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Association between DRD2/ANKK1 TaqIA Allele Status and Striatal Dopamine D2/3 Receptor Availability in Alcohol Use Disorder

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Abstract

Background: The association between blunted dopaminergic neurotransmission and alcohol use disorder (AUD) is well-known. In particular, the impairment of postsynaptic dopamine 2 and 3 receptors (DRD2/3) in the ventral and dorsal striatum during the development and maintenance of alcohol addiction has been investigated in several positron emission tomography (PET) studies. However, it is unclear whether these changes are the result of adaptation or genetic predisposition. **Methods**: Here we investigated the association between *DRD2*/ankyrin repeat and kinase domain-containing 1 (*ANKK1*) TaqIA allele (*rs1800497*) status and striatal DRD2/3 availability measured by 18F-fallypride PET in 12 AUD patients and 17 sex-matched healthy controls. Age and smoking status were included as covariates. **Results**: Contrary to our expectations, TaqIA allele status was not associated with striatal DRD2/3 availability in either group and there was no significant difference between groups, possibly due to the relatively small sample size (N = 29). **Conclusions**: Nonetheless, this is the first *in vivo* study investigating the relationship between dopamine receptor availability and genetic factors in AUD. The pitfalls of assessing such relationships in a relatively small sample are discussed. **Clinical Trial Registration**: The published analysis is an additional, post hoc analysis to the preregistered trial with clinical trial number NCT01679145 available on https://clinical-trials.gov/ct2/show/NCT01679145.

Keywords: alcohol use disorder; dopamine D2 and D3 receptor availability; DRD2/ANKK1 TaqIA allele status; 18F-fallypride PET

1. Introduction

Alcohol use disorder (AUD) is a complex and chronic disorder with high costs for society. The pathogenesis of AUD involves an interplay of social, individual, and biological factors; approximately 50% of the etiology of alcohol dependence is attributed to genetic influence [1].

Chronic alcohol intake is associated with lower dopamine receptor availability. In particular, reduced availability of dopamine D2 and D3 receptors (DRD2 and DRD3, respectively) in the striatum of patients with alcohol dependence compared to healthy controls (HCs) has been demonstrated by several positron emission tomography (PET) studies [2–9] and described in recent reviews [10,11]. However, it is unclear whether these changes reflect neuroadaptations to excess acute striatal dopamine release during regular substance use or are the result of genetic predisposition for substance use disorder. Several potential contributing genetic factors have been discussed in the literature. The Taq1A polymorphism of the dopamine D2 receptor *DRD2* gene is a SNP (*rs1800497*) giving rise

to the *DRD2* allele (*A2, rs1800497 (C)*). The minor allele (*A1, rs1800497 (T)*) has been associated with a reduction of dopamine receptor availability and therefore been suggested to contribute to the development of substance use disorders also other mental disorders [12–15].

More precisely, the minor allele (*A1, rs1800497 (T)*) has been linked to alcohol dependence in several studies [16–19] although the effect size was found to be small [20].

Inconsistencies in published results have cast doubt on the functional relevance of the *DRD2/ANKK1 TaqIA* allele in AUD, as there is limited evidence that *rs1800497* affects DRD2 role. A recent meta-analysis of 62 studies including 16294 participants found that the positive association between DRD2 and alcoholism was due to low allele frequencies in control subjects rather than changes in *DRD2* gene expression [21]. On the other hand, single nucleotide polymorphism (SNP) of the *DRD2/ANKK1* (*rs1800497*) *TaqI* allele was shown to be linked to reduced striatal DRD2/3 availability [22], which has also been reported in obesity, schizophrenia, and schizoaffective disorder [23]. Addition-

ally, *TaqIA* allele status was found to be associated with higher DRD2/3 availability in patients with major depressive disorder and lower availability in HCs [24].

To date there have been no *in vivo* studies investigating DR availability and *DRD2/ANKK1 TaqIA* allele status in AUD, except for a postmortem study reporting a link between the binding characteristics of DRD2 in the cerebral cortex and AUD [16]. We previously showed that dorsostriatal DRD2/3 availability was reduced in AUD patients and individuals at high risk for developing AUD compared to HCs [3].

With the present study we aim to examine the *DRD2/ANKK1 TaqIA* allele status to investigate if the DRD2/3 availability (measured by 18F fallypride-PET) is moderated by the presence of the *minor allele (A1, rs1800497 (T))* and whether this relationship depends on group affiliation (AUD versus healthy controls).

2. Materials and Methods

This study was part of a multicenter project investigating behavioral, genetic, and neuroimaging changes associated with reward-based learning and its relevance to the maintenance of alcohol dependence (http://www.lead-studie.de; clinical trial number NCT01679145). The study was approved by the local ethics committee (Charité–Universitätsmedizin Berlin; EA1/245/11) and all subjects gave written, informed consent before participating. Recruitment took place between 2014 and 2016. The main objective was to investigate striatal DRD2/3 availability by PET as well as glutamate level in the prefrontal cortex by magnetic resonance imaging (MRI) in patients with or at high risk of developing alcohol dependence, as well as in HCs. As we also planned to explore the neurochemical changes in AUD with potential predisposing genetic factors, blood samples were collected from the study participants for biochemical analysis and genotyping. The specific research question of the association between *DRD2/ANKK1 (rs1800497) TaqI allele* status and DRD2/3 availability was evaluated in a post hoc analysis. The assessed samples constituted a subsample of the above mentioned study cohort for which PET and genetic data were available.

2.1 Sample

The sample consisted of 17 HCs and 13 detoxified, abstinent patients diagnosed with AUD. One male AUD patient had to be excluded during the process because he showed an extremely unlikely value for receptor availability at limbic striatum (<1). We therefore considered the entire measurement invalid and excluded all data points for that participant. Therefore, the final sample consisted of 12 diagnosed AUD patients.

Subjects were matched for age, sex, education, handedness, and smoking status (Table 1, Ref. [25–27]). AUD patients were recruited from hospitals in Berlin and had

met Diagnostic and Statistical Manual for Mental Disorders, 4th Edition (DSM-IV) criteria for AUD for at least 3 years (American Psychiatric Association, 2000) prior to the start of the study. The patients were diagnosed at the participating institutions by a trained clinician and their diagnosis was later confirmed with the Composite International Diagnostic Interview (CIDI) [28,29] during testing. AUD patients had been abstinent for about 34.4 days and at least 72 h when they were tested, had mild withdrawal symptoms (Clinical Institute Withdrawal Assessment for Alcohol score <3), and were free of any psychotropic medication for at least 4 half-lives [30]. There was one outlier in the AUD group with an abstinence duration of 96 days, which did not influence the results. HCs were recruited via local online platforms and did not meet DSM-IV criteria for AUD in the standardized telephone screening, which was confirmed with the CIDI.

All study participants with any other current substance use or dependence apart from alcohol and nicotine were excluded from the study. Healthy controls were instructed not to drink any alcoholic beverages in the 24 h before PET scanning. Compliance was verified by urine screening and breath alcohol tests. Participants with any contraindications for MRI, current pregnancy or nursing, or any neurologic or major psychiatric disorder according to DSM-IV criteria were also excluded from the study [26,27,31].

The study consisted of 3 separate testing sessions. In the first session, participants were asked to complete clinical questionnaires such as the Alcohol Dependence Scale or Obsessive Compulsive Drinking Scale, and underwent blood sampling for the genetic analyses MRI and PET scanning took place in the second and third sessions, respectively [25,26].

2.2 DNA Extraction and Genotyping

DNA was semi-automatically extracted from whole blood with the Chemagen Magnetic Separation Module (Perkin Elmer, Boston, MA, USA). Genotyping was performed with the Infinium Psych Array Bead Chip (Illumina, San Diego, CA, USA). Three subjects (two male AUD subjects and one female healthy control) were excluded from the final sample as outliers in the genome-wide association study. We examined the status of the *rs1800497* SNP, which is also known as the *TaqIA* polymorphism of the *DRD2* gene. The minor allele is a C-to-T substitution (i.e., *A1, rs1800497[T]*) that is associated with a reduced number of dopamine binding sites in the brain and is thought to play a role in alcoholism, smoking, and certain neuropsychiatric disorders [16]. As there are 2 different allele types, 3 genotypes were possible (*A1/A1, A1/A0,* and *A0/A0*). Because of the very limited prevalence of *A1/A1* in the HC (n = 0) and AUD (n = 1) groups, we pooled *A1/A1, A1/A0,* and *A1* into a single group. The allele frequencies are shown in Table 2.

Table 1. Sample characteristics (n = 29).

Variable	Group		p value for test statistic
	AUD (n = 12)	HC (n = 17)	
Demographic characteristics			
Sex, no. of females/males	1/11	2/15	0.77[c]
Education, years	15.1 (3.6)	14.8 (3.3)[§]	0.62[a]
Age, years	44.5 (9.3)	45.4 (8.9)	0.80[b]
Smoker, %	83	59	0.16[c]
Clinical characteristics			
Duration of abstinence, days	34.4 (23.6)	-	-
Severity of alcohol dependence[†]	17.7 (5.8)	3.5 (3.8)	<0.0001*[a]
Craving[‡]	13.7 (8.0)	3.8 (3.3)	<0.0001*[a]

Data are shown as mean (standard deviation) unless otherwise indicated.

[†]Determined using the Alcohol Dependence Scale [25].

[‡]Determined using the Obsessive Compulsive Drinking Scale [26,27].

[§]Information was not available for 1 subject.

*Significant difference; [a]Wilcoxon rank-sum test; [b]t test; [c]χ^2 test.

Abbreviations: AUD, alcohol use disorder; HC, healthy control.

Table 2. Means and standard deviations of 18F-fallypride BP_{ND}[a].

	Associative striatum	Limbic striatum	Sensorimotor striatum
HC–A− (n = 7)	24.58 (2.77)	24.39 (1.85)	30.75 (2.02)
HC–A+ (n = 10)	24.33 (3.18)	24.56 (2.84)	29.03 (2.55)
AUD–A− (n = 8)[b]	19.74 (3.90)	22.66 (2.06)	25.24 (2.92)
AUD–A+ (n = 4)	22.56 (1.08)	23.55 (1.61)	27.57 (0.51)

*Data are shown as mean (standard deviation).

Abbreviations: AUD, alcohol-use disorder; A−, no risk allele; A+, at least 1 risk allele; HC, healthy control.

[a]BP_{ND} estimates the ratio of DRD2/3-bound 18F-fallypride to nondisplaceable 18F-fallypride at equilibrium.

[b]One AUD subject was excluded for extremely low means (m = 0) of 18F-fallypride BP_{ND} in the limbic striatum.

2.3 MRI and PET Data Acquisition and Processing

For MRI, T1-weighted images were acquired using a 3 Tesla Verio scanner (Siemens, Munich, Germany) with a magnetization prepared rapid gradient echo (MPRAGE) sequence (isotropic resolution = 1.0 mm, repetition time = 2.3 s, echo time = 3.03 ms, inversion time = 900 ms, and flip angle = 9°).

PET scanning was performed using a time-of-flight PET/computed tomography (CT) system (Gemini TF 16; Philips Medical Systems, Amsterdam, The Netherlands) [32]. Dynamic PET imaging was started simultaneously with intravenous injection of 18F-fallypride [33,34]. Data were acquired over 4 h in 3 blocks with breaks in between. A low-dose CT scan for attenuation correction was performed before each block [35]. PET images were reconstructed using the iterative line of response–row action maximum likelihood algorithm of the scanner software with default parameter settings (3 iterations, 33 subsets, and "normal" relaxation). Head motion during the PET acquisition was corrected frame-by-frame using the realign tool of the Statistical Parametric Mapping software package [36]. Thereafter, all PET frames were coregistered to each subject's MPRAGE MRI.

The 2-step simplified reference tissue method was used to obtain voxel-by-voxel parametric maps of the nondisplaceable binding potential (BP_{ND}) of 18F-fallypride. This method reduces statistical noise using a global rate constant of tracer clearance from the reference region [37]. BP_{ND} estimates the ratio of DRD2/3-bound 18F-fallypride to nondisplaceable 18F-fallypride at equilibrium [38]. The superior longitudinal fasciculus as defined by Johns Hopkins University Laboratory of Brain Anatomical MRI [39] was used as a reference region to maximize the statistical power [40]; the cerebellum was considered unsuitable as a reference region given the cerebellar atrophy that occurs in AUD [41].

Each subject's T1 images and BP_{ND} map were normalized to Montreal Neurological Institute (MNI) anatomic space using the unified segmentation approach [42]. We used the limbic, associative, and sensorimotor striatum as regions-of-interest (ROIs) predefined in MNI space [43,

44]. The BP_{ND} for each ROI was averaged between the left and right hemispheres to reduce the number of comparisons for primary analyses and because we did not have a priori hypotheses about laterality effects. We included age and smoking status as covariates in our analyses as both of these variables were shown to be independently associated with altered dopamine receptor availability [7,45]. One AUD subject was excluded from further analysis due to extremely low means (m = 0) of 18F-fallypride BP_{ND}.

2.4 Statistical Analysis

We used R (R 4.0.0, Bell Laboratories, Murray Hill, NJ, USA) and lme4 (Version: 1.1-25, https://cran.r-pro ject.org/web/packages/lme4/index.html) to perform linear mixed effects analyses of the relationship between SNP status and DRD2/3 availability across prespecified striatal regions. We entered phenotype (AUD patients versus HCs) and SNP status (risk SNP versus no risk SNP) into the model as fixed effects; intercepts for subjects and brain site were entered as random effects. Visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality. The *p* values were obtained by likelihood ratio tests of the full model with versus without the effect in question (maximum likelihood). *DRD2/ANKK1TaqIA genotype* distribution did not significantly differ from Hardy–Weinberg equilibrium ($p > 0.05$, $\chi^2 = 0.723$, df = 1).

3. Results

Means and standard deviations of 18F-fallypride BP_{ND} for all group combinations are shown in Table 2. The data structures of the 3 ROIs are displayed as boxplots in Fig. 1.

In the basic model (receptor availability ~1 + brain site + phenotype [HC/AUD] + [1 | ID]), 61% (interclass correlation coefficient) of the total variance in receptor availability was accounted for by person clustering. There was a significant improvement in model fit with a nested data structure (logLik $\Delta\chi^2[1]$ = 31.8, $p < 0.0001$); both the Akaike information criterion (AIC) and Bayesian information criterion (BIC) were smaller with this model (AIC: 446.26 versus 416.46; BIC: 456.13 versus 428.79).

As expected, we found a significant main effect of phenotype status (0 = HCs; 1 = AUD patients) ($\chi^2[1]$ = 11.49, $p < 0.001$), indicating that BP_{ND} across brain regions was lower in AUD patients compared to controls. Including *DRD2/ANKK1 TaqIA* allele status did not significantly improve model fit ($\chi^2[1]$ = 0.276, $p = 0.5996$), indicating that the *DRD2/ANKK1 TaqIA* allele does not predict BP_{ND} either alone or through interaction with group.

4. Discussion

The results of this study demonstrate that DRD2/3 availability is reduced in the associative, sensorimotor, and limbic striatum of AUD patients compared to HCs. This supports our hypothesis and is in line with our previous finding of reduced striatal DRD2/3 availability in AUD patients compared to individuals at high risk of AUD and HCs [3,46]. Contrary to our hypothesis we did not find an association between the presence of the minor allele (risk allele) of the *Taq1A* polymorphism of the dopamine D2 receptor *DRD2* gene and striatal DRD2/3 availability in AUD patients versus HCs. This result must be interpreted with caution because of the limited effect size and relatively small sample size.

Several studies have reported a link between *DRD2/ANKK1 TaqIA* allele status (*rs1800497*) and alcohol-related behaviors [16–20]. While *DRD2/ANKK1 TaqIA* allele status and striatal DRD2/3 availability have been investigated in clinical studies [22–24,47] and postmortem samples [48,49] ours is the first study to examine *DRD2/ANKK1 TaqIA* allele (*rs1800497*) and DRD2/3 status in AUD patients *in vivo*. However, a study in patients with schizophrenia found no association between DRD2 availability and *TaqIA* allele status by single photon emission computed tomography [50].

Despite the well-known contribution of dopaminergic impairment to AUD and other substance use disorders [3,5,8,9,51,52], few studies have examined the relationship between DRD2/3 and genetic predisposing factors in AUD, possibly because of power issues in PET studies, which may also have contributed to the null result in this study. Therefore, our findings are noteworthy as other studies might have experienced similar limitations.

Our study population consisted of 12 AUD patients and 17 HCs, which is a typical sample size for PET studies but caused statistical difficulties when calculating allele frequencies in the same sample. Because there were 2 different allele types and hence, 3 genotypes (*A1/A1*, *A1/A0*, and *A0/A0*), the compared groups were relatively small. To minimize the effect of small sample size on the results and because of the lack/very small number of homozygous alleles in the HC (n = 0) and AUD (n = 1) groups, we pooled *A1/A1* and *A1/A0* into a single group. Many other studies have experienced the issue of insufficient data for homozygous alleles, especially in HCs [23,50–52], and have thus been unable to detect between-group differences in the effect of DRD2/3 status for the 3 genotypes of *DRD2/ANKK1 TaqIA rs1800497*, as was the case in our study. Moreover, even after pooling homozygotes and heterozygotes, the compared subgroups were still relatively small, resulting in power issues in the statistical analyses. One way to overcome this limitation in the future is to pool the results of comparable PET studies with genetic data to increase the sample size.

As the molecular mechanism by which *TaqIA* allele status influences DRD2 expression is unknown, the functional relevance of any observed associations must be interpreted with caution. Preclinical studies have reported the regulation of *ANKK1* expression by dopamine [53,54], but no direct interaction has been demonstrated thus far.
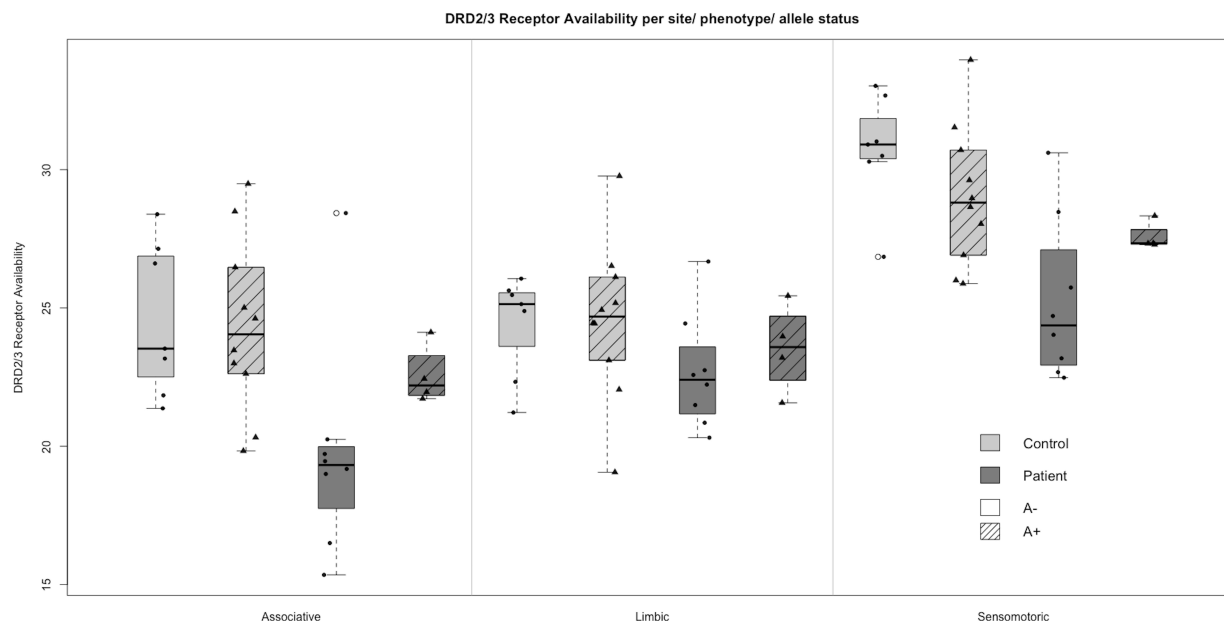
Fig. 1. DRD2/3 Receptor Availability per site/ phenotype/ allele status. Dopamine receptor D2 and 3 availability in the associative, sensorimotor and limbic striatum and allele status in individuals with AUD and HC. The D2/3 availability was measured in BP_{ND} of 18F-fallypride for each of the three different ROIs and shown for patients (AUD) and controls (HC). *A−*, no risk allele; *A+*, at least 1 risk allele.

As described above, low allele frequencies in control subjects may drive associations between *DRD2* gene status and AUD [16]. However, in this study the allele frequency in HCs was comparable to that in AUD patients for the subgroup with no risk allele (A–: HC, n = 8 and AUD, n = 7), and was higher in HCs than in AUDs in the risk allele subgroup (A+: HC, n = 10 and AUD, n = 4).

Another factor potentially contributing to our results was the use of 18F-fallypride as the radioligand; this high-affinity DRD2/3 antagonist is nonselective for DRD2 and may be displaced by endogenous dopamine, although the density of DRD3 in the ventral and especially dorsal striatum is relatively low [55]. Moreover, endogenous dopamine may have influenced data acquisition although there were no stimuli during the scanning and the participants were not actively drinking at the time of the study. The use of specific DRD2 radiotracers such as [11C]NMB may improve the specificity of the results [23].

5. Conclusions

We were able to show that DRD2/3 availability is reduced in the associative, sensorimotor, and limbic striatum of AUD patients compared to HCs. This supports our previous finding of reduced striatal DRD2/3 availability in AUD patients compared to individuals at high risk of AUD and HCs [3,46]. Contrary to our expectations we did not find any association between *DRD2/ANKK1 TaqIA* allele status and striatal DRD2/3 availability, although this result must be interpreted with caution because of the limited effect size and relatively small sample size.

Clarifying the association between *DRD2/ANKK1 TaqIA* allele status and DRD2/3 availability is important for resolving the longstanding controversy of whether dopaminergic impairment in the striatum of individuals with alcohol dependence is the result of compensatory downregulation or genetic predisposition. To this end, additional investigations that combine data from different PET studies are needed to overcome the issue of low statistical power inherent in this type of research.

6. Limitations and Future Perspectives

The main limitation of this study was the issue of the lack of power. This problem stems from our limited sample size (N = 29) which may be appropriate for a PET study but is complicated by investigating three different genotypes in two subgroups (AUD versus HC). Nonetheless, similar studies in this research field, possess comparable sample sizes such as Eisenstein *et al.* (n = 39; n = 18) and Savitz *et al.* (N = 36) [23,24]. Other studies provide larger samples, but investigated healthy controls only such as Pohjalainen *et al.* and Hirvonen *et al.* (both used the same sample of N = 54) [12,51,52]. To be able to evade this issue it is important to pool data between research groups. Therefore, the dataset of this study is available online (https://osf.io/t4brz/) to make it accessible to other researchers of this field.

Author Contributions

GS, JG, TG, MS and EF conceived the study; GS, EF, LF, JG, AH, MS, TG, TZ and RB drafted the manuscript;

GS, EF, LF, TZ, TG and RB analyzed and interpreted the data; LF created the figures; EF and JG supervised the study; TZ, AH, TG, RB, AH and MS revised the manuscript for important intellectual content. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

Clinical Trial Registration

Preprint is available on https://psyarxiv.com/kx9ma; Dataset and analysis are available on https://osf.io/t4brz/; The published analysis is an additional, post hoc analysis to the preregistered trial with clinical trial number NCT01679145 available on https://clinical-trials.gov/ct2/show/NCT01679145.

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Conflict of Interest

The authors declare no conflict of interest.

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