1 to 10, where 1 indicates the lowest possible impairment and 10 indicates the highest possible impairment. Level of impairment was defined according to the “care level (Pflegestufe)” used by the care insurance for long-term care. Each person is assigned to either none or a specific grade of care. If a care level is assigned, each patient is categorized into one of four levels ranging from 0–3, with people in three needing the highest and in zero the lowest level of care. For all patients who had provided the respective informed consent all formal medical diagnoses were retrieved from the medical records in their GPs’ practice. For a formal diagnosis of dementia, we analyzed the ICD-10 codes: F00, F01, F02, F03, G30 and G31. For a formal diagnosis of any coronary heart disease we analyzed ICD-10 codes I20-I25. The sample is described in detail in Table 1.

Analysis of agAAB levels

We used an enzyme-linked immunosorbent assay (ELISA) to detect agAAB. Peptides were directed against the α1-adrenergic receptor loop 1 and β2-adrenergic receptor loops 1 and 2. Modified peptides were bound to 96-well streptavidin-coated plates. Peptides were coupled to preblocked streptavidin-coated 96-well plates (Perbio Science, Bonn, Germany). Patient serum was added in a 1:100 dilution and incubated for 60 min. As detection antibody a horseradish peroxidase conjugated anti-human IgG antibody was used (Biomol, Hamburg, Germany). Antibody binding was visualized by the 1-Step Ultra TMB ELISA (Perbio Science, Bonn, Germany). The absorbance was measured at 450 nm against 650 nm with an SLT Spectra multiplate reader (TECAN, Crailsheim, Germany).

Statistics

For descriptive statistics, categorical variables were described by proportions, metric variables by mean and standard deviations, and calculating the summarization of the analyzed subsample and the total DelpHi-MV-sample. We compared the descriptive statistics among four groups: 1) no agAAB, 2) only α1-AR-agAAB, 3) only β2-AR-agAAB, and 4) both agAAB. The groups were tested on differences with Fisher’s exact test in the categorical variables and one-factorial ANOVAs in the metric variables. Note, however, that the p-values resulting from these tests are only for reference and must be treated cautiously as they do not reflect the structure of the sample that is clustered by the GPs. Thus, to take the stochastic dependency of the data on the GP into account, we ran logistic regressions with the antibody status being the dichotomous outcome variable including random effects for the GP. The predictors in these regressions were age, sex, the MMSE score, the diagnosis of a dementia before screening (dichotomous) and the diagnosis of coronary heart disease. This model was calculated three times with the prevalence of agAAB overall, α1-AR-agAAB or β2-AR-agAAB being the dichotomous outcome variable. The p-value was set to 0.05 (two-sided). All of the analyses were performed with STATA 13/SE (STATA Inc., College Station, Texas).

RESULTS

In 40 (42%) of the samples, agAAB was detected above threshold. In 29 (31%) samples, α1-AR-agAAB was detected; 19 (66%) samples had solely α1-AR-agAAB, while 10 (34%) also had β2-AR-agAAB. We found β2-AR-agAAB in 21 (22%) of the samples; 11 (52%) had solely β2-AR-agAAB, and 10 (48%) also had α1-AR-agAAB. There was no statistically significant association between any of these antibodies and the presence of a formal diagnosis of dementia. However, the demented patients who had coronary heart disease were more likely (OR = 4.23, 95% CI: 1.50–11.96, \( p = 0.006 \), adjusted for age, sex and cluster) to have α1-AR-agAAB than those without CHD. In contrast, the association between coronary heart disease and β2-AR-agAAB was statistically not significant (OR: 1.58; CI: 0.12–20.21, \( p = 0.726 \)).

There were no associations between the presence of agAAB with any of the psychometric variables or sociodemographic variables.

The results of the analyses are presented in Table 2.
Table 1
Descriptive statistics for people screened positive for dementia in primary care

<table>
<thead>
<tr>
<th></th>
<th>Total Sample (n = 348)</th>
<th>Sample Analyzed (n = 95)</th>
<th>agAAB negative (n = 55)</th>
<th>β2-AR-agAAB positive only (n = 11)</th>
<th>α1-AR-agAAB positive only (n = 19)</th>
<th>Both agAAB (n = 10)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (female), n (%)</td>
<td>211 (60%)</td>
<td>59 (62.1%)</td>
<td>36 (65.5%)</td>
<td>4 (36.3%)</td>
<td>11 (57.9%)</td>
<td>8 (80%)</td>
<td>0.204*</td>
</tr>
<tr>
<td>Age, mean (SD) MMSE</td>
<td>80.2 (5.7)</td>
<td>79.8 (4.8)</td>
<td>79.4 (4.6)</td>
<td>78.7 (5.8)</td>
<td>80.4 (4.8)</td>
<td>82.1 (4.2)</td>
<td>0.311 b</td>
</tr>
<tr>
<td>Score, mean (SD)</td>
<td>22.3 (5.2)</td>
<td>21.9 (5.0)</td>
<td>21.2 (5.0)</td>
<td>22.9 (4.8)</td>
<td>22.7 (5.5)</td>
<td>24.2 (3.4)</td>
<td>0.243 b</td>
</tr>
<tr>
<td>None to mild (score 20–30), n (%)</td>
<td>238 (73%)</td>
<td>70 (73.7%)</td>
<td>39 (70.9%)</td>
<td>8 (72.7%)</td>
<td>14 (73.7%)</td>
<td>9 (90%)</td>
<td></td>
</tr>
<tr>
<td>Moderate (score, 10–19), n (%)</td>
<td>78 (23.9%)</td>
<td>25 (26.3%)</td>
<td>16 (29.1%)</td>
<td>3 (26.3%)</td>
<td>5 (26.3%)</td>
<td>1 (10%)</td>
<td></td>
</tr>
<tr>
<td>Severe (score, 0–9), n (%)</td>
<td>10 (3.1%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0.563*</td>
</tr>
<tr>
<td>Formal diagnosis of dementia, n (%)</td>
<td>134 (38.6%)</td>
<td>38 (40%)</td>
<td>23 (41.8%)</td>
<td>6 (54.5%)</td>
<td>5 (26.3%)</td>
<td>4 (40%)</td>
<td>0.473*</td>
</tr>
<tr>
<td>Formal diagnosis of CHD, n (%)</td>
<td>130 (37.4%)</td>
<td>29 (30.5%)</td>
<td>13 (23.6%)</td>
<td>4 (36.3%)</td>
<td>8 (42.1%)</td>
<td>4 (40%)</td>
<td>0.345*</td>
</tr>
</tbody>
</table>

*p-value from Fisher’s exact test, two-tailed; b p-value from one-factorial ANOVA.

Table 2
Logistic regression analyses of factors associated with the presence of agAAB

<table>
<thead>
<tr>
<th>Ref. cat.</th>
<th>agAAB OR (95%-CI) p</th>
<th>α1-AR-agAAB OR (95%-CI) p</th>
<th>β2-AR-agAAB OR (95%-CI) p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex female</td>
<td>0.58 (0.19–1.77) 0.399</td>
<td>0.98 (0.87–1.11) 0.151</td>
<td>0.81 (0.22–3.07) 0.762</td>
</tr>
<tr>
<td>Age</td>
<td>1.04 (0.93–1.16) 0.543</td>
<td>0.26 (0.04–1.62) 0.953</td>
<td>1.02 (0.74–1.43) 0.875</td>
</tr>
<tr>
<td>Cognitive impairment (MMSE)</td>
<td>1.08 (0.99–1.19) 0.085</td>
<td>1.00 (0.90–1.10) 0.953</td>
<td>1.11 (0.99–1.24) 0.081</td>
</tr>
<tr>
<td>Diagnosis of dementia (ICD-10) yes</td>
<td>0.76 (0.28–2.03) 0.585</td>
<td>0.35 (0.06–2.21) 0.264</td>
<td>1.70 (0.07–39.69) 0.742</td>
</tr>
<tr>
<td>Coronary heart disease (ICD-10) yes</td>
<td>2.74 (1.00–7.49) 0.049</td>
<td>4.23 (1.50–11.96) 0.006</td>
<td>1.58 (0.12–20.21) 0.726</td>
</tr>
</tbody>
</table>

Results from mixed logistic regression models with random effects for the general practitioner; MMSE, Mini-Mental State Examination.

DISCUSSION

The prevalence of agAAB in our sample of a community-based group of subjects with dementia was slightly lower than in the sample of people with dementia recruited for treatment in a clinical setting [10]. In our sample, 42% tested positive for any antibody, in contrast to a proportion of 50% in the clinical sample. Specifically, in our sample, 31% were positive for α1-AR-agAAB with 34% also harboring β2-AR-agAAB compared to frequencies of 44% and 73%, respectively, in the clinical sample. Restricting the analyses to mild to moderate cases of dementia in the clinical sample raised the prevalence of agAAB [10]. The high prevalence of agAAB in patients with dementia suggests a potential causal relationship. In contrast, there was no association between the severity of cognitive impairment and the frequency of AABs in our analyses. It seems likely that several additional factors are required to render the agAAB pathogenic, including the leakiness of the blood-brain barrier, inflammatory mediators, and antibody affinity. This would fit well into the increasingly recognized model of a “second hit” in patients with antibody-mediated diseases of the brain. In the present study, we were able to find an association between the presence of α1-AR-agAAB and prevalent coronary heart disease in patients with dementia. This is in line with the current literature indicating that α1-AR-agAAB may contribute to vascular lesions and increased plaque formation in people with dementia [13] and with research based on rat models indicating that the pathological significance of these antibodies in pathologies of the human central nervous system are linked to impairments of brain vasculature, such as stroke and dementia [14].

The role of agAAB against β2-AR is not understood. As shown by Ni et al. [24], the activation of β2-AR by the selective agonist clenbuterol stimulates γ-secretase and enhances the production of amyloid-β 40 and 42. It can be assumed that agAAB against β2-AR may act as an agonist and stimulate...
amyloid-β production in a similar way. It cannot be excluded that these antibodies are also involved in the pathomechanisms of increased amyloid-β release. Further experiments must be performed to demonstrate that agAAB against β2-AR activate the target cells and intracellular cascade-like agonists.

Immunoadsorption as therapy has shown its efficacy in diseases where there is a clear causal relation between the disease and the antibody (for example: cardiomyopathy). In dementia, one pilot study reported positive results when targeting agonistic autoantibodies (agAAB) directed against G-protein-coupled receptors (GPCR). However, the sample size was very small and selective, and only eight patients out of 31 positive for autoantibodies underwent immunoabsorption [14].

There are limitations to our analyses that might reduce the generalizability of our results. First, there might be a selection bias in the sample under analysis. While 348 subjects were identified as eligible for blood sampling, only 1/3 of them were included in the analyses. Approximately a third declined to participate and a quarter dropped out. However, drop-out analyses of the total study did not reveal any selection bias, and the high proportion of people declining to participate can be attributed to the design of the DelpHi-study, whose focus was not the collection of biosamples but rather an intervention close to routine care. Second, the sample under analysis consisted of people who screened positive for dementia, and the formal diagnosis of dementia was collected from the medical record of the GP. We neither controlled for nor conducted guideline-oriented diagnostics for our trial. This is problematic in two ways: 1) We do not know the rate of false positives is in our sample. We might have underestimated or overestimated the prevalence in people with dementia, and this might explain differences in the data reported in other studies. However, the presence of agAAB is not specific to people with dementia, and our aim was to estimate the presence of the biomarker in people affected by dementia in routine care. 2) We were not able to analyze our results according to the type of dementia. Previous analyses have shown that the majority of the sample had received a formal diagnosis of “unspecified dementia” (F03:53–69% [25–27]). There were too few people having been diagnosed with, for example, “vascular dementia” (F01:17–24% [25–27]). Further studies that analyze the association between dementia and these biomarkers should focus on different types of dementia diagnosed according to the gold standard. Our study has reached its goal by describing the prevalence in the GP-based population, underlining the need for further studies.

Conclusions

This is the first study reporting data on the high prevalence of agAAB, α1-AR-agAAB, and β2-AR-agAAB in patients with dementia identified in a routine care setting. Previous findings were restricted to highly selective samples. We identified an association between α1-AR-agAABs and prevalent coronary heart diseases but no associations with other demographic or psychopathological factors.

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