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SYNGAP1 DNA methylation in alcohol dependence and its potential function as a biomarker for diagnostic, disease severity and therapy outcome

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Index of Abbreviations

5-MeC 5-methylcytosine
AD ..... Alcohol dependence
AMPAR ..... α-amino- 3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors
ATP ..... Adenosine triphosphate
AUD ..... Alcohol use disorder, Alcohol Use Disorder Identification Test
AUDIT ..... Alcohol Use Disorder Identification Test
CaMKII ..... calcium/calmodulin-dependent protein kinase II
DALYs ..... Disability Adjusted Life Years
DNA ..... Deoxyribonucleic acid
DNAm ..... DNA methylation
DNAmS ..... DNAm of SYNGAP1
DNMTs ..... DNA methyltransferases
dNTP ..... Desoxyribonucleoside triphosphate
DSM-5 ..... US-American Diagnostic and Statistical Manual of Mental Disorders
EDTA ..... ethylenediaminetetraacetic
GDAP1 ..... Ganglioside-induced differentiation-associated protein 1
GSI ..... Global Severity Index
GxE ..... Gene-enviroment interaction
HPA axis ..... Hypothalamic-pituitary-adrenal axis
ICD-10 ..... International Statistical Classification of Diseases and Related Health Problems – 10th Revision
LTP ..... Long-term potentiation
MDD ..... Major depressive disorder
ncRNA ..... non-coding RNA
NMDAR ..... N-methyl-D-aspartate receptors
OCDS ..... Obsessive-Compulsive Drinking Scale
OPRM1 17
PCR ..... polymerase chain reaction
PPi ..... pyrophosphate
PPM1G ..... 3´-protein-phosphatase-1G
PSD ..... Postsynaptic density
QE ..... Qualifizierte Entzugsbehandlung
R^2 ..... Coefficient of determination
SAM ..... S-adenosyl methionine
SAP ..... Synapse associated protein
SCL-90-R ..... 90-item revised Symptom Checklist
SD ..... Standard deviation
SG31 ..... Subgroup including participants aged 31 years and older
SYNGAP1 ..... Synaptic Ras-GTPase-activating protein
THC ..... Tetrahydrocannabinol

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1 Introduction

1.1 Alcohol use disorder (AUD) and alcohol dependence (AD)

Alcohol is the world´s most prevalent substance of dependence, with 63.5 million estimated cases of alcohol dependence in 2015 (Peacock et al., 2018). In 2012, in Germany, 3.4% of the population aged 18 to 64 suffered from alcohol dependence (Pabst et al., 2013). Three million deaths result from harmful alcohol use in 2016, making up 5.3% of all deaths worldwide (World Health Organization, 2018). In that year, 5.1% of the global DALYs (summing up the years lived with disability and years of life lost) are caused by AUD (World Health Organization, 2018), making alcohol use one of the most important preventable risk factors for global burden of disease (Rehm et al., 2009). Its major health risk is partly based on the large number of physical secondary diseases it causes or promotes, such as different cancer entities and infectious diseases, damage of the cardiovascular and/or gastrointestinal system (Rehm et al., 2017; World Health Organization, 2019). In addition, harmful alcohol use increases the risk of being involved in violence and accidents that lead to injury and death (Morandi et al., 2015; Rehm et al., 2017). Furthermore, AUD is often associated with mental comorbidities such as depression and anxiety, personality disorders and ADHS (Batra et al., 2016; Rehm et al., 2017) contributing to its impairment severity. Economically, the costs caused by alcohol consumption are of substantial relevance. Exemplarily for Germany, they are estimated to sum up to 57 billion Euro per year (Bundesgesundheitsministerium, 2022).

1.1.1 Definition of AD

Diagnostic criteria of alcohol dependence according to the ICD-10 (International Statistical Classification of Diseases and Related Health Problems – 10th Revision) are alcohol craving, difficulty to control the consumption, physical withdrawal symptoms, tolerance, increasing neglect of other activities in order to obtain and to consume alcohol and the persistent consumption of the

substance, even though adverse consequences are experienced (Kiefer et al., 2020). Three of the six criteria must be fulfilled at the same time within the past 12 months (Batra et al., 2016). Clinical observations and laboratory markers can provide further indications for a harmful alcohol use (Batra et al., 2016).

The term AUD is used in the US-American Diagnostic and Statistical Manual of Mental Disorders (DSM-5). The DSM-5 considers alcohol abuse/harmful alcohol use and alcohol dependence to be different dimensions on the same disorder continuum, whereas ICD-10 defines them as separate diseases. In this study, ICD-10 criteria were applied. However, literature also applies AUD criteria so that both AD and AUD are named in the present study when referring to the literature.

1.1.2 Etiology of AD

The etiology of AD/AUD is complex and presents a subject of diverse current research. In a meta-analysis of five adoption and 12 twin studies, AUD´s genetic heritability was estimated to account 50% (Verhulst et al., 2015) and a number of genes of susceptibility and resilience-related genes have been discovered (Egervari et al., 2021). Environmental factors such as influence of the peer group, availability of alcohol, and age at first drink have been described as moderators of the development of dependence (Rietschel & Treutlein, 2013). Epigenetic mechanisms play a pivotal role for gene-environment interactions (GxE) and will be outlined in Chapter 1.2 and 1.3.

Alcohol affects the neurotransmitter systems of dopamine, GABA, glutamate, serotonin, endogenous cannabinoids and opioids such as systems of neuroendocrine allostasis (Kranzler & Soyka, 2018).
Addiction and its neurological changes result in a vicious circle consisting of intoxication, withdrawal, and craving ((Koob & Volkow, 2016), Figure 1). The dependent individual´s brain underlies neuroplastic changes, also referred to as neuroadaptation. Neuroadaptation happens due to conditioned learning

processes as well as direct substances´ effect (Batra et al., 2016). Alcohol influences transcriptional activity, alternative splicing, protein translation, neuromodulators, and epigenetic regulation in the brain (Egervari et al., 2021). In consequence, circuitries that physiologically regulate stress, motivation, and mood are altered, possibly favoring dysfunctional reward seeking behavior and facilitating relapse (Batra et al., 2016; Koob & Volkow, 2016; Seo & Sinha, 2014). Moreover, withdrawal causes an imbalance in the altered neurotransmitter allostasis with adverse symptoms (vegetative symptoms, dysphoria, anxiety) favoring recurring drinking (Batra et al., 2016; Koob & Volkow, 2016).

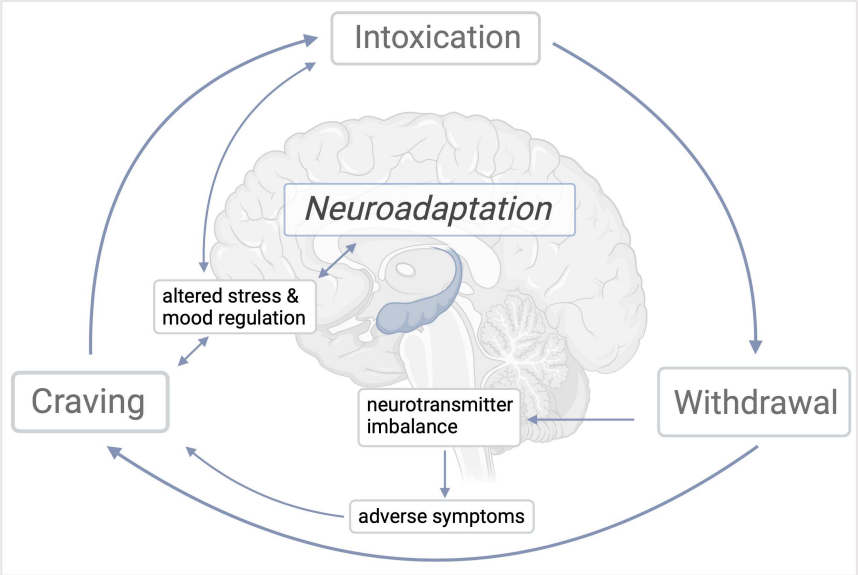


Figure 1 The vicious circle of addiction. *Alcohol influences neuronal circuitries, affecting the regulation of stress and mood, favoring craving and drug-seeking behavior (Batra et al., 2016; Koob & Volkow, 2016; Seo & Sinha, 2014). Withdrawal causes an imbalance in the neurotransmitter systems, leading to adverse symptoms and drinking (Batra et al., 2016; Koob & Volkow, 2016). Neuroadaptation leads to an imprint of this dysregulation in the brain. Created with BioRender.com.*

1.1.3 Therapy of AD and its challenges

The so-called qualified withdrawal treatment (in German: qualifizierte Entzugsbehandlung, short: QE) represents the S3 guideline-recommended therapy for AD (Deutsche Gesellschaft für Psychiatrie und Psychotherapie, Psychosomatik und Nervenheilkunde & DG Sucht, 2020). Besides the physical

detoxication, QE patients are provided educational insights into the disease. Furthermore, the diagnostic and therapy of psychiatric and somatic comorbidities are taken into account (Batra et al., 2016). Essential aim of this treatment program is the stabilization of abstinence by offering interdisciplinary therapies and referring to longer-lasting support systems after the discharge, e.g., social or medical rehabilitation (Deutsche Gesellschaft für Psychiatrie und Psychotherapie, Psychosomatik und Nervenheilkunde & DG Sucht, 2020).

Common withdrawal symptoms of alcohol are nausea, perspiration, hypertension, tremor, depressed mood, and anxiety. Seizures and delirium tremens are severe and potentially lethal consequences and their prevention is a focus in withdrawal treatment (Witkiewitz et al., 2019). The gold standard for pharmacotherapy of withdrawal symptoms are benzodiazepines (Batra et al., 2016; Witkiewitz et al., 2019). Pharmacotherapy for relapse-prevention with different mechanisms of action exist, such as Acamprosate, Nalmefene and Disulfiram (Kranzler & Soyka, 2018; Lanz et al., 2023; Plosker, 2015). Relatively new paths in psychiatric therapies are being tested in AD (e.g., neuromodulating techniques such as deep-brain stimulation and neurofeedback) though reliable evidence is lacking to this day (Witkiewitz et al., 2019).

Worldwide, only one in six individuals with AUD (including alcohol abuse, AD and AUD) receives any treatment, with a gradient from high to low-income countries (Mekonen et al., 2021). 85% of those undergoing a detoxification experience a relapse if no following treatment is admitted after withdrawal (Batra et al., 2016). Relevant factors promoting relapse include older age, impaired physical health, craving, negative emotions and stress (Sliedrecht et al., 2019). The psychological components of the dependence – conditioning, habits, and the functionality of the alcohol consumption – might add to the relapse risk as well as adverse withdrawal symptoms and mental comorbidities (Batra et al., 2016).

1.2 Epigenetics

Epigenetics describes the – reversible – modulation of genomic transcription and expression and, in consequence, gene function without changing the Deoxyribonucleic acid (DNA) sequence itself (Edelmann et al., 2025). Genotypically equivalent cells can thereby vary considerably in phenotypes (Novik et al., 2002). Epigenetic mechanisms are transgenerationally transmittable (Fitz-James & Cavalli, 2022).
These modulations differ physiologically between individuals. Potential reasons for disparities are various and include age, sex, ethnicity (Seale et al., 2022; Solomon et al., 2022; Zhang et al., 2011) and inheritance (Chong & Whitelaw, 2004). Already during the fetal period, factors like maternal nutrition and exposure to noxious substances passing the placental barrier affect the organism through epigenetic mechanisms (Romani et al., 2015). Furthermore, changes in epigenetics occur over a lifetime, due to aging (Fuke et al., 2004; Seale et al., 2022) or environmental factors such as diet (Feil & Fraga, 2012), chemical pollutants (Romani et al., 2015) or toxic substances, e.g. tobacco smoke (Breitling et al., 2011) or alcohol (Longley et al., 2021). Several mechanisms are known to contribute to this plasticity of genome. To the most studied ones belong DNA methylation (DNAm), histone modification, chromatin remodeling and non-coding RNA (ncRNA)(Figure 2 (Romani et al., 2015)) which are tightly interlinked.

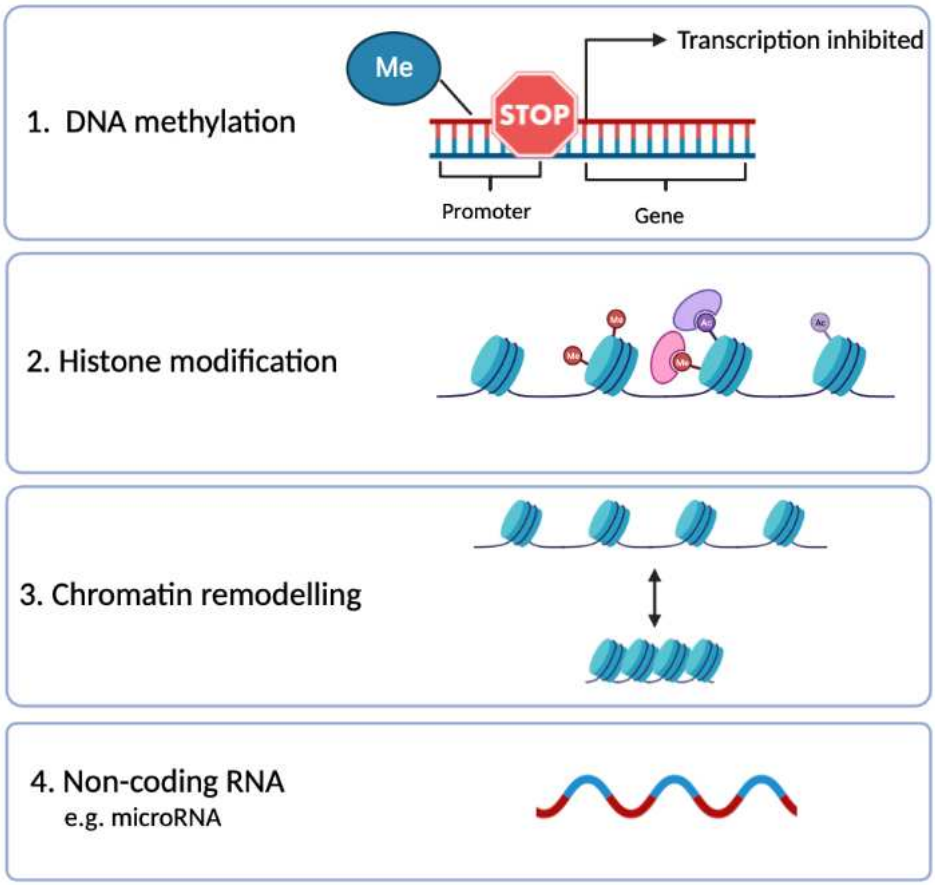


Figure 2 Four mechanisms of epigenetic modifications. *DNA methylation, histone modification, chromatin remodeling and non-coding RNA belong to the most studied epigenetic mechanisms. They are tightly interlinked and modulate reversibly the genomic transcription (Novik et al., 2002; Romani et al., 2015). Created with BioRender.com.*

DNAm (Figure 2.1) is, to date, the most-studied epigenetic modification (Campagna et al., 2021). A methyl group of the methyl donor S-adenosyl methionine (SAM) is added to cytosine nucleotides by DNA methyltransferases (DNMTs), forming a 5-methylcytosine (5-MeC, Figure 3).

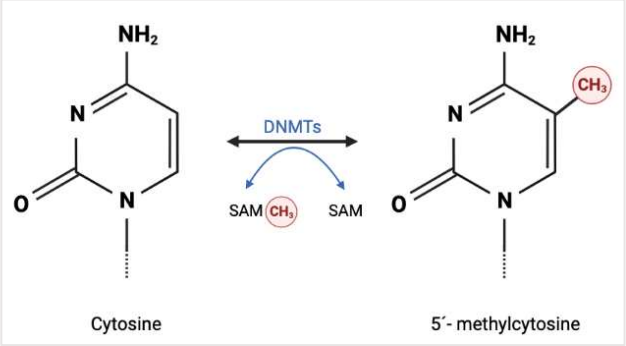


Figure 3 DNA methylation. *A methyl group (CH_3) is covalently added by DNMT by the methyl donor SAM to the cytosine. Created with BioRender.com.*

DNAm takes place more often than not at CpG sites, that is, DNA regions where guanin nucleotides follow cytosine nucleotides (Novik et al., 2002). CpG sites can be found in intra- or intergenic regions and in promotor regions of the DNA (Romani et al., 2015). DNAm is a rather stable epigenetic modification; DNMTs maintain methylation levels but can, however, also lead to new methylations (Romani et al., 2015). DNAm interacts with transcription factors, both attracting them and being attracted by them, regulating gene expression negatively or positively, also depending on where the DNAm is located, and lead to transcription elongation and modulate splicing (Brenet et al., 2011; Kaplun et al., 2022; Li & Tollefsbol, 2021). DNA methylation is essential for cell specificity in identity and function and embryonic development (Li & Tollefsbol, 2021). Significantly altered methylation patterns can be found in a number of sicknesses as in cancer (Nishiyama & Nakanishi, 2021; van Engeland et al., 2003) or psychiatric disorders such as schizophrenia (Magwai et al., 2021; Mahgoub & Monteggia, 2013), depression (Mahgoub & Monteggia, 2013), and substance use disorders (Andersen et al., 2015; Mahgoub & Monteggia, 2013).

1.3 Epigenetics in AD

Epigenetics have a major influence on the development and maintenance of AD/AUD. NcRNA show distinctly altered expression of ncRNA in several pathways and interactions (Zhu et al., 2022). Histone modifications have been

suggested to be associated with alcohol seeking and depressive symptoms during withdrawal in rodent models (Chen et al., 2019; Simon-O'Brien et al., 2015; Warnault et al., 2013).

AD is associated with altered DNAm (Longley et al., 2021). Several single genes have been identified as differentially methylated in AD/AUD patients, e.g. the serotonin transporter in women but not in men (Philibert et al., 2008), *Ganglioside-induced differentiation-associated protein 1* (*GDAP1*) (Brückmann et al., 2016), *3´-protein-phosphatase-1G* (*PPM1G*) (Ruggeri et al., 2015) and the *μ-opioid receptor* gene (*OPRM1*) (Zhang et al., 2012). Differentially methylated CpG sites within networks of genes involved in immunological and inflammatory functions have been discovered in AUD/AD patients (Liu et al., 2018; Lohoff et al., 2021; Longley et al., 2021). In AD mice, DNA methyltransferase inhibitors reduced alcohol intake (Barbier et al., 2015; Warnault et al., 2013). Although a lot of progress has been made in identifying affected pathways, single genes and networks, distinct results that could signify the function of a biomarker or therapy target have been scarce.

A limited number of studies investigated DNAm in alcohol-dependent patients throughout withdrawal treatment (Philibert et al., 2014; Witt et al., 2020). In our research group, Brückmann et al. conducted in 2017 a genome-wide analysis of DNAm in $CD3^+$ T-cells of AD patients before and after undergoing QE, using the Illumina 450 K array. They discovered that, before treatment, patients had a lower global DNAm level compared to control individuals which approximated those of controls after treatment (Brückmann et al., 2017). In addition, a set of 59 CpG sites was identified as differentially methylated between controls and patients. Of these, seven approximated the controls´ DNAm level after therapy, among them *Synaptic Ras-GTPase-activating protein* gene (*SYNGAP1*) (Brückmann et al., 2017). These findings on the dynamics of DNAm of *SYNGAP1* suggested a promising linkage between its biological function and AD. Furthermore, a possible usage of its DNAm in the diagnostic and monitoring of AD was implicated.

1.3.1 The physiological function of SYNGAP1

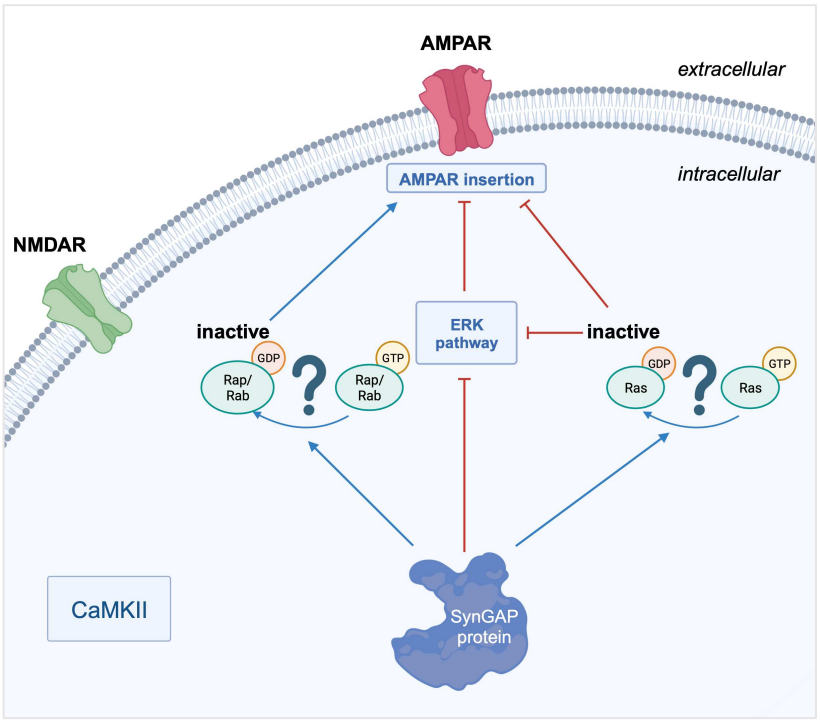
The *SYNGAP1* gene encodes for the **syn**aptically localized **G**TPase-**a**ctivating **p**rotein, that is SynGAP protein, first characterized in 1998 (Chen et al., 1998; Kim et al., 1998). *SYNGAP1* expression focuses on the brain, especially the cortex and hippocampus (Araki et al., 2020), although low levels are also detectable in other tissues like lung and kidney (Chen et al., 1998). It is localized primarily in the postsynaptic density (PSD) of excitatory neurons, being interlinked in synapse associated protein (SAP) families and in networks involving N-methyl-D-aspartate receptors (NMDAR) (Kim et al., 1998). The PSD is an area on the postsynaptic membrane enriched with proteins of signaling and scaffolding (Kong et al., 2022).

The activity of SynGAP is controlled by the calcium/calmodulin-dependent protein kinase II (CaMKII) (Chen et al., 1998) which is activated by inflowing calcium through NMDA receptors (Chen et al., 1998; Yamauchi, 2005). SynGAP regulates the insertion of α-amino- 3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR) – and thus the induction of long-term potentiation (LTP) – presumably including the regulation of the small GTP-binding proteins Ras, Rap and Rab and the ERK pathway (Figure 5) (Kilinc et al., 2018; Kim et al., 1998; Krapivinsky et al., 2004; Rumbaugh et al., 2006; Wang et al., 2013; Zhu et al., 2002). It furthermore acts on a spatial level, dispersing from dendritic spines after neuronal activation and promotes LTP through impacting the cytoskeleton and size of dendritic spines (Araki et al., 2015; Koeberle et al., 2017).
Alternative transcriptional start sites, posttranslational modifications such as splicing (Araki et al., 2020; Gamache et al., 2020) lead to several structural isoforms of SynGAP which are specific in function, abundance during brain development and brain regions (Araki et al., 2020; McMahon et al., 2012).

In summary, SynGAP is imbedded in a complex network of signaling cascades with numerous set screws regulating AMPAR membrane insertion and synaptic plasticity. It fulfills both signaling in neurotransmitter signaling, morphology of

synapses and scaffolding of protein networks. SynGAP promotes AMPAR insertion and LTP induction in activated neurons while providing for a reduced/ stable number of AMPARs during baseline activity.

a)



b)

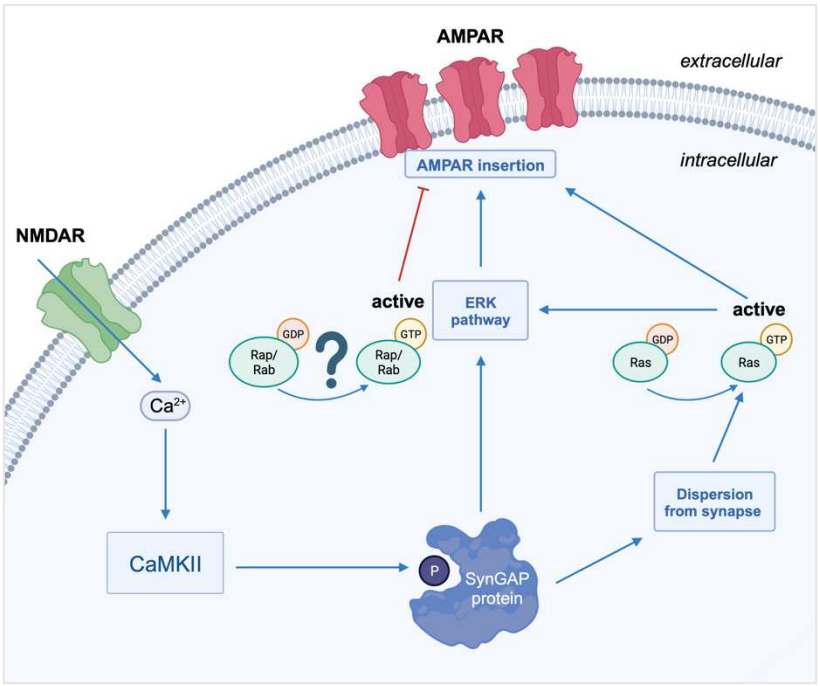


Figure 4 Molecular function of SynGAP protein *during a) baseline activity and b) neuronal activation. SynGAPs activity is controlled by the CAMKII (Chen et al., 1998) which is activated by inflowing Ca^{2+} through NMDA receptors (Yamauchi, 2005). SynGAP regulates, via various intracellular signal cascades and spatial conformation changes, the AMPAR-insertion and thus LTP-induction (Araki et al., 2015; Kilinc et al., 2018; Kim et al., 1998; Koeberle et al., 2017; Krapivinsky et al., 2004; Rumbaugh et al., 2006; Wang et al., 2013; Zhu et al., 2002). Created with BioRender.com.*

1.3.2 SYNGAP1 in disease

Loss-of-function variants of *SYNGAP1* mutations lead to mild to severe intellectual disabilities, often associated with autism spectrum disorder, epilepsy, and other neurodevelopmental variations (Araki et al., 2020) such as innate risk-taking behavior (Kilinc et al., 2018).
SYNGAP1 seems to be crucial for the early neuronal development by negatively regulating excitatory neurons (Llamosas et al., 2020). A disturbed *SYNGAP1* function in early neuronal development is assumed to cause long-lasting synapse dysmorphia, resulting in a deranged ability of balancing excitation and inhibition in neuronal networks (Ozkan et al., 2014). *SYNGAP1* knockout in mice results in impaired LTP and memory (Araki et al., 2015). Kim et al. reported death of SynGAP mutant mice by P7 (Kim et al., 2003).
DNAm of *SYNGAP1* showed significant correlations with IQ scores in a study examining changes in DNA methylation after malnutrition in early childhood (Peter et al., 2016).

In the past years, associations between *SYNGAP1* and mental and physical disorders have emerged. Its DNAm (though analyzed at a different CpG site than in our study) was differential in relation to serum lipid profiles (Sayols-Baixeras et al., 2016). In human hepatocellular cancer, *SYNGAP1* expression was upregulated (Calvisi et al., 2011). *SYNGAP1* has been identified as differentially expressed in the anterior cingulate cortex of mice with depression (Wei et al., 2021) and concerning schizophrenia, *SYNGAP1* was suggested as a susceptibility single nucleotide polymorphism (SNP) (Niu et al., 2019).
Recently, links between *SYNGAP1* and substance use disorder have been reported. Qvist et al. discovered THC(Tetrahydrocannabinol) -induced changes of SynGAP protein in the amygdala and pre-frontal cortex of male adolescent rats (Qvist et al., 2022).
Kong et al. in 2022 firstly described a connection between alcohol and *SYNGAP1*: In a mouse model, the group discovered SynGAP to be one of seven hub proteins related to alcohol withdrawal. They reported a significantly

downregulation of SynGAP in brain tissue of mice undergoing withdrawal (Kong et al., 2022).

1.4 Study aim

AD is a severe chronic psychiatric disorder contributing substantially to the global burden of disease. The effectiveness of therapy options is often far from ideal, and biomarkers are missing. Molecular processes in AD have been object of research of numerous studies; nevertheless, a wide understanding of them is still missing to date. A deeper understanding would open new possibilities for biomarkers for AD and therapy targets that could alleviate the burden of disease on individuals and societies.

DNAm of *SYNGAP1* has been detected as a potentially suitable biomarker for diagnostic and therapy outcome of AD. In order to validate these findings and further investigate its function for monitoring disease severity, we analyzed the DNAm of *SYNGAP1* of AD patients in clinically applicable tissues – i.e. peripheral venous whole blood and saliva – in a longitudinal study over the course of 12 months after therapy start and compared these levels to healthy controls.

We hypothesize that DNAm in *SYNGAP1* in whole blood and saliva differs between the patient and control group and furthermore correlates with the severity of AD and success of withdrawal therapy.

A robust correlation would signify a novel instrument for monitoring the course of AD such as giving insights in the underlying molecular processes. It would further open new perspectives on this epigenetic alteration “as a possible therapeutic target, enabling personalized therapy options and [thus] a more effective health care” (Edelmann et al., 2025).

2 Material and methods

2.1 Study collective

Our study collective consisted of 64 patients and 83 healthy control individuals. The patients were diagnosed with AD according to ICD-10. They underwent a three-weeks in-patient alcohol treatment program, corresponding the QE in the Department of Psychiatry at the University hospital in Tübingen. At study inclusion, patients were detoxified.

Both groups were matched for sex, age, and smoking behavior as far as possible. Zhang et al. showed that DNAm patterns differ significantly from ethnicity (Zhang et al., 2011). Therefore, we limited the study collective to subjects of European descent.

Exclusion criteria were a current substance use other than alcohol or nicotine and acute psychiatric disorders such as schizophrenia or psychosis. Comorbidity with major depressive disorder (MDD) is very prevalent in AD patients (Rehm et al., 2017) and was not applied as an exclusion criterion for this study.

The study was approved by the ethics committee of the University of Tübingen (Projekt-Nr. 264/2018 BO2) and conducted according to the Declaration of Helsinki. All study subjects gave their informed written consent into participating in this study.

2.2 Sample materials

Sample materials in which DNAmS was identified were venous peripheral blood in ethylenediaminetetraacetic (EDTA) and saliva collected in Oragene® DNA Collection Kits. Sample materials were collected at study inclusion (T1), after three weeks – in case of the patients after the withdrawal treatment– (T2), after six months (T3) and after 12 months (T4; Table 1). All samples were stored at -80°C until further usage.

Table 1 The four time points of sampling. *At all time points, saliva and whole blood were collected and participants completed the questionnaires.*

Time point	Time after study start
T1	0
T2	three weeks
T3	six months
T4	12 months

2.3 Questionnaires

All study subjects were asked to complete the following questionnaires at the sampling time points to collect information about alcohol related variables.

2.3.1 90-item revised Symptom Checklist (SCL-90-R) and Global Severity Index (GSI)

As AD is a disease with numerous comorbidities contributing to the subjects´ impairment, the overall well-being in AD patients is an important and informative variable to assess.
Designed by Derogatis in 1977, SCL-90-R is a self-administering tool used to inquire 90 symptoms experienced by the subject in the past seven days, differentiated to the severity of impairment on a five-point scale (Supplement 8.5.1). Answers are included in the calculation of nine scales of impairment by the reported symptoms (e.g. somatization, phobic anxiety, depression, and other). The GSI score gives an overview over the psychological distress over all scales. It is calculated by the sum of all intensity scores divided by the number of items (= 90) (Mercier et al., 1992).
The SCL-90-R has been proven to be an adequate scale for psychological distress and impairment in subjects with AUD/AD (Lucht et al., 2002; Mercier et al., 1992).

2.3.2 Alcohol Use Disorder Identification Test (AUDIT)

To identify the extent and riskiness of alcohol consumption and its change over time, study participants were asked to fill out the AUDIT questionnaire (Supplement 8.5.2). It assesses the drinking frequency, amount, and adverse consequences to social life and safety over the past year and as lifetime experiences. It intends to identify hazardous drinking behavior. Intensity/ frequency must be assigned on a five-point-scale (Saunders et al., 1993).
A review analyzing AUDITs sensitivity and specificity reported in multiple studies summarized a high quality in these criteria (Williams, 2014).

2.3.3 Obsessive-Compulsive Drinking Scale (OCDS)

The OCDS (German version) psychometrically measures alcohol craving over the last seven days. Two dimensions – obsession and compulsion – can be evaluated differentiated from each other. However, according to a study by Mann and Ackermann in 2000, their additional information content is rather low compared to the overall score (Mann & Ackermann, 2000). Thus, in this study, the overall score will be used.

2.4 Analysis of *SYNGAP1* methylation level

In order to analyze the DNA methylation level of *SYNGAP1*, the DNA was first isolated from the saliva and blood samples, followed by a bisulfite conversion. The targeted DNA sequence was amplified with a polymerase chain reaction (PCR) and the sequence was read using pyrosequencing.

2.4.1 DNA isolation from peripheral EDTA whole blood

The DNA was isolated from EDTA-blood samples using the QIAamp® DNA Blood-Maxi Kit (Qiagen, Hilden) according to the manufacturer's instructions and stored at -20° until proceeding.

2.4.2 DNA isolation from saliva

The extraction of DNA from saliva samples collected in Oragene® DNA Collection Kits was performed using the DNA-Genotek® Kit (DNA Genotek, Ottawa, Ontario, Canada), according to the manufacturer's instructions and stored at -20°C until further usage.

2.4.3 DNA quantitation

The DNA concentration obtained from both blood and saliva was measured using the Qubit® 2.0 Fluorometer (ThermoFisher scientific, Waltham, MA, USA) with a fluorescence-based technique.
The stock solution was diluted to 50 ng DNA/µl and stored at -20°C until further usage. If the initially isolated DNA concentration undercut 50 ng/µl, it was diluted to 25 ng/µl. In case of a concentration below 25 ng/µl, no dilution was prepared.

2.4.4 Bisulfite conversion of the DNA to identify DNAm of SYNGAP1

With the bisulfite conversion, unmethylated cytosine nucleotides were converted to uracil nucleotides, while methylated cytosine nucleotides remained unchanged (Table 2, (QIAGEN, 2019)). In the following PCR, uracil nucleotides were converted into thymine nucleotides(Li & Tollefsbol, 2021). The relation of thymine to cytosine nucleotides at the CpG site were later calculated by the pyrosequencing software, giving information about the methylation level.

Table 2 Example for the change in a DNA sequence of CpG sites before and after bisulfite conversion and after PCR *for methylated and unmethylated cytosines, respectively.*

	DNA sequence		
	before bisulfite conversion	after bisulfite conversion	after PCR
Unmethylated Cs	CGT CGA CGA	UGT UGA UGA	TGT TGA TGA
Methylated Cs	CGT CGA CGA	CGT CGA CGA	CGT CGA CGA

Note: C= Cytosine, G=Guanin, T=Thymine, A=Adenine, U=Uracil.

The bisulfite conversion was performed using the EpiTect® Fast DNA Bisulfit Kit (Qiagen, Hilden) according to the manufacturer´s manual. 50 ng/ml DNA-solutions or 25 ng/ml DNA solutions were incubated with a working mix containing bisulfite solution and DNA protect buffer (Table 3), then incubated in the thermocycler according to the program in Table 4.

Table 3 Working solution for bisulfite conversion *with its components for 50 and 25 ng/µl DNA, respectively.*

	Concentration of DNA dilution	
Component	DNA 50 ng/µl	DNA 25 ng/µl
Bisulfite solution (µL)	85	85
DNA Protect buffer (µl)	35	35
Water (µl)	10	/
DNA (µl)	10	20

Note: Components are provided by the manufacturer.

Table 4 Thermocycler program for bisulfite conversion.

Step	Temperature (°C)	Duration	Function
1	95	5 min	Denaturation
2	60	15 min	Incubation
3	95	5 min	Denaturation
4	60	15 min	Incubation
5	20	Forever	Storage

The bisulfite converted DNA was stored at -20°C until further usage.
The bisulfite conversion was performed either with the peqSTAR 96 Universal Gradient Thermocycler (PEQLAB, Darmstadt, Germany) or Biometra Thermocycler T-Gradient ThermoBlock (Analytik Jena GmbH+Co. KG, Jena, Germany).

2.4.5 Amplification of SYNGAP1 using PCR

For the amplification of *SYNGAP1*, a PCR was conducted to obtain sufficient DNA for the sequencing.
The bisulfite converted DNA was added to a solution containing forward and reverse primer (Table 5). Master mix, Coralload and water are provided in the PyroMark® Kit (Qiagen, Hilden). Primers were ordered at Metabion international AG (Planegg, Germany). The PCR was performed in the Biometra Thermocycler T-Gradient ThermoBlock (Analytik Jena GmbH+Co. KG, Jena, Germany) according to the program displayed in Table 6.

Table 5 Composition of PCR preparation.

Components	Volume (µl)
Master Mix	12.5
H_2O	7
Coralload	2.5
Forward Primer	1
Reverse Primer	1
DNA	1

Note. Volume of components is given per PCR tube (total of 25 µl/tube).

Table 6 PCR program for SYNGAP1 amplification.

Step	Temperature (°C)	Duration	Function	
1	95	15 min	PCR activation	
2	94	30 sec	Denaturation	45 repeats
3	62	30 sec	Annealing	
4	72	30 sec	Extension	
5	72	10 min	Final extension	
6	8	Forever	Storage	

The PCR products were stored at -20°C until proceeding.

2.4.6 Gel electrophoresis

Gel electrophoresis for quality assessment of the PCR products was conducted in 2% agarose gel in Trisborat-EDTA (TBE) with a 200 bp ladder and 100 V/ 200 mA for 35 minutes.

The outcome of the gel electrophoresis was documented by photography using UV light (E-Box – PEQLAB, Darmstadt, Germany; example in Supplement 8.3)

2.4.7 Pyrosequencing

After amplification of the bisulfite converted DNA and quality control, the analysis of DNAmS was conducted by pyrosequencing. For this purpose, the assay for the CpG site to be analyzed had been designed by Kiriakos Kotziaeroglou and reported in his internship report in our research group in 2018 (primer sequence: Table 10; pyrosequencing assay: Supplement 8.2). The PCR amplified DNA template was biotin marked by the reverse primer (Table 10). Biotin allowed the binding to streptavidin sepharose beads (GE-Healthcare, Amersham, UK), in a solution containing binding buffer. By drawing the solution with vacuum through a membrane on the PCR workstation, the beads and the DNA template bound to them were held back at the membrane due to their size. Subsequently, the beads and the DNA were washed with ethanol, denaturation buffer and washing buffer, being then placed on a well plate with a solution of annealing buffer and sequencing primer. The solution was incubated for two minutes at 80°C, enhancing the annealing of the primer to the single strand DNA. After cooling down at room temperature for 15 minutes, the well plate was inserted in the PyroMark® Q24.
The PyroMark® had beforehand been loaded with the PyroMark® Q24 Cartridge containing the chemicals listed in Table 7.

***Table 7 Components of enzyme and substrate mix used for** pyrosequencing and their function in the reaction.*

Reagent mix	Chemical	Function
Enzymes	DNA polymerase	Incorporation of dNTPS
	ATP Sulfurylase	Conversion of pyrophosphate to ATP
	Luciferase	Generation of light signal
	Apyrase	Degradation of ATP and unincorporated dNTPS
Substrates	adenosine 5´phosphosulfate (APS)	Generation of ATP
	luciferin	Substrate for luciferase
	dNTPS	

Starting the PyroMark® assay, the template was incubated with the enzymes and substrates (Table 7). Then, the first of four dNTPS according to the dispensation order (corresponding the complementary bases of the DNA sequence, Supplement 8.1 and 8.2) was added to the reaction. In case of dispensation of a nucleotide complementary to the base in the template strand, it was incorporated by the DNA polymerase. Each incorporation released one molecule pyrophosphate (PPi) which was converted to adenosine triphosphate (ATP). ATP drove the conversion of luciferin to oxyluciferin, generating a light signal which was detected by the PyroMark® and shown as a peak in the Pyrogram® example of a Pyrogram in Supplement 8.4). The intensity of light was proportional to the incorporated desoxyribonucleoside triphosphates (dNTPs). Remaining ATPs and dNTPs were degraded by apyrase (QIAGEN, 2016). The dispensation of the next dNTP could take place. The dispensation order was followed one by one, building up the sequence in the Pyrogram®.

As mentioned above, the bisulfite conversion had transformed the unmethylated cytosines to thymines, whereas methylated cytosines had been left unchanged.

The pyrosequencer was able to analyze – via detection of the light signal strength – how many dGTPs (complementary to the cytosines) were being incorporated at the on hand CpG site: Afterwards, dATPs were being dispensed, the light signal again detected and the number of thymines at this site was traced. Given the relation of those two figures, the software calculated the methylation level of the CpG site (Higashimoto et al., 2023).

2.5 Statistic evaluation

For the statistic evaluation, IBM® SPSS Statistics, Version 28.0.1.1 (14), was used. Plots were created with SPSS and edited with Adobe® Illustrator 2023 (27.5). Graphics were made using BioRender.com.
For all tests a significance level of $\alpha \leq 0{,}05$ was applied. Distribution of the values per group, variable and time point of sampling was analyzed applying the Shapiro-Wilk-test. Outlying values remained in the data and non-parametric tests (Wilcoxon-signed rank test for dependent samples or Mann-Whitney U test for independent samples, respectively) were applied. For analyzing the correlation between variables, the Spearman-Rho correlation was assessed.

2.6 Materials

2.6.1 Chemicals, tools and manufacturer

The devices, tools and reagents used in the laboratory and their manufacturers are listed in Table 8.

***Table 8 Devices and chemicals** used for the methods.*

Devices and chemicals	Manufacturer
AE-Buffer	Qiagen, Hilden, Germany
Agarose	GE Healthcare, Amersham, UK
Boric acid krist.	MERCK, KGaA, Darmstadt, Germany
Butterfly-needles	Safety multifly®-Needle SARSTEDT AG & Co. KG, Nümbrecht, Germany
Cyan coularant	SYBR®Safe DNA Gel Stain – lifetechnologies™, Carlsbad, CA, USA
DNA ladder (100bp)	Promega, Fitchburg, WI, USA
EDTA	Titriplex® - MERCK KGaA, Darmstadt
EDTA tubes	S-Monovette® - SARSTEDT, Nürnbrecht, Germany
Elektrophorese combs	PEQLAB, Darmstadt, Germany
EpiTect® Fast DNA Bisulfit Kit	Qiagen, Hilden, Germany
Eppendorf reaction tubes	Eppendorf, Hamburg, Germany
Acetic acid	SIGMA-ALDRICH, St. Louis, MO, USA
Ethanol 100%	AnalaR NORMAPUR Ethanol absolut – VWR Prolab, Darmstadt, Germany
Falcons	BD, Heidelberg, Germany
Fluorometer	Qubit® 2.0 Fluorometer – ThermoFisher scientific, Waltham, MA, USA
Gel documentation	E-Box – PEQLAB, Darmstadt, Germany
Gel combs	PEQLAB, Darmstadt, Germany
Gel chamber	PerfectBlue™ Gelsystem Mini M, VWR Peqlab, Darmstadt, Germany

Heating block	RSM-10HS – Phoenix Instruments, Garbsen, Germany
Heating cubicle	Binder GmbH, Tuttlingen, Germany
Loading Dye	Blue/Orange 6x Loading Dye – Promega, Fitchburg, WI, USA
Magnesium acetat	MERCK, KGaA, Darmstadt, Germany
Multi pipette	Multipipette® plus – Eppendorf Ag, Hamburg, Germany
Sodium chlorid	Carl Roth, Karlsruhe, Germany
Sodium hydroxid	Carl Roth, Karlsruhe, Germany
Oragene® DNA Collection Kits	OrageneDiscover DNAgenotek, Ottawa, ON, Kanada
PCR MasterMix	PyroMark® PCR-MasterMix, 2x - Qiagen Hilden, Germany
PCR plaque	96 Multiply PCR-Platte – SARSTEDT, Nürnbrecht, Germany
PCR tubes	8 strip-reaction tube (0,2ml) + domed caps – Biometra, Göttingen, Germany
PCR work station	VWR International GmbH, Darmstadt, Germany
PerfectSpin Mini	Peqlab, Darmstadt, Germany
prepIT L2P	DNAgenotek, Ottawa, ON, Kanada
Primers	Metabion international AG, Planegg, Germany
PyroMark Q24 Gold Reagents	Qiagen, Hilden, Germany
Pyrosequencer PyroMark® Q24	Qiagen, Hilden, Germany
QIAamp® DNA Blood Maxi-Kit	Qiagen, Hilden, Germany
Qubit™ dsDNA BR Buffer	ThermoFisher scientific, Waltham, MA, USA
Qubit™ dsDNA BR Reagent	ThermoFisher scientific, Waltham, MA, USA
RNase-free water	Promega, Fitchburg, WI, USA
Hydrochloric acid	MERCK, KGaA, Darmstadt, Germany
Plate shaker	DPC MicroMix® 5 – DPC, Los Angeles, CA, USA
Sepharose beads	GE-Healthcare, Amersham, UK

SYBR®Safe DNA Gel Stain	ThermoFisher scientific, Waltham, MA, USA
Power supply for electrophoresis	Electrophresis Power Supply EPS 300, GE Healthcare, Amersham, UK
Thermocyclers	peqSTAR 96 Universal Gradient Thermocycler – PEQLAB, Darmstadt, Germany Biometra Thermocycler T-Gradient ThermoBlock (Analytik Jena GmbH+Co. KG, Jena, Germany)
Tris	Trizma®-base – SIGMA-ALDRICH, St. Louis, MO, USA
Tween®20	Carl Roth, Karlsruhe, Germany
Vortex device	Vortex-Genie 2 – Scientific Industries, NY, USA
Scale	Mettler PM 4600 DeltaRange® - Mettler Toledo, Gießen, Germany
Water	Ampuwa®, Fresenius Kabi Deutschland, Bad Homburg v.d.H., Germany
Water bath	GFL®, Burgwedel, Germany
Centrifuge large	HERAEUS Multifuge 3L-R – Heraeus Holding, Hanau, Germany
Centrifuge small	HERAEUS Fresco 21 Micozentrifuge – Heraues Holding, Hanau, Germany

2.6.2 Buffers and their components

The composition of buffers used for the methods are listed in

Table 9.

Table 9 Buffers and their components.

Buffer	Components
TBE (Trisborat-EDTA) 1x	90 mM Tris 90 mM Boric acid 2 mM EDTA
Binding Buffer	10 mM Tris 10 mM sodium chloride 1 mM EDTA 0,1% Tween®20 pH 7,6 (adjust with 1 M HCl)
Annealing Buffer	20 mM Tris 2 mM Mg-Acetat pH 7,6 (adjust with 100% acetic acid)
Denaturation Solution	0,2 M caustic soda
Washing Buffer	10 mM Tris pH 7,6 (adjust with 100% acetic acid)

2.7 Primers of *SYNGAP1*

Gene localization of *SYNGAP1*: cg02652579
chr6:33386818-33387117 5'pad=0 3'pad=0 strand=+
Further information on its surrounding sequence can be found in Supplement 8.1.
The primers used for PCR (forward and reverse) and sequencing (sequence) and their annealing temperature are listed in Table 10.

Table 10 Primers of SYNGAP1.

Primer	Primer sequence	Annealing temperature
Forward	5´-GAG GGG TTA ATG AGA GGT AGA GAG GTG-3´	63.40°C
Reverse	5´-Biotin-CCC CAC TTC CCT ACC CTA AAA CC-3´	63.6°C
Sequence	5´TTG TTT GGT GGT GGG GAT GTT-3´	65.15°C

3 Results

Author´s note: Prior to the publication of this doctoral thesis, parts of the data had been published in the following publication: Edelmann S, Kummer C, Pasche S, Zimmermann M and Nieratschker V (2025) Epigenetic regulation of SYNGAP1 in alcohol use disorder in whole blood and saliva. Front. Psychiatry 16:1661760. doi: 10.3389/fpsyt.2025.1661760
The relevant subchapters are preceded by a separate note.

3.1 Characteristics of the study collective

Author´s note: Data concerning characteristics of the study collective (i.e. number, age, sex and smoking status) had been, prior to the publication of this doctoral thesis, published in the following: Edelmann S, Kummer C, Pasche S, Zimmermann M and Nieratschker V (2025) Epigenetic regulation of SYNGAP1 in alcohol use disorder in whole blood and saliva. Front. Psychiatry 16:1661760. doi: 10.3389/fpsyt.2025.1661760

In total, 147 participants were included in the study. The age of patients ranged from 21 to 71 years at study inclusion, with an average age of 49.80 years (Standard deviation (SD) = 11.467). The age of control individuals ranged from 20 to 69 years, with an average age of 40.64 years (SD = 13.731; Table 11). The difference in age between the groups was of high statistical significance (Z= -4.112, p <.001).
We included 25 female and 39 male patients and 55 female and 28 male control individuals (Table 11).
The smoking status differed between the two groups, with 71.86 % (n=46) of patients smoking compared to only 4.82 % (n=4) of control individuals (Table 11).

Table 11 Descriptive analysis of characteristics of the study collective *(sex, average age, and smoking status) divided by group.*

Characteristics	Group	
	Patients (N=64)	Control individuals (N=83)
Female	N= 25	N= 55
Male	N= 39	N= 28
Average age (years)	49.80 ± *11.47*	40.64 ± *13.73*
Smoker	N= 46	N= 4

Note. Average age (years) ± standard deviation. N= Number of samples.

The patients were divided into subgroups of relapsed and abstinent. In order to identify relapses, the last day of drink at T3 and T4 (reported in the OCDS questionnaire, see Supplement 8.5.3) was used. If the number of days indicated a consumption of alcoholic beverages since the withdrawal treatment (i.e. if undercutting approx. 180 days since the last drink at T3 or 365 days since T4, respectively) and if the AUDIT score exceeded 15 at that time, a relapse was assumed. For our analysis, the patients were grouped starting from T1, according to their alcohol related variables at T3 and/or T4 if available. At T3, data for eleven relapsed and four abstinent patients was available. At T4, the number dropped to seven (relapsed) and four (abstinent), respectively (Table 12).

***Table 12 Number of relapsed and abstinent patients** at T3 and T4.*

	Patients´ subgroups	
Time	Relapsed	Abstinent
T3	11	4
T4	7	3

Outlying values remained in the data and non-parametric tests were applied for their analysis.

The distribution of the values per group, variable and time of sampling was analyzed applying the Shapiro-Wilk-test. DNAmS both in blood and saliva were partly normally distributed whereas the questionnaire scores, days since last drink and age where mostly not normally distributed (Table 13).

Table 13 Report of normal distribution *per group, variable, and time point.*

Variable	Time	Group	
		Patients	Controls
DNAmS in EDTA	T1	.811	.754
	T2	.592	.497
	T3	.094	.545
	T4	.081	.024
DNAmS in saliva	T1	.004	.265
	T2	.074	.013
	T3	.722	.066
	T4	.024	.537
GSI-Score	T1	.02	<0.001
	T2	<0.001	<0.001
	T3	.053	<0,001
	T4	0.026	0.002
OCDS score	T1	.313	<0.001
	T2	.423	<0.001
	T3	.657	<0.001
	T4	.552	<0.001
AUDIT-score	T1	.018	<0.001
	T2	.283	<0.001
	T3	.655	<0.001
	T4	.740	<0.001
Days since last drink	T1	<0.001	<0.001
	T2	<0.001	<0.001
	T3	<0.001	<0.001
	T4	<0.001	<0.001
Age		.438	<0.001

Note: p-values of > 0,05 imply a normal distribution of the values. Values implying no normal distribution are highlighted in blue.

As described above (Chapter 2.3.3), the OCDS assesses an overall score as well as a separate obsession and compulsive score. A highly significant correlation was identified for all combinations ($p < .001$) both if analyzed over both groups and for the two groups separately (Supplement 8.6). Therefore, the overall score was used.

3.2 Comparison of alcohol related variables over time

Author´s note: Data concerning the scores of AUDIT and OCDS at T1 and T2 had been published in the following publication: Edelmann S, Kummer C, Pasche S, Zimmermann M and Nieratschker V (2025) Epigenetic regulation of SYNGAP1 in alcohol use disorder in whole blood and saliva. Front. Psychiatry 16:1661760. doi: 10.3389/fpsyt.2025.1661760.

With the three questionnaires, three different alcohol related variables were measured: the subjective impairment due to physical and psychological symptoms (GSI = Global severity index in SCL-90-R), risk of alcohol consumption (AUDIT) and alcohol craving (OCDS). The questionnaire scores are displayed for both groups and all time points in Table 14 and Figure 5 a-c, for the relapsed and abstinent subgroups in Table 15 and Figure 5 d-f.

***Table 14 Scores of GSI, AUDIT and OCDS** over time in patients and controls.*

	Group											
	Patients						Controls					
	GSI		AUDIT		OCDS		GSI		AUDIT		OCDS	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
T1	1.12	0.73	24.13	7.29	21.28	7.59	0.21	0.21	2.93	2.30	2.00	2.66
T2	0.55	0.48	20.54	7.69	13.27	6.21	0.19	0.21	3.09	2.39	1.63	2.24
T3	0.86	0.71	16.6	8.71	13.87	9.52	0.22	0.21	2.89	2.54	1.69	2.18
T4	0.87	0.88	23.00	8.41	15.50	9.09	0.21	0.18	2.15	2.20	1.65	2.05

Note. M= Mean (%), SD=Standard deviation of the mean (%).

Table 15 Scores of GSI, AUDIT and OCDS *over time in relapsed and abstinent patients.*

	Subgroup											
	Relapsed patients						Abstinent patients					
	GSI		AUDIT		OCDS		GSI		AUDIT		OCDS	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
T1	1.11	0.80	27.54	5.14	23.00	6.73	0.85	0.48	22.80	6.02	20.60	3.13
T2	0.51	0.49	24.00	2.12	13.50	4.34	0.25	0.21	24.50	2.12	12.80	5.12
T3	1.04	0.74	22.64	7.03	17.46	8.00	0.38	0.35	9.00	5.03	4.00	5.66
T4	0.54	0.72	21.60	5.23	15.00	4.79	0.57	0.42	-	-	7.33	5.69

Note. M = Mean (%), SD = Standard deviation of the mean (%).

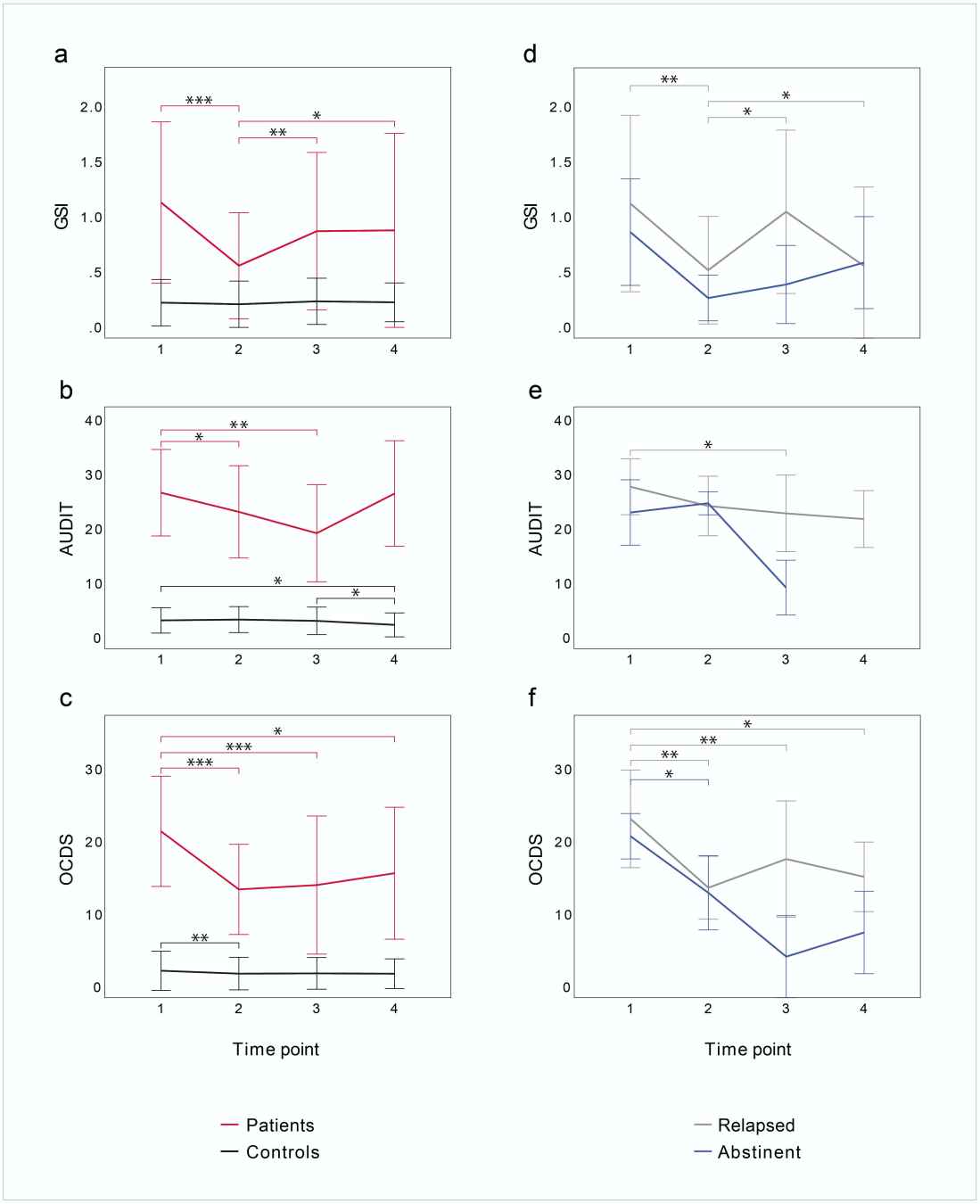


Figure 5 Overview of the line plots of mean questionnaire scores over sampling time *compared between patients and controls (a-c) and relapsed and abstinent patients (d-f), respectively. Error bars indicate standard deviation of the mean. Significance in changes over time is described by * indicating p ≤ 0.05, ** indicating p ≤ 0.01 and *** indicating p ≤ 0.001, in the corresponding color. Significant differences between the groups/ subgroups are stated in the main text. GSI: psychological distress. AUDIT: alcohol consumption. OCDS: alcohol craving.*

3.3 Comparison of alcohol related variables between groups and subgroups

Author´s note: Data concerning the scores of AUDIT and OCDS at T1 and T2 had been published in the following publication: Edelmann S, Kummer C, Pasche S, Zimmermann M and Nieratschker V (2025) Epigenetic regulation of SYNGAP1 in alcohol use disorder in whole blood and saliva. Front. Psychiatry 16:1661760. doi: 10.3389/fpsyt.2025.1661760.

To control for the validity of alcohol related variables concerning the difference between patients and controls, significance tests were applied at each time point for all three questionnaires. Mann-Whitney-U test was used since they showed no normal distribution (Table 13).
Visualized in Figure 5 a-c, the scores of the three questionnaires differed at all time points between patients and control individuals, patients´ scores being higher at all times (GSI T1: Z = -8.579 , p < .001; GSI T2: Z = -4.975, p < *.001; GSI T3:* Z = -3.941, p < .001; GSI T4: Z = -2.198, p = .028; AUDIT T1: Z = -9.904, p < .001; AUDIT T2: Z = -6.362, p < .001; AUDIT T3: Z = -5.666, p < .001; AUDIT T4: Z = -4.065, p < .001; T1: Z = -10.028, p < .001; OCDS T2: Z = -9.110, p < .001; OCDS T3: Z = -4.555, p < .001; OCDS T4: Z = -4.856, p < .001, Supplement 8.7).

3.3.1 Psychological distress

Comparing the development of severity of psychological distress (GSI) over time in patients, a decrease from T1 to T2 was identified (Z=-5.406, p <.001). Furthermore, distress increased from T2 to T3 and T2 to T4 (T2/T3: Z =-2.7, p =.007; T2/T4: Z = -2.547, p = .011; see Table 14 and Figure 6 a).
When dividing the patients` group into subgroups – relapsed and abstinent patients – distress decreased between T1 to T2 in both subgroups but only significantly in relapsers (relapsed: Z = -2.667, p = .008; abstinent: Z = -1.826, p = .068, Figure 6 b).
A subsequent increase of distress between T2 and T3 was revealed in relapsing patients (Z = 2.395, p =.017) as well as between T2 and T4 (Z = -1.992, p = .046). Abstinent patients´ distress levels remain without significant changes at a rather stable level.
Comparing the subgroups of relapsed and non-relapsed patients at each time, psychological distress differed only slightly but not significantly at T3, with relapsed patients reporting the higher distress levels (T3: Z = -1.828, p = .068).

In control individuals, psychological distress remained stable over time (Figure 6 a).

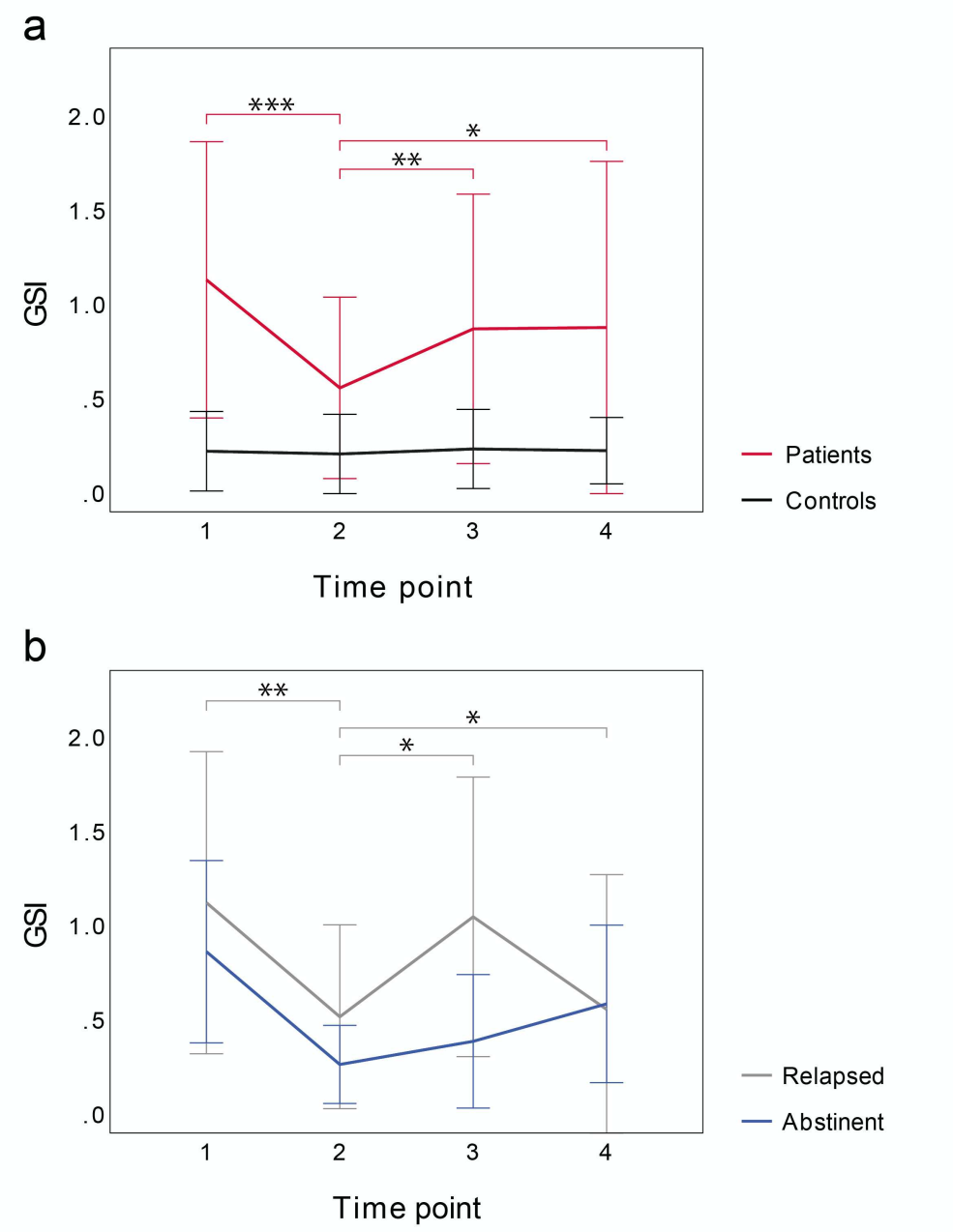


Figure 6 Line plots of mean GSI scores *over sampling time, compared between patients and controls (a) and relapsed and abstinent patients (b), respectively. Error bars indicate standard deviation of the mean. Significance in changes over time is described by * indicating p ≤ 0.05, ** indicating p ≤ 0.01 and *** indicating p ≤ 0.001, in the corresponding color of the group/ subgroup. Significant differences between the groups/ subgroups are stated in the main text.*

3.3.2 Alcohol consumption

Regarding the alcohol consumption of patients, a decrease in the AUDIT score from T1 to T3 (T1/T2: *Z* = -2.340, *p* = .019; T1/T3: *Z* = *-2.987*, *p* = .003, Figure 7 a) was detected and remained unchanged towards T4. At T4, it almost met T1´s level again (Table 14).

Separating the patient group in relapsed and abstinent patients, a decrease of alcohol consumption over time was reported by relapsed patients from T1 to T3 (Z = -2.497, *p* = .013, Table 7 b) and showed a trend of decrease between T1 and T4 (Z = -1.826, *p* = .068, Table 7 b).

In abstinent patients, alcohol consumption did not change significantly after therapy and over the course of the following six months. For T4, not enough data of abstinent patients was available.

When comparing the alcohol consumption between the subgroups of relapsed and abstinent patients, at T3 (Table 15), relapsed patients reported a higher consumption than abstainers (T3: Z = -2.681, *p* = .003). At T1 and T2, abstainers and relapsers reported a similar alcohol consumption (Table 15, Figure 7 b).

The alcohol consumption of control individuals, visually, seemed unchanged (Figure 7 a). However, a decrease over time was identified statistically (T1/T4: Z = -2.555, *p* = .011; T3/T4 Z = -2.514, *p* = .012).

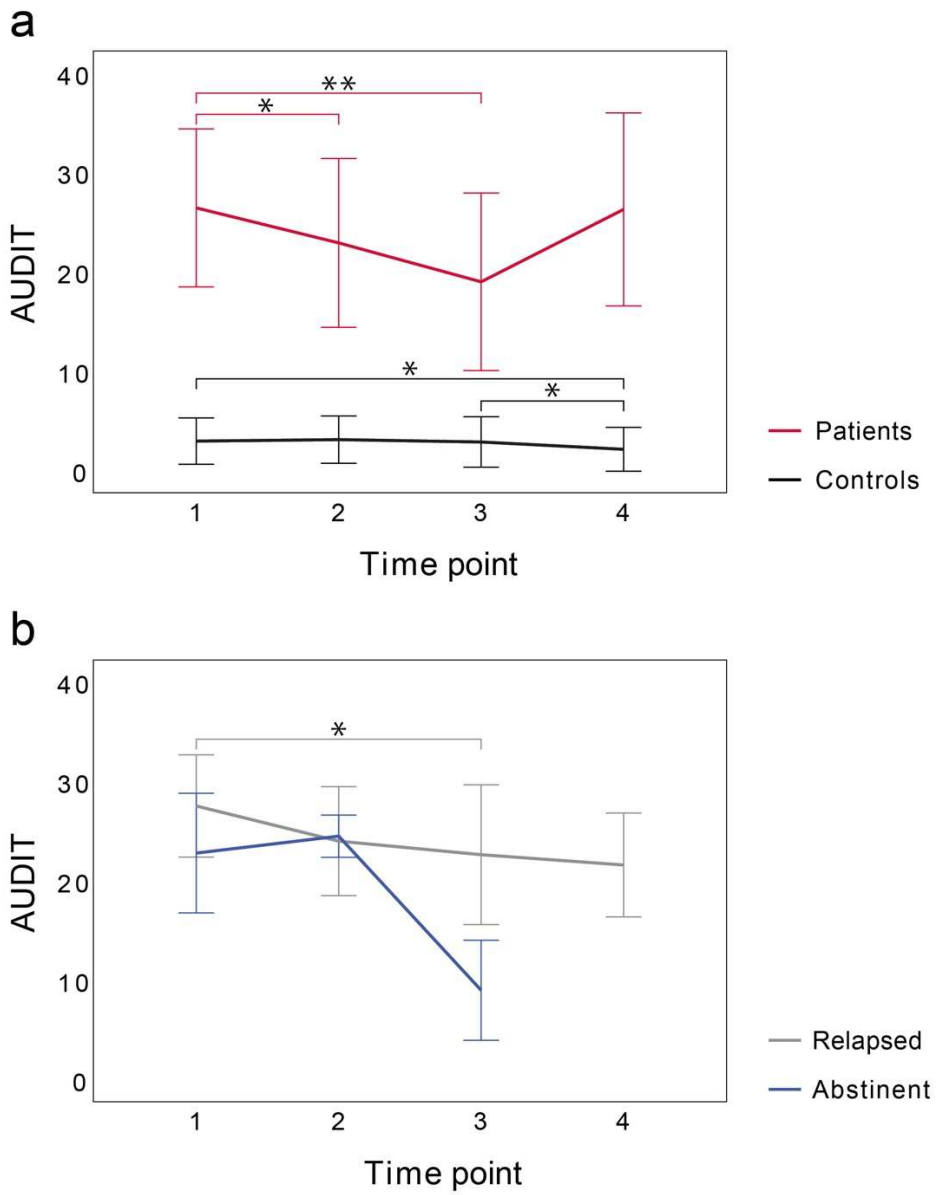


Figure 7 Line plots of mean AUDIT scores *over sampling time, compared between patients and controls (a) and relapsed and abstinent patients (b), respectively. Error bars indicate standard deviation of the mean. Significance in changes over time is described by * indicating p ≤ 0.05, ** indicating p ≤ 0.01 and *** indicating p ≤ 0.001, in the corresponding color of the group/ subgroup. Significant differences between the groups/ subgroups are stated in the main text.*

3.3.3 Alcohol craving

Over the course of time, alcohol craving (OCDS score) of patients decreased significantly after T1 (T1/T2: Z = -5.814, p <.001; T1/T3: Z = -3.268, p < .001; T1/T4: Z = -2.161, p = .031. Figure 8 a). After T2, the score remained without significant changes.

Separating the patient group in its two subgroups and analyzing the development of alcohol craving over time, the decreasing trend from T1 to all later time points of assessment was replicated in relapsed patients (T1/T2: Z = -2.938, p = .003; T1/T3: Z = -2.716, p = .007; T1/T4: Z = -2.120, p = .034, Figure 8 b).

In abstinent patients, craving decreased between T1 and T2 and then, slightly, between T2 and T3 (T1/T2: Z = -2.032, p = .042; T2/T3: Z = -1.826, p = .068). When comparing alcohol craving of abstinent and relapsed patients with each other, no difference was found at T1, T2 or T4. However, at T3, abstinent patients experienced significantly lower craving than relapsers (Z = -2.424, p = .015).

Control individuals reported decreasing alcohol craving between T1 and T2 (Z = -2.680, p=.007) and then remained without further dynamics (Figure 8 a).

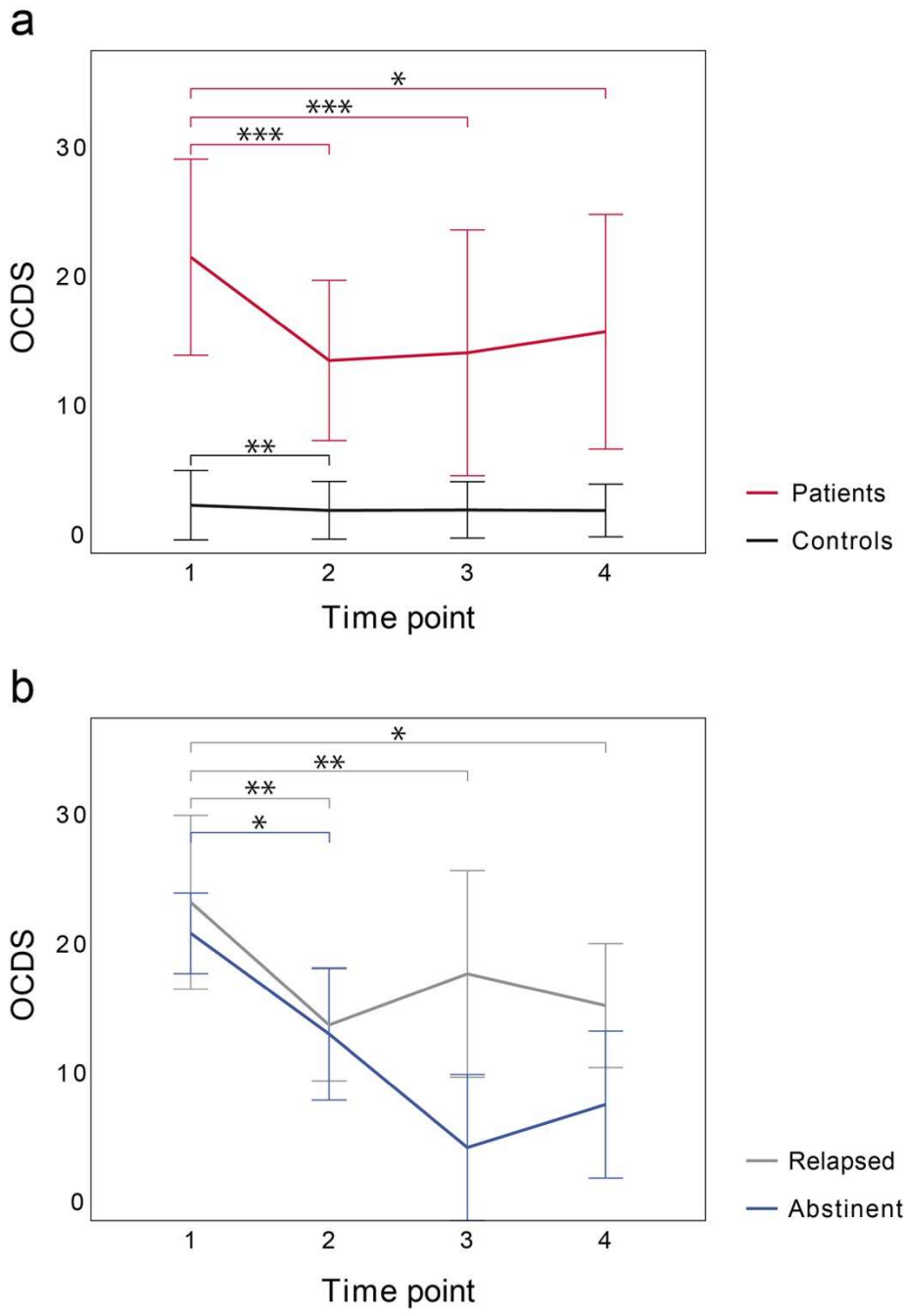


Figure 8 Line plots of mean OCDS scores *over sampling time, compared between patients and controls (a) and relapsed and abstinent patients (b), respectively. Error bars indicate standard deviation of the mean. Significance in changes over time is described by * indicating p ≤ 0.05, ** indicating p ≤ 0.01 and *** indicating p ≤ 0.001, in the corresponding color of the group/ subgroup. Significant differences between the groups/ subgroups are stated in the main text.*

3.3.4 Correlation between alcohol related variables

In order to analyze the interdependence of psychological distress, alcohol consumption and alcohol craving, correlations between the corresponding questionnaires were identified assessing the Spearman-Rho correlation. For the three variables over the two groups and four times, a highly significant correlation ($p < .001$) was reported in all combinations (Figure 9 and Supplement 8.8).

The same was reported when correlating only the patients´ scores.

When assessing the correlation of control individuals´ scores separately, severity of psychological symptoms (GSI) and alcohol craving (OCDS) did not correlate (Supplement 8.8).

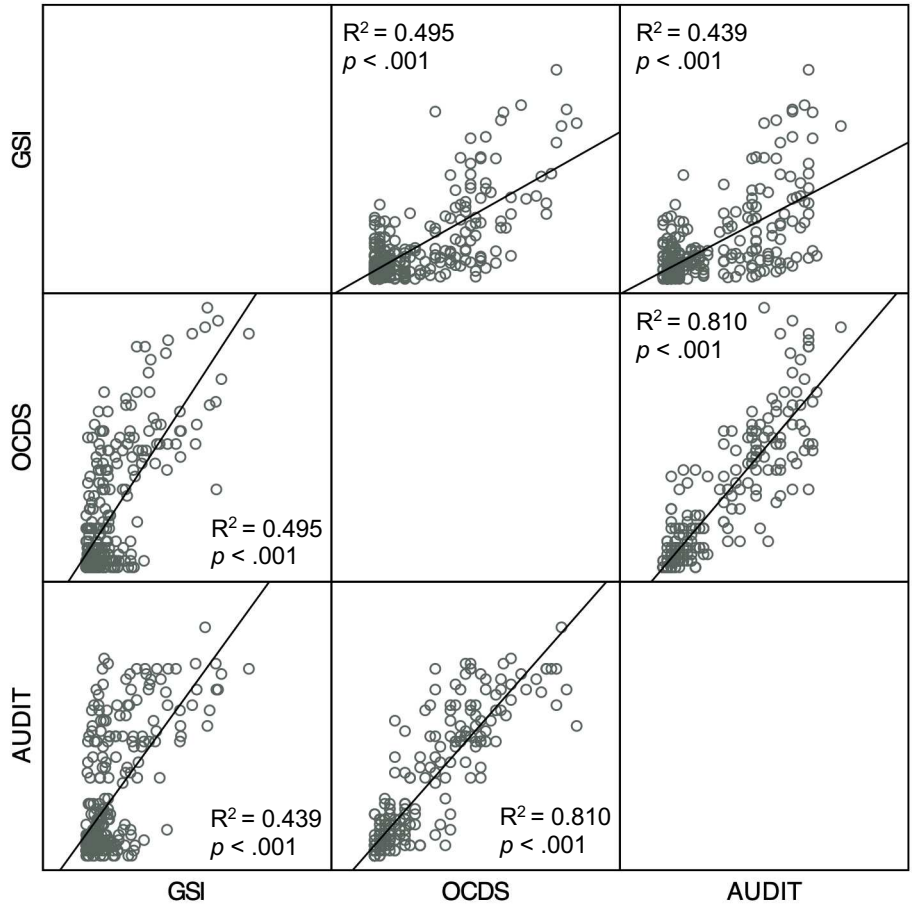


Figure 9 Scatterplot matrix for correlations between questionnaire scores *over both groups and all time points. Psychological distress (GSI), alcohol craving (OCDS) and alcohol consumption (AUDIT) are positively and statistically significantly correlated in each combination, for the statistical analysis see Supplement 8.8. In the diagonal, histograms of distribution of the corresponding score are outlined.* R^2*= Coefficient of determination.*

3.4 DNAm level of *SYNGAP1* in blood

Author´s note: Data concerning the scores of DNAm levels in blood at T1 and T2 had been published in the following publication: Edelmann S, Kummer C, Pasche S, Zimmermann M and Nieratschker V (2025) Epigenetic regulation of SYNGAP1 in alcohol use disorder in whole blood and saliva. Front. Psychiatry 16:1661760. doi: 10.3389/fpsyt.2025.1661760.

The DNAmS in whole blood of patients and control individuals was analyzed at each sampling time. The mean DNAm level ranged between 75 and 78% with a standard deviation between 3.7 and 5.19% (Table 16). The number of analyzed samples decreased over time in both groups towards T4 and are displayed in Table 16.

Table 16 Descriptive analysis of DNAmS in blood of patients and controls *at the four time points of sampling.*

Time	Group					
	Patients			Controls		
	M	SD	N	M	SD	N
T1	77.80	4.78	59	76.01	3.77	78
T2	76.56	5.19	48	75.05	4.27	72
T3	75.73	4.74	16	75.74	4.74	53
T4	76.44	4.91	11	76.24	4.22	30

Note. M= Mean DNAmS (%), SD= standard deviation (%), N= number of analyzed samples.

At T1, the mean DNAmS of patients amounted 77.80% which was 1.79% higher than that of controls individuals (Z = -2.269, *p* = .023, Figure 10 and Table 16).

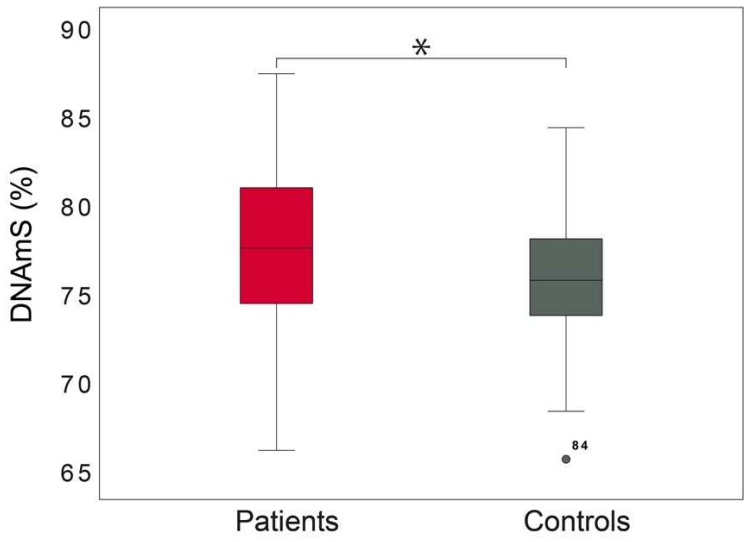


Figure 10 DNAmS (%) in blood of patients and control individuals at T1, *p = .023. Boxes represent the interquartile range. Whiskers indicate the standard deviation of the mean [%].*

At the later time points, DNAmS between groups ranged from 75 to 76% and did not differ significantly (Table 16).

Regarding the development over time, mean DNAmS in patients´ blood decreased from 77.80% at T1 to 75.73% at T3, accounting a change of 2.07% (T1/T3: Z= -2.275, *p* = .023; T2/T3 Z= -2.045, *p* = 0.041, Figure 11). The rest of the changes were not significant.

In control individuals, a decrease by 0.96% from T1´s level – 76.01% – towards T2 was detected (T1/T2: *Z* = *-2.026, p* = .043). Afterwards, the level increased by 1.19% towards T4, then amounting 76.24% (T2/T4: *Z* = -2.141, *p* = .032, Figure 11).

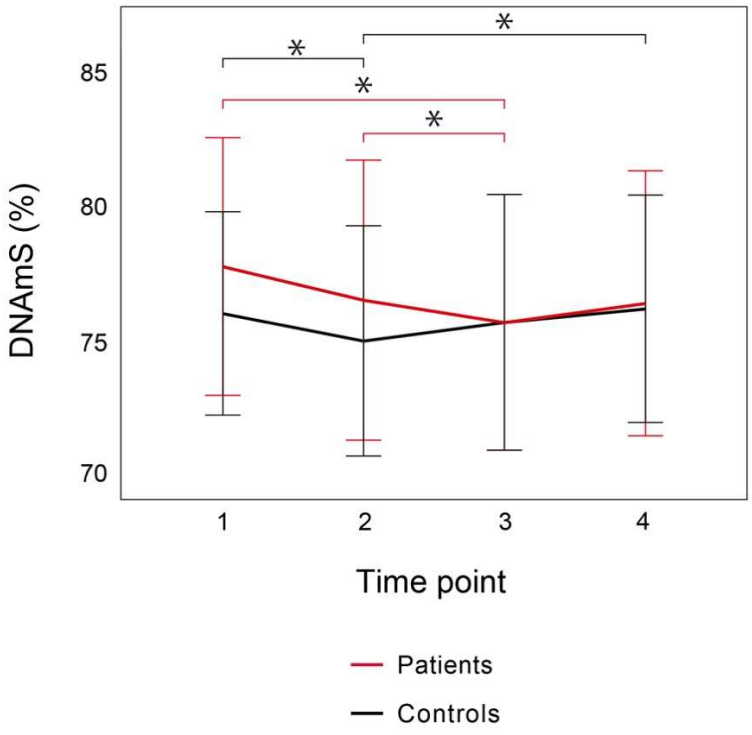


Figure 11 Line plots of mean DNAmS (%) in blood *over sampling time, compared between patients and controls. Error bars indicate standard deviation of the mean. Significance in changes over time is described by * indicating p ≤ 0.05 in the corresponding color of the group. Significant differences between the groups are stated in the main text.*

The mean DNA methylation levels of *SYNGAP1* in blood of relapsed patients ranged from 75 and 77%, those of abstinent patients between 78 and 81% (Table 17). The standard deviation was between 3 and 6%. 13 samples of relapsed patients were analyzed at T1, decreasing to six samples at T4. The samples of abstainers accounted five at T1 and three at T4, respectively (Table 17).

Table 17 Descriptive analysis of DNAmS in blood of relapsed and abstinent patients *at T1-T4.*

Time	Subgroup					
	Relapsed			Abstinent		
	M	SD	N	M	SD	N
T1	77.21	4.81	13	80.81	5.57	5
T2	76.81	5.49	12	79.01	4.01	5
T3	75.19	4.78	11	78.79	3.13	4
T4	75.05	4.62	6	78.39	5.40	3

Note. M= Mean DNAmS (%), SD= standard deviation (%), N= Number of analyzed samples.

Comparing the subgroups of abstinent and relapsed patients, abstainers´ DNAmS were approximately 3% higher at each time point. However, the differences were not significant.

Over the course of study time, the levels varied only slightly without significant changes (Figure 12).

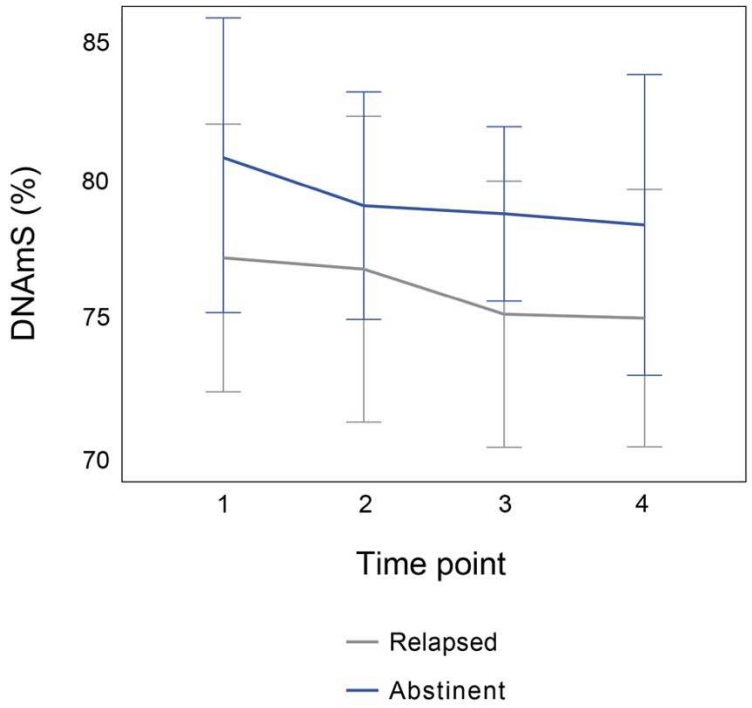


Figure 12 Line plots of mean DNAmS (%) in blood *over sampling time, compared between relapsed and abstinent patients. Error bars indicate standard deviation of the mean.*

When integrating the control individuals´ average DNAmS in the comparison of subgroups (Table 16 and 17), it was non-significantly lower than the abstinent patients´ level, ranging on a similar level as the relapsed patients´ DNAmS (Figure 13).

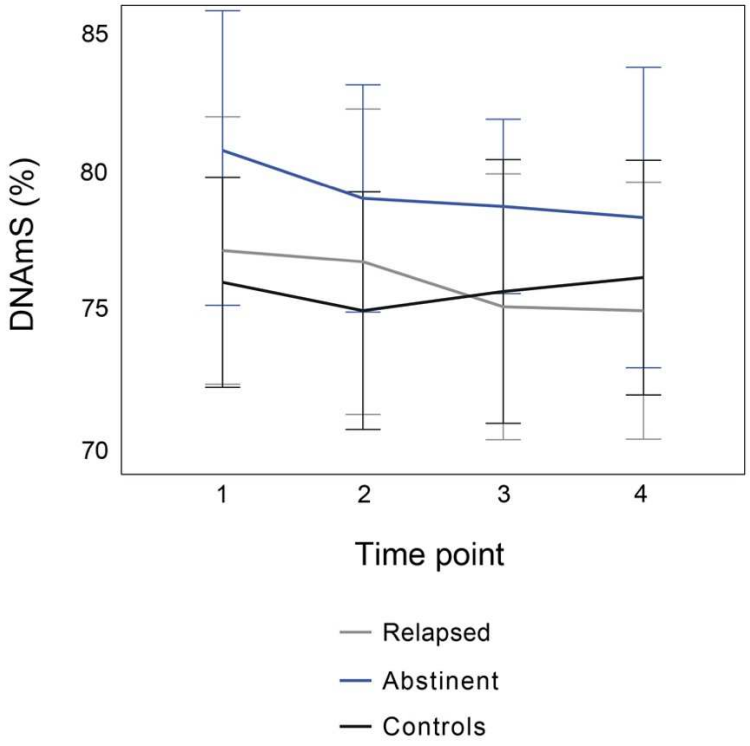


Figure 13 Overview of line plots of mean DNAmS (%) in blood of control individuals, abstinent and relapsed patients *over sampling time. Error bars indicate standard deviation of the mean (%). Significances are not displayed in this figure.*

3.4.1 Age as a confounding factor for DNAmS in blood

The mean age of the patient group in this study was 49.80 years (standard deviation = 11.47 years, Table 11), that of the control group 40.64 years (standard deviation = 13.73, Table 11), the difference being of significant difference (Z= -3.961, p <.001).
For normalization of the variable age, the values were centered (individual age minus the mean age of all study subjects). The age (centered) between the patient and control group differed significantly (Z: -4.112, p <.001, Figure 14 a).

A weak positive correlation between age (centered) and DNAmS in blood at T1 was detected (Spearman's ρ = .203, p = .018; Figure 15 a). At later time points, the variables did not correlate. Because of the correlation at study start, a confounding effect of age interfering with our hypothesis was to be controlled for.
In order to create an age-subgroup in which the DNAmS in blood and age (centered) do not correlate, a threshold was found at the age of 30 years. When excluding individuals aged 30 and younger (that is, two patients and 28 control individuals) and analyzing only the subgroup with subjects aged 31 years and older (SG31) the difference in age between patients and control individuals was not significant anymore (Z: -1.429, p = .153, Figure 14 b).
In SG31, DNAmS in blood at T1 did not correlate with age (centered) anymore (Spearman's ρ = .134, p = .166, Figure 15 b).

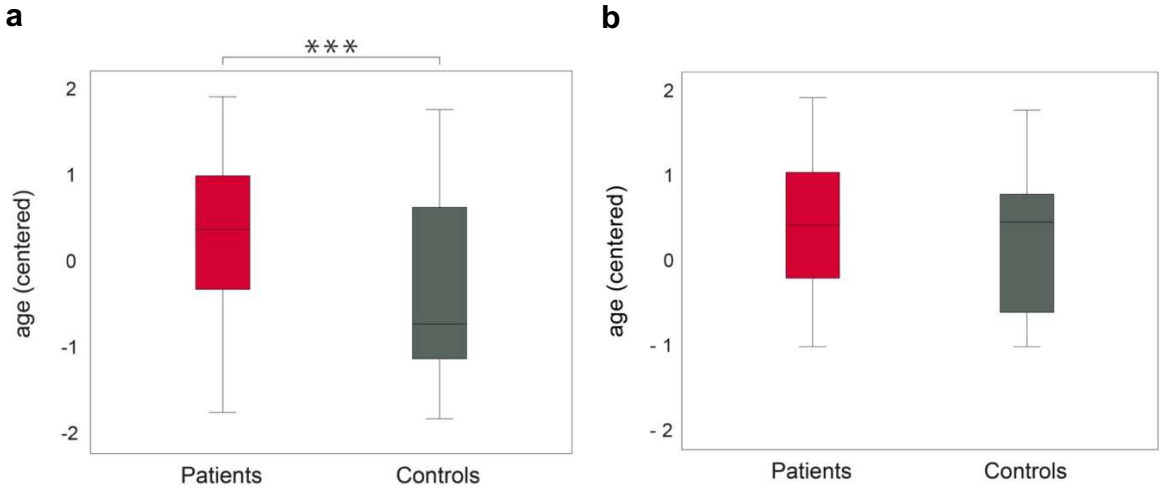


***Figure 14 Comparison of boxplots displaying the age (centered) per patients and control individuals**, comparing all age groups (a) and SG31 (b). Boxes represent the interquartile range; whiskers indicate the standard deviation of the mean. *** indicate p < .001.*

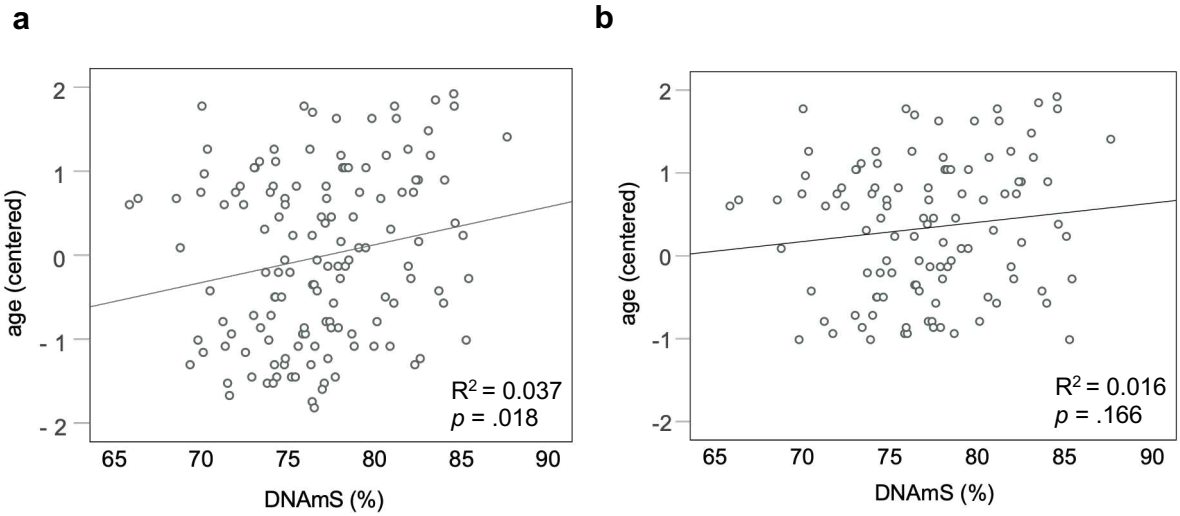


***Figure 15 Comparison of scatterplots displaying the correlation of age (centered) and DNAmS (%) in blood**, of all age groups (a) and SG31 only (b). Coefficient of determination (R^2). Age (centered) and DNAmS (%) in blood are significantly correlated when including participants of all ages.*

3.4.2 DNAmS in blood of participants aged 31 and older

The mean DNAmS in blood of both patients and controls in the SG31 were in the range of roughly 75 to 78% with a standard deviation between 4 and 5% (Table 18).

***Table 18 Descriptive analysis of DNAmS in blood of patients and controls in SG31** at T1-T4.*

Time	Group					
	Patients			Controls		
	M	SD	N	M	SD	N
T1	77.84	4.86	57	76.37	3.94	51
T2	76.28	5.09	46	74.94	4.10	47
T3	75.60	4.88	15	75.91	4.89	34
T4	76.44	4.91	11	75.80	4.44	23

Note. M= Mean DNAmS (%), SD= standard deviation (%), N= number of analyzed samples.

Compared to the dynamics including the participants of all ages (Figure 10), a similar pattern was observed in the dynamics of DNAmS in blood of participants of SG31 (Figure 16).

DNAmS in blood of the patients´ subgroup decreased from 77.84% at T1 to 75.60% at T3 ($Z = -2.329$, $p = .020$, Figure 16) and then remained unchanged. In control individuals older than 30, a significant decrease from T1 and T2 was detected ($Z = -2.458$, $p = .014$, Figure 16). Afterwards, the levels persisted stable.

However, the significantly higher DNAmS level of patients at T1 was not replicated in this age-subgroup. Also at later time points, the DNAmS did not differ significantly between the patients and controls.

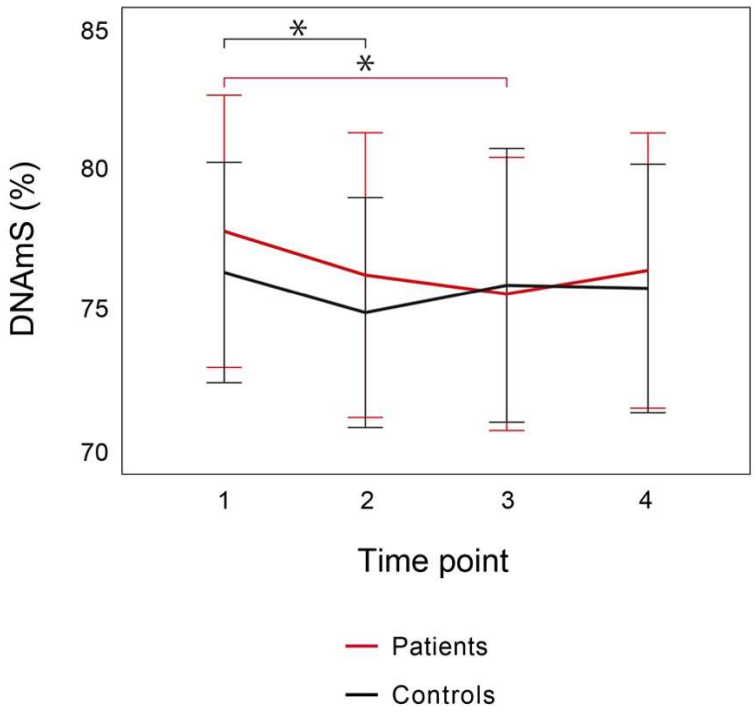


Figure 16 Line plots of mean DNAmS (%) in blood of patients and control individuals aged 31 and older *over sampling time. Significance in changes over time is described by * indicating p ≤ 0.05 in the corresponding color of the group. Error bars indicate standard deviation of the mean (%).*

In the SG31, the DNAmS in relapsed patients ranged between 75 and 77% with a standard deviation of the mean of approximately 5%. In abstainers, DNAmS were in the range of 78 to 81% (Table 19).

***Table 19 Descriptive analysis of DNAmS in relapsed and abstinent patients in SG31** at T1-T4.*

Time	Subgroup					
	Relapsed			Abstinent		
	M	SD	N	M	SD	N
T1	77.28	5.01	12	80.81	5.57	5
T2	76.46	5.63	11	79.09	4.09	5
T3	74.93	4.96	10	78.79	3.13	4
T4	75.05	4.62	6	78.39	5.40	3

Note. M= Mean DNAmS (%), SD= standard deviation (%), N= Number of analyzed samples.

In SG31, DNAmS of abstainers were circa 3% higher at each time point compared to relapsed patients. However, statistically, the differences were not significant.

Over the course of the study time, both subgroups maintained a stable DNAmS.

3.4.3 Sex and smoking as confounding factors for DNAmS in blood

The variable sex was distributed unevenly between the two groups, with 60.94% of the patients being male compared to only 33.73% of the control group. DNAmS of both groups, exemplified by T1, was in similar ranges with a median DNAmS of females of 76.42% and a median for males of 77.14% (Supplement 8.9).

71% of patients were smokers, in contrast to only 5% of the control group. Analyzed over both groups, smokers had a significantly higher DNAmS (77.78%) compared to the non-smokers´ level (76.15%; Z = -2.333, p = .020, Figure 17)

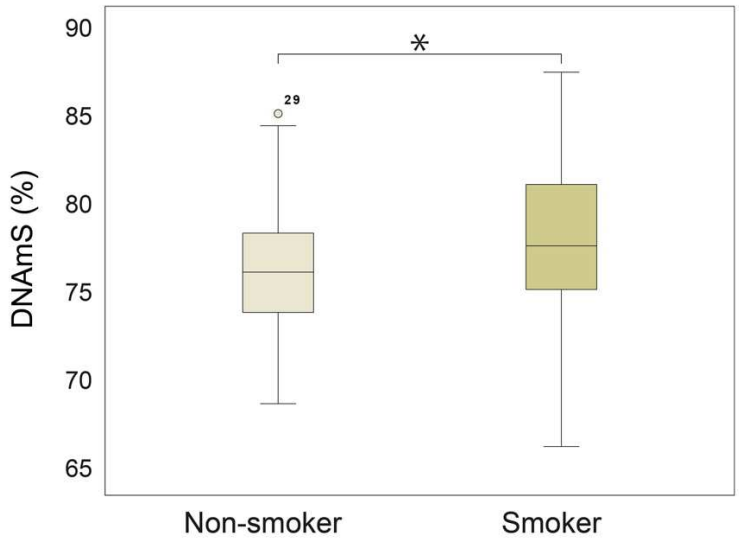


Figure 17 Boxplot of DNAmS (%) in blood of smokers and non-smokers at T1. *Boxes represent the interquartile range. Whiskers indicate the standard deviation of the mean.*

3.4.4 Association of DNAmS in with alcohol related variables

When correlating the DNAmS in blood with the questionnaire scores of both groups and all time points, weak correlations of DNAmS with GSI and OCDS score were detected (GSI score: Spearman's ρ = .152, *p* = .004; OCDS score: Spearman's ρ = .110, *p* = .036; Figure 18, Supplement 8.10). The AUDIT score did not correlate with DNAmS.

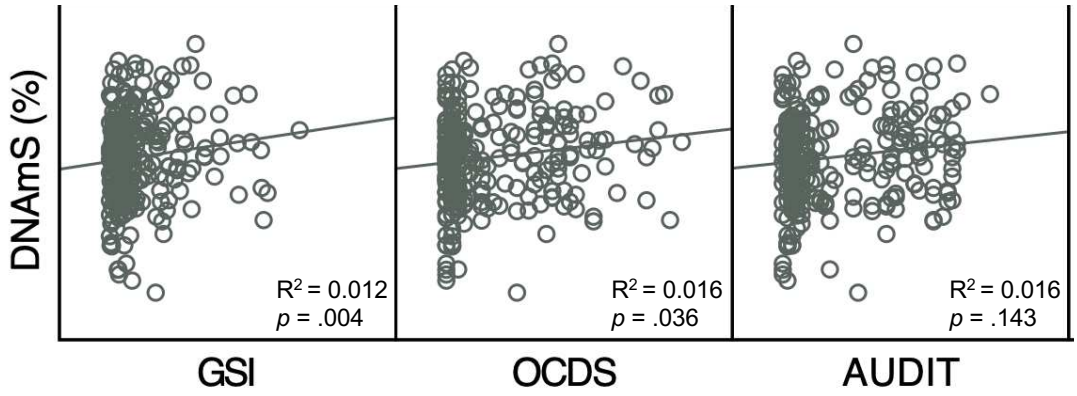


Figure 18 Scatterplot matrix for correlations between DNAmS in blood (%) and questionnaire scores *over both groups and all time points. Psychological distress (GSI, Spearman's ρ = .152,* p *= .004) and alcohol craving (OCDS, Spearman's ρ = .110,* p *= .036) are positively and statistically significantly correlated with DNAmS (%) in blood.* R^2*= Coefficient of determination.*

When correlating the DNAmS in blood with the questionnaire scores of both patients and control individuals of SG31 over all time points, OCDS and AUDIT score did not correlate with DNAmS in blood. However, a weak positive correlation was detected with GSI score (GSI score: Spearman's ρ = .135, p = .026, Figure 19).

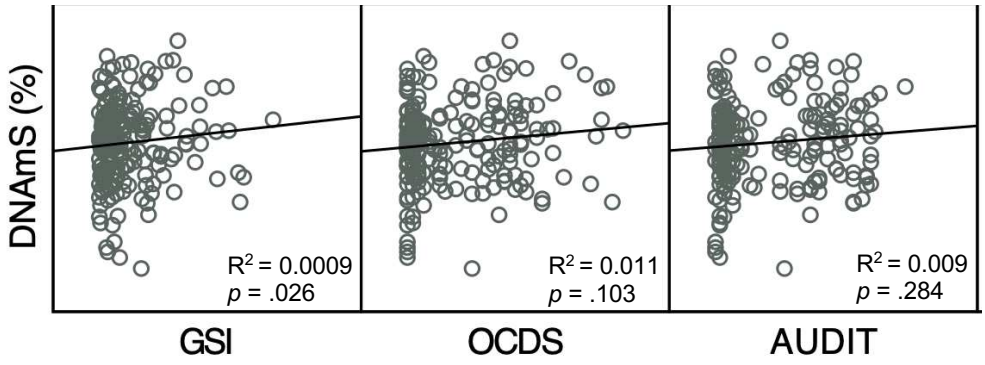


Figure 19 Scatterplot matrix for correlations between DNAmS in blood (%) and questionnaire scores of SG31 *at all time points. DNAmS and GSI score (psychological distress) showed a weak correlation.* R^2*= Coefficient of determination.*

3.5 DNAm level of *SYNGAP1* in saliva

Author´s note: Data concerning the scores of DNAm levels in saliva at T1 and T2 had been published in the following publication: Edelmann S, Kummer C, Pasche S, Zimmermann M and Nieratschker V (2025) Epigenetic regulation of SYNGAP1 in alcohol use disorder in whole blood and saliva. Front. Psychiatry 16:1661760. doi: 10.3389/fpsyt.2025.1661760.

In saliva samples, the DNAmS was analyzed at each time point in both groups. The mean DNAmS in saliva ranged between 87 and 88.1%, being approximately 12% higher than in the blood samples. The standard deviation, in contrast, is smaller and amounts 2 to 3 % (Table 20).

Table 20 Descriptive analysis of DNAmS in saliva of patients and controls at T1-T4.

Time	Group					
	Patients			Controls		
	M	SD	N	M	SD	N
T1	87.29	2.91	49	87.29	2.78	56
T2	87.36	2.26	42	87.19	2.72	49
T3	87.97	2.81	13	87.14	2.49	48
T4	86.14	2.01	10	88.10	2.57	33

Note. M=Mean DNAmS (%), SD= Standard deviation (%), N= number of analyzed samples.

The DNAmS in saliva of the patient and control group were in similar ranges at all time points, not differing significantly (Table 20, Figure 20).
Furthermore, in patients, the DNAmS remained at a stable level over the course of the study time (Figure 20).
In the control group, DNAmS was stable from T1 to T3 at approximately 87% and then increased towards T4, with a significant difference of 0.81% between T1 and T4 (Z = -2.116, *p* = .034, Figure 20). The rest of the changes between the time points per group did not differ significantly.

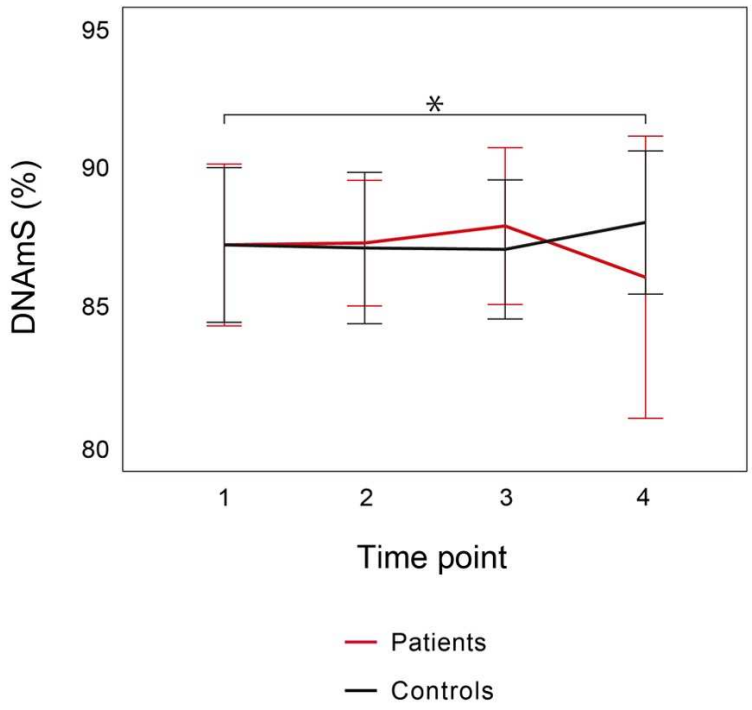


Figure 20 Line plots of mean DNAmS (%) in saliva of patients and control individuals *over sampling time. Significance in changes over time is described by * indicating p ≤ 0.05 in the corresponding color of the group. Error bars indicate standard deviation of the mean.*

The mean DNA methylation level of *SYNGAP1* in saliva of relapsed patients ranged from 84 to 88%. The abstainers DNAmS was between 88 and 90%, exceeding the level of the overall patient group by up to 3%. The standard deviation ranged between 1 and 6% (Table 21).

Table 21 Descriptive analysis of DNAmS in saliva of abstinent and relapsed patients *at T1- T4.*

Time	Subgroup					
	Relapsed			Abstinent		
	M	SD	N	M	SD	N
T1	88.44	2.49	13	88.06	2.39	5
T2	87.54	2.66	12	88.56	1.52	5
T3	87.01	2.95	8	90.20	1.37	4
T4	84.44	5.63	6	90.31	2.64	2

Note. M=Mean DNAmS (%), SD= Standard deviation (%), N= Number of analyzed samples.

Statistically, the DNAmS in saliva did not differ significantly between the subgroups at all four time points.

However, the DNAmS of relapsed patients decreased while those of abstinent patients *increased* over the course of sampling time: Over the course of sampling time, DNAmS in saliva of relapsed patients decreased by 4% between T1 and T4, then reached a level of 84.44% (Z = -1.992, *p* = .046; Figure 21). In abstainers, a trend of increase was revealed from 88.06% and T1 and 90.20% at T3 (T1/T3: Z = -1.826, *p* = .068; T2/T3: Z = -1.826, *p* = .068). The rest of the dynamics were not of statistical significance.

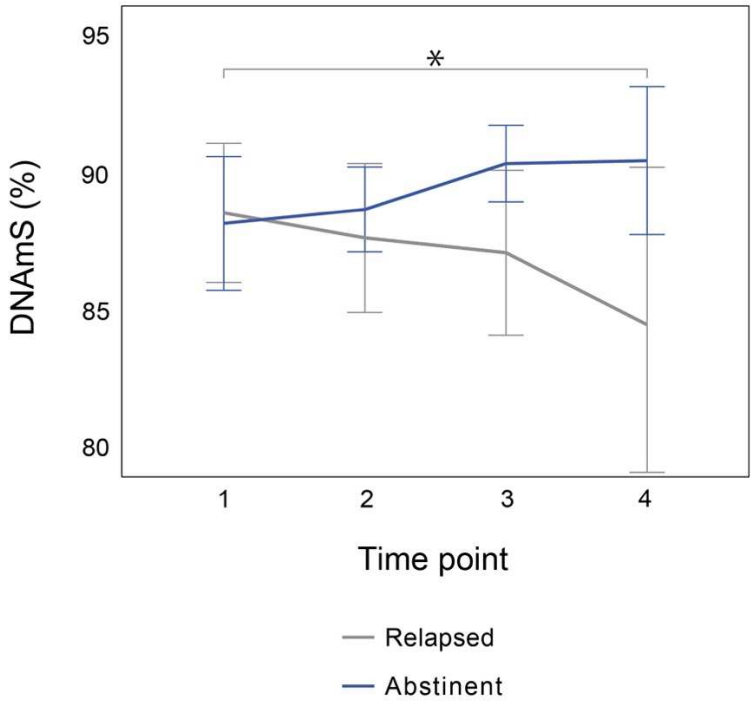


Figure 21 Line plots of mean DNAmS (%) in blood of relapsed and abstinent patients *over sampling time. Significance in changes over time is described by * indicating p ≤ 0.05 in the corresponding color of the group. Error bars indicate standard deviation of the mean.*

Comparing the patients´ subgroups with the control individuals, the control group and relapsed subgroup were in similar levels at time points T1 to T3. At T4, the level of the relapsed subgroup drops below that of the control group without a significant difference.

The abstainers´ DNAmS in saliva was significantly higher than the two other (sub-)groups only at T3, where it exceeded the levels of relapsers and control individuals by 3%, corresponding to a DNAmS of 90.2% (Z = -2.370, *p* = .018, Figure 22).

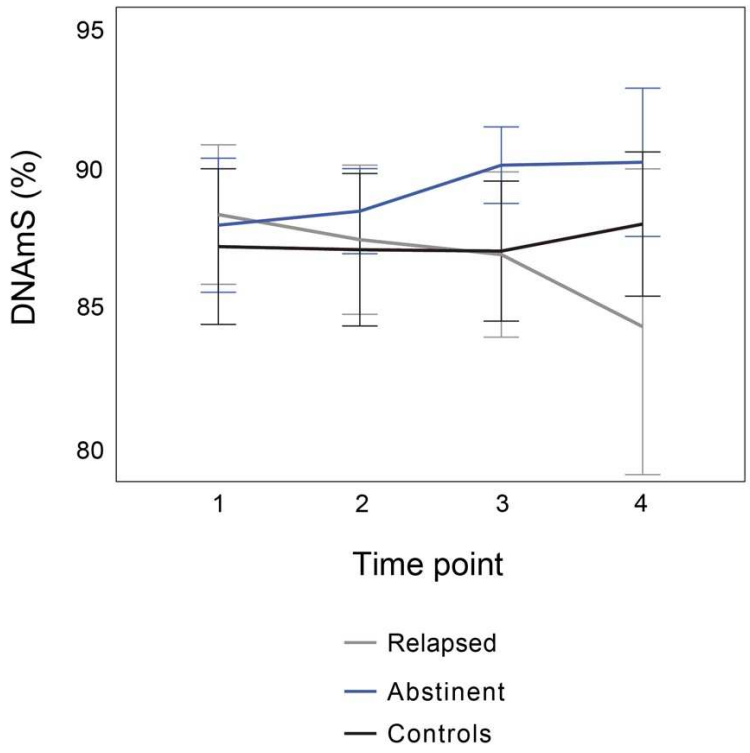


Figure 22 Overview of line plots of mean DNAmS (%) in saliva of control individuals, abstinent and relapsed patients *over sampling time point. Error bars indicate standard deviation of the mean. Significances are not displayed.*

3.5.1 Age, sex, and smoking status as a confounding factor for DNAmS in saliva

DNAmS in saliva did not correlate with age (centered, Spearman's ρ = -.026, *p* = .790).
DNAmS at T1 was very similar comparing the levels of male (median: 87.78%) and female (median: 87.76%) study participants (Supplement 8.11).
Also, regarding the smoking status, the DNAmS at T1 were in similar ranges comparing the median of smoking participants of 87.71% and that of non-smoking study participants of 87.79% (Supplement 8.12).

3.5.2 Association of DNAmS in saliva with alcohol related variables

When assessing the correlation between the DNAmS in saliva and the alcohol related variables over both groups and all time points, weak negative correlations were detected with the OCDS (Spearman's ρ = -.130, *p* = .025; Figure 23) and with the AUDIT score (Spearman's ρ = -.145, *p* = .031; Figure 23). GSI did not correlate with DNAmS in saliva.

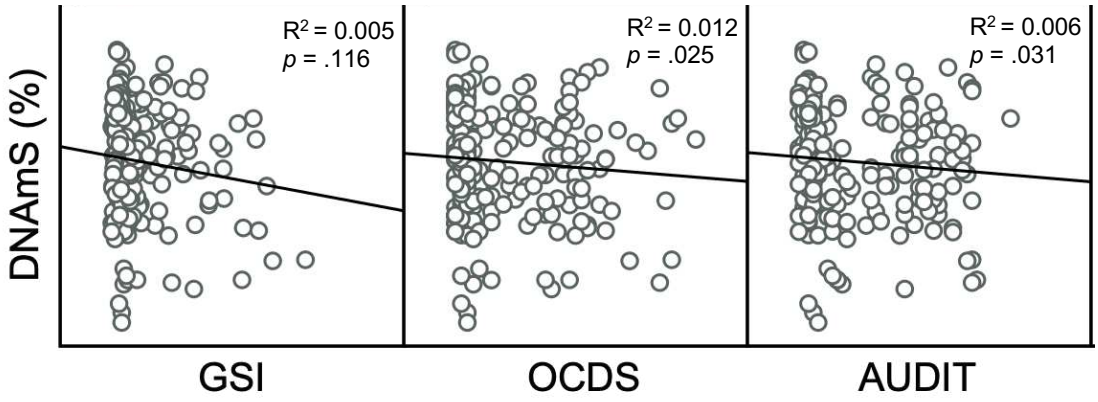


Figure 23 Scatterplot matrix for correlations between DNAmS in saliva (%) and questionnaire scores *over both groups and all time points. Alcohol craving (OCDS overall score, Spearman's ρ = -.130,* p *= .025) and alcohol consumption (AUDIT score, Spearman's ρ = -.145,* p *= .031) are positively and statistically significantly correlated with DNAmS (%) in saliva.* R^2*= Coefficient of determination.*

4 Discussion

AD is a severe disease with extensive health consequences for individuals and social and economic burdens for the society (Peacock et al., 2018; Rehm et al., 2009; World Health Organization, 2018, 2022).

Well studied therapy options are, for instance in the German health care system, widely available. Still, relapses are often experienced after completion of withdrawal treatment, especially if no further treatment is attended. For these cases, Batra et al. stated a relapse rate of 85% (Batra et al., 2016). In our study we observed a relapse rate of 73% after six months and 70% 12 months after therapy, approximately in line with the literature.

Efforts are made to develop a broader understanding of AD, its etiology, and physical correlates, aiming for progress parameters, biomarkers and, in the long run, for developing individually adapted therapy options.

Potential biomarkers have been identified in former studies (Brückmann et al., 2017; Liu et al., 2018).

The present study aimed to validate the significance of one of these candidate biomarkers, i.e. the DNA methylation (DNAm) level of a particular CpG site in the promotor region of *SYNGAP1* (Brückmann et al., 2017) in association with established questionnaires assessing alcohol related variables.

4.1 Questionnaires

As expected, in all questionnaires at all four time points, scores for harmful alcohol consumption, psychological distress, and alcohol craving, respectively, were significantly higher in AD patients compared to the control group (Edelmann et al., 2025). We thus concluded that they provided an adequate scale for alcohol related variables, as had been shown in previous studies (Mann & Ackermann, 2000; Mercier et al., 1992; Williams, 2014).
Interpreting the interplay between alcohol related variables, a strong correlation between psychological distress, alcohol consumption and craving was observed both in control individuals and AD patients. However, questions of causality remained unanswered in this study and would represent an interesting subject for further investigations.

4.1.1 Alcohol related variables in control individuals

Psychological distress, alcohol consumption and craving were, as expected, significantly lower at all four times in control individuals compared to AD patients. While well-being of control individuals remained constant, their reported alcohol consumption decreased between study inclusion and twelve months afterwards and between six and twelve months. Alcohol craving declined over the first three weeks after study inclusion. One possible explanation for this reduced consumption and craving could be an increased sensitivity due to study participation. Unfortunately, literature discussing this specific effect in control individuals is, to our knowledge, not available.

In summary, well-being of controls remained stable while an interesting decrease of alcohol consumption and craving was observed. Developing an explanation for this trend would have to be subject of further research.

4.1.2 Alcohol related variables in patients

In patients, an overall higher scoring of alcohol related variables was observed compared to the control group. They reported an improvement of psychological well-being after therapy. This development is the intended and expected outcome of undergoing a withdrawal and could therefore be interpreted as an indication for therapy success.
Over the following year, their psychological distress increased. This trend was also observed in a study by Sander and Jux (Sander & Jux, 2006). One explanation could be the confrontation with manifold challenges when returning to everyday routines, in contrast to the stay in the protected framework of an inpatient setting. Situations where conditioning had been happening before therapy are encountered that contain so-called drug-related *cues*. Cues are stimuli eliciting a conditioned behavior and exert a recurring pressure of dependence (Sinha & Li, 2007), negatively influencing the well-being of AD patients in attempt of abstinence.

Alcohol consumption of patients decreased significantly over the six months after therapy start, which can be attributed to the period of abstinence within therapy and the lasting effect of it over the following six months (Edelmann et al., 2025).
Craving for alcohol decreased, as expected, significantly over therapy, and remained at a stable level afterwards (Edelmann et al., 2025).
Even though increasing trends in some alcohol related variables were revealed after the end of therapy, their levels remained for the following twelve months below those at therapy start.

The QE (=*Qualifizierte Entzugsbehandlung*, qualified withdrawal treatment) is the recommended and evidence-based withdrawal therapy in Germany (Deutsche Gesellschaft für Psychiatrie und Psychotherapie & Suchttherapie, 2020) and attended by our patient collective. In addition to physical withdrawal, this treatment includes psychoeducation, various forms of therapy and motivating for a lasting abstinence (Batra et al., 2016). Its effect, compared to a

limitation to physical withdrawal, has been proven in several studies (e.g. (Bauer & Hasenöhrl, 2000) and also reflected in the long-lasting reduction of alcohol consumption and alcohol craving surveyed in this study, suggesting the success of withdrawal therapy. Patients´ recurring psychological distress after therapy are in line with the high risk of relapse stated in the literature (Batra et al., 2016).

4.1.3 Alcohol related variables in abstinent vs. relapsed patients

One objective of this study was to identify differences between relapsed and abstinent patients. To approach this purpose, the patients were grouped into subgroups of relapsed and abstinent patients. The limited reliability due to the small number of samples per subgroup should be emphasized at this point. Nevertheless, interesting first trends became visible.

Although the differences were not significant, the trend of initially lower scoring being associated with abstinence indicates a promising use of questionnaires in the clinical context as a predictor for successful therapy outcome, i.e., abstinence. Six months after therapy, abstinent patients reported higher levels of all alcohol related variables, alcohol consumption and craving differing significantly. This contrast might reflect the distress connected to recurring alcohol consumption affecting only relapsing patients.
This connection corresponds to a prospective study published by Bottlender and Soyka in 2004. They observed that, in subjects with AD undergoing an outpatient treatment, alcohol craving at the end of treatment (assessed with OCDS) was significantly higher in patients who, after 12 months, had experienced a relapse (Bottlender & Soyka, 2004). Soyka et al. replicated this result in a study analyzing follow-ups over 24 months after therapy onset (Soyka et al., 2010). Similar findings are available for GSI (Engel et al., 2015; Lucht et al., 2002). To our knowledge, for AUDIT, corresponding studies have not been published at present. However, a positive correlation between past

alcohol intake (Tuithof et al., 2014) or severity of dependence (Yoshimura et al., 2021) and risk of relapse has been observed.

12 months after therapy start, abstinent and relapsed patients reported similar levels of psychological distress and alcohol craving. More information about follow-up treatments (e.g. outpatient psychotherapy), adherence to medication, rehabilitation measures and life circumstances as well as the status of abstinence or relapse, respectively, in the course of time would be very interesting to better understand this trend since it has been observed that patients without further treatment after withdrawal are in high risk of relapsing (Batra et al., 2016). Unfortunately, information about these circumstances was not available for our study collective.

Psychological distress and alcohol craving showed similar dynamics over time in both subgroups. Psychological distress may be a major motive leading to alcohol consumption (van Gils et al., 2021).

Alcohol craving decreased in both of our subgroups. This is an indicator that, despite experienced distress and recurring drinking by part of the collective, QE´s positive effects remained over the first year after treatment as observed by former studies (Bauer & Hasenöhrl, 2000). This effect could – and should – be reinforced by follow-up treatments to establish long-lasting abstinence.

4.2 DNA methylation level of *SYNGAP1* and its potential function as a biomarker for AD

In the study on which this work was based, Brückmann et al. analyzed the DNAm of *SYNGAP1* (DNAmS) in $CD3^+$ T-cells of AD patients. An intriguing dynamic was revealed: Initially, DNAmS was significantly lower in AD patients compared to control individuals, and, after therapy, reverted to a level not differing from the control level anymore (Brückmann et al., 2017). These findings were to be validated in the present study using tissues which are more comfortable to sample and analyze than specific immune cells: venous peripheral whole blood and saliva. The most promising finding in the present study was the altered DNAmS in patients´ blood before therapy compared to the control group´s level when including individuals of all ages (Edelmann et al., 2025). This endorsed Brückmann et al.´s discovery, although we detected a higher instead of a lower methylation (Brückmann et al., 2017). The opposite direction might be explainable with the different sample tissues since DNAm varies widely in the different materials (Harlaar & Hutchison, 2013). This finding has to be regarded carefully due to a potential confounding effect of the age variable.
Further interesting results were obtained, opening new insights on *SYNGAP1* and its function in AD.

4.2.1 SYNGAP1

SynGAP protein, encoded by *SYNGAP1*, is involved in major protein networks, predominantly in excitatory neurons of the cortex and hippocampus (Araki et al., 2020). Among them are the postsynaptic density (Araki et al., 2020) and NMDAR networks (Kim et al., 1998) where it fulfills both signaling and scaffolding functions (Gross, 2022). It is crucial for the brain development (Araki et al., 2020), regulation of neuronal excitability (Kilinc et al., 2018) and synaptic plasticity (Llamosas et al., 2021). Both the hippocampus and cortex are brain regions affected by addiction and AD in particular, as is the synaptic function

(Egervari et al., 2021; Koob & Volkow, 2016; Peyton et al., 2021). Therefore, *SYNGAP1* is a gene worth investigating with regards to its changes associated with AD.

4.2.2 Molecular consequences of DNA (de-)methylation of SYNGAP1

Author´s note: A briefer overview of the molecular reasoning of DNA (de-)methylation of SYNGAP1 and its potential consequences had been, prior to the publication of this doctoral thesis, published in the following: Edelmann S, Kummer C, Pasche S, Zimmermann M and Nieratschker V (2025) Epigenetic regulation of SYNGAP1 in alcohol use disorder in whole blood and saliva. Front. Psychiatry 16:1661760. doi: 10.3389/fpsyt.2025.1661760

The DNAm level of a gene or CpG site must be considered in relative terms, taking into account the function of the corresponding DNA segment, the analyzed tissue and the cell type. Generally, not the concrete level of a certain gene or CpG site is crucial for the proper function of the analyzed gene. Rather, the deviation from the physiological level should be considered as an indicator for a relevant change in DNAm. A precise DNAm, in interplay with many other regulatory factors, is needed for the correct maturation and function of the corresponding gene, cell and tissue, for example the central nervous system (Moore et al., 2013). Therefore, it is often not the DNAm level itself which is reported by researchers but rather the alteration from control levels.
In cancer research, for example, different cancer entities have been correlated to hypomethylation (Gaudet et al., 2003; Li & Zhang, 2014). Gaudet et al. created a mice line depleted of a functional DNA methyltransferase 1 allele, presenting a global DNAm of 10% of the wild type. As a result, the animals developed aggressive T cell lymphomas, implying a protective character of DNAm (Gaudet et al., 2003). On the other hand, *hyper*methylation in promoter regions of tumor suppressor genes can contribute to a higher risk for the development and progression of cancer (Baylin & Jones, 2016) since the expression of protective factors are reduced by DNAm. This demonstrates that

DNAm is part of a complex system, which must be carefully considered and put into perspective of the respective research context.

A negative correlation between the DNAm of CpG sites in promotor regions and gene expression was reviewed and validated by Brenet et al. (Brenet et al., 2011). Due to the localization of the here analyzed CpG site in the promotor region of *SYNGAP1,* a suppressed gene expression is expected in case of DNAm (Brenet et al., 2011; Reik & Dean, 2001).
Thus, an increased DNAmS suggests the consequence of a reduced abundance of SynGAP in the central nervous systems synapses, compared to a baseline level. This triggers signal cascades that have not yet been conclusively uncovered. However, evidence has been presented that they include Ras, Rab and Rap proteins, the ERK pathway and changes in SynGAPs localization (Araki et al., 2015; Kim et al., 1998; Krapivinsky et al., 2004; Oh et al., 2004; Wang et al., 2013). Literature is consistent in the finding that the cascades downstream of a changed activity of SynGAP (compared to baseline activity) favor the insertion of AMPARs in the postsynaptic membrane (Figure 25), resulting in LTP induction (Araki et al., 2015; Kilinc et al., 2018; Rumbaugh et al., 2006) and enhanced excitability.

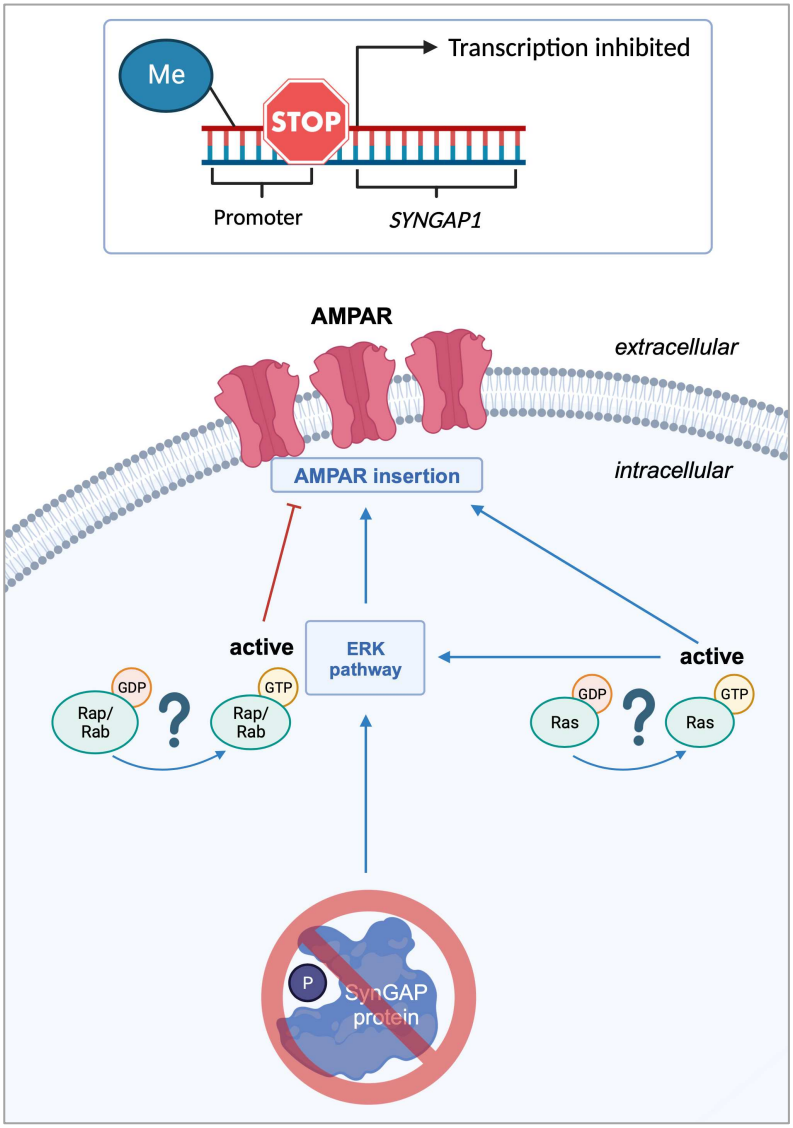


Figure 24 Schematic display of the consequence of DNAm of SYNGAP1. *DNAm of SYNGAP1 leads to a decreased abundance of SynGAP. Different signaling cascades result in an increased insertion of AMPARs in the postsynaptic membrane, favoring LTP induction (Araki et al., 2015; Kilinc et al., 2018; Kim et al., 1998; Krapivinsky et al., 2004; Oh et al., 2004; Rumbaugh et al., 2006; Wang et al., 2013). Created with BioRender.com.*

This enhanced excitability through higher DNAmS (Figure 24) effects the synapse in the same direction as an activation of the neuron (see Chapter 1.3.1, Figure 4).

On the other hand, a reduced DNAmS would lead to an increased expression of SynGAP. This would represent a stimulus to the signal cascades mimicking the state of baseline activity, resulting in a reduced insertion of AMPARs

(Figure 25). This consideration is consistent with a finding by Rumbaugh et al. made in cultured neurons presenting a SynGAP overexpression: They reported a decreased number of inserted AMPARs in the synapse membrane and a resulting downregulated excitability (Rumbaugh et al., 2006).

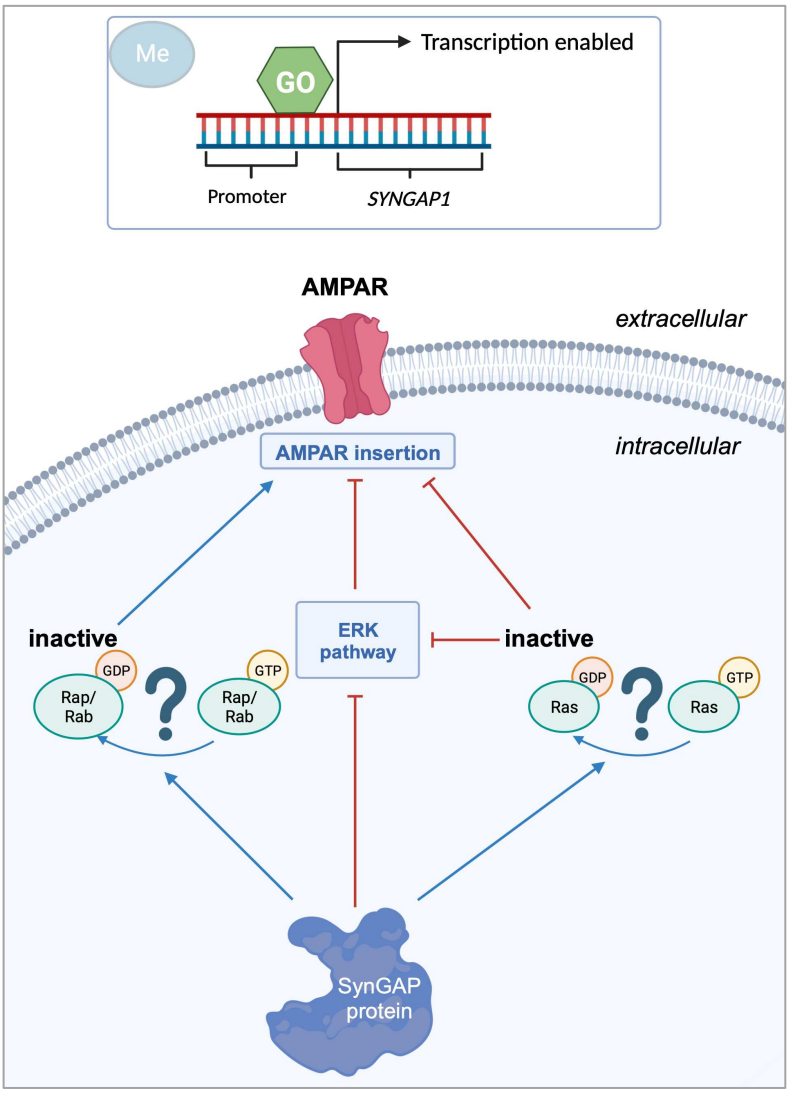


Figure 25 Schematic display of the consequence of DNA demethylation of SYNGAP1. *The heightened abundance of SynGAP protein and its downstream cascades lead to an inhibited AMPAR insertion (Rumbaugh et al., 2006). Created with BioRender.com.*

4.2.3 Tissue-specificity of DNAmS and the challenge of selecting a suitable sample material

Epigenetic marks vary fundamentally between individuals and different somatic tissues (Cabrera-Mendoza et al., 2023; Edelmann et al., 2025; Hannon et al., 2015b). The choice of a tissue and cell type providing robust information about an epigenetic attribute concerning the respective object of research is substantial and challenging (Edelmann et al., 2025; Harlaar & Hutchison, 2013).

Brückmann et al. reported a DNAmS in $CD3^+$ T-cells of, roughly, 50 to 70% (Brückmann et al., 2017). In our peripheral venous whole blood samples, the DNAmS were in ranges of 72 to 83% and, in saliva samples, in ranges of 85 to 91% (Edelmann et al., 2025).
Hannon et al. created an online tool, presenting references for researchers of the DNAm levels of Illumina 450K probes in blood and four brain regions (prefrontal cortex, entorhinal cortex, superior temporal gyrus and cerebellum) and the correlation between them (Hannon et al., 2015a). The database was fed with the information on the tissues of over 70 individuals (Hannon et al., 2015b). The tool displays a DNAmS in blood of approximately 70 to 85%, compatible with our findings. In the brain material, they state a DNAmS of 40 to 65% in the cerebellum and 70 to 80% in the prefrontal cortex, entorhinal cortex, and superior temporal gyrus (Hannon et al., 2015b). Furthermore, a trend of correlation between DNAmS in blood and the prefrontal cortex was displayed ($r = 0.219$, $p = 0.061$(Hannon et al., 2015a)).
It is interesting that a trend of correlation between DNAmS in blood was found with the prefrontal cortex´ DNAmS since the cortex is a brain region where *SYNGAP1* is highly expressed (Araki et al., 2020). Furthermore, the prefrontal cortex is especially intertwined in the neurocircuitry of addiction in the phase of craving (Edelmann et al., 2025): A glutamate-mediated activation of the PFC takes place when facing drug-associated cues, sending projections to the striatum. It is attributed to a central position in the controlling of craving (Koob & Volkow, 2016). Simultaneously, its activation decreases – probably due to dysregulated GABA-signaling – and impedes decision making and self-

regulation (Koob & Volkow, 2016). This potentially favors poor decision making and drug-seeking behavior that promotes relapsing.
If the model of the biochemical functioning of SynGAP displayed here is accurate, a neuronal activation during craving would be correlated with an increase in DNAmS, which would enable glutamatergic activity (Figure 24, (Edelmann et al., 2025)). “This [model] is coherent with our finding of a significantly higher DNAm[S] in blood of patients compared to control individuals [when analyzing all age groups] and substantiates the potential as a possible diagnostic biomarker” (Edelmann et al., 2025).

As mentioned above, Hannon et al.´s online tool displays a trend of correlation between DNAmS in blood and the prefrontal cortex ($r = 0.219$, $p = 0.061$(Hannon et al., 2015a), where *SYNGAP1* is highly expressed (Araki et al., 2020).
Considering this connection and the fact that the use of brain material itself for diagnostics and monitoring is not possible, the use of blood biomarkers, i.e., potentially DNAmS, is a feasible approach. Regarding saliva, to the best of our knowledge, the present study was the first to investigate DNAmS to date. Saliva is a tissue easily and non-invasively to collect and relatively uncomplicated in storage, containing DNA of good quality and thus being a promising supplementary diagnostic material to blood (Zhang et al., 2016). Whole blood itself is a widely used diagnostic tissue in the medical field. These two materials, suitable for clinical use, were chosen for our study in order to control for their efficiency as a biomarker.

Blood has been successfully used as a medium for biomarker identification, for example in cancer diagnosis (e.g. (Birse et al., 2017; Duffy, 2019). Among them, circulating DNAm has been discussed as a promising biomarker for early detection of various tumor entities such as hepatocellular carcinoma and colorectal cancer (Luo et al., 2020; Xu et al., 2017).
In psychiatric and neurodegenerative disorders, the blood brain barrier had been discussed, though not finally understood, as a potential obstacle for

circulating biomarkers comparing their expression in cerebrospinal fluid and blood (van den Berg et al., 2020).
It is possible that the stated challenges also apply to Hannon et al.´s discovery of only a trend of correlation between the DNAmS in the prefrontal cortex and blood(Hannon et al., 2015a). A limiting factor for the expressiveness of DNAmS in blood could as well be the limited expression of *SYNGAP1* in peripheral tissue (Chen et al., 1998). However, peripheral blood has been used for the analysis of DNAm alterations in AD, where findings consistent with literature have been made, thus implying that whole blood might be an adequate sample material for our study aim (Witt et al., 2020).

4.2.4 Control for age, sex and smoking as confounding factors

Age (Seale et al., 2022), sex (Solomon et al., 2022) and exposure to noxious substances such as tobacco smoke (Breitling et al., 2011) are known to have a sensitive influence on epigenetics.
A confounding factor is a variable that, preexisting before the study, correlates with both the independent and the dependent variable of a study and carries the risk of confounding an actual effect (Vetter & Mascha, 2017). In our case, the independent variable was the allocation to the study groups. Despite targeted recruitment intending to obtain balanced groups concerning three crucial variables for epigenetic configuration– sex, age, and smoking status –, an uneven distribution remained, differing widely between the patient and control group (Edelmann et al., 2025). We therefore controlled for their correlation with the dependent variable, i.e. DNAmS in blood or saliva, respectively.
Regarding the DNAmS in blood, a correlation with age was observed. However, the coefficient of determination (R^2) was close to zero, suggesting that there was no important linear correlation between the variables (Pardoe, 2021). Still, we controlled for the age variable to explore whether it can provide interesting insides. When excluding the study subjects aged 30 years and younger, the correlation disappeared. It therefore remains to cautiously consider the unequal distribution of age as a possible confounding factor that may have suggested a

spurious statistical effect (Vetter & Mascha, 2017). The results and differences between the age-subgroups will be discussed in the following chapters.

In saliva, age did not show confounding effects concerning DNAmS.
It was also found that the variable sex was not a confounding factor for either blood or saliva samples.
In contrast, DNAmS was significantly higher in smokers compared to non-smokers in the blood samples (77.78% vs. 76.15%) but not in saliva samples. Alcohol and tobacco use disorders are generally strongly correlated (Jackson et al., 2003) which also applied to our study collective(Edelmann et al., 2025): 71% of patients were smokers, in contrast to only 5% of the control individuals. Therefore, measured differences in DNAmS could as well be caused by AD and not by smoking. Consistent with this consideration is the finding that DNAmS in the blood of both smokers *and* AD patients was significantly higher than in non-smokers or in the control group, respectively. Furthermore, in saliva, DNAmS did not differ between the patient and control group or between smokers and non-smokers. These findings substantiated the assumption that smoking as a widespread comorbidity of AD is not to be treated as a confounding factor in order to prevent the masking of actual differences.

4.2.5 Association of SYNGAP1 DNA methylation in blood with age

Another crucial finding in the present study was the significantly positive correlation between age and DNAmS in blood at study start – and not at later time points–, although the R^2 close to zero suggested that it might not be linear (Pardoe, 2021). Nevertheless, considering a potential biological effect of DNAmS – or SynGAP protein, respectively – on age, and vice versa, could lead to interesting insights.
Nyffeler et al. revealed a decrease of SynGAP correlated to aging in hippocampal brain slices of mice (Nyffeler et al., 2007). Other components connected to SynGAP in the post-synaptic density (PSD) in excitatory neurons also decreased with age, such as CaMKII. They suggested that aging has a

major impact on synaptic morphology, resulting in a reduced efficiency of its activity. Additionally, LTP – which is affected by SynGAPs activity among others – has been long known to function unchanged, but decays more rapidly in aged individuals than in young ones (Nyffeler et al., 2007).
The result of decreased SynGAP in higher age is consistent with our finding of a positive correlation between DNAmS and age and the model displayed in Figure 24: Higher DNAmS results in a lower abundance of SynGAP proteins. However, lower levels of SynGAP would induce a higher insertion of AMPAR (Figure 24). The seemingly contradictory reduced synaptic efficiency in older individuals described by Nyffeler et al. might be a result of other alterations in the complex PSD This interaction would be an interesting aspect of further research investigating *SYNGAP1* and its role in aging.

In Brückmann et al.´s study's cohort, the groups were matched more closely for age than in the present work. The control collective in the present study was, in average, six years younger than the control collective in Brückmann et al.´s study, while our patient collective´s mean age was two years older (Brückmann et al., 2017). The lack of comparability in age between the studies may have contributed to the divergent results, among other possibly reasons such as the choice of sample material, as discussed above.

A control for the age variable was conducted by excluding participants aged 30 and younger, resulting in age-comparable groups. The promising difference in DNAmS in blood between the patient and control group – discussed in detail in Chapter 4.2.6 – thereby disappeared.

Concluding, a correlation between DNAmS and age does not provide a complete consistency with our considerations of the biological function of SynGAP and findings in the literature. Due to this disparity, the R^2 close to zero and the fact that age correlated with DNAmS at study start only, further research on this topic would be needed.

4.2.6 Evaluation of DNAmS as a potential diagnostic biomarker for AD

Considering the biochemical level of the altered DNA methylation in patient´s blood before therapy to the control group when including individuals of all ages, the higher DNAmS suggests a reduced abundance of SynGAP, resulting in an elevated excitability (Figure 24), presumably mainly in the cortex due to its high expression there. As described above, the prefrontal cortex might play a role in control of craving (Koob & Volkow, 2016). At study start, the AD patients of our study collective have been hospitalized between one and three days before and were detoxified. They most probably were already experiencing withdrawal symptoms and craving which start six to 24 hours after the last alcohol consumption (Muncie et al., 2013). Given that *SYNGAP1* is highly expressed in the cortex, a higher DNAmS, elevated excitability, and cortical activity may be associated with craving periods.

NMDARs adapt during chronic alcohol intake and excessively re-activated during withdrawal, leading to hyperexcitability. This effect is called excitotoxicity and leads to toxic effects on cells/neurons (Hughes, 2009). Kong et al. reported a significant downregulation of SynGAP in mice undergoing alcohol withdrawal (Kong et al., 2022). Again, these findings substantiate our model of elevated excitability during withdrawal considering the biochemical mechanisms of SynGAP proteins: fewer SynGAP proteins – due to increased DNAmS – lead to an increase in AMPARs. The upregulation of NMDARs might potentiate this effect (Figure 24 and Figure 4 in Chapter 1.3.1).

However, the difference between the groups is detectable before/at therapy start and not at later time points. In order to be able to interpret the possible usage as a biomarker, it would be necessary to assess the different drinking patterns and investigate their association with DNAmS; cessation and re-starting of drinking, the amounts of alcohol impacting of DNAmS, chronic vs. binge-drinking might have different impacts.

The reversion of the alteration reported by Brückmann et al. was confirmed as well: The DNAmS level in patients´ blood decreased over therapy, approaching the control individuals´ level (Figure 26).

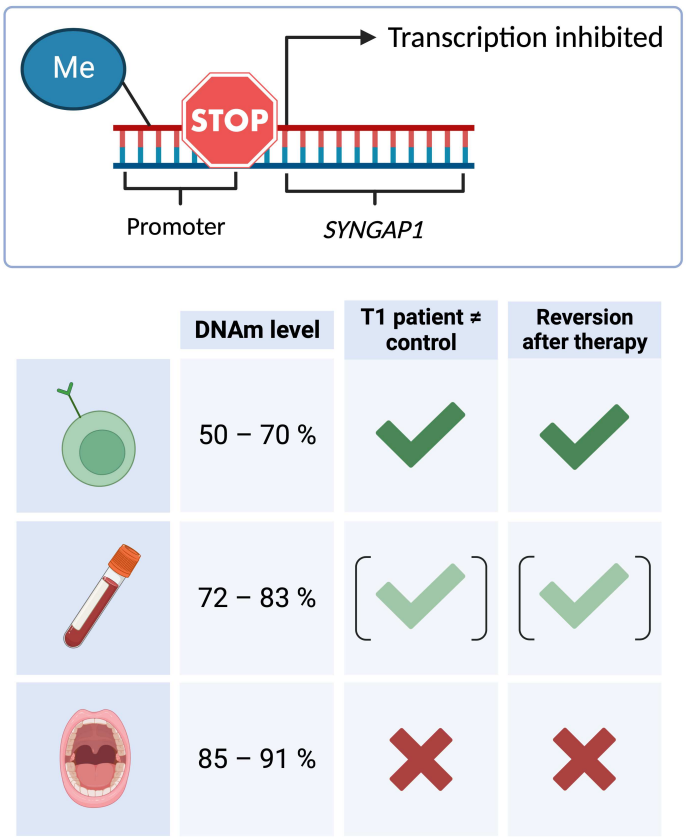


Figure 26 Summary evaluation of DNAmS as a diagnostic biomarker for AD in the different tissues. *The differential DNA methylation at T1 and reversion after withdrawal treatment are detectable in CD3⁺ T-cells: (Brückmann et al., 2017). In blood, promising findings were made in our study but need validation in a future work. In saliva, DNAmS did not show promising characteristics for a usage as diagnostic biomarker for AD. Created with BioRender.com.*

The expressive power of the reversion must be regarded cautiously, because DNAmS also decreased significantly over the same period of time in the control group (both if assessing all participants and aged 31 and older only). This suggests that certain fluctuations in DNAmS in blood over time may be physiological. Brückmann et al. reported the control individuals´ DNAmS at T1 only and not at T2, so that their development over time cannot be compared (Brückmann et al., 2017).

For DNAmS in saliva, no differences were observed between the control group and patients, suggesting that it is not a suitable marker to use for the diagnosis of AD (Figure 26).

4.2.7 SYNGAP1 DNA methylation and disease severity

Biomarkers for disease severity in substance use disorders have been missing, impeding personally adapted treatment development. However, they would be highly desirable to enable the improvement of therapy outcomes.
We assessed the alcohol related variables psychological distress (GSI in SCL-90), alcohol craving (OCDS) and alcohol consumption (AUDIT). They reflected adequately the differences between the two study collectives in accordance with literature (Mann & Ackermann, 2000; Mercier et al., 1992; Williams, 2014). All variables were significantly higher in patients than in the control group. After completion of the withdrawal treatment, psychological distress, alcohol craving and alcohol consumption had significantly decreased. Six months after therapy, patients experienced a recurring psychological distress, reflecting the high risk of relapse (Batra et al., 2016). Due to the small sample number when dividing the patients into subgroups of relapsed and abstinent patients, only few results remained. Still, six months after therapy, relapsed patients reported a higher alcohol consumption and craving and a trend of higher psychological distress indicating that recurring drinking affected their well-being substantially.

For DNAmS in blood, a strong positive correlation with psychological distress (GSI score) and a weak positive correlation with alcohol craving (OCDS score) was observed but none with alcohol consumption (AUDIT score). The correlation with alcohol craving ceased when including the participants aged 31 and older only.
In saliva, on the other hand, DNAmS correlated weakly with distress and alcohol consumption, but not with craving.
A possible explanation for the positive correlation between DNAmS and craving has been outlined in Chapter 4.2.2: The intracellular mechanism in times of suppressed SynGAP expression may possibly be associated with an elevated excitability in the prefrontal cortex during craving (Koob & Volkow, 2016).

Concerning the neurocircuitry of psychological distress, the amygdala is the

brain region known to be deeply involved in stress and anxiety response. It is regulated by both the hippocampus and the medial prefrontal cortex (the regions of high SynGAP expression) (Araki et al., 2020; Sanford et al., 2015). Furthermore, the hippocampus and prefrontal cortex show high affinity to the stress hormone cortisol and project glutaminergic feedback to the hypothalamus, thus regulating the hypothalamic-pituitary-adrenal axis (HPA axis) (Vachon-Presseau, 2018). When major acute stress is experienced – especially stress that the individual perceives as uncontrollable – functioning of the prefrontal cortex can be impaired (Arnsten, 2009), potentially contributing to a subsequent vicious circle of dysregulated HPA axis and further stress. Stress that activates glutaminergic projections in the hippocampus and prefrontal cortex (Vachon-Presseau, 2018), might be associated with a higher DNAmS. Higher DNAmS results in a decreased abundance of SynGAP and promotes the insertion of AMPARs and LTP (Kilinc et al., 2018)(Figure 25). This activation would feedback negatively on the HPA axis. However, the axis is regulated by other brain regions as well (Vachon-Presseau, 2018).
Another perspective is the reduced hippocampal volume of individuals with harmful alcohol use (Wilson et al., 2017). This could potentially reduce negative feedback of this brain region in times of stress, resulting in a stimulation of the HPA axis.

The correlations between DNAmS and questionnaire scores were mainly weak with low R^2 values, and not consistent over all variables. Conclusions should thus be drawn with caution. Still, the results provide interesting suggestions to further insights into the biological function of DNAmS in AD.

4.2.8 DNAmS and therapy response

AD patients very frequently experience relapses, with rates up to 85% according to literature (Batra et al., 2016). In our study collective, we observed a relapse rate of 73% six months and 70% 12 months after therapy, respectively. Response biomarkers aim to detect ineffective treatment attempts, in order to

adjust therapy options (Califf, 2018) and achieve a positive outcome without avoidable delays. DNAmS was, neither in blood nor saliva, significantly different between relapsed and abstinent patients nor between abstainers before therapy and at later time points.
It is to emphasize that, at the later time points, very few patients´ samples were available which could have led to a reduced statistical power.
However, the present results of the small collective suggest that DNAmS in blood or saliva has no major potency as a biomarker for therapy outcome.

4.2.9 Conclusion of DNAmS in blood and saliva as a biomarker for AD

The potential use of DNAm of the analyzed CpG site in *SYNGAP1* in blood as a biomarker for diagnostic, disease severity and therapy outcome of AD showed interesting trends but could not be finally validated. The results of the present study suggested that DNAmS in saliva does not have high potential as a biomarker for AD.
SYNGAP1 expression has been identified as correlated to alcohol withdrawal in mice´ brains (Kong et al., 2022), implying a connection between AD and this gene. However, gene expression underlies a complex network of regulatory factors which has been object of intense research (Pope & Medzhitov, 2018). Different classes of DNA binding transcription factors are essential in the regulation of gene expression. They interact with activating or repressing co-factors (Pope & Medzhitov, 2018). Other epigenetic mechanisms, such as histone acetylation, chromatin remodeling and non-coding RNA, contribute substantially to the control of gene expression (Figure 2, Chapter 1.2.) (Ferlier & Coulouarn, 2022; Romani et al., 2015). DNAm represents only one of the regulatory adjusting screws (Ferlier & Coulouarn, 2022). Thus, it is possible that relevant influences on *SYNGAP1* are induced by regulatory factors other than DNAm. Unfortunately, literature has been limited to gene expression or DNAm of *SYNGAP1*. Investigations into additional mechanisms related to *SYNGAP1* expression represent a necessary topic of research to provide a more complete picture of its regulation in general and specifically in association with alcohol

consumption and AD.

Furthermore, *SYNGAP1* is mainly expressed in the brain (Araki et al., 2020; Chen et al., 1998) and its DNA methylation between the brain and peripheral blood do not correlate significantly, only showing a trend of correlation between the prefrontal cortex and blood (Hannon et al., 2015a). Hence, DNAmS might be altered in AD patients' *brains* but not in their peripheral blood – or saliva – and may therewith not be visible in our study. Although blood has been identified as a powerful medium for isolating biomarkers, it shows limited informative value and correlation concerning processes in the central nervous system (Birse et al., 2017; Duffy, 2019; van den Berg et al., 2020). Thus, one important challenge for future studies remains the identification of a sample material that meets the requirement of both high expressive power and convenient access and analysis (Edelmann et al., 2025).

A promising finding was the correlation of the age variable with DNAmS in blood at study start. This correlation potentially had a confounding effect. We discussed a possible biological interplay of the two variables but could not find a conclusive connection with the function of *SYNAGP1/* SynGAP in literature. Therefore, the correlation and biological link should be re-validated.

An important limitation of our study was the high drop-out rate in the patient group six and 12 months after therapy start. It was therefore not possible to make statements about the potential association of DNAmS with relapse and abstinence. Interestingly, in blood, a non-significant difference between the DNAmS of abstinent and relapsed patients was detected. A follow-up study including more patients at later study time points would be highly desirable to further evaluate this trend.

5 Summary

5.1 Summary

Alcohol dependence (AD) contributes significantly to the global burden of disease. Genetic and environmental factors play a role in its development. High relapse rates show that a deeper understanding of the pathomechanism and new therapeutic approaches are urgently needed. In 2017, Brückmann et al. identified in an epigenome-wide study in $CD3^+$ T-cells differentially DNA methylated CpG sites in 59 genes from AD patients compared to controls (Brückmann et al., 2017). These included *SYNGAP1*: The DNA methylation level of *SYNGAP1* (DNAmS) of AD patients was initially significantly lower than that of control individuals and, after a three-week inpatient withdrawal treatment, approached that of the control group. In the present study, the differential DNAmS was to be validated in an independent subject collective and in more easily accessible material (saliva and peripheral venous whole blood) in order to determine its function as a biomarker for diagnostic, disease severity and therapy success in AD.

The study cohort consisted of 64 patients undergoing a three-week inpatient withdrawal treatment in the Department of Psychiatry at the University hospital in Tübingen and 84 control subjects, at four time points (0, 3 weeks, 6 months, and 12 months). The DNA was isolated from both saliva and blood, a bisulfite conversion was performed and the DNAmS was analyzed by pyrosequencing. Data on alcohol-related variables was collected with questionnaires – AUDIT questionnaire for alcohol consumption, OCDS for alcohol craving and GSI in SCL90-R for psychological distress.

The questionnaires reflected reliably the difference in alcohol-related variables between patients and control individuals. Correlations with DNAmS and alcohol-related variables were mostly weak and not consistent over all variables. However, they opened interesting insights into the biological function of *SYNGAP1* in times of craving and distress. The pattern of DNAmS observed in $CD3^+$ T-cells could not be validated in saliva. In blood, DNAmS was significantly higher in patients than in controls at study start – in contrast to Brückmann et al.´s work, where it was lower. After withdrawal therapy it had approached the

controls level. A potential confounding factor was found in the age variable since it correlated with DNAmS in blood at therapy start. After controlling for this by forming age subgroups, the difference between patients and controls before therapy could not be replicated. Significant differences in the DNAmS comparing the subgroups of relapsed vs. abstinent patients six or twelve months after therapy start were not detected in blood nor in saliva.
We found coherent links between *SYNGAP1*s biological function and both age and DNAmS and discussed possible roles for Ads pathomechanism.
Concerning an association of DNAmS with relapse or abstinence, a high dropout rate of patients at later study time points reduced the statistical power and no conclusions could be drawn.
In summary, in this study, DNAmS in saliva did not show patterns of a suitable biomarker for diagnostic, disease severity or therapy progress of AD. In blood, a promising difference between the groups at study start was discovered, although relativized by a potential confounding effect of age. Insights in the biological function of *SYNGAP1* and a potential pathomechanism for AD were found. A larger and more closely age-matched study collective would be needed to validate the differential DNAmS in blood of AD patients at study start and differentiate it from age effects. Given the link between the DNAm of *SYNGAP1* and its cellular function, further investigation would be a promising step towards a novel biomarker for AD and ultimately a potential therapeutic target.

5.2 Zusammenfassung

Die Alkoholabhängigkeit (AD) trägt erheblich zur weltweiten Krankheitslast bei. Genetische sowie umweltbedingte Faktoren spielen in ihrer Entstehung eine Rolle. Hohe Rückfallquoten zeigen, dass ein tieferes Verständnis des Pathomechanismus und neue therapeutische Ansätze dringend erforderlich sind. Im Jahr 2017 identifizierten Brückmann et al. in einer epigenomweiten Studie an $CD3^+$-T-Zellen differenziell DNA-methylierte CpG-Stellen in 59 Genen von AD-Patienten im Vergleich zu Kontrollen (Brückmann et al., 2017). Dazu gehörte *SYNGAP1*: Das DNA-Methylierungslevel von *SYNGAP1* (DNAmS) war bei AD-Patienten zunächst signifikant niedriger als bei Kontrollpersonen und näherte sich nach einer dreiwöchigen stationären Entzugsbehandlung dem der Kontrollgruppe an. In der vorliegenden Studie sollte das differentielle DNAmS in einem unabhängigen Probandenkollektiv und in leichter zugänglichem Material (Speichel und peripheres venöses Vollblut) validiert werden, um dessen Funktion als Biomarker für Diagnose, Krankheitsschwere und Therapieerfolg bei AD zu bewerten.

Die Studienkohorte bestand aus 64 Patienten, die sich einer dreiwöchigen stationären Entzugsbehandlung in der Psychiatrischen Universitätsklinik Tübingen unterzogen, und 84 Kontrollpersonen, und zwar zu vier Zeitpunkten (0, drei Wochen, sechs Monate und zwölf Monate). Die DNA wurde aus Speichel und Blut isoliert, eine Bisulfit-Konvertierung wurde durchgeführt und die DNAmS wurde durch Pyrosequenzierung analysiert. Daten zu alkoholbezogenen Variablen wurden mit Fragebögen erhoben – AUDIT-Fragebogen für den Alkoholkonsum, OCDS für Alkoholcraving und GSI in SCL90-R für psychische Belastung.

Die Fragebögen spiegelten zuverlässig den Unterschied zwischen Patienten und Kontrollpersonen in Bezug auf alkoholbezogene Variablen wider. Die Korrelationen mit DNAmS und alkoholbezogenen Variablen waren meist schwach und nicht über alle Variablen hinweg konsistent. Sie eröffneten jedoch interessante Einblicke in die biologische Funktion von *SYNGAP1* bei Craving und psychischen Stress. Das in $CD3^+$-T-Zellen beobachtete DNAmS-Muster

konnte im Speichel nicht validiert werden. Im Blut war die DNAmS bei den Patienten zu Beginn der Studie deutlich höher als bei den Kontrollen – im Gegensatz zu der Arbeit von Brückmann et al. Nach der Therapie hatte es sich dem Level der Kontrollen angenähert. Ein potenzieller Störfaktor wurde in der Altersvariable gefunden, da sie zu Studienbeginn mit der DNAmS im Blut korrelierte. Nachdem dies durch die Bildung von Altersuntergruppen kontrolliert worden war, konnte der Unterschied zwischen Patienten und Kontrollen vor der Therapie nicht reproduziert werden. Signifikante Unterschiede in der DNAmS zwischen den Untergruppen der rückfälligen und abstinenten Patienten sechs oder zwölf Monate nach Therapiebeginn wurden weder im Blut noch im Speichel festgestellt.

Wir fanden kohärente Zusammenhänge zwischen der biologischen Funktion von *SYNGAP1* und sowohl dem Alter als auch der DNAmS und erörterten mögliche Rollen für den Pathomechanismus der AD. Bezüglich einer Assoziation von DNAmS mit Rückfällen oder Abstinenz wurde die statistische Aussagekraft durch eine hohe Dropout-Rate von Patienten zu späteren Studienzeitpunkten eingeschränkt, so dass keine Schlussfolgerungen gezogen werden konnten.

Zusammenfassend lässt sich sagen, dass der DNAmS in Speichel in dieser Studie kein Muster eines geeigneten Biomarkers für die Diagnose, den Schweregrad der Erkrankung oder den Therapieverlauf von AD zeigte. Im Blut wurde ein vielversprechender Unterschied zwischen den Gruppen zu Beginn der Studie entdeckt, der allerdings durch einen potenziell störenden Effekt des Alters relativiert wurde. Es wurden Einblicke in die biologische Funktion von *SYNGAP1* und in einen möglichen Pathomechanismus für AD gefunden. Eine größere und genauer alters-gematchte Studienkohorte ist erforderlich, um die differentielle DNAmS im Blut von AD-Patienten zu Studienbeginn zu validieren und von Alterseffekten zu unterscheiden. Angesichts des Zusammenhangs zwischen der DNAm von *SYNGAP1* und seiner zellulären Funktion ist eine weitere Untersuchung ein vielversprechender Schritt auf dem Weg zu einem neuartigen Biomarker für AD und zu einem potenziellen therapeutischen Ziel.

6 List of references

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7 Erklärung zum Eigenanteil

Die Arbeit wurde in der Universitätsklinik für Psychiatrie und Psychotherapie Tübingen unter Betreuung von Prof. Dr. Vanessa Nieratschker durchgeführt. Weitere Betreuung wurde durch Dr. Susanne Edelmann geleistet.

Die Konzeption der Studie erfolgte durch Prof. Dr. Vanessa Nieratschker.

Die Rekrutierung, Entnahme von Blut- und Speichelproben sowie deren DNA-Isolierung und Bisulfitkonvertierung wurden seit 2020 fortlaufend von medizinischen Doktoranden der AG Molekulare Psychiatrie – Philipp Plessing, Tobias Löffler, Henning Plättner, Dominic Gaisbauer – durchgeführt. Von Oktober 2022 bis April 2023 übernahm ich diese Aufgaben. Die Rekrutierung wurde teilweise durch ÄrztInnen der Station 23 der Psychiatrie Tübingen unterstützt.
Die weiteren Arbeitsschritte bis zur Sequenzierung der DNA-Methylierung von *SYNGAP1* wurden, für alle analysierten Proben, von mir durchgeführt. Sämtliche Versuche wurden – nach Einarbeitung durch Dr. Susanne Edelmann und Sarah Pasche – von mir eigenständig durchgeführt.

Die statistische Auswertung erfolgte eigenständig durch mich, in regelmäßiger Rücksprache mit Dr. Susanne Edelmann.
Bei der Verwendung von Adobe Illustrator unterstützte mich Benjamin Geiger.

Vor Publikation dieser Dissertation erfolgte die Publikation von Teilen der Daten, der Ergebnisse sowie der Diskussion in folgender Publikation, in der ich als Zweitautorin geführt werde: Edelmann S, Kummer C, Pasche S, Zimmermann M and Nieratschker V (2025) Epigenetic regulation of SYNGAP1 in alcohol use disorder in whole blood and saliva. Front. Psychiatry 16:1661760. doi: 10.3389/fpsyt.2025.1661760. Dieses betrifft folgende Daten: Merkmale der Studienpopulation (d. h. Anzahl, Geschlecht, Raucherstatus, Alter), DNAmS

sowohl im Blut als auch im Speichel zu T1 und T2 sowie die AUDIT- und OCDS-Werte zu T1 und T2.
In der vorliegenden Dissertation habe ich die Stellen, an denen Daten oder Inhalte vorkommen, die bereits im genannten Paper publiziert wurden, kenntlich gemacht. Dies erfolgte durch den Kapiteln und Unterkapiteln vorangestellte Anmerkungen („Autor´s note“) oder durch Zitationen.
Die statistische Auswertung in der Publikation von Edelmann et al. erfolgte zum Teil mit anderen statistischen Methoden, sodass sich manche Ergebnisse und deren Diskussion von denen in dieser Dissertation unterscheiden. In der genannten Publikation erfolgte weder eine Einteilung in die Untergruppen „relapsed“ und „abstinent“ noch in Alters-Untergruppen.

Ich versichere, nach der Beurteilung und vor Publikation dieser Dissertation keine weiteren Änderungen als das Einfügen von Zitationen und Anmerkungen bzgl. der publizierten Daten sowie die Korrektur weniger Zeichensetzungsfehler vorgenommen zu haben.

Ich versichere, das Manuskript selbständig verfasst zu haben und keine weiteren als die von mir angegebenen Quellen verwendet zu haben.

Tübingen, den 07.07.2026

8 Supplement

8.1 Sequence of *SYNGAP1*

***Syngap1* / cg02652579**

>hg19_dna range=chr6:33386818-33387117 5'pad=0 3'pad=0 strand=+ repeatMasking=none

CATCCTGTGACCCAAAAATGGAGAGAGGGGCCAATGAGAGGCAGAGAGG
T
GGGAGGAGGTGCAGAGAAAAAAGCTCTTGCCACTGCCCCCACCCAATATT
TCCCCTCCCTTCAAGGTCTGAGGTCATTGGTCTGGTGGTGGGGATGCCTC
GGAGTTTCCCAGGCTGACGTCAGGGTTATACCTCTCGGAGGTTCTAGGGC
AGGGAAGTGGGGAGGGGAGGTTCCCTGAGATCTCTGTCTTTGAGTTTGGA
GTTTGTCTCTGAGACAGATTTCGGAGGGGTCTTTCAGTATTTGTGTGGAG

Bisulfite converted:

TATTTTGTGATTTAAAAATGGAGAGAGGGGTTAATGAGAGGTAGAGAGGT
GGGAGGAGGTGTAGAGAAAAAAGTTTTTGTTATTGTTTTTATTTAATATT
TTTTTTTTTTTTAAGGTTTGAGGTTATTGGTTTGGTGGTGGGGATGTTTC
GGAGTTTTTTAGGTTGACGTTAGGGTTATATTTTTCGGAGGTTTTAGGGT
AGGGAAGTGGGGAGGGGAGGTTTTTTGAGATTTTTGTTTTTGAGTTTGGA
GTTTGTTTTTGAGATAGATTTTGGAGGGGTTTTTTAGTATTTGTGTGGAG

Primer:

Fw: 5´-GAG GGG TTA ATG AGA GGT AGA GAG GTG-3´ 27 bp
63,40°C

Rev: 5´-Biotin-CCC CAC TTC CCT ACC CTA AAA CC-3´ 32 bp
63,60°C

Seq: 5´TTG TTT GGT GGT GGG GAT GTT-3´ 21 bp
65,15°C

CG: cpg sites

8.2 Pyrosequencing assay

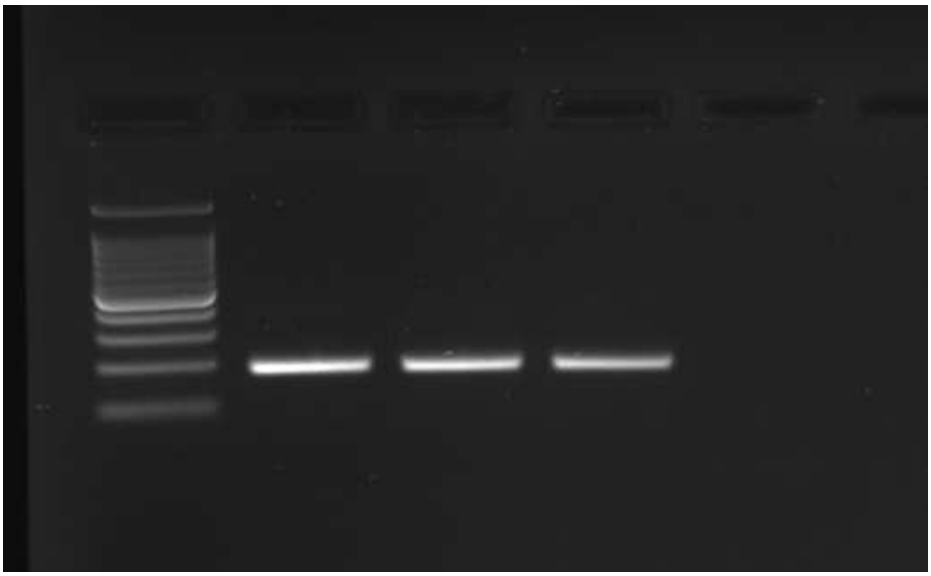
Sequenze to analyze:

TYGGAGTTTTTTAGGTT

Dispensation Order:

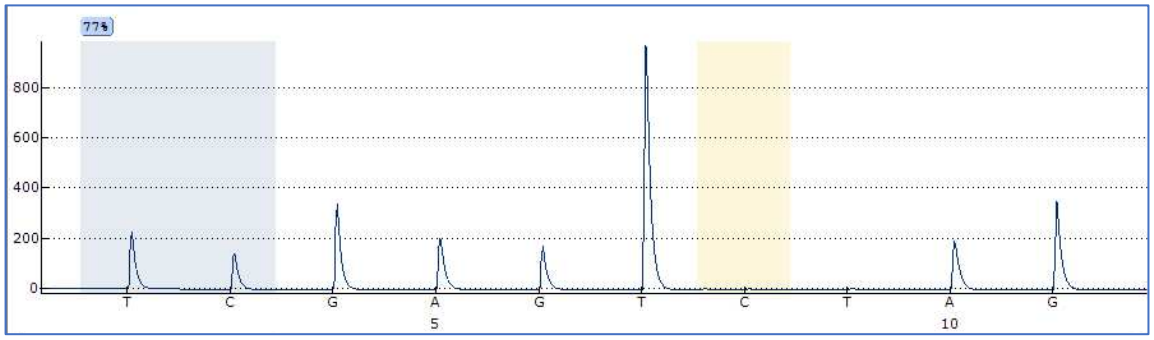
ATCGAGTCTAG

8.3 Example of a result of gel electrophoresis



Supplement 8.3 Example of a result of gel electrophoresis for quality assessment of the PCR. The first well on the left contains the DNA ladder marking the PCR product´s length. The 2nd to 4th wells contain PCR products with a distinct band at approx. 200 bp, indicating a successful amplification. The last well (no band) contains the mastermix without DNA (negative control). Showing no band, it proves the purity of the chemicals used for the PCR.

8.4 Example of a result of pyrosequencing



Supplement 8.4 Example of a result of pyrosequencing of SYNGAP1. Y-axis: Light intensity caused by incorporation of a nucleotide and the degradation of released ATP. X-axis: Position of dispensation order. For the CpG site in the promotor region of *SYNGAP1* (on position 1), a methylation level of 77% was identified in this example. At position 8, a bisulfite conversion control is performed.

8.5 Questionnaires

8.5.1 SCL-90-R

Interne Version
AG Molekulare Psychiatrie

Probandennummer:
Datum:

SCL-90-R

Anleitung
Sie finden auf diesem Blatt eine Liste von Problemen und Beschwerden, die man manchmal hat. Bitte lesen Sie jede Frage sorgfältig durch und entscheiden Sie, wie sehr Sie **in den letzten sieben Tagen** durch diese Beschwerden gestört oder bedrängt worden sind. Überlegen Sie bitte nicht erst, welche Antwort „den besten Eindruck" machen könnte, sondern antworten Sie so, wie es für Sie persönlich zutrifft. Machen Sie bitte hinter jeder Frage nur ein Kreuz in das Kästchen mit der für Sie am besten zutreffenden Antwort. Streichen Sie versehentliche Antworten deutlich durch und kreuzen Sie danach das richtige Kästchen an.
Bitte beantworten Sie jede Frage!

Beispiel:
Frage: Wie sehr litten Sie in den letzten sieben Tagen unter Rückenschmerzen?

Wenn bei Ihnen als Antwort auf diese Frage am besten „sehr stark" zutrifft, dann kreuzen Sie bitte das Kästchen [4] = „sehr stark" an.

Stärke Ihrer Zustimmung:
überhaupt nicht | ein wenig | ziemlich | stark | sehr stark
0 | 1 | 2 | 3 | X

Alle Ihre Antworten werden selbstverständlich vertraulich behandelt.

Wie sehr litten Sie in den letzten sieben Tagen unter...? (überhaupt nicht / ein wenig / ziemlich / stark / sehr stark)		Wie sehr litten Sie in den letzten sieben Tagen unter...? (überhaupt nicht / ein wenig / ziemlich / stark / sehr stark)	
1. Kopfschmerzen	0 1 2 3 4	21. Schüchternheit oder Unbeholfenheit im Umgang mit dem anderen Geschlecht	0 1 2 3 4
2. Nervosität oder innerem Zittern	0 1 2 3 4	22. der Befürchtung, ertappt oder erwischt zu werden	0 1 2 3 4
3. immer wieder auftauchenden unangenehmen Gedanken, Worten oder Ideen, die Ihnen nicht mehr aus dem Kopf gehen	0 1 2 3 4	23. plötzlichem Erschrecken ohne Grund	0 1 2 3 4
4. Ohnmachts- oder Schwindelgefühlen	0 1 2 3 4	24. Gefühlsausbrüchen, gegenüber denen Sie machtlos waren	0 1 2 3 4
5. Verminderung Ihres Interesses oder Ihrer Freude an Sexualität	0 1 2 3 4	25. Befürchtungen, wenn Sie alleine aus dem Haus gehen	0 1 2 3 4
6. allzu kritischer Einstellung gegenüber anderen	0 1 2 3 4	26. Selbstvorwürfen über bestimmte Dinge	0 1 2 3 4
7. der Idee, daß irgend jemand Macht über Ihre Gedanken hat	0 1 2 3 4	27. Kreuzschmerzen	0 1 2 3 4
8. dem Gefühl, daß andere an den meisten Ihrer Schwierigkeiten Schuld sind	0 1 2 3 4	28. dem Gefühl, daß es Ihnen schwerfällt, etwas anzufangen	0 1 2 3 4
9. Gedächtnisschwierigkeiten	0 1 2 3 4	29. Einsamkeitsgefühlen	0 1 2 3 4
10. Beunruhigung wegen Achtlosigkeit und Nachlässigkeit	0 1 2 3 4	30. Schwermut	0 1 2 3 4
11. dem Gefühl, leicht reizbar und verärgerbar zu sein	0 1 2 3 4	31. dem Gefühl, sich zu viele Sorgen machen zu müssen	0 1 2 3 4
12. Herz- und Brustschmerzen	0 1 2 3 4	32. dem Gefühl, sich für nichts zu interessieren	0 1 2 3 4
13. Furcht auf offenen Plätzen oder auf der Straße	0 1 2 3 4	33. Furchtsamkeit	0 1 2 3 4
14. Energielosigkeit oder Verlangsamung in den Bewegungen oder im Denken	0 1 2 3 4	34. Verletzlichkeit in Gefühlsdingen	0 1 2 3 4
15. Gedanken, sich das Leben zu nehmen	0 1 2 3 4	35. der Idee, daß andere Leute von Ihren geheimsten Gedanken wissen	0 1 2 3 4
16. Hören von Stimmen, die sonst keiner hört	0 1 2 3 4	36. dem Gefühl, daß andere Sie nicht verstehen oder teilnahmslos sind	0 1 2 3 4
17. Zittern	0 1 2 3 4	37. dem Gefühl, daß die Leute unfreundlich sind oder Sie nicht leiden können	0 1 2 3 4
18. dem Gefühl, daß man den meisten Menschen nicht trauen kann	0 1 2 3 4	38. der Notwendigkeit, alles sehr langsam zu tun, um sicher zu sein, daß alles richtig ist	0 1 2 3 4
19. schlechtem Appetit	0 1 2 3 4	39. Herzklopfen oder Herzjagen	0 1 2 3 4
20. Neigung zum Weinen	0 1 2 3 4	40. Übelkeit oder Magenverstimmung	0 1 2 3 4

SCL-90-R

Wie sehr litten Sie in den letzten sieben Tagen unter...?	überhaupt nicht	ein wenig	ziemlich	stark	sehr stark
41. Minderwertigkeitsgefühlen gegenüber anderen	0	1	2	3	4
42. Muskelschmerzen (Muskelkater, Gliederreißen)	0	1	2	3	4
43. dem Gefühl, daß andere Sie beobachten oder über Sie reden	0	1	2	3	4
44. Einschlafschwierigkeiten	0	1	2	3	4
45. dem Zwang, wieder und wieder nachzukontrollieren, was Sie tun	0	1	2	3	4
46. Schwierigkeiten, sich zu entscheiden	0	1	2	3	4
47. Furcht vor Fahrten in Bus, Straßenbahn, U-Bahn oder Zug	0	1	2	3	4
48. Schwierigkeiten beim Atmen	0	1	2	3	4
49. Hitzewallungen und Kälteschauern	0	1	2	3	4
50. der Notwendigkeit, bestimmte Dinge, Orte oder Tätigkeiten zu meiden, weil Sie durch diese erschreckt werden	0	1	2	3	4
51. Leere im Kopf	0	1	2	3	4
52. Taubheit oder Kribbeln in einzelnen Körperteilen	0	1	2	3	4
53. dem Gefühl, einen Klumpen (Kloß) im Hals zu haben	0	1	2	3	4
54. einem Gefühl der Hoffnungslosigkeit angesichts der Zukunft	0	1	2	3	4
55. Konzentrationsschwierigkeiten	0	1	2	3	4
56. Schwächegefühl in einzelnen Körperteilen	0	1	2	3	4
57. dem Gefühl, gespannt oder aufgeregt zu sein	0	1	2	3	4
58. Schweregefühl in den Armen oder den Beinen	0	1	2	3	4
59. Gedanken an den Tod und ans Sterben	0	1	2	3	4
60. dem Drang, sich zu überessen	0	1	2	3	4
61. einem unbehaglichen Gefühl, wenn Leute Sie beobachten oder über Sie reden	0	1	2	3	4
62. dem Auftauchen von Gedanken, die nicht Ihre eigenen sind	0	1	2	3	4
63. dem Drang, jemanden zu schlagen, zu verletzen oder ihm Schmerz zuzufügen	0	1	2	3	4
64. frühem Erwachen am Morgen	0	1	2	3	4
65. zwanghafter Wiederholung derselben Tätigkeit wie Berühren, Zählen, Waschen	0	1	2	3	4

Wie sehr litten Sie in den letzten sieben Tagen unter...?	überhaupt nicht	ein wenig	ziemlich	stark	sehr stark
66. unruhigem oder gestörtem Schlaf	0	1	2	3	4
67. dem Drang, Dinge zu zerbrechen oder zu zerschmettern	0	1	2	3	4
68. Ideen oder Anschauungen, die andere nicht mit Ihnen teilen	0	1	2	3	4
69. starker Befangenheit im Umgang mit anderen	0	1	2	3	4
70. Abneigung gegen Menschenmengen, z. B. beim Einkaufen oder im Kino	0	1	2	3	4
71. einem Gefühl, daß alles sehr anstrengend ist	0	1	2	3	4
72. Schreck- und Panikanfällen	0	1	2	3	4
73. Unbehagen beim Essen oder Trinken in der Öffentlichkeit	0	1	2	3	4
74. der Neigung, immer wieder in Erörterungen oder Auseinandersetzungen zu geraten	0	1	2	3	4
75. Nervosität, wenn Sie alleine gelassen werden	0	1	2	3	4
76. mangelnder Anerkennung Ihrer Leistungen durch andere	0	1	2	3	4
77. Einsamkeitsgefühlen, selbst wenn Sie in Gesellschaft sind	0	1	2	3	4
78. so starker Ruhelosigkeit, daß Sie nicht stillsitzen können	0	1	2	3	4
79. dem Gefühl, wertlos zu sein	0	1	2	3	4
80. dem Gefühl, daß Ihnen etwas Schlimmes passieren wird	0	1	2	3	4
81. dem Bedürfnis, laut zu schreien oder mit Gegenständen zu werfen	0	1	2	3	4
82. der Furcht, in der Öffentlichkeit in Ohnmacht zu fallen	0	1	2	3	4
83. dem Gefühl, daß die Leute Sie ausnutzten, wenn Sie es zulassen würden	0	1	2	3	4
84. sexuellen Vorstellungen, die ziemlich unangenehm für Sie sind	0	1	2	3	4
85. dem Gedanken, daß Sie für Ihre Sünden bestraft werden sollten	0	1	2	3	4
86. schreckenerregenden Gedanken und Vorstellungen	0	1	2	3	4
87. dem Gedanken, daß etwas ernstlich mit Ihrem Körper nicht in Ordnung ist	0	1	2	3	4
88. dem Eindruck, sich einer anderen Person nie so richtig nahe fühlen zu können	0	1	2	3	4
89. Schuldgefühlen	0	1	2	3	4
90. dem Gedanken, daß irgend etwas mit Ihrem Verstand nicht in Ordnung ist	0	1	2	3	4

8.5.2 AUDIT

	Code	*Datum*	Termin

AUDIT

Bitte lesen Sie jede Aussage und **kreuzen Sie** die für Sie zutreffendste Antwort **an**.

Als Maßeinheit für 1 Drink = ein 0,33 l Glas/Dose/Flasche Bier
ein 0,2 l Glas Wein
ein 2cl Glas Rum, Whiskey, Schnaps, usw.

1. Wie oft trinken Sie Alkohol?
- ☐ Nie
- ☐ 1mal im Monat oder seltener
- ☐ 2 bis 4mal im Monat
- ☐ 2 bis 3mal in der Woche
- ☐ 4 oder mehrmals in der Woche

2. An den Tagen, an denen Sie trinken, wie viele Drinks konsumieren Sie dann typischerweise?
- ☐ 1-2
- ☐ 3-4
- ☐ 5-6
- ☐ 7-9
- ☐ 10 oder mehr

3. Wie oft trinken Sie 6 oder mehr Drinks pro Tag?
- ☐ Nie
- ☐ Seltener als 1 mal im Monat
- ☐ Jeden Monat
- ☐ Jede Woche
- ☐ Jeden Tag/fast jeden Tag

4. Wie oft konnten Sie im letzten Jahr nicht mehr aufhören zu trinken, nachdem Sie einmal angefangen hatten?
- ☐ Nie
- ☐ Seltener als 1mal im Monat
- ☐ Jeden Monat
- ☐ Jede Woche
- ☐ Jeden Tag/fast jeden Tag

5. Wie oft konnten Sie im letzten Jahr wegen des Trinkens nicht das tun, was von Ihnen normalerweise erwartet wurde?
- ☐ Nie
- ☐ Seltener als 1mal im Monat
- ☐ 1mal im Monat
- ☐ 1mal in der Woche
- ☐ Fast täglich

6. Wie oft haben Sie im letzten Jahr morgens Alkohol gebraucht, um nach einem starken Alkoholkonsum wieder in die Gänge zu kommen?
- ☐ Nie
- ☐ Seltener als 1mal im Monat
- ☐ Jeden Monat
- ☐ Jede Woche
- ☐ Jeden Tag/fast jeden Tag

7. Wie oft hatten Sie im letzten Jahr Schuldgefühle oder ein schlechtes Gewissen nach dem Trinken?
- ☐ Nie
- ☐ Seltener als 1mal im Monat
- ☐ Jeden Monat
- ☐ Jede Woche
- ☐ Jeden Tag/fast jeden Tag

8. Wie oft konnten Sie sich im letzten Jahr nicht mehr an den vorangegangenen Abend erinnern, weil Sie getrunken hatten?
- ☐ Nie
- ☐ Seltener als 1mal im Monat
- ☐ Jeden Monat
- ☐ Jede Woche
- ☐ Jeden Tag/fast jeden Tag

9. Wurden Sie oder jemand anderes infolge Ihres Trinkens schon einmal verletzt?
- ☐ Nein
- ☐ Ja, aber nicht im letzten Jahr
- ☐ Ja, im letzten Jahr

10. War ein Verwandter, Freund oder Arzt um Ihr Trinken besorgt oder haben sie Ihnen geraten, Ihren Alkoholkonsum zu verringern?
- ☐ Nein
- ☐ Ja, aber nicht im letzten Jahr
- ☐ Ja, im letzten Jahr

8.5.3 OCDS (German version)

Obsessive Compulsive Drinking Scale (OCDS-G)

Die folgenden Fragen beziehen sich auf Ihren Alkoholkonsum und auf Gedanken, Vorstellungen, Impulse oder Bilder, die mit dem Trinken von Alkohol zusammenhängen.
Bitte kreuzen Sie jeweils die Aussage an, die am ehesten auf Sie zutrifft.
Falls nicht anders angegeben, beziehen sich die Fragen auf den Zeitraum der vergangenen sieben Tage.

1. Wenn Sie keinen Alkohol trinken, wie viel Ihrer Zeit wird dann von Vorstellungen, Gedanken, Impulsen oder Bildern beansprucht, die etwas mit dem Trinken zu tun haben?

[ ] Keine
[ ] Weniger als eine Stunde am Tag
[ ] 1-3 Stunden am Tag
[ ] 4-8 Stunden am Tag
[ ] Mehr als 8 Stunden am Tag

2. Wie häufig treten diese Gedanken oder Vorstellungen auf?

[ ] Niemals
[ ] Nicht häufiger als achtmal am Tag
[ ] Häufiger als achtmal am Tag, aber die meisten Stunden des Tages sind frei davon
[ ] Mehr als achtmal am Tag und während der meisten Stunden des Tages
[ ] Die Gedanken treten so häufig auf, dass man sie nicht mehr zählen kann, und es vergeht kaum eine Stunde, in der sie nicht auftreten

3. Wie stark werden Ihre berufliche Tätigkeit oder Ihr soziales Verhalten von diesen Vorstellungen, Gedanken, Impulsen oder Bildern beeinflusst? Gibt es etwas, was sie deswegen nicht tun oder nicht können?(Falls sie gerade nicht berufstätig sind: Wie sehr wäre Ihre berufliche Tätigkeit dadurch beeinflusst, falls Sie arbeiten würden?)

[ ] Die Gedanken an Alkohol beeinflussen mich überhaupt nicht - ich arbeite oder verhalte mich völlig normal.
[ ] Die Gedanken an Alkohol beeinflussen mein soziales Verhalten oder meine beruflichen Tätigkeiten in geringem Maße, mein Auftreten oder meine Leistung insgesamt ist jedoch nicht beeinträchtigt.
[ ] Die Gedanken an Alkohol beeinflussen mein soziales Verhalten oder meine berufliche Leistung eindeutig, ich komme aber noch damit zurecht.
[ ] Die Gedanken an Alkohol beeinträchtigen mein soziales Verhalten oder meine berufliche Leistung ganz erheblich.
[ ] Die Gedanken an Alkohol beeinträchtigen mein soziales Verhalten oder meine Arbeitsleistung vollständig.

4. Wenn Sie keinen Alkohol trinken, wie sehr leiden Sie dann unter den Vorstellungen, Gedanken, Impulsen oder Bildern, die mit dem Trinken zu tun haben bzw. wie sehr werden Sie dadurch gestört.

[ ] Keine Belastung oder Störung
[ ] Geringfügig, selten und nicht besonders störend
[ ] Mäßig, häufig und störend; ich kann aber noch damit zurechtkommen
[ ] Stark, sehr häufig und sehr störend
[ ] Extrem stark, fast durchgängig, alles andere wird beeinträchtigt.

5. Wenn Sie keinen Alkohol trinken, wie sehr bemühen Sie sich dann, diesen Gedanken zu widerstehen, Sie nicht zu beachten oder Ihre Aufmerksamkeit auf etwas Anderes zu lenken? (Geben Sie das Ausmaß Ihrer Bemühungen um Widerstand gegen diese Gedanken an, nicht den Erfolg oder Misserfolg, den Sie dabei haben.)

[ ] Ich habe so selten derartige Gedanken, dass es nicht notwendig ist, dagegen anzugehen. Wenn Sie aber auftauchen, bemühe ich mich immer, diesen Gedanken zu widerstehen.

[ ] Ich versuche meistens, diesen Gedanken zu widerstehen.

[ ] Ich unternehme einige Anstrengungen, um diesen Gedanken zu widerstehen.

[ ] Ich lasse allen derartigen Gedanken freien Lauf, ohne zu versuchen, sie zu kontrollieren. Dabei habe ich allerdings ein ungutes Gefühl.

[ ] Ich lasse diesen Gedanken freien Lauf.

6. Wenn Sie keinen Alkohol trinken, wie erfolgreich können Sie dann diese Gedanken beenden oder sie zerstreuen?

[ ] Es gelingt mir stets vollkommen, diese Gedanken zu beenden oder sie zu zerstreuen.

[ ] Gewöhnlich kann ich diese Gedanken mit einiger Anstrengung und Konzentration beenden oder zerstreuen.

[ ] Manchmal kann ich diese Gedanken beenden oder sie zerstreuen.

[ ] Ich kann diese Gedanken nur ganz selten beenden und sie nur sehr schwerlich zerstreuen.

[ ] Selbst für kurze Momente kann ich solche Gedanken nur selten zerstreuen.

7. Wie viele „drinks“ nehmen Sie täglich zu sich? (Denken Sie an die letzten Wochen, in denen Sie Alkohol getrunken haben.)

[ ] Keinen

[ ] Weniger als einen „drink“ (entspricht *weniger als 0,33 Liter Bier oder 1/8 Liter Wein oder 30 ml Schnaps)*

[ ] 1-2 „drinks“ täglich (entspricht *maximal 0,66 Liter Bier oder 1/4 Liter Wein oder 60 ml Schnaps*)

[ ] 3-7 „drinks“ täglich (entspricht *bis 2,5 Liter Bier oder bis 1 Liter Wein oder bis 200 ml Schnaps*)

[ ] 8 oder mehr „drinks“ täglich (entspricht *mehr als 2,5 Liter Bier oder mehr als 1 Liter Wein oder mehr als 200 ml Schnaps*)

8. An wie vielen Tagen in der Woche trinken Sie Alkohol? (Denken Sie an die letzten Wochen, in denen Sie Alkohol getrunken haben.)

[ ] An keinem

[ ] An nicht mehr als einem Tag

[ ] An 2-3 Tagen die Woche

[ ] An 4-5 Tagen die Woche

[ ] An 6-7 Tagen die Woche

9. Wie stark wird Ihre berufliche Tätigkeit durch das Trinken von Alkohol beeinflusst? Gibt es etwas, was Sie wegen Ihres Trinkens nicht tun oder nicht können?
(Falls Sie gerade nicht berufstätig sind: Wie sehr wäre Ihre berufliche Tätigkeit beeinflusst, falls Sie arbeiten würden?)

[ ] Das Trinken beeinflusst mich beruflich überhaupt nicht – ich arbeite völlig normal.

[ ] Das Trinken beeinflusst meine beruflichen Tätigkeiten in geringem Maße, meine Arbeitskraft insgesamt st jedoch nicht beeinträchtigt.

[ ] Das Trinken beeinflusst meine berufliche Leistung eindeutig, ich komme aber noch damit zurecht.

[ ] Das Trinken beeinträchtigt meine berufliche Leistung ganz erheblich.

[ ] Das Trinken beeinträchtigt meine Arbeitsleistung völlig.

10. Wie stark wird Ihr soziales Verhalten durch das Trinken von Alkohol beeinflusst? Gibt es etwas, was Sie wegen Ihres Trinkens nicht tun oder nicht können?

[ ] Das Trinken beeinflusst mein soziales Verhalten überhaupt nicht – ich verhalte mich völlig normal.

[ ] Das Trinken beeinflusst mein soziales Verhalten in geringem Maße, mein Auftreten insgesamt ist jedoch nicht beeinträchtigt.

[ ] Das Trinken beeinflusst mein soziales Verhalten eindeutig, ich komme aber noch damit zurecht.

[ ] Das Trinken beeinträchtigt mein soziales Verhalten ganz erheblich.

[ ] Das Trinken beeinträchtigt mein soziales Verhalten völlig.

11. Wenn Sie ein alkoholisches Getränk trinken möchten, aber daran gehindert wären, wie ängstlich oder ungehalten würden Sie dann werden?

[ ] Ich würde überhaupt nicht ängstlich oder gereizt.

[ ] Ich würde in geringem Maße ängstlich oder gereizt.

[ ] Angst oder Reizbarkeit würden ansteigen, aber noch zu beherrschen sein.

[ ] Angst oder Reizbarkeit würden für mich sehr stark und störend.

[ ] Angst oder Reizbarkeit würden mich völlig überwältigen.

12. Wie sehr bemühen Sie sich, dem Trinken von Alkohol zu widerstehen?
(Geben Sie das Ausmaß Ihrer Bemühungen um Widerstand gegen das Trinken an, nicht den Erfolg oder Misserfolg , den Sie dabei haben.)

[ ] Ich trinke so minimal, dass es nicht notwendig ist, dagegen anzugehen. Wenn ich doch trinke, bemühe ich mich immer, dem Trinken zu widerstehen.

[ ] Ich versuche meistens, dem Trinken zu widerstehen.

[ ] Ich unternehme einige Anstrengungen, um dem Trinken zu widerstehen.

[ ] Ich lasse dem Trinken meistens freien Lauf, ohne zu versuchen, es zu kontrollieren. Dabei habe ich ein ungutes Gefühl.

[ ] Ich lasse dem Trinken völlig freien Lauf.

13. Wie stark ist Ihr Drang, Alkohol zu trinken?

[ ] Ich verspüre keinen Drang.

[ ] Ich verspüre etwas Drang, Alkohol zu trinken.

[ ] Ich verspüre starken Drang, Alkohol zu trinken.

[ ] Ich verspüre sehr starken Drang, Alkohol zu trinken.

[ ] Der Drang zum Trinken ist völlig überwältigend und nicht zu beeinflussen.

14. Wie viel Kontrolle haben Sie über Ihr Trinkverhalten?

[ ] Ich habe mein Trinkverhalten völlig unter Kontrolle.

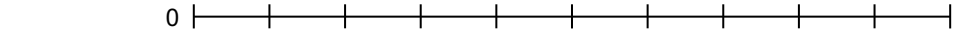
[ ] Gewöhnlich kann ich mein Trinkverhalten unter willentlicher Kontrolle halten.

[ ] Ich kann mein Trinkverhalten nur unter Schwierigkeiten kontrollieren.

[ ] Ich muss trinken und kann es nur unter Schwierigkeiten hinausschieben.

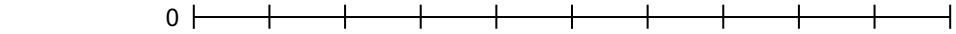
[ ] Ich bin kaum in der Lage, das Trinken auch nur für kurze Zeit hinauszuschieben.

15. Wie stark war während der letzten sieben Tage Ihr Verlangen nach Alkohol (der Wunsch nach Alkohol, während der Zeit, in der sie nicht tranken) im Durchschnitt?



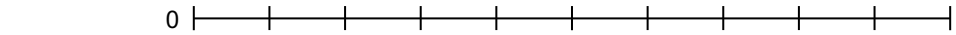
nicht vorhanden sehr stark

16. Denken Sie bitte einmal an den Moment innerhalb der letzten sieben Tage zurück, als das Verlangen nach Alkohol am stärksten war. Wie stark war dieses Verlangen?



nicht vorhanden sehr stark

17. Wie häufig hatten Sie während der letzten sieben Tage Verlangen nach Alkohol (den Wunsch nach Alkohol, während der Zeit, in der sie nicht tranken)?



nie
immer

18. Wann haben Sie zuletzt Alkohol getrunken?

. Vor ☐☐☐ Tagen

Bitte prüfen Sie, ob sie alle Fragen beantwortet haben.
Herzlichen Dank!

8.6 Correlation between OCDS overall, obsession and compulsion score

Correlation between the OCDS subscores overall, obsession, and compulsion, analyzed over both groups.

Korrelationen

			OCDS overall	OCDS compulsion	OCDS obsession
Spearman-Rho	OCDS_overall	Korrelationskoeffizient	1.000	.985**	.864**
		Sig. (2-seitig)	.	<.001	<.001
		N	402	402	402
	OCDS_compulsion	Korrelationskoeffizient	.985**	1.000	.806**
		Sig. (2-seitig)	<.001	.	<.001
		N	402	402	402
	OCDS_obsession	Korrelationskoeffizient	.864**	.806**	1.000
		Sig. (2-seitig)	<.001	<.001	.
		N	402	402	402

**. Die Korrelation ist auf dem 0,01 Niveau signifikant (zweiseitig).

Correlation between the OCDS subscores overall, obsession, and compulsion, analyzed in patients only.

Korrelationen

			OCDS overall	OCDS obsession	OCDS compulsion
Spearman-Rho	OCDS overall	Korrelationskoeffizient	--		
		Sig. (2-seitig)	.		
		N	139		
	OCDS obsession	Korrelationskoeffizient	,888**	--	
		Sig. (2-seitig)	<,001	.	
		N	139	139	
	OCDS compulsion	Korrelationskoeffizient	,883**	,605**	--
		Sig. (2-seitig)	<,001	<,001	.
		N	139	139	139

**. Die Korrelation ist auf dem 0,01 Niveau signifikant (zweiseitig).

Correlation between the OCDS subscores overall, obsession, and compulsion, analyzed in controls only.

Korrelationen

			OCDS_overall	OCDS_obsession	OCDS_compulsion
Spearman-Rho	OCDS_overall	Korrelationskoeffizient	1.000	.481**	.987**
		Sig. (2-seitig)	.	<.001	<.001
		N	263	263	263
	OCDS_obsession	Korrelationskoeffizient	.481**	1.000	.384**
		Sig. (2-seitig)	<.001	.	<.001
		N	263	263	263
	OCDS_compulsion	Korrelationskoeffizient	.987**	.384**	1.000
		Sig. (2-seitig)	<.001	<.001	.
		N	263	263	263

**. Die Korrelation ist auf dem 0,01 Niveau signifikant (zweiseitig).

8.7 Difference between alcohol related variables between control and patient group

Teststatistiken[a]

	GSI1	GSI2	GSI3	GSI4	AUDIT1	AUDIT2	AUDIT3	AUDIT4	OCDS1	OCDS2	OCDS3	OCDS4
Mann-Whitney-U-Test	306,500	912,000	149,500	100,500	21,000	42,500	19,500	,000	36,500	156,500	112,000	20,500
Wilcoxon-W	3081,500	4072,000	1919,500	661,500	3507,000	1170,500	1504,500	351,000	3606,500	3559,500	1882,000	840,500
Z	-8,625	-5,020	-3,941	-2,198	-9,923	-6,371	-5,666	-4,065	-10,118	-9,122	-4,555	-4,856
Asymp. Sig. (2-seitig)	<,001	<,001	<,001	,028	<,001	<,001	<,001	<,001	<,001	<,001	<,001	<,001
Exakte Sig. [2*(1-seitige Sig.)]				,027[b]				<,001[b]				

a. Gruppenvariable: group

b. Nicht für Bindungen korrigiert.

8.8 Correlation between the alcohol related variables

Correlation between the questionnaire scores of GSI (in SCL-90-R), OCDS and AUDIT analyzed over both groups.

Korrelationen

			GSI	OCDS	AUDIT
Spearman-Rho	GSI	Korrelationskoeffizient	--		
		Sig. (2-seitig)	.		
		N	379		
	OCDS	Korrelationskoeffizient	.516**	--	
		Sig. (2-seitig)	<.001	.	
		N	377	402	
	AUDIT	Korrelationskoeffizient	.557**	.877**	--
		Sig. (2-seitig)	<.001	<.001	.
		N	299	310	310

**. Die Korrelation ist auf dem 0,01 Niveau signifikant (zweiseitig).

Correlation between the questionnaire scores of GSI (in SCL-90-R), OCDS and AUDIT patients only.

Korrelationen

			GSI	OCDS	AUDIT
Spearman-Rho	GSI	Korrelationskoeffizient	1,000	,541**	,384**
		Sig. (2-seitig)	.	<,001	<,001
		N	136	135	101
	OCDS	Korrelationskoeffizient	,541**	1,000	,631**
		Sig. (2-seitig)	<,001	.	<,001
		N	135	139	102
	AUDIT	Korrelationskoeffizient	,384**	,631**	1,000
		Sig. (2-seitig)	<,001	<,001	.
		N	101	102	102

**. Die Korrelation ist auf dem 0,01 Niveau signifikant (zweiseitig).

Correlation between the questionnaire scores of GSI (in SCL-90-R), OCDS and AUDIT controls only.

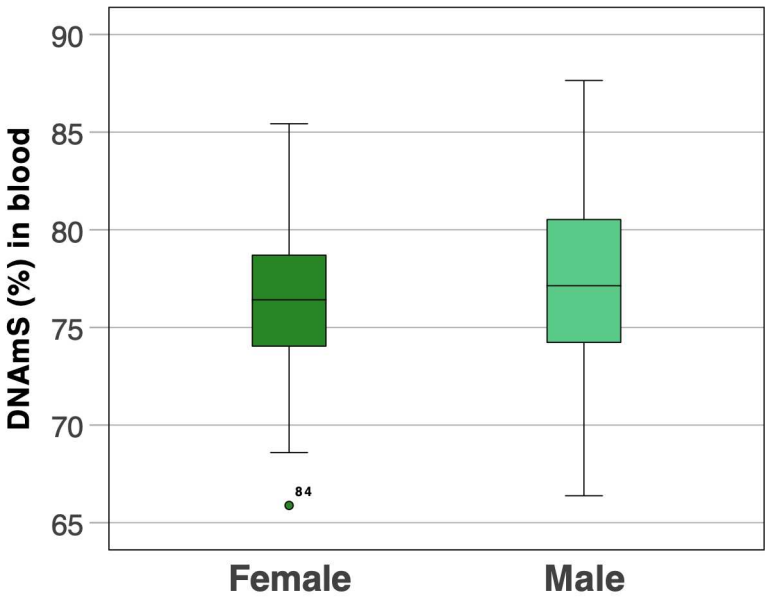
Korrelationen

			GSI	OCDS	AUDIT
Spearman-Rho	GSI	Korrelationskoeffizient	1,000	,006	,170*
		Sig. (2-seitig)	.	,923	,016
		N	245	244	200
	OCDS	Korrelationskoeffizient	,006	1,000	,719**
		Sig. (2-seitig)	,923	.	<,001
		N	244	265	210
	AUDIT	Korrelationskoeffizient	,170*	,719**	1,000
		Sig. (2-seitig)	,016	<,001	.
		N	200	210	210

*. Die Korrelation ist auf dem 0,05 Niveau signifikant (zweiseitig).

**. Die Korrelation ist auf dem 0,01 Niveau signifikant (zweiseitig).

8.9 Comparison of DNAmS in blood between female and male participants



Supplement 8.9 Boxplot of DNAmS in blood of female vs. male study participants at T1 (Z = -.850, *p* = .395). Boxes represent the interquartile range; whiskers indicate the standard deviation of the mean [%].

8.10 Correlation between DNAmS in blood and questionnaire scores

Correlation between DNAmS in blood and questionnaire scores analyzed over both groups.

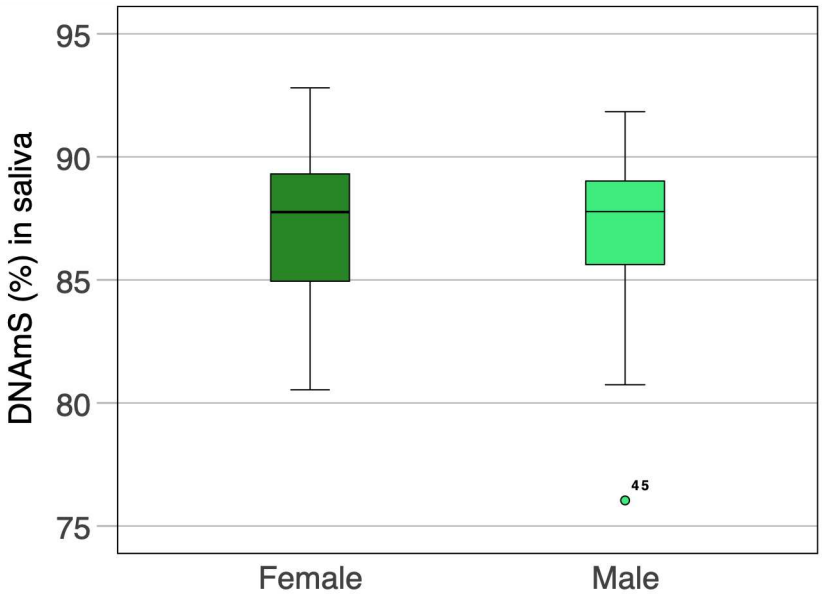
Korrelationen

			GSI	OCDS	AUDIT	EDTAMet
Spearman-Rho	GSI	Korrelationskoeffizient	1.000	.516**	.557**	.152**
		Sig. (2-seitig)	.	<.001	<.001	.004
		N	379	377	299	349
	OCDS	Korrelationskoeffizient	.516**	1.000	.877**	.110*
		Sig. (2-seitig)	<.001	.	<.001	.036
		N	377	402	310	361
	AUDIT	Korrelationskoeffizient	.557**	.877**	1.000	.087
		Sig. (2-seitig)	<.001	<.001	.	.143
		N	299	310	310	287
	EDTAMet	Korrelationskoeffizient	.152**	.110*	.087	1.000
		Sig. (2-seitig)	.004	.036	.143	.
		N	349	361	287	367

**. Die Korrelation ist auf dem 0,01 Niveau signifikant (zweiseitig).

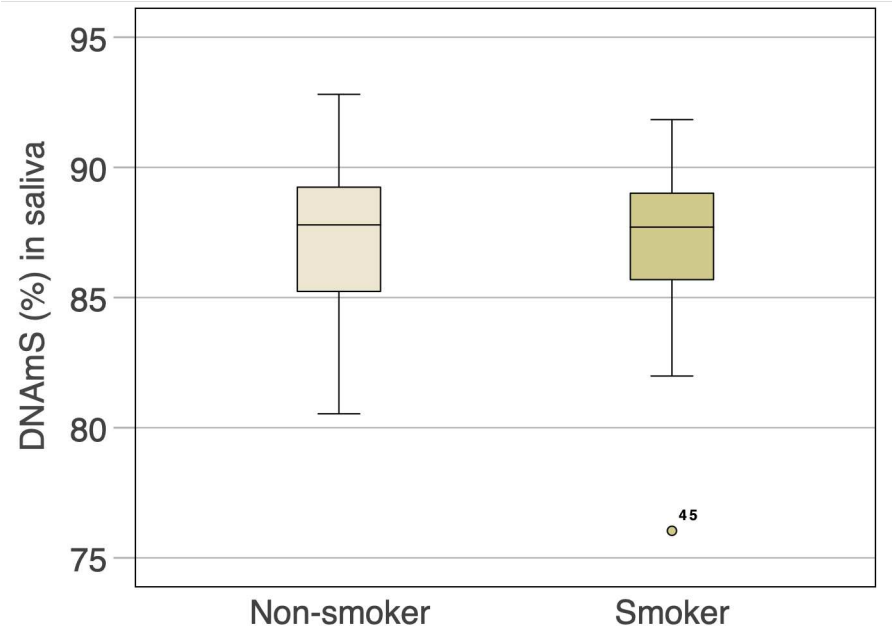
*. Die Korrelation ist auf dem 0,05 Niveau signifikant (zweiseitig).

8.11 Comparison of DNAmS in saliva between female and male participants



Supplement 8.11 Boxplot of DNAmS in saliva of female and male study participants at T1. Boxes represent the interquartile range; whiskers indicate the standard deviation of the mean). Z = -.154, *p* = .878

8.12 Comparison of DNAmS in blood between smokers and non-smokers



Supplement 8.12 Boxplot of DNAmS (%) in saliva of smokers and non-smokers at T1 (boxes represent the interquartile range; whiskers indicate the standard deviation of the mean). (Z = -.143, *p* = .886; Figure 21).