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Neuroimaging and behavioral biomarkers for neuropsychiatric diseases

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To my mother, in loving memory.

I will always be grateful for your support and love.

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Zusammenfassung

Bis heute ist die Früherkennung und Diagnose vieler neuropsychiatrischer Erkrankungen eine Herausfoderung, was häufig zu Unter- oder Fehldiagnosen führt. Um das Leiden der Patienten zu lindern und die ökonomische Belastung zu verringern, hat sich die neuropsychiatrische Forschung in den letzten zwei Jahrzehnten zunehmend auf die Entwicklung von Biomarkern konzentriert. Während Biomarker unterschiedlichen Zwecken dienen und aus einer Vielzahl von Modalitäten abgeleitet werden können, bewertet diese Arbeit den aktuellen Stand der Entwicklung von Biomarkern für chronische und nicht-chronische neuropsychiatrische Erkrankungen am Beispiel der verhaltensabhängigen frontotemporalen Demenz (bvFTD), der postpartalen Depression (PPD) und der Anpassungsstörung (adjustment disorder - AjD) im Wochenbett.

Im Detail wurde die Vorhersagekraft soziodemographischer und klinisch-anamnestischer Informationen neben Online-Erhebungen von Stimmung und Stress bewertet (Studie 1) sowie frühe strukturelle und funktionelle Hirnveränderungen (Studie 2) in PPD und AjD untersucht. Darüber hinaus wurden räumliche Korrelationen struktureller und funktioneller Neurobildgebung mit Neurotransmitterdichte als Biomarker für Neurotransmitter-Vulnerabilität bei bvFTD evaluiert (Studie 3).

Die erste Studie zeigte, dass PPD, AjD und gesunde Kontrollen anhand der Online-Erhebungen von Stimmung und klinisch-anamnestischer Information (d.h. postnatale Depressions- und Bindungsscores) präzise differenziert werden können. Dahingegen konnte die zweite Studie keine robusten strukturellen und funktionellen Hirnveränderungen in PPD und AjD nachweisen. Die dritte Studie ergab, dass eine verminderte funktionale Konnektivität in fronto-temporalen und fronto-parietalen Regionen in bvFTD mit der Verteilung von Rezeptoren und Transportern des γ-Aminobuttersäure-basierten, norepinephrinergen, und serotonergen Neurotransmitter-systems sowie deren kodierender mRNA-Genexpression kolokalisiert war, welches auf eine spezifische Neurotransmitter-Vulnerabilität in Patienten mit bvFTD hinweist.

Zusammenfassend zeigten die ersten beiden Studien den Nutzen der Kombination aus Online-Erhebungen und maschinellem Lernen für die Früherkennung von PPD und AjD. Zudem zeigte die dritte Studie, dass räumliche Korrelationen von Hirnstruktur und -funktion mit Neurotransmitterdichte zur Beurteilung der Neurotransmitter-Vulnerabilität genutzt werden können. Zukünftig könnten diese Ansätze der Biomarkerentwicklung auch für die Früherkennung und Diagnose anderer neuropsychiatrischer Erkrankungen von Nutzen sein.

Summary

To date, early recognition and diagnosis of many neuropsychiatric diseases remains challenging, resulting in frequent under- or misdiagnosis. To reduce the suffering of patients and to lower the economic burden, neuropsychiatric research began to focus on biomarker development in the last two decades. While biomarkers can serve different purposes and can be derived from a variety of modalities, this thesis evaluates the current state of biomarker development for chronic and non-chronic neuropsychiatric diseases at the examples of behavioral variant frontotemporal dementia (bvFTD), postpartum depression (PPD), and adjustment disorder (AjD) in the postpartum period.

More specifically, the predictive value of socio-demographic and clinical-anamnestic information in addition to remote mood and stress assessments was evaluated (study 1), and early structural and functional brain alterations (study 2) were examined in PPD and AjD. Moreover, spatial correlations of structural and functional neuroimaging with neurotransmitter density were evaluated as neurotransmitter vulnerability biomarker in bvFTD (study 3).

While the first study demonstrated that PPD, AjD, and healthy controls can be accurately differentiated using remote mood assessments and clinical-amnestic information (i.e., postnatal depression and attachment scores), the second study revealed no robust early structural and functional brain alterations in PPD and AjD. The third study showed that reduced functional connectivity in frontotemporal and frontoparietal regions in patients with bvFTD co-localized with the distribution of receptors and transporters of γ-aminobutyric acid-ergic, norepinephrinergic, and serotonergic neurotransmitter systems, and their encoding mRNA gene expression, indicating a specific neurotransmitter vulnerability in patients with bvFTD.

In summary, the first two studies demonstrated the value of utilizing remote assessments in combination with machine learning for early recognition of PPD and AjD. Moreover, the third study demonstrated the potential of spatial correlations of brain structure and function with neurotransmitter density to assess neurotransmitter vulnerability. In the future, these biomarker development approaches may also prove useful for the early recognition and diagnosis of other neuropsychiatric diseases.

List of abbreviations

5-HT1a	Serotonin 1a receptor
5-HT1b	Serotonin 1b receptor
5-HT2a	Serotonin 2a receptor
5-HTT	Serotonin transporter
5-HTTLPR	Serotonin-transporter-linked promoter region
AD	Alzheimer's disease
ADHD	Attention deficit hyperactivity disorder
AjD	Adjustment disorder
BAC	Balanced accuracy
bvFTD	Behavioral variant frontotemporal dementia
C9orf72	Chromosome 9 open reading frame 72
COMT	Catechol-O-methyl transferase
CSF	Cerebrospinal fluid
D1	Dopamine D1 receptor
DAT	Dopamine transporter
DP	Digital phenotyping
DTI	Diffusion tensor imaging
EPDS	Edinburgh Postnatal Depression Scale
ESR1	Estrogen receptor gene
EWS	Ewing's sarcoma protein
fALFF	Fractional amplitude of low frequency fluctuations
FC	Functional connectivity
FDOPA	Fluorodopa
fMRI	Functional magnetic resonance imaging
FDG-PET	Fluorodeoxy glucose positron emission tomography
FTD	Frontotemporal dementia
FUS	Fused-in-sarcoma
GABA	γ-aminobutyric acid
GABAa	γ-aminobutyric acid type A receptor
GCor	Global correlation
GMV	Gray matter volume
GRN	Progranulin
HC	Healthy control
IL	Interleukin
LCor	Local correlation
MAO-A	Monoamine oxidase A
MAPT	Microtubule-associated protein tau
MDD	Major depressive disorder
mGLUR5	Metabotropic glutamate receptor type 5
ML	Machine learning
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
MPAS	Maternal Postnatal Attachment Scale
NET	Norepinephrine transporter
NfL	Neurofilament
nfvPPA	Non-fluent variant primary progressive aphasia
PET	Positron emission tomography
PMS	Premenstrual syndrome
PPA	Primary progressive aphasia
PPD	Postpartum depression
RNA	Ribonucleic acid
rsfMRI	Resting state functional magnetic resonance imaging
SLC6A4	Serotonin transporter gene
sMRI	Structural magnetic resonance imaging
SSRI	Selective serotonin reuptake inhibitor
STin2VNTR	Serotonin transporter intron 2 variable number tandem repeat
svPPA	Semantic variant primary progressive aphasia
TAF15	TATA-binding protein associated factor 15
TDP-43	TAR-DNA-binding protein-43
VAChT	Vesicular acetylcholine transporter

Table of contents

1 Introduction

Neuropsychiatric diseases comprise a spectrum of disorders, which exhibit neurological and psychiatric features (1–4). For example, neurodegenerative diseases such as Parkinson's and Alzheimer's disease (AD) are often accompanied by psychiatric symptoms such as depression or anxiety (4–6). Similarly, many mental disorders such as schizophrenia and depression are commonly accompanied by neurobiological changes such as structural and functional brain alterations and/or induced by an underlying neurobiological mechanism (7). Mental disorders alone have a global prevalence of 10% and contributed 14% to the total of years lived with disability in the last three decades, leading to a high societal and economic burden of an estimated 5 trillion US dollars in 2019 (8–11). To reduce this burden and particularly the suffering of patients and their caretakers, early recognition is crucial (12–14). Ideally, individuals at risk should be identified before disease onset or patients should be diagnosed at an early stage of the disease, who can subsequently receive interventions and/or treatment. Depending on the age of onset, interventions could already start during childhood or early adolescence (15). Thereby, the development of the disorder can either be prevented or the magnitude of the symptoms can be significantly reduced (12).

1.1 Biomarkers: concept, definition, and types

Early disease recognition and disease course prognosis may be facilitated by the utilization of biomarkers (16,17). Biomarkers were originally defined as a "characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" by the Biomarkers Definitions Working Group in 2001 (18). However, this definition holds several limitations as it excludes subjective parameters and additional, not-listed processes (19). Therefore, the Food and Drug Administration and National Institutes of Health Biomarker Working Group re-defined the term in 2016 into a "defined characteristic measured as an indicator of normal biological processes, pathogenic processes, or biological responses to an exposure or intervention, including therapeutic interventions" (20). Importantly, biomarkers are contrary to clinical endpoints, which describe "how a patient feels, functions, or survives" (18,20).

Furthermore, biomarkers can serve different purposes, dividing them into several categories: susceptibility/risk, predictive, diagnostic, monitoring, prognostic, response,

and safety (20). Whereas susceptibility or risk biomarkers estimate the potential of an individual to develop a disorder, predictive biomarkers are focused on the identification of individuals who are reactive to a specific environmental agent or medical product. In contrast, diagnostic biomarkers indicate the presence of a disease or a subtype of a disease and should not overlap between disorders. While monitoring biomarkers determine the status of a condition or assess the response to a specific environmental agent or medical product and are often measured repeatedly, prognostic biomarkers estimate the recurrence, course, and the outcome of a disease. In addition, response biomarkers are utilized to detect a biological response after exposure to a specific environmental agent or medical product. Whereas a pharmacodynamic biomarker measures the biological activity of a specific environmental agent or medical product, for example to assess proof-of-concept (17,20), a surrogate endpoint biomarker is often used as a "substitute for how a patient feels, functions, or survives" (18,20). Finally, safety biomarkers evaluate the potential toxicity after exposure to a specific environmental agent or medical product (17,20).

1.1.1 Biomarkers in the field of neuropsychiatry

In contrast to other medical disciplines, the field of neuropsychiatry has only started to discover the potential of biomarkers in the last two decades (16). Biomarkers for neuropsychiatric diseases can be derived from a variety of modalities such as behavioral information including socio-demographic and clinical-anamnestic information, digital phenotyping (DP; i.e., data collected with smartphones and wearables such as smartwatches) (21), and neuropsychological testing (16), omics (e.g., genomics, proteomics, transcriptomics, metabolomics and epigenetics), peripheral (i.e., blood-based) and cerebrospinal fluid (CSF)-derived information, and neuroimaging (e.g., structural and functional magnetic resonance imaging) (19). Although the biomarker development in the field is promising, there are several issues which need to be taken into consideration. One concern arises from the early recognition or prediction of mental illnesses in children and adolescents as they might receive drug treatment already in pre-symptomatic or subclinical stages, potentially causing adverse effects. A further issue concerns differing disease prevalences between ethnicities, potentially leading to discrimination against minorities with specific genetic predispositions. Finally, many potential biomarkers have small effect sizes and lack accuracy when used for prediction (16). However, machine learning (ML) shows promises for overcoming the issue of insufficient accuracy by combing multiple modalities, taking advantage of the ensemble learning principle. This principle states that the combination of multiple models leads to

errors canceling each other out in the integrated ensemble, meaning a combination of models can reduce overfitting and improve prediction accuracy (22). For example, Koutsouleris and colleagues (23) developed a cybernetic model (i.e., a model requiring an interaction of clinicians with a ML algorithm). The cybernetic model combined several models including the expert prediction of a clinician as separate modality, clinical-neurocognitive information, polygenic risk scores, and structural MRI (sMRI). In line with the ensemble learning principle, the cybernetic model significantly outperformed either classifier alone.

1.1.2 Biomarkers for chronic vs. non-chronic neuropsychiatric diseases

Depending on the chronicity of the disease, certain biomarker types might be more valuable in comparison to other types. Patients with a chronic condition persistently exhibit severe symptoms and gravely impaired function (24). For example, frontotemporal dementia (FTD) is a neurodegenerative (i.e., chronic) disease comprising behavioral and cognitive changes (25). Risk, monitoring, prognostic, and predictive biomarkers could be more valuable to enable early recognition, to assess disease stability or progression, to estimate the disease course or recurrence of episodes (e.g., for psychosis or depression), and to evaluate the favorability of several treatment options. In contrast, non-chronic neuropsychiatric conditions are either temporary by definition, for example adjustment disorder (AjD) is defined as a difficulty to adjust within a period of three to six months after the occurrence of a stressor (26), or constitute a temporary form of a disease such as non-chronic depression (i.e., depression with an illness duration of less than two years) (27). Non-chronic or temporary conditions might similarly benefit from risk and predictive biomarkers and additionally from diagnostic biomarkers, since patients with non-chronic diseases can benefit from early intervention and treatment. Postpartum depression (PPD) exemplifies an event-related condition (i.e., childbirth), which is treatable, but has the potential to develop into a chronic illness (28,29). Both FTD and PPD are often under- or misdiagnosed (28,30), illustrating the necessity of reliable and valid diagnostic biomarkers for these conditions.

To summarize, biomarkers for neuropsychiatric diseases can serve different purposes such as risk prediction or disease course prognosis and can be derived from a variety of modalities. FTD, a neurodegenerative disease, and PPD, a childbirth-related, often temporary disease with the potential to transition into a chronic form of depression, serve as examples of underdiagnosed neuropsychiatric diseases with a strong need for biomarkers.

1.2 Biomarkers for postpartum depression

Approximately one out of seven women develop PPD after childbirth, with even higher prevalences in developing countries (28,31). According to the DSM-5, PPD is a major depressive disorder (MDD) developing within four weeks after childbirth (32). However, in clinical practice disease onsets of three to 12 months after childbirth might be considered (33). Common symptoms are sadness, anxiety, irritability, sleep disturbance (i.e., beyond sleep disturbances caused by taking care of the infant) and tiredness, lack of interest, and low self-esteem. These symptoms are often accompanied by self-blame and feelings of humiliation (31,34,35). Moreover, mothers can display negligence or develop a hostile attitude towards their infants and/or exhibit reduced receptiveness to their infants' needs (28,36). Maternal depression can further have detrimental effects on the mental health of their children. For example, PPD has been associated with difficulties in early emotional regulation and social behavior, and an increased risk for depression, anxiety, and attention deficit hyperactivity disorder (ADHD). Additionally, the cognitive development (e.g., language development, general ability to learn) of infants can be negatively affected (28,37,38). AjD is another condition which can occur in the postpartum period and can present with similar symptoms. In the DSM-5, AjD is defined as developing within three months after exposure to a stressor and as resolving within six months after the stressor is removed, not meeting criteria for depression or any other mental disorders (32). Importantly, PPD and AjD are different from postpartum blues, which is a mild mood disturbance experienced by 80% of mothers lasting up to ten days after delivery (39).

Although PPD is treatable, a significant number of women continue to experience depressive symptoms up to two years postpartum. While some women start to exhibit depressive symptoms later in the postpartum period, making the PPD diagnosis debatable considering diagnostic criteria, others develop a chronic form of PPD (40). Moreover, approximately 40% of women will relapse during a subsequent pregnancy or even unrelated to pregnancy. If untreated, depressive episodes are likely to recur (35). Unfortunately, PPD is often not recognized by significant others and frequently missed during routine follow-up visits. Additionally, feelings of guilt and shame hinder women from reporting their symptoms (41). This results in up to 50% of cases not being diagnosed (42). Whereas in 33% of the cases the depressive episode begins already during pregnancy, in 27% of the cases the episode starts even before pregnancy (43). The failure to recognize PPD stresses the need for routine care screening during pregnancy and after delivery. A small study found that screening and subsequent

treatment for PPD produces feasible cost-effectiveness ratios with $13,857 per quality-adjusted life-year and $10,182 per remission (44). For example, one potential screening tool is the Edinburgh Postnatal Depression Scale (EPDS), which includes ten questions about the feelings in the past seven days aimed at pregnant women or women after childbirth (45). However, more screening tools and robust early biomarkers are needed.

1.2.1 Pathology, etiology, and potential biomarkers for postpartum depression

To date, the etiology of PPD remains unknown. However, risk factors have been identified, which include history of depression, stressful life events, neuroticism, low self-esteem, antenatal depression and anxiety, postpartum blues, poor marital relationship, and poor social support, with sociodemographic factors only having a weaker association with PPD (46–49). Although the heritability of perinatal depression is around 50% (46,50) and genes involved in major depression have been found to be involved in PPD, many only show a weak association with PPD, resulting in no clear genetic basis for PPD (51). Two polymorphisms (i.e., 5-HTTLPR and STin2VNTR) of the serotonin transporter (5-HTT) gene (SLC6A4) are among the most widely studied genetic causes. Whereas the short allele of the 5-HTTLPR polymorphism seems to be linked to an increased risk for PPD, the long allele is associated with PPD symptoms after childbirth (22,46,52). In contrast, the STin2VNTR polymorphism seems to be more generally linked to depressive disorders and mental health issues (46). Other genes examined in the context of PPD include glucocorticoid receptor complex regulating genes, monoamine oxidase A (MAO-A) and catechol-O-methyl transferase (COMT) genes, and the estrogen receptor gene (ESR1) (46,52,53).

Moreover, the relevance of reproductive hormones and other hormones involved in reproduction including estrogen, progesterone, prolactin, oxytocin, and testosterone is frequently discussed in the literature (46). While reproductive hormones generally increase throughout pregnancy and drop to low levels immediately after childbirth, there is evidence that some women are vulnerable to hormonal shifts across the lifespan (35,54). Whereas there is no clear involvement in the development of PPD for estrogen, lower levels of prolactin, progesterone's metabolite allopregnanolone, and oxytocin have been associated with a higher risk of PPD. For testosterone, there is suggestive evidence that women with PPD have higher serum levels during late pregnancy or early postpartum, but other studies failed to replicate these findings (46).

Next to a potential interplay of thyroid hormones and reproductive hormones (46,53,55), other peripheral biomarkers have been evaluated. For example, elevated levels of

proinflammatory cytokines (e.g., IL6, IL-1β, and tumor necrosis factor-alpha) in late pregnancy and early postpartum period have been associated with a higher risk for PPD (56–61). Studies examining MDD found that proinflammatory cytokines such as IL-6 can 'hyperactivate' the hypothalamic-pituitary-adrenal axis, which may cause depression if this hyperactivation is prolonged (46,53,55,62,63). Moreover, low levels of the brain-derived neurotrophic factor in pregnancy and PPD women have been associated with depressive symptoms and suicide risk, respectively (46,53). Additionally, low levels of the serotonin precursor tryptophan in the first few days postpartum were associated with PPD (64). For dietary-related peripheral biomarkers, low vitamin D and zinc levels have been linked to an increased risk of developing PPD and with higher symptom severity, respectively (46,53,55). However, there was only mixed evidence for other dietary-related markers such as serum cholesterol, vitamin B12, gut microbiome, long-chain polyunsaturated fatty acids status, folate, and kynurenine level (46,53).

Currently, there are few imaging studies in women with PPD with largely inconsistent results. Reviews by Duan (65) and Horáková (66) identified affected regions including the medial prefrontal cortex, the anterior cingulate cortex, and the amygdala. While these regions were mainly identified based on functional MRI (fMRI) studies, sMRI and metabolite (i.e., positron emission tomography [PET] and magnetic resonance spectroscopy) studies showed inconsistent results. However, sMRI studies point towards regional increases in grey matter volume (GMV) and metabolite studies show inconsistent results across the γ-aminobutyric acid (GABA)-ergic, glutamatergic and serotonergic neurotransmitter systems (65,66). Given the inconsistent results and the low number of studies on neuroimaging in PPD (i.e., 26 studies), more research including larger samples is needed to clarify these inconsistencies across the postpartum period.

In summary, while the etiology of PPD remains unknown, there are a few candidate biomarkers comprising genes, reproductive hormones, and other peripheral markers with mixed results for neuroimaging. So far, most studies only have small sample sizes and from a genetic point of view, there is no candidate gene or genetic risk score available (yet) (46,50).

1.2.2 Treatment of postpartum depression

The treatment of PPD routinely includes psychosocial, psychological, pharmacological, and somatic interventions. While psychosocial interventions include nondirective counseling and peer support, psychological treatment comprises cognitive behavior and

interpersonal therapy. Pharmacological interventions include selective serotonin reuptake inhibitors (SSRIs) in addition to other antidepressants in case of ineffectiveness of SSRIs. Generally, SSRIs are preferable as they have minimal transfer into breastmilk. Nevertheless, antidepressants should only be taken into consideration in more severe cases or if psychosocial or psychological interventions are not sufficient. For severe cases of PPD, additionally somatic treatments such as electroconvulsive therapy can be considered. Nonetheless, women in the postpartum period mostly prefer psychosocial and psychological interventions (28,34,35,38).

In summary, PPD is a serious condition affecting one out of seven mothers and their children. Despite being treatable, PPD is often not recognized due to a lack of screening during routine care and women not seeking help caused by feelings of guilt and shame. The etiology of PPD remains unknown, stressing the need for effective biomarkers facilitating early recognition.

1.3 Biomarkers for frontotemporal dementia

Frontotemporal dementia (FTD) is a neurodegenerative disease characterized by the degeneration of frontal and temporal lobes, constituting one of a cluster of pathologies referred to as frontotemporal lobar generation. In people under the age of 65, FTD is one of the most common types of dementia with a prevalence of 0.01 to 4.6 per 1000 persons (67–70). In addition to the early mean onset age of 56 years, FTD is marked by a short survival time, which can range from three to 12 years (71,72). The heterogenous disease has several clinical manifestations: the behavioral variant (bvFTD), two main variants of primary progressive aphasia (PPA), and the more recently recognized and rare right temporal variant (rtvFTD) (67,70,71,73–76). bvFTD is the most common subtype accounting for about half of the cases and is depicted by rapidly progressing alterations in behavior and personality. Common symptoms include apathy, disinhibition (e.g., substance abuse, gambling), altered eating behavior, loss of empathy, stereotyped attitude regarding spoken language and motor functions, and impaired executive functions (e.g., deficits in attention span and working memory, planning and problem-solving skills, reduced mental flexibility) (67,70–77). Semantic variant PPA (svPPA) progresses more slowly and has two slightly differing manifestations depending on which hemispheric anterior temporal lobe is affected more strongly by cortical atrophy. Whereas the involvement of the left anterior temporal lobe leads to language alterations such as failing to recognize meaning of words, the involvement of the right anterior temporal lobe induces behavioral alterations in eating behavior, disinhibition, and poor

insight similar to bvFTD (67,70–77). Non-fluent variant PPA (nfvPPA) is the second most common and most heterogeneous manifestation, which includes symptoms of impaired speech ultimately becoming non-fluent with either combined agrammatism and apraxia of speech or agrammatism alone. In advanced stages, patients can display apathy or disinhibition, but compared to svPPA, they usually show smaller deficits in socioemotional functioning (67,70–77). Logopenic variant PPA (lvPPA), which is usually more common in AD and rarely occurs in FTD, is an additional clinical manifestation, which leads to minimal verbal output and fluency disruption, leaving grammatical and motor aspects of language intact (67,71). Finally, the right lobe variant (rtvFTD) is distinct from the other subtypes and difficult to distinguish at onset. Symptoms commonly include behavioral alterations such as disinhibition and obsessive personality, episodic memory disturbances, spatial, anterograde, and topographic disorientation with language impairments such as difficulties in lexical retrieval and anomie with the progression of the disease (67,71,78). Interestingly, while the behavioral and semantic variants are predominantly male, the non-fluent variant is predominantly female (67,71). Due to its symptom overlap with other dementias such as AD and psychiatric diseases such as schizophrenia, MDD, borderline personality disorder, obsessive-compulsive disorder, and bipolar affective disorder, FTD is often misdiagnosed (67,71,71,74,75). Especially in very young patients with the behavioral variant, psychotic symptoms such as delusions and hallucinations are common, making the differentiation from psychiatric diseases even more difficult. These symptoms in addition to a high education and a family history of psychiatric illness often lead to an initial psychiatric diagnosis in up to 50% of the cases (71,73,79).

Despite FTD being a neurodegenerative disease, early recognition is crucial to start symptom and patient management as early as possible. Due to the heterogeneity of the disease, the diagnosis, and especially the differentiation between subtypes, usually requires multiple steps consisting of considering multiple modalities such as neuropsychological testing, laboratory testing, and neuroimaging (e.g., MRI). However, the patient might have already been misdiagnosed with a psychiatric disease in the early stages of the disease (67,73). Based on the number of fulfilled criteria, a degree of probability (i.e., possible, probable or definitive) can be assigned (67,74). For example, a definitive diagnosis of bvFTD requires the presence of clinical symptoms, clinical manifestations, a demonstrable functional decline, cortical changes visible with neuroimaging, and finally pathogenetic mutations or other histopathological evidence for FTD (67).

1.3.1 Pathology, etiology, and potential biomarkers for frontotemporal dementia

Since one of the core symptoms of FTD includes impairments of executive function and language, neuropsychological testing is essential for diagnosis and should include multiple cognitive domains. Assessments for working memory (e.g., n-back tasks), verbal fluency (e.g., Animal Naming), inhibition (e.g., Stroop test), and decision-making (e.g., Iowa Gambling Task) are relevant for bvFTD. The neuropsychological profile of svPPA should be assessed using confrontation naming (e.g., Boston Naming Test), single word comprehension (e.g., Aachen Aphasia Test), category fluency (e.g., Animal Naming, F-A-S test), and word-picture matching (e.g., Semantic Word Picture Matching). In contrast, sentence reading and ordering, and syntax and grammar assessment (e.g., Northwestern Anagram Test, picture description tasks) should be considered for nfvPPA (74). However, social cognition and behavioral changes including apathy, stereotypical behaviors, and changes in eating behavior can mainly be assessed during a clinical interview (67,74).

Next to neuropsychological testing constituting behavioral biomarkers, few risk factors have been identified. For example, autoimmune disorders (excluding thyroid-related disorders) are common in svPPA patients. Additionally, learning disabilities in patients and first-degree relatives might constitute a risk factor (71). Moreover, risk factors might differ in sporadic and genetic forms of FTD. For example, lower education and impaired cardiovascular health were associated with the sporadic form of FTD and specifically with the bvFTD phenotype. For genetic FTD, a potential association between earlier age at disease onset and heart diseases was discovered (80). Therefore, presence of autoimmune disorders, education, age at onset, and cardiovascular diseases constitute potential risk factors for FTD.

In 40% of FTD cases there is a family history of dementia with 10% expressing an autosomal dominant inheritance pattern (67,70–72,77,78,81,82). Whereas bvFTD is genetic in almost 50% of the cases, PPA is genetic in only 12% of the cases, with svPPA being least likely to be genetic (67,71,72,83). Three genetic loci account for most cases of genetic FTD: microtubule-associated protein tau (MAPT) and progranulin (GRN) on chromosome 17, and chromosome 9 open reading frame 72 (C9orf72) (67,72,77,78,81,83). More than 80 MAPT mutations, which are common in bvFTD and svPPA, can induce neurotoxicity through three main mechanisms including tau loss of function, tau neurotoxic gain of function, and tau mislocalization into post-synaptic spines (67,72). GRN with over 70 mutations, which is common in bvFTD and can occur in nfvPPA, causes TAR-DNA-binding protein-43 (TDP-43) pathology (67,72). In contrast,

hexanucleotide expansions cause C9orf72 mutations, which are common in bvFTD and rare in PPA. These mutations induce pathology through loss of function of C9orf72 protein, toxic gain of function of ribonucleic acid (RNA) foci, and toxic gain of function of dipeptide repeat proteins (67,72).

Considering peripheral and CSF biomarkers, the most promising biomarker for FTD are neurofilament (NfL) CSF and blood markers, since they can differentiate bvFTD, nfvPPA, and svPPA from psychiatric disorders and are associated with the rate of clinical progression and survival. However, NfL marker cannot differentiate FTD from other neurodegenerative diseases (67,74,81,84). Additionally, FTD is associated with changes in several neurotransmitter systems such as GABAergic and glutamatergic neurotransmission (67), showing potential for biomarkers assessing neurotransmitter vulnerability.

Neuroimaging is used to increase the degree of probability of a diagnosis with FTD. The clinical manifestations show differences across several modalities. bvFTD is marked by early bilateral anterior brain atrophy as measured by sMRI and glucose hypometabolism as measured by Fluorodeoxyglucose-PET (FDG-PET), which is typically more pronounced in the right hemisphere. Potentially even before symptom onset, affected brain areas include the medial prefrontal, orbitofrontal, dorsolateral, and anterior cingulate cortex, basal ganglia, and the frontal insula (67,82,84,85). While structural connectivity as measured by diffusion tensor imaging (DTI) is mostly affected in the uncinate fasciculus, paracallosal cingulum and genu of the corpus callosum (84,85), functional connectivity (FC) measures show disruptions of intrahemispheric connectivity in salience, temporal, and basal ganglia networks (67,85). In svPPA, atrophy and glucose hypometabolism affect mostly the language-dominant (i.e., typically left) hemisphere including temporoparietal junction, anterior cingulate and orbitofrontal cortex, subiculum, insula, entorhinal cortex, hippocampus, and amygdala (67,82,84). Additionally, DTI shows changes in anterior and inferior temporal white matter and bilateral uncinate fasciculi and resting-state fMRI (rsfMRI) shows reduced connectivity in the left temporal lobe (84). In nfvPPA, atrophy and glucose hypometabolism occurs asymmetrically in left inferior frontal gyrus and insula, premotor and motor cortex, dorsolateral and medial prefrontal cortex, medial and lateral temporal lobe, orbitofrontal cortex, thalamus, striatum, and supplementary motor area. With disease progression, atrophy will spread to dorsolateral and medial prefrontal cortex, medial and lateral temporal lobe, orbitofrontal cortex, thalamus, and striatum (67,82,84). Moreover, DTI shows damage to left anterior temporal and orbitofrontal white matter (84). Importantly,

atrophy and glucose hypometabolism can even occur five to ten years before symptom onset in genetic FTD (67).

In line with the heterogeneity of the clinical manifestations, the neuropathology underlying FTD is similarly diverse. Several proteinopathies can occur in FTD including TDP-43, tauopathies (i.e., involving tau), and FET-related pathologies consisting of the fused in sarcoma (FUS), Ewing's sarcoma protein (EWS), and TATA-binding protein associated factor 15 (TAF15). These proteins are thought to misfold, leading to toxic accumulations in neuronal and glial cells, originating in distinct regions and spreading in a prion-like manner within neural networks. Although some patients only exhibit one proteinopathy, many feature two or three, making it difficult to distinguish clinical subtypes based on proteinopathy (67,70,74–78). The loss of function and toxic gain of function requires several genetic hits to alter proteostasis and cause protein accumulation, which sensitizes the cells for insults and leads to cell death (67,70,74–78).

Neuropsychological testing, neuroimaging, genetic testing, and CSF and peripheral biomarkers show promises as biomarkers for FTD. However, differentiation of subtypes and discrimination from other dementias and psychiatric diseases is still difficult, stressing the need for effective and robust diagnostic biomarkers.

1.3.2 Treatment of frontotemporal dementia

In addition to the difficulty of diagnosing FTD, treatment options are still limited, focusing on symptom and patient management. Next to a few ongoing pharmacological trials targeting tauopathies, progranulin deficiency, and neuroinflammation processes, there are other new treatments including gene-editing therapies and non-invasive brain stimulation (67,74,77,81). However, the current standard treatment includes physical, occupational, and speech therapies in addition to support for the caregiver and facilitating patient management (67,72,73,76,77,86). To improve the patient's quality of life, symptoms are managed with antidepressants such as SSRIs for depressive symptoms and potentially even for impulsivity, disinhibition, and repetitive behaviors. Furthermore, neuroleptics can improve cognitive and behavioral symptoms (67,71–73,76,77,81,86).

In summary, FTD is a heterogeneous disease constituting several clinical manifestations and pathologies. The diagnosis of the disease is still complex and requires multiple modalities to increase the degree of probability of the diagnosis. Moreover, clinical subtypes are not easily distinguishable from each other and from other diseases such as psychiatric diseases and AD, especially in the early stages. Therefore, there is a clear

need for effective and robust biomarkers to facilitate the (differential) diagnosis and potentially shed more light on the underlying neuropathological mechanism.

1.4 Aim of the thesis

Early recognition and diagnosis can reduce the suffering of patients and lower the economic burden considerably (8–14). Currently, many neuropsychiatric diseases are frequently under- or misdiagnosed (28,30). To enable early recognition and diagnosis of neuropsychiatric diseases, there is a need for robust biomarkers in the field (16,17). The overall aim of this thesis was to demonstrate the potential of utilizing biomarkers in the field of neuropsychiatry. More specifically, the aim was to evaluate neuroimaging and behavioral biomarkers for chronic and non-chronic neuropsychiatric diseases. Whereas PPD is a typically treatable disease with a potential to turn into a chronic form, especially if unrecognized (40), AjD in the postpartum period constitutes as example for a non-chronic disease (26). In contrast, bvFTD characterized by neurodegeneration of frontal and temporal lobes and continuous deterioration of symptoms serves as example for a chronic disease (67,70). First, demographic, clinical-anamnestic, and digital biomarkers were evaluated in a classification framework as early diagnostic biomarkers for PPD and AjD (study 1). Next, early alterations of brain structure and function were examined in PPD and AjD (study 2). Finally, structural and functional brain alterations in bvFTD compared to healthy controls (HC) were evaluated to assess neurotransmitter vulnerability using spatial correlations with neurotransmitter maps from a healthy volunteer population (study 3).

1.4.1 Early identification of postpartum depression using demographic, clinical, and digital phenotyping

The goal of the first study (87) was to evaluate socio-demographic and clinical-anamnestic information collected within 12 weeks postpartum for early identification of PPD and AjD. While the initial and final diagnostic interviews and the collection of socio-demographic and clinical-anamnestic information were conducted in person, bi-daily mood and stress assessments and follow-up EPDS and Maternal Postnatal Attachment Scale (MPAS) were collected remotely. After examining univariate differences regarding socio-demographic and clinical-anamnestic factors between PPD, AjD, and HC, three separate logistic regression classifiers including mood, postnatal depression and attachment scores were trained in a first cohort and validated in a second, independent cohort. Compared to HC, women with PPD and AjD more frequently displayed a

personal and familial psychiatric history, premenstrual syndrome (PMS), subjective birth-related psychological traumas, postpartum blues, and reduced breastfeeding after 12 weeks postpartum. However, there were no differences between women with PPD and AjD. Moreover, mood and stress scores showed significantly different patterns for patients and controls. Interestingly, PPD and AjD were only distinguishable regarding mood and stress scores after four and five weeks, respectively. Finally, all classifiers achieved high balanced accuracies (BACs) for the differentiation of PPD, AjD, and HC in both cohorts (PPD-HC: $BAC_{exploration}$ = 87%, $BAC_{validation}$ = 93%; AjD-HC: $BAC_{exploration}$ = 91%, $BAC_{validation}$ = 79%; PPD-AjD: $BAC_{exploration}$ = 76%, $BAC_{validation}$ = 73%). Thereby, the first study demonstrated the potential of postnatal depression and attachment scores in combination with remote mood assessments as early diagnostic biomarkers for PPD and AjD.

1.4.2 Examining early structural and functional brain alterations in postpartum depression through multimodal neuroimaging

The second study (88) aimed at examining early structural and functional brain alterations in postpartum women. More specifically, GMV was extracted from sMRI and FC measures including the fractional amplitude of low frequency fluctuations (fALFF), local correlation (LCor), and global correlation (GCor) were obtained from rsfMRI. sMRI and rsfMRI were collected within the first week postpartum in a subsample of women from the first study. There were no group differences between PPD, AjD and HC regarding GMV and FC measures. However, there was a significant association between LCor and EPDS score at 12 weeks postpartum in the left superior medial frontal gyrus. While there were no robust early structural and functional brain alterations in PPD and AjD, these results suggest that brain alterations might not precede symptoms but co-occur with them.

1.4.3 Resting-state alterations in behavioral variant frontotemporal dementia are related to the distribution of monoamine and GABA neurotransmitter systems

The goal of the third study (89) was to evaluate structural and functional brain alterations as neurotransmitter vulnerability biomarker for bvFTD. fALFF as derived from rsfMRI was spatially correlated with the non-pathological distribution of 11 receptors and transporters of the serotonergic, GABAergic, norepinephrinergic, dopaminergic, and μ-opioid neurotransmitter system, and their coding messenger RNA (mRNA) gene expression. Whereas GMV was reduced in anterior caudate, medial and lateral prefrontal, thalamic,

insular, and temporal regions, fALFF was reduced in frontoparietal and frontotemporal regions in bvFTD patients compared to controls. These fALFF alterations co-localized with the distribution of the GABAergic, norepinephrinergic, and serotonergic neurotransmitter system, and their encoding mRNA gene expression. This co-localization was further associated with cognitive symptoms (verbal fluency) and disease severity (Mini Mental State Examination). In contrast, alterations of GMV did not co-localize with any neurotransmitter system, indicating that the observed co-localization of fALFF with these neurotransmitter systems was not driven by underlying atrophy. Thus, fALFF co-localization with the non-pathological distribution of these neurotransmitter systems can function as biomarker for neurotransmitter vulnerability.

1.5 Ethics vote

Study 1 and 2 were performed in accordance with the positive vote of the Institutional Review Board of the Medical Faculty of RWTH Aachen University (EK 208/15). Study 3 was approved by local ethics committees of all universities involved in the German Consortium for Frontotemporal Lobar Degeneration (Ethics Committee University of Ulm approval number 20/10).

2 Early identification of postpartum depression using demographic, clinical, and digital phenotyping

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Abstract

Postpartum depression (PPD) and adjustment disorder (AD) affect up to 25% of women after childbirth. However, there are no accurate screening tools for either disorder to identify at-risk mothers and enable them to benefit from early intervention. Combinations of anamnestic, clinical, and remote assessments were evaluated for an early and accurate identification of PPD and AD. Two cohorts of mothers giving birth were included in the study ($N = 308$ and $N = 193$). At baseline, participants underwent a detailed sociodemographic-anamnestic and clinical interview. Remote assessments were collected over 12 weeks comprising mood and stress levels as well as depression and attachment scores. At 12 weeks postpartum, an experienced clinician assigned the participants to three distinct groups: women with PPD, women with AD, and healthy controls (HC). Combinations of these assessments were assessed for an early an accurate detection of PPD and AD in the first cohort and, after pre-registration, validated in a prospective second cohort. Combinations of postnatal depression, attachment (for AD) and mood scores at week 3 achieved balanced accuracies of 93 and 79% for differentiation of PPD and AD from HC in the validation cohort and balanced accuracies of 87 and 91% in the first cohort. Differentiation between AD and PPD, with a balanced accuracy of 73% was possible at week 6 based on mood levels only with a balanced accuracy of 73% in the validation cohort and a balanced accuracy of 76% in the first cohort. Combinations of in clinic and remote self-assessments allow for early and accurate detection of PPD and AD as early as three weeks postpartum, enabling early intervention to the benefit of both mothers and children.

Introduction

The postpartum period poses the highest risk to women for developing a mental disorder[1], with postpartum depression (PPD) being the most frequent one[2]. PPD is defined as a major depressive disorder occurring in direct relation (within 4 weeks postpartum) to childbirth in the DSM-5[3]. Early diagnosis and treatment of PPD can substantially improve the outcome, prevent relapse, and minimize the associated emotional and financial burden[4]. Maternal mental health is a reliable predictor of child's cognitive development and subsequent achievements[5]. The risk of a mother-to-child transmission of the vulnerability to depression[6,7], through genetic as well as other factors such as depression-related effects on parenting[8], is particularly high. Successful treatment of maternal depression alleviates the risk of childhood behavioral problems[9].

PPD is often overlooked during postnatal visits, missing the critical window for early intervention[10,11]. One reason is that low mood in the early postpartum period is largely deemed "normal" with 50–80% of new mothers experiencing initial sadness (i.e., postpartum blues), primarily due to dramatically plunging hormone levels at parturition[12]. Adjustment disorder (AD) in reaction to postpartum stress is another postpartum condition with

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similar symptoms. The crucial difference to PPD is that the severity of AD does not meet the criteria for depression at any time point. In the clinical context, AD needs to be considered as an important differential diagnosis to PPD[13].

History of mental illness, vulnerability to hormonal changes, psychological and social distress, baby blues, premenstrual syndrome (PMS), unwanted pregnancy, traumatic birth experience and stressful life events are all associated with an increased risk of PPD[11,12,14]. It is of crucial importance to evaluate the relative and combined predictive value of these factors for development of PPD. Previous studies aiming at prediction of PPD focused either on time points in the late postpartum period (e.g., after 8–32 weeks)[15] or only on single time points, thereby ignoring symptom dynamics or convolving PPD with major depression or AD[16]. Detailed in-clinic assessments are costly and burdensome, providing the likely reason for the cross-sectional nature of most previous studies. Online remote self-assessments may provide an easy means of obtaining the relevant information on symptom dynamics in individual patients.

Here, we recruited two cohorts of mothers giving birth and followed them longitudinally over 12 weeks to explore whether an accurate prediction of PPD is feasible based on socio-demographic and clinical-anamnestic information as well as early symptom dynamics using remote mood and stress assessments. Data from the first cohort were used to identify combinations of demographic and clinical data achieving highest accuracy for early identification and differentiation of PPD and AD using a machine learning approach. In this cohort, we identified and trained the optimal model for individual diagnostic prediction. The model and approach were preregistered and evaluated against an independent validation cohort to obtain unbiased performance estimates of the proposed algorithm.

Methods

First cohort and study design

To identify the best predictors of PPD, a first cohort of 308 mothers (mean age = 31.7 ± 4.76) was recruited following childbirth at the University Hospital Aachen between November 2015 and June 2018. The current project was part of the Risk of Postpartum Depression (RiPoD) study conducted at the University Hospital Aachen. The main exclusion criteria were a depressive episode (according to a clinical interview) at the time of recruitment and specific child health conditions (for details see supplementary material). The recruitment was conducted at the Department of Gynecology and Obstetrics within the first two to five days postpartum. Out of a total recruitment pool of ~1000 births per year, 50–60% of women were contacted (30% were directly excluded based on some exclusion criteria due to close collaboration with the obstetrics department) of which 50% were willing to participate and met the inclusion criteria. Written informed consent was obtained from all participants. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. All procedures involving human subjects/patients were approved by the Institutional Review Board of the Medical Faculty of RWTH Aachen University (EK 208/15). The study design comprised follow-up for 12 weeks with evaluation at five time points each three weeks apart (T0-T4) (Fig. S1). Evaluations were conducted at the clinic for T0 and T4 and via remote online questionnaires for T1 to T3. All women were asked to complete mood and stress assessments (scale from one to ten, ten being high) online on a bi-daily basis. Remote assessments were sent via e-mail. If three consecutive assessments were missed, a reminder was sent via e-mail, which allowed for close monitoring of the participation.

A clinical interview was conducted at T0 to ascertain current conditions. At T4, an experienced psychiatrist conducted a second clinical interview for a final diagnosis. Based on this interview, participants were assigned into one of three groups: healthy controls (HC, $N = 247$, 80.2%) without any sign of depression during the whole observation period, and women meeting DSM-5 criteria for PPD ($N = 28$, 9.1%) or AD ($N = 33$, 10.7%)[3]. In case of a depression, the Hamilton Depression Rating Scale[17] was administered. Clinical interviews were based on the DSM-5[3].

An sociodemographic-anamnestic questionnaire was used to obtain additional information about personal and socioeconomic status, psychiatric history, current pregnancy, child, breastfeeding at T0, postpartum blues (T4), PMS[18] (T4), subjective quality of support at home (T4), and breastfeeding at T4 (Table 1, Table S1). The Stressful Life Events Screening Questionnaire[19] was collected to assess encounter with stressful life events (T0) (Table 1). The Edinburgh Postnatal Depression Scale (EPDS)[20] was collected at all time points (T0-T4). Maternal attachment was evaluated from T1 through T4 using the Maternal Postnatal Attachment Scale (MPAS)[21].

Second cohort

For the second cohort, further referred to as validation cohort, 193 mothers (mean age = 32.7 ± 4.78) were recruited between November 2018 and January 2020 following the same protocol and study design as for the first cohort (Fig. S1). The prevalence rates in the validation cohort were 76.2% for HC ($N = 147$), 8.29% for PPD ($N = 16$), and 15.5% for AD ($N = 30$).

Table 1 Sociodemographic and anamnestic data for the first and second cohort.

Sociodemographic/anamnestic variable	First cohort				Second cohort			
	HC	PPD	AD	Statistical test	HC	PPD	AD	Statistical test
Age (in years)	31.9 ± 4.61 *N* = 247	30.4 ± 5.51 *N* = 28	31.4 ± 5.09 *N* = 33	$X^2(2, N= 308) = 2.60$ $p = .27$	33.1 ± 4.31 *N* = 146	31.8 ± 7.04 *N* = 16	31.5 ± 5.42 *N* = 30	$X^2(2, N= 193) = 3.40$ $p = 0.18$
Education (years)	13.8 ± 2.89 *N* = 240	12.4 ± 2.85 *N* = 27	13.4 ± 4.67 *N* = 33	$X^2(2, N= 300) = 3.04$ $p = 0.22$	14.6 ± 3.22 *N* = 139	14.3 ± 2.96 *N* = 16	14.08 ± 2.56 *N* = 26	$X^2(2, N= 179) = 0.77$ $p = 0.68$
Personal psychiatric history (no/yes)	220/27	16/12	19/14	$X^2(2, N= 308) = 34.5$ $p < 0.001$ [b,1,2]	118/26[c]	6/10	15/15	$X^2(2, N= 190) = 24.2$ $p < 0.001$ [b,1,2]
Familial psychiatric history (no/yes)	194/53	16/12	18/15	$X^2(2, N= 308) = 13.3$ $p = 0.001$ [b,1,2]	112/33	8/8	65/14	$X^2(2, N= 193) = 10.8$ $p = 0.005$ [b,**2**]
Birth complications (no/yes)	209/37	20/8	24/9	$X^2(2, N= 307) = 5.57$ $p = 0.062$	121/26	10/6	25/5	$X^2(2, N= 193) = 3.80$ $p = 0.15$
Subjective birth-related psychological traumas (no/yes)	215/29	19/9	20/13	$X^2(2, N= 305) = 21.1$ $p < 0.001$ [b,1,2]	124/14	12/4	23/7	[a]
PMS (no PMS/mild PMS/PMS)	111/84/29	4/12/12	7/16/10	$X^2(4, N= 285) = 27.9$ $p < 0.001$ [b,**1**]	83/44/14	3/4/8	9/14/6	$X^2(2, N= 185) = 26.6$ $p < 0.001$ [b,a]
Postpartum blues (no/yes)	151/93	8/20	7/26	$X^2(2, N= 305) = 27.7$ $p < 0.001$ [b,1,2]	102/45	0/16[c]	9/21	$X^2(2, N= 193) = 39.4$ $p < 0.001$ [b,a]
Stressful life events								
(number)	0.81 ± 1.27	1.46 ± 1.71	1.19 ± 1.18		1 ± 1.35	2.44 ± 2.06	1.97 ± 1.88	
(no/yes)	144/103	11/17	12/20	$X^2(2, N= 307) = 7.78$ $p = 0.020$	71/74	2/14	8/22	$X^2(2, N= 191) = 11.5$ $p = 0.003$ [b,**1**]
Breastfeeding T4 (no/yes)	63/182	14/14	8/25	$X^2(2, N= 306) = 7.62$ $p = 0.022$ [b,**1**]	41/96	9/7	9/20	$X^2(2, N= 182) = 4.56$ $p = 0.10$
Hamilton Depression Rating Scale T4	--	13.2 ± 2.88 *N* = 27	--		--	14.6 ± 4.18 *N* = 16	–	–

AD adjustment disorder, *HC* healthy controls, *PMS* premenstrual syndrome, *PPD* postpartum depression.
[a]No statistical analysis possible due to low expected cell counts.
[b]Bonferroni-corrected significant difference ($p < 0.05$) between [1] HC and PPD, [2] between HC and AD and/or [3] between PPD and AD.
[c]Significant group difference ($p < 0.05$) between first and validation cohort.

Univariate analyses of the first cohort

All data were analyzed using MATLAB R2018a, Python Jupyter Notebook 5.6.0, IBM SPSS Statistics 22 and jamovi 1.0.5.0[22]. Chi-square tests were performed to compare categorical sociodemographic-anamnestic variables across the groups in the first cohort. For continuous variables, logistic regressions were computed. Weekly mood and stress levels were calculated by averaging the corresponding bi-daily assessments. Mood-stress difference scores were calculated as the difference between both z-transformed variables to estimate individual discrepancies between perceived stress and mood (i.e., z-score mood minus z-score stress). Changes from baseline and the preceding week were computed for these variables. Dynamic changes in mood, stress, mood-stress difference, MPAS, and EPDS were analyzed using mixed effects repeated-measures analyses of variance (ANOVA) with week as within-subject and group as between-subject variable including an interaction term. Only post-hoc pairwise group comparisons (i.e., chi-square tests for categorical and binomial logistic regression for continuous sociodemographic-anamnestic variables, and independent samples t tests for mixed effects repeated-measures ANOVAs) were corrected for multiple testing using Bonferroni correction. The sample size was calculated as adequate for all univariate tests with a power of 0.8 and small to moderate effect sizes. Receiver operating characteristic (ROC) curves and their associated area under the curve (AUC) (within-sample) for differentiation between the three groups were computed for each measure per week.

Identification of most predictive combinations in the first cohort

Next, we aimed to evaluate if and which combinations of sociodemographic and clinical-anamnestic factors, mood, stress, MPAS and EPDS allow for an accurate differentiation between HC, PPD and AD in the first cohort. To that end, we used a logistic regression classifier (MATLAB built-in mnrfit and mnrval functions, no parameter optimization needed) performing 1000 repetitions of strict threefold cross-validation. The classification was performed for each pair-wise group comparison separately and oversampling was applied to the PPD and AD groups. Low-variance variables (family status, breastfeeding T0, education, completed professional education, income, and psychiatric diagnosis in previous pregnancy), i.e., variables with low group cell counts (less than 80% of expected cell counts >5), were excluded from the analysis in the whole sample (see Table 1 and Table S1). Independent samples t tests were performed in the training data to select the baseline variables to be included in the classifier ($p < 0.05$).

To identify the most sensitive combinations for early identification of PPD, the following nine feature combinations were evaluated: [1] baseline sociodemographic-anamnestic data alone, [2] mood scores, [3] stress scores, [4] mood-stress difference scores, [5] mood scores incl. changes (change to baseline and to preceding week), [6] stress scores incl. change scores, [7] mood-stress difference scores incl. changes, [8] combination of mood and stress scores incl. changes, [9] and combination of mood, stress, and mood-stress difference scores incl. changes. Combinations [1] to [9] were evaluated either alone or in combination with EPDS scores, MPAS scores or both. In addition, all combinations with features [2] to [9] were evaluated with and without inclusion of baseline sociodemographic-anamnestic information. The baseline sociodemographic-anamnestic information alone (i.e., feature combination [1]) served as null model for comparison with best performing models.

Balanced accuracies, sensitivities, specificities, positive and negative predictive values as well as ROC curves including the AUC were computed. The best performing combination (high balanced accuracy at earliest possible time-point) for each pair-wise comparison was selected for replication analysis. A logistic regression was computed for the selected combination using all participants. These results of the first cohort along with the validation plan were pre-registered on https://osf.io/ecmrp?view_only=6feb8e89818445a0b675621c8f22ba82. The obtained coefficients were applied to the prospectively collected validation cohort.

Application to the validation cohort

The selected and preregistered model as trained on the first dataset was then used to predict diagnoses in the independent validation cohort (Table S2). The class probability p for the validation cohort was obtained using the following standard logistic regression formula, where β denotes the coefficients and X the included features:

$$p = \frac{1}{1 + e^{-X\beta}}$$

As for the validation cohort, we computed balanced accuracy, sensitivity, specificity, AUC, ROC, and positive and negative predictive value by comparing predicted versus actual group labels. To obtain a chance level spread estimate for the classifier, we randomly permuted the "predicted" labels 1000 times across the validation cohort recomputing all performance measures and their 95% confidence interval.

Results

Sociodemographic-anamnestic and baseline group comparisons

In the first cohort, PPD and AD were associated with personal ($p < 0.001$ for HC vs. PPD and HC vs. AD) and familial psychiatric history ($p = 0.036$ for HC vs. PPD, $p = 0.009$ for HC vs. AD), subjective birth-related psychological traumas ($p = 0.024$ for HC vs. PPD, $p < 0.001$ for HC vs. AD), and postpartum blues ($p = 0.003$ for HC vs. PPD, $p < 0.001$ for HC vs. AD) (Table 1, S1 and S2). A higher PMS prevalence ($p = .012$ for HC vs. PPD) and reduced breastfeeding at T4 were observed in PPD compared to HC ($p = 0.021$). No differences were seen between PPD and AD. Similar effects were observed in the validation cohort for all sociodemographic-anamnestic factors (Table 1, Table S1; for odds ratios see Table S3).

Univariate analyses of the first cohort

The average participation over a total of 84 days of observation was 40 responses with a maximum of 45 responses, with no significant differences between the subsamples (HC: M = 40, max = 45; AD: M = 40, max = 44; PPD: M = 40, max = 45; $F(2, 305) = 0.33$, $p = 0.717$). Both PPD and AD showed a distinct pattern in weekly mood, stress, and mood-stress difference scores over the course of 12 weeks (significant time by diagnosis interactions – mood: $F(13.8,1303) = 16.3$, $p < 0.001$; stress: $F(11.3,1026) = 9.85$, $p < 0.001$; mood-stress difference: $F(13.1,1162) = 17.3$, $p < 0.001$) (Fig. 1A-C). The groups differed significantly in mood and mood-stress difference at all weeks ($p = 0.004$ for mood-stress baseline, all other $p < 0.001$) (see Tables S4 and S5). For stress, the difference was significant at all weeks except for baseline (all $p < 0.001$, see Table S6).

PPD had significantly lower mood levels compared to HC at all weeks except for baseline (Fig. 1A). AD had significantly lower mood relative to HC from baseline until week 6 reaching the highest difference at week 2. PPD had lower mood compared to AD from week 4 through week 12. Stress levels were significantly higher in PPD compared to HC from week 2 through week 12 and compared to AD between week 5 and week 12. AD had higher stress levels relative to HC from week 1 until week 4 (Fig. 1B). Mood-stress difference differed significantly between HC and PPD from week 1 through week 12, between HC and AD from week 1 through week 6, and between PPD and AD from week 4 through week 12 (Fig. 1C).

Both EPDS and MPAS showed significant time by diagnosis interactions (EPDS: $F(6.87,1034) = 34.4$, $p < 0.001$; MPAS: $F(5.35,805) = 8.24$, $p < 0.001$) with a significant between-group difference at all weeks (all $p < 0.001$) (Fig. 1D, E). EPDS scores were significantly lower in HC compared to PPD and AD at all time-points (T0-T4) ($p < 0.001$). The difference between PPD and AD was significant from T2 until T4 with higher EPDS scores in PPD women ($p < 0.001$). MPAS scores were significantly lower at all time points (T1–T4) in PPD ($p < 0.001$) and AD ($p < 0.001$ for T1-T3, $p = 0.008$ for T4) compared to HC. Lower MPAS scores were observed in PPD compared to AD at T4 ($p = 0.001$).

Prediction in the first cohort

Next, we evaluated which combinations of sociodemographic-anamnestic, mood, stress, EPDS, and MPAS data allow for reliable differentiation between PPD, AD, and HC. The outcomes of all evaluated combinations are summarized in Tables S7–14. For differentiation of PPD from HC, a high balanced accuracy of 87% was achieved at week 3 using a combination of baseline EPDS and follow-up EPDS and mood levels at week 3 (Table 2, Fig. 2A, and Table S7). The best early differentiation between AD and HC with a 91% balanced accuracy was also achieved at week 3 using a combination of baseline EPDS and follow-up EPDS, MPAS and mood scores at week 3 (Table 2, Fig. 2B, and Table S8). A reasonable differentiation of AD and PPD with a balanced accuracy of 76% was only achieved at week 6 using only the mood levels (Table 2, Fig. 2C, and Table S9). Logistic regression coefficients were trained with these combinations using the first cohort and applied to predict the diagnostic labels in the validation cohort (Table S2). The null model (i.e. sociodemographic-anamnestic information alone) performed inferior compared to the best performing models for all group comparisons (HC-PPD: BA = 0.72, HC-AD: BA = 0.75, AD-PPD: BA = 0.48; Table S9, Feature Combination 1).

Prediction in the validation cohort

The validation cohort had an average participation of 37 responses with a maximum of 45 responses for the remote assessments with no differences between the subgroups (HC: M = 38, max = 45; AD: M = 38, max = 43; PPD: M = 34, max =43; $F(2, 190) = 1.51$, $p = 0.223$). The classifier trained on the first cohort for differentiation of HC and PPD reached a high balanced accuracy of 93% in the validation cohort with a sensitivity of 88% and specificity of 99% (Table 2, Fig. 2D). The classifier differentiating HC and AD reached a balanced accuracy of 79% with a high specificity (98%) but only moderate sensitivity (60%) (Table 2, Fig. 2E). For PPD and AD differentiation, the selected classifier reached a balanced accuracy of 73%, again with high specificity (90%) but only low sensitivity (56%) (Table 2, Fig. 2F).

Discussion

Here, we adopted a within- and out-of-sample validation study design to identify combinations of

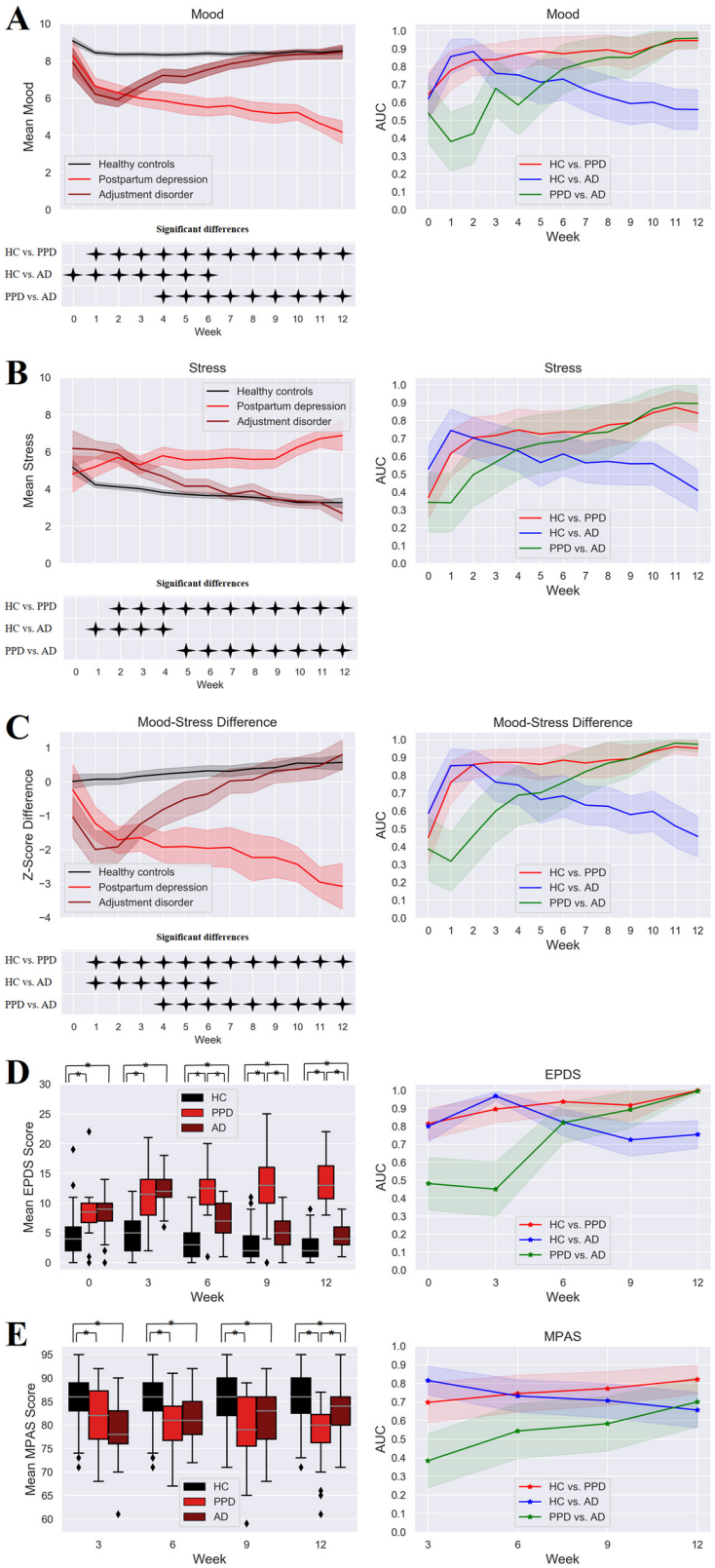

Fig. 1 Mood, stress, mood-stress difference, EPDS, and MPAS scores. Weekly mood (**A**), stress, (**B**) and mood-stress difference scores (**C**) incl. 95% confidence intervals, results of the simple effects analyses, and within-sample AUCs incl. 95% confidence interval for each group comparison. EPDS (**D**) and MPAS (**E**) mean scores and associated within-sample AUCs for each time point and group separately incl. their standard error and 95% confidence interval. Statistically significant *t* tests for group comparisons are marked with *.

Table 2 Results of prediction for the first and validation cohort.

	HC vs. PPD			HC vs. AD			PPD vs. AD		
	Cross-validation (1st cohort)	Out-of-sample validation	Chance (95% CI)	Cross-validation (1st cohort)	Out-of-sample validation	Chance (95% CI)	Cross-validation (1st cohort)	Out-of-sample validation	Chance (95% CI)
Balanced accuracy	0.87	0.93	0.50 (0.44; 0.59)	0.91	0.79	0.50 (0.45; 0.57)	0.76	0.73	0.50 (0.35; 0.67)
Sensitivity	0.85	0.88	0.11 (0.00; 0.27)	0.88	0.60	0.12 (0.03; 0.23)	0.76	0.56	0.28 (0.07; 0.50)
Specificity	0.89	0.99	0.90 (0.89; 0.92)	0.94	0.98	0.88 (0.86; 0.90)	0.76	0.90	0.73 (0.63; 0.83)
PPV	0.45	0.88	0.10 (0.00; 0.25)	0.66	0.86	0.18 (0.05; 0.33)	0.66	0.75	0.32 (0.08; 0.58)
NPV	0.98	0.99	0.91 (0.89; 0.92)	0.98	0.92	0.83 (0.81; 0.85)	0.79	0.79	0.68 (0.59; 0.78)
AUC	0.91	0.98	0.50 (0.34; 0.65)	0.97	0.92	0.50 (0.39; 0.62)	0.79	0.88	0.50 (0.31; 0.68)

Balanced accuracy, sensitivity, specificity, positive and negative predictive value obtained for the validation cohort (out-of-sample validation). For comparison, results of the first cohort aside with chance-level classification for the validation cohort are also reported.
AD adjustment disorder, *AUC* area under the curve, *CI* confidence interval, *HC* healthy controls, *PPD* postpartum depression, *PPV* positive predictive value, *NPV* negative predictive value.

sociodemographic-anamnestic and clinical factors allowing for early and accurate identification and differentiation of PPD and AD in two large cohorts of postpartum women. In both cohorts high accuracy was achieved at week 3 for identification of PPD and AD compared to HC using a simple combination of EPDS, mood, and MPAS (for AD) assessments. In contrast, differentiation of PPD and AD was possible only from week 6 based solely on mood levels.

In both cohorts, the prevalence of PPD was slightly lower than the 10–20 % reported in the literature[23,24]. As the focus of our study was on prediction of PPD, we purposely excluded women with manifest depression at the time of inclusion in the study, which may explain the lower prevalence. Furthermore, studies estimating early prevalence of PPD may have included women with AD. Although there is an increased risk for PPD within the first postpartum year[25], meaning that some women may develop PPD after four to six weeks (i.e. late onset), this was not the case for our sample. In line with previous research, we found postpartum blues, psychiatric history, subjective birth-related psychological traumas, and PMS to be significant risk factors for PPD[14,26,27].

Interestingly, no differences between the PPD and AD groups were found with respect to risk factors, suggesting that similar mechanisms may be involved in the generation of initial depressive symptoms in both groups. Over the observation period, stress levels continuously increased in women with PPD whilst they normalized after about five weeks in AD. Descriptively, mood levels in AD followed the stress levels normalizing only after about seven weeks. The temporal delay is in line with the interpretation that reductions in stress may contribute to the recovery observed in mood. The increase in stress levels and the simultaneous decline in mood levels in PPD may indicate the contribution of stress-mediated components in line with previous studies reporting parenting stress among the most important postpartum factors[28,29]. Whilst not a causal factor on its own, parenting stress is likely to increase vulnerability to depression in high-risk individuals.

Similarly, PPD and AD displayed distinct temporal courses of EPDS and attachment scores as measured by MPAS. The EPDS temporal dynamics were highly similar to the observed stress and mood levels. The initially lowest attachment scores were found to increase in AD while PPD maintained the low attachment levels throughout the study. These observations underscore the necessity of longitudinal monitoring of both measures to better characterize the dynamic relationship between depressed mood and maternal attachment[30,31]. Differences in MPAS and EPDS remained significant between AD and HC at all time points. According to recent findings, child neurodevelopment is affected by maternal

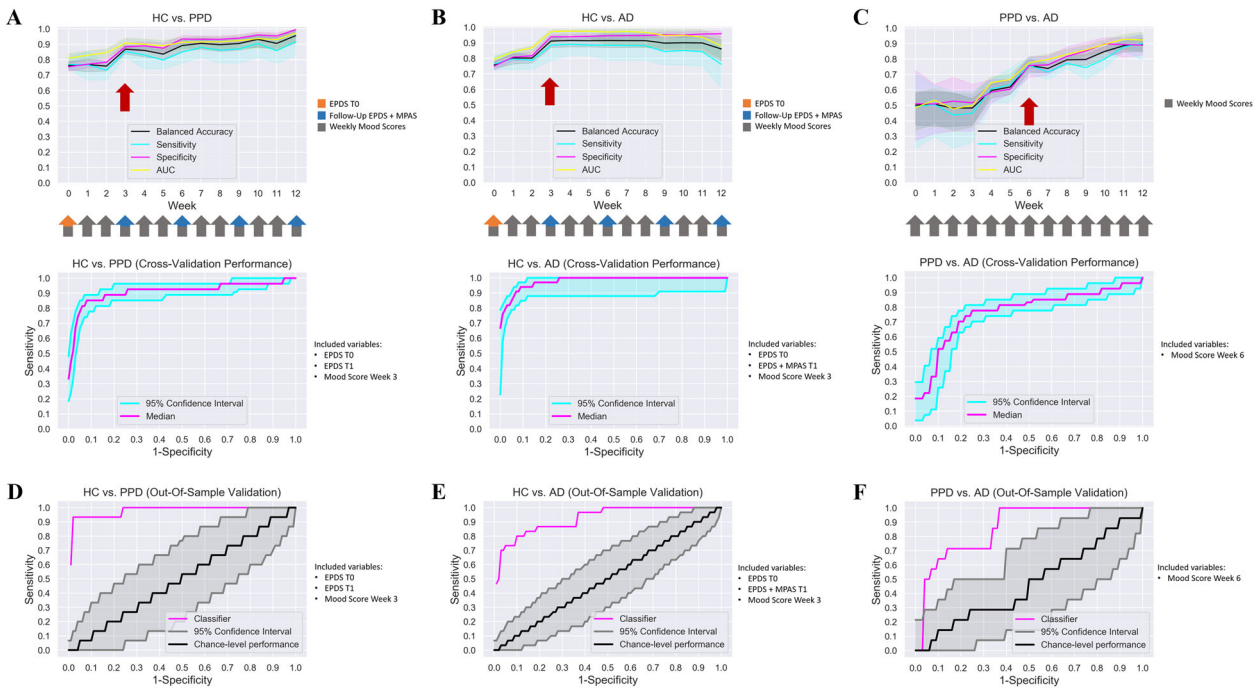

Fig. 2 Results of machine learning analysis. Balanced accuracy, sensitivity, specificity and out-of-sample AUC for each group comparison are displayed for the first cohort (**A**–**C**). For HC vs. PPD (**A**), the values are displayed for EPDS at baseline and follow-up incl. mood scores. For HC vs. AD (**B**), the values are displayed for EPDS at baseline, EPDS and MPAS at follow-up incl. mood scores. For PPD vs. AD (**C**), the values are displayed for mood scores. (**D**–**F**) AUCs obtained for the validation cohort are displayed for the classifier selected based on results from the first cohort aside with chance-level performance.

depressive symptoms even when they do not exceed clinical thresholds[32,33]. Our observations emphasize the need for further detailed evaluation of potential consequences also for the AD group.

A combination of baseline EPDS and week 3 remote follow-up EPDS, and mood scores achieved about 90% balanced accuracy for early identification of PPD as compared to HC. The same combination with addition of MPAS achieved a similar accuracy for early identification of AD. Both findings were largely confirmed in the validation cohort with an accuracy reduction from 90 to 80% seen only for differentiation of AD and HC. None of the evaluated combinations allowed for an accurate early differentiation between PPD and AD with all classifiers performing close to chance level until week 5. A reasonable differentiation of both groups was only achieved through mood scores at week 6 with a moderately high accuracy but a high specificity for PPD as confirmed in the validation cohort. Our classification results suggest that a simple stepwise procedure including remote mood, EPDS, and MPAS assessments may be a promising approach towards early identification of PPD. Whilst week 3 remote testing provided a high accuracy and a particularly high specificity for detection of both populations at risk, week 6 data additionally allowed for further differentiation between PPD and AD. In particular, the addition of mood scores led to a substantial increase in balanced accuracies for all group differentiations compared to all other feature combinations (e.g., addition of stress scores). Interestingly, the classifiers performed superior for the out-of-sample prediction in several cases. As we applied a strict cross-validation procedure the differences in prediction may simply reflect random variation in the accuracy of our model.

Three potential limitations need to be mentioned. First, as we did not register the reason for refusal during recruitment, we cannot exclude a bias based on the differences between women willing and women unwilling to participate. However, according to a recent study, there are no differences in motivation and willingness to participate between healthy controls and patients with psychiatric mood disorders[34]. Therefore, we do not expect any significant bias regarding the exclusion of women with PPD or AD based on their refusal to participate in the study. A potential bias introduced by the recruitment after childbirth vs. before childbirth may be a second limitation. However, the main goal of the current study was the identification of a risk group through a method, which could be easily applied in routine care. Prediction before childbirth may be more difficult to incorporate into

routine care as it may require the transfer of information between multiple institutions (e.g. gynecologist and hospital). Third, oversampling was applied only to the cross-validation in the first cohort, but not to the training of the classifier for prediction in the validation cohort, resulting in a potential bias of the logistic regression classifier due to asymmetric group sizes. However, considering that the highly similar results for the cross-validation and the out-of-sample (with the out-of-sample validation results being even superior at times), these findings indicate a minor influence of the asymmetric group sizes on the outcomes of our study.

In summary, by means of a longitudinal approach we identify and validate combinations of remote assessments allowing for early and accurate identification and differentiation of PPD and AD using a step-wise procedure. By administering the EPDS and mood assessments in-clinic immediately after childbirth and a second assessment remotely after three weeks, these findings can be easily translated into routine care. The behavioral and clinical time courses over 12 weeks provided important insight into the development and interaction of mood, stress, and maternal sensitivity in the first weeks postpartum.

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Author contributions
L.H. performed all analyses and wrote the manuscript. S.B.E., N.C., and J.D. designed the overall study. N.C., P.S., U.H., E.S., T.W.G., and S.S. conducted the clinical studies. All authors reviewed and commented on the manuscript.

Data availability
The data of this study are not publicly available due to privacy and ethical restrictions. Data to support the findings of this study are available upon reasonable request.

Code availability
The computer code used for the prediction analysis is available upon reasonable request.

Conflict of interest
J.D. is a former employee and current consultant for F.Hoffmann-La Roche. All authors report no conflicts of interest with respect to the work presented in this study.

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3 Examining early structural and functional brain alterations in postpartum depression through multimodal neuroimaging.

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Own contributions

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Examining early structural and functional brain alterations in postpartum depression through multimodal neuroimaging

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Postpartum depression (PPD) affects approximately 1 in 10 women after childbirth. A thorough understanding of a preexisting vulnerability to PPD will likely aid the early detection and treatment of PPD. Using a within-sample association, the study examined whether the brain's structural and functional alterations predict the onset of depression. 157 euthymic postpartum women were subjected to a multimodal MRI scan within the first 6 days of childbirth and were followed up for 12 weeks. Based on a clinical interview 12 weeks postpartum, participants were classified as mentally healthy or having either PPD or adjustment disorder (AD). Voxel-based morphometry and resting-state functional connectivity comparisons were performed between the three groups. 13.4% of women in our study developed PPD (n = 21) and 12.1% (n = 19) adjustment disorder (AD). The risk factors for PPD were a psychiatric history and the experience and severity of baby blues and the history of premenstrual syndrome. Despite the different risk profiles, no differences between the PPD, AD and control group were apparent based on structural and functional neuroimaging data immediately after childbirth. At 12 weeks postpartum, a significant association was observed between Integrated Local Correlation (LCor) and the Edinburgh Postnatal Depression Score (EPDS). Our findings do not support the notion that the brain's structural and resting-state functional alterations, if present, can be used as an early biomarker of PPD or AD. However, effects may become apparent if continuous measures of symptom severity are chosen.

Postpartum depression (PPD) is a disorder with the onset occurring within the first four weeks postpartum[1]. The onset of depression within the first four weeks postpartum is typically rapid, affecting particularly those with an increased sensitivity to reproductive hormone fluctuation[2,3]. Other factors such as alterations in the production of corticotropin-releasing hormone[4] and accelerated immune responses[5] are also thought to play a role. A history of PPD has been found to increase the mother's risk of further depressive episodes[6] and be associated with the child's behavioral and emotional problems[7]. The depression-related effects on parenting[8] significantly heighten the risk of a mother-to-child transmission of the susceptibility to depression[9,10]. An early detection of PPD, coupled with appropriate treatment measures, can not only help prevent a relapse of the condition, but also minimize the attendant emotional and financial burdens[11].

Despite PPD being a major public health concern, about 50% of the cases go undetected, thus failing to receive evidence-based forms of treatment[12]. The earliest stages of PPD are frequently overlooked due to the commonplace nature of baby blues (sudden feelings of sadness within the first few days postpartum), affecting up to 80% of new mothers[13,14]. Another likely event linked to childbirth is adjustment disorder (AD), which is a maladaptive reaction to identifiable psychosocial stressors[1]. While baby blues only last for a brief period of time and cease within the first few days of childbirth, AD can occur up to 3 months after the exposure to psychosocial

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stressors[1]. The symptom severity of AD does not meet the criteria for depression at any time point, distinguishing the condition from PPD[1]. While neither AD nor baby blues have the debilitating effects of clinical depression, both should be regarded as important differential diagnoses of PPD.

Studies in major depression (MDD) have identified functional as well as structural abnormalities in the hippocampus, the amygdala, as well as the subgenual cingulate cortex (for a meta-analysis, see[15]). In contrast to studies related to MDD, imaging studies with regard to PPD are rare and frequently underpowered (for detailed reviews, see[16,17]), with only one imaging study to date including more than 14 patients[18] and none pertaining to the structural changes in PPD[17]. In addition, the majority of the resting-state studies included PPD patients within 8 to 12 weeks postpartum (e.g.[18–22]), thereby missing any early alterations with potential prognostic value and likely mixing up early- and late-onset cases, which are thought to represent different etiologies[2]. The 4-week postpartum time frame is deemed to distinguish the so-called early-onset[2] or hormone-sensitive phenotype of PPD[3] from the later-onset phenotype, in which stress-inducing psychosocial factors are thought to play a more central role[2]. According to the DSM-5 criteria[1], only an early onset can be considered as real PPD, whereas the later onset should be classified as MDD.

In spite of these limitations, functional abnormalities in PPD have been reported in the amygdala, the insula, and the orbitofrontal and dorsomedial prefrontal cortices (for a review, see[16]). However, with respect to both MDD and PPD, it is still unclear if the reported structural and functional alterations are present early in the disease course, potentially preceding the clinical symptoms, or if they develop as a consequence of the disease. An understanding of these time courses is essential to establish the diagnostic and prognostic value of the respective neuroimaging alterations.

Our study sought to detect the structural and functional brain alterations in PPD (based on the DSM-5 criteria) on the basis of multimodal neuroimaging data obtained shortly after childbirth (i.e. 1 to 6 days after delivery). The project was part of a longitudinal study aiming at early recognition of PPD (RiPoD, Risk of Postpartum Depression) in a large cohort of women who were not depressed at childbirth.

Methods

Study participants. The data of 157 postpartum women were used in the present study. The inclusion criteria were no depression (according to the clinical interview) at the time of recruitment, being between 18 and 45 years of age, being in the early postpartum period (1 to 6 days following childbirth), and being eligible for magnetic resonance imaging (MRI). The exclusion criteria were severe birth- and pregnancy-related complications (e.g. HELLP, eclampsia), alcoholic or psychotropic substance dependency or use during pregnancy, history of psychosis or manic episodes, and lack of sufficient command of German or English. None of the participants met the DSM-5 criteria for depression at the time of recruitment. The exclusion criteria based on the child's condition were very premature birth (less than 29 weeks of gestation), very low birth weight (less than 1000 g), genetic defects (e.g. trisomy), or a pathological assessment on the basis of the German Child Health Test (U2).

Procedure. Upon recruitment at the Department of Gynecology and Obstetrics, University Hospital Aachen, written informed consent was obtained from all participants. A 12-week monitoring period (T0 to T4) commenced with a clinical-anamnestic interview (T0) based on the DSM-5 criteria[1] to obtain anamnestic and pregnancy-related information, as well as information on the current and previous psychiatric diagnoses. Additionally, a functional MRI (fMRI) assessment was conducted at T0. At time points T1 to T3, participants received links to the online platform SurveyMonkey, where they were required to fill in several questionnaires (for more detailed information on the study procedure, see supplemental information). At T4, participants were re-invited to the University Hospital Aachen for a final clinical interview (including some questionnaires) when a diagnosis of PPD or AD was made according to the DSM-5 criteria[1]. Additionally, if depressive symptomatology was present during the observational period, the Hamilton Depression Rating Scale 21 (HDR-S-21)[23] was administered. Those who were not diagnosed with PPD or AD are referred to here as healthy controls (HC).

The study was approved by the local Ethics Committee of the University Hospital RWTH Aachen and was conducted according to the declaration of Helsinki.

MRI procedure. The MRI scanning was conducted using a 3 Tesla Prisma MR Scanner (Siemens Medical Systems, Erlangen, Germany) located in the Medical Faculty of RWTH Aachen University. Functional images were acquired for an 11-min resting-state sequence with an echo-planar imaging (EPI) T2*-weighted contrast sequence sensitive to blood oxygen level-dependent (BOLD) contrast (34 slices, TR = 2.2 s, TE = 28 ms, FoV = 192 × 192 mm^2, flip angle = 90°, voxel resolution = 3.0 × 3.0 × 3.0 mm^3). T1-weighted structural images were acquired by means of a three-dimensional magnetization-prepared rapid acquisition gradient echo imaging (MPRAGE) sequence (4.12 min; 176 slices, TR = 2.3 s, TE = 1.99 ms, TI = 900 ms, FoV = 256 × 256 mm^2, flip angle = 9°, voxel resolution = 1 × 1 × 1 mm^3).

Behavioral analyses. Group comparisons regarding demographic and clinical variables were conducted using chi-squared (χ^2) tests for categorical variables and univariate analyses of variance (ANOVAs) for continuous variables. The analysis was conducted using IBM Statistics 25 (SPSS, Chicago, IL). Results are considered significant if $p < 0.05$.

Resting-state preprocessing and analyses. Resting-state fMRI (rsfMRI) were preprocessed using SPM12 toolbox[24] implemented in Matlab 2020a (MathWorks, Inc., Natick, MA). Images were realigned, unwarped, and co-registered to the structural image, spatially normalized using structural information, and smoothed by a Gaussian convolution kernel with 6 mm full-width at half maximum (FWHM). A gray matter

(GM) mask was applied to reduce all analyses to GM tissue. Images were further processed in the CONN toolbox version 18.b[25]. First principal components for white matter (WM) and cerebrospinal fluid (CSF) signals as well as 24 motion parameters (Friston-24) were regressed out before computing voxel- and region-based measures of interest. Global Correlation (GCor) was calculated as the average of bivariate correlations between the BOLD signal of a given voxel and every other voxel[25]. Integrated Local Correlation (LCor) was computed as the average bivariate correlation between each voxel and its neighboring voxels weighted by a Gaussian convolution with 6 mm FWHM[26]. Fractional Amplitude of Low Frequency Fluctuations (fALFF) was calculated at each voxel as the root mean square of the BOLD signal amplitude in the analysis frequency band (here 0.01 – 0.08 Hz) divided by the amplitude in the entire frequency band[27].

Voxel-based analyses. Voxel-wise group comparisons were performed in SPM12 using a flexible-factorial design for each modality with group as a factor and age as a covariate. Pair-wise t-contrasts were evaluated comparing PPD, AD and HC. All contrasts were evaluated for significance using an exact permutation-based cluster threshold (1000 permutations permuting group labels) ($p < 0.05$) combined with an uncorrected voxel-threshold of $p < 0.01$. In addition, we explored if any of the contrasts survived whole-brain voxel-wise family-wise error (FWE) correction ($p < 0.05$).

Regions of Interest (ROI) analyses. ROI analyses were performed in the CONN toolbox using the 100 regions Schaefer atlas[28] in combination with 16 subcortical regions (right and left nucleus accumbens, amygdala, caudate, hippocampus, pallidum, putamen, thalamus, and ventral diencephalon) from the Neuromorphometrics atlas (http://neuromorphometrics.com). All pairwise group t-contrasts (i.e. HC > PPD, PPD > HC, HC > AD, AD > HC, AD > PPD, PPD > AD) were calculated for all regions in addition to the network-based statistics based on intensity.

A threshold of $p < 0.01$ was applied at an uncorrected level for ROI-to-ROI connections combined with a permutation-based family-wise error (FWE)-corrected cluster threshold of $p < 0.05$ applied at network level.

In addition, we explored if the rsfMRI data at baseline correlated with EPDS at T4 as a continuous measurement of depressive symptomatology (in contrast to a binary assignment based on the diagnosis). Therefore, a multiple regression analysis was conducted using EPDS score at T4 and age as covariates. A whole-brain voxel-wise FWE correction ($p < 0.05$) was applied.

Structural data preprocessing and analysis. The structural data were preprocessed using the Computational Anatomy Toolbox (CAT12) implemented in Matlab 2020a (MathWorks, Inc., Natick, MA). The default settings of CAT12 were applied for spatial registration, segmentation and normalization with modulation. Normalized gray matter tissue volumes were smoothed with an 8 mm FWHM Gaussian kernel. After preprocessing, data were analyzed using the SPM12 toolbox implemented in Matlab 2020a (MathWorks, Inc., Natick, MA). To compare GM volumes between the groups (HC, AD, PPD), a univariate ANOVA was conducted controlling for age and total intracranial volume (TIV). T-Contrasts were used for pair-wise group comparisons. An exact permutation-based cluster threshold ($p < 0.05$) was applied combined with an uncorrected threshold of $p < 0.01$. In addition, we explored if any of the contrasts survived whole-brain voxel-wise family-wise error (FWE) correction ($p < 0.05$).

Additionally, we explored if the structural data at baseline correlated with EPDS at T4 as a continuous measurement of depressive symptomatology (in contrast to a binary assignment based on the diagnosis). Therefore, a multiple regression analysis was conducted using EPDS score at T4 and age as well as TIV as covariates. A whole-brain voxel-wise FWE correction ($p < 0.05$) was applied.

Results

Behavioral analyses. *Demographic and clinical characteristics.* Demographic, anamnestic and clinical characteristics for PPD, AD and HC are reported in Table 1. The prevalence of PPD in our study was 13.4% and the prevalence of AD was 12.1%. The EPDS score at T0 was significantly lower in HC compared to women with AD or PPD ($p < 0.001$). At T4, HC again showed significantly lower EPDS scores compared to the AD and the PPD groups ($p < 0.001$). Also, there was a significant interaction between EPDS score and group with women in the PPD group showing an increase in EPDS scores from T0 to T4, while women in the AD and HC groups showed a decrease in EPDS scores from T0 to T4, $F(2,151) = 40.86$, $p < 0.001$. Women with PPD and AD reported more often to have had a psychiatric history compared to HC ($p < 0.001$), while women with PPD had a psychiatric history more often than their counterparts with AD ($p < 0.001$). PMS severity was significantly higher in women with PPD compared to HC ($p = 0.031$). These women also experienced baby blues more often compared to HC ($p < 0.001$) and the baby blues they experienced were more severe in comparison to those experienced by HC ($p = 0.001$). This pattern was also apparent in the AD group: compared to HC, they experienced baby blues more often ($p < 0.001$) and in a more severe form ($p = 0.001$). Birth-related psychological or physical trauma were reported significantly more often by the AD women (36.8%) compared to PPD (14.2%) and HC (8.8%) ($p = 0.002$). Also, the children of women with AD were relocated to another ward significantly more often (52.6%) than those of women with PPD (19.0%) and healthy mothers (25.9%) ($p = 0.034$).

Resting-state analyses. No significant between-group differences were identified in pair-wise group comparisons for any of the voxel-wise or region-based rsfMRI measures. Applying the FWE-corrected voxel-wise threshold of $p < 0.05$ did not yield significant between-group differences either.

In additional correlational analyses, we explored if the rsfMRI measures correlated with continuous EPDS scores at T4. A whole-brain FWE-corrected significant positive correlation with EPDS score at T4 was observed

Variable	HC (N = 117)	PPD (N = 21)	AD (N = 19)	Statistical test
Age (M, SD)	31.97 (4.82)	31.24 (6.12)	30.68 (5.06)	F (2, 31.30) = .609, p > .05
Length of pregnancy in days (M, SD)	273.32 (14.18)	273.52 (13.81)	265.63 (23.19)	F (2, 30.78) = .982, p > .05
Birthweight of child (in gram)	3302.72 (563.13)	3320.95 (545.07)	2956.47 (911.98)	F (2, 30.85) = 1.31, p > .05
Birth mode (yes/no)				
Spontaneous	79/117	8/21	13/19	χ^2 (2) = 6.87, p = .032*[1,3]
Ventouse	4/117	4/21	0/19	χ^2 (2) = 10.15, p = .006*[1,3]
Planned C-Section	20/117	5/21	2/19	χ^2 (2) = 1.24, p > .05
Emergency C-section	14/117	4/21	4/19	χ^2 (2) = 1.63, p > .05
Married (yes/no)	82/35	14/7	13/6	χ^2 (2) = .11, p > .05
Psychiatric history (yes/no)	20/96	12/9	6/13	χ^2 (2) = 15.98, p < .001 *[1,2,3]
Depression	17/20	10/12	4/6	χ^2 (2) = 12.15, p = .002 *[1,2,3]
Other	3/20	2/12	2/6	χ^2 (2) = 3.84, p > .05
Stressful life events (yes/no)	58/59	13/8	13/6	χ^2 (2) = 3.02, p > .05
Number of stressful life events (M, SD)	0.93 (1.32)	1.67 (2.13)	1.74 (1.79)	F (2, 26.56) = 2.68, p > .05
Baby blues (yes/no)	40/76	16/5	14/5	χ^2 (2) = 19.75, p < .001 *[1,2]
Severity of baby blues (N = 60)	7.84 (3.80)	13.14 (2.61)	13.22 (5.04)	F (2, 13.46) = 12.14, p = .001 *[1,2]
PMS (yes/no)	47/54	12/5	11/6	χ^2 (2) = 4.66, p > .05
Severity of PMS (M, SD)	7.07 (7.49)	12.47 (6.82)	7.94 (6.31)	F (2, 126) = 3.57, p = .031 *[1]
EPDS score T0 (M, SD)	4.50 (3.10)	8.19 (4.01)	9.42 (5.42)	F (2, 29.01) = 14.12, p < .001*[1,2]
EPDS score T4 (M, SD)	2.55 (2.17)	13.55 (4.50)	5.68 (4.46)	F (2, 26.56) = 59.30, p < .001*[1,2,3]
HDR-S-21 score T4 (M, SD)	–	13.74 (3.74)	–	–
Breastfeeding T0 (yes/no)	102/15	17/4	18/1	χ^2 (2) = 1.71, p > .05
Birth-related psychological or physical trauma (yes/no)	9/103	3/18	7/12	χ^2 (2) = 12.40, p = .002 *[1,2,3]
Relocation of child to another ward (yes/no)	30/86	4/17	10/9	χ^2 (2) = 6.78, p = .034 *[2,3]

Table 1. Demographic and anamnestic data for all groups. *HC* healthy controls, *PPD* postpartum depression, *AD* adjustment disorder, *M* Mean, *SD* Standard deviation, *PMS* premenstrual syndrome, *EPDS* Edinburgh Postnatal Depression Scale, *HDR-S-21* Hamilton Depression Rating Scale 21. *Games-Howell/Bonferroni-corrected significant difference between [1]HC and PPD, between [2]HC and AD, and/or between [3]AD and PPD.

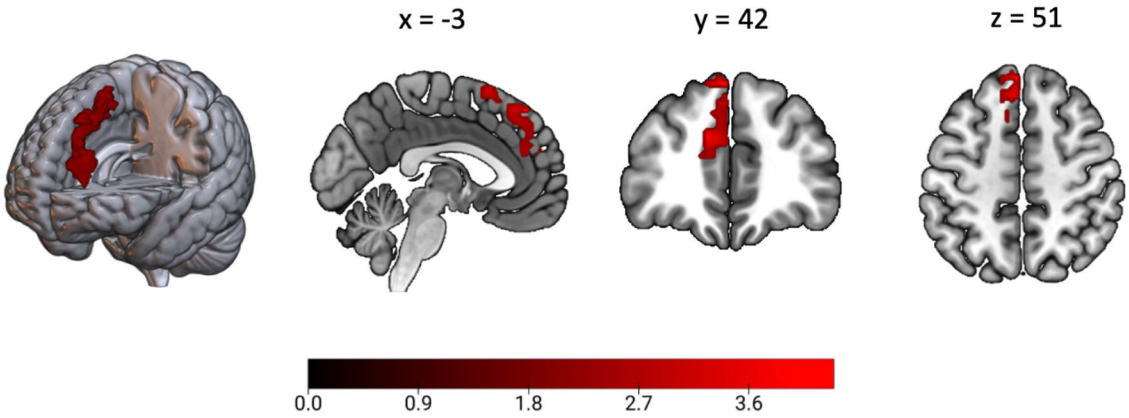

Figure 1. Significant positive correlation with EPDS score at T4 in the left superior medial frontal gyrus. For visualization purposes the region showing a significant whole-brain corrected voxel-wise association with EPDS at T4 is displayed at p < .01 uncorrected at voxel-level.

for LCor in the left superior medial frontal gyrus (T(1.0, 144.0) = 5.13, MNI(x,y,z) = −3, 42, 51) (Fig. 1). No other significant associations were observed.

Structural data analyses. No significant between-group differences were identified in pair-wise group comparisons for any of the voxel-wise or region-based rsfMRI measures using the cluster-correction threshold combined with a liberal voxel-wise threshold. Similarly, no contrast showed significant between-group differences when applying the FWE-corrected voxel-wise threshold of p < 0.05. In additional correlational analyses,

we sought to determine if the structural measures correlated with continuous EPDS scores at T4 and observed no significant associations.

Discussion

This study sought to explore early alterations in brain structure and function in PPD. The participants were recruited within a very narrow time frame following childbirth and before any clinical manifestation of PPD. The 13.4% PPD prevalence in our sample was well within the range (8% to 26%) indicated in the literature[29]. Studies that use only self-assessment tools to measure depression usually have a higher prevalence of PPD[30] as they likely also include AD cases. In the present study, a clinical interview helped separate the cases of AD (which had a 12.1% prevalence) from those of PPD. The risk factors associated with PPD were found to be a psychiatric history, the experience and severity of baby blues and severity of PMS[31–33]. Earlier studies have shown that previous depressive episodes lead to higher depressive symptomatology shortly after childbirth (as measured with the EPDS)[34,35]. Also, a prior depressive episode and higher EPDS scores after childbirth have been found to be associated with a diagnosis of PPD[35]. In addition to the experience of PMS and baby blues being more severe in our sample of women with PPD, baby blues were also reported more often by these women, indicating a sensitivity to estrogen-mediated epigenetic changes in the early onset of PPD (for detailed reviews, see[2,17]). In contrast, women with AD reported considerably more often to have found childbirth a traumatic experience and their children were more often relocated to another ward, highlighting the reactive nature of AD symptoms. Given the transitory nature of AD, women with AD showed a decrease in their EPDS scores toward the end of the observational period. In contrast to these clear differences between the groups in terms of risk profiles and clinical scales, neither the structural nor the resting-state data could differentiate between PPD, AD, and HC. This might have been due to the fact that our study participants were not depressed when the multimodal imaging data were acquired. Multimodal neuroimaging approaches are a promising method for translating self-reported symptoms or symptoms assessed by means of clinical interviews into a neurobiological model. Applying machine-learning techniques to VBM, rsfMRI, and task-based fMRI data, a recent study has, for the first time, deciphered distinct brain signatures of schizophrenia and depression[36]. This novel approach has provided compelling evidence that the combination of neuroimaging and clinical data carries high discriminatory value in disentangling differential diagnoses. However, similar to previous studies that reported alterations in brain connectivity in PPD and assessed clinically depressed women after several weeks or months postpartum[21,22], this study also has used data from acute psychiatric disorders[36]. Additionally, the sample sizes in these studies were small and the groups were heterogeneous in terms of onset time (e.g.[21,22,36,37]), limiting the generalizability of the findings.

The female brain undergoes dynamic neuroplastic processes during pregnancy and the postpartum period (for a review, see[38]) with decreases in GM volume in a number of brain regions (e.g. hippocampus, cingulate cortex, medial orbitofrontal cortex, insula)[39], which have been shown to play key roles in social processes[40], emotion regulation[41], stress processing[42], as well as being linked to the development of depression[15]. While these changes are thought to be adaptive, preparing new mothers for their new role, their possible contribution to the development of mental disorders cannot be ruled out[43] as the changes in brain structure and the development of postpartum psychiatric disorders co-occur in time. However, while these adaptive processes in a postpartum brain were detectable in the structural data[39], there was no indication of them (at least as suggested by our data) being more pronounced in women who were going to develop PPD. Research in MDD suggests dynamic changes in brain structure and function based on the state of depression (remission vs. manifest symptoms), treatment response or the severity of symptoms. For instance, in MDD, the effects of psychotherapy or pharmacotherapy are thought to be reflected in the activity patterns of the dorsal lateral prefrontal cortex and the precuneus (for a review, see[44]). Additionally, mean GM volume increases have been reported in remitted patients[45] with the increases being particularly pronounced in the subgenual prefrontal cortex and the amygdala[46,47]. These and other studies[48,49] indicate that alterations in brain structure and function may regress once the remission of MDD is achieved. According to these findings and our results, the observed alterations reflect a state biomarker of depression that co-occurs with the development of symptomatology. However, it is difficult to draw definite conclusions in this regard as research of trait markers or preexisting vulnerabilities with respect to both MDD and PPD is scarce. Our results indicate that trait markers, if existent, are subtle in comparison to state characteristics and may only be clearly identifiable in large groups. In our study, the only significant association between LCor and EPDS scores was observed at T4, suggesting that the effects of depressive conditions may become apparent when more sensitive continuous symptom severity measures are chosen.

In summary, the present findings support previous studies with regard to the prevalence and risk factors of PPD. Despite the disparate risk profiles of the groups in our study, no differences between the PPD, AD and control group were apparent based on the structural and functional neuroimaging data. The results indicate that if early structural or functional alterations in PPD or AD exist, they are either too subtle to be detected with the sample sizes used in our study or develop later in the disease course. More optimized longitudinal designs following larger cohorts of women from the beginning of pregnancy through childbirth into the late postpartum period may help address these questions more directly.

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Author contributions

P.S. performed structural imaging analyses and correlation analyses of both structural and resting-state imaging with clinical data (i.e. EPDS), and wrote the manuscript. L.H. conducted all resting-state analyses. N.C. and S.B.E. designed the study. N.C., J.D., S.S., P.S., U.H., and E.S. conducted the clinical study. All authors reviewed and commented on the manuscript.

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Competing interests

The authors declare no competing interests.

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4 Resting-state alterations in behavioral variant frontotemporal dementia are related to the distribution of monoamine and GABA neurotransmitter systems

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Own contributions

Writing the manuscript, preparing figures, performing data analyses, and contributing to interpretation of results.

RESEARCH ARTICLE

Resting-state alterations in behavioral variant frontotemporal dementia are related to the distribution of monoamine and GABA neurotransmitter systems

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Abstract

Background: Aside to clinical changes, behavioral variant frontotemporal dementia (bvFTD) is characterized by progressive structural and functional alterations in frontal and temporal regions. We examined if there is a selective vulnerability of specific neurotransmitter systems in bvFTD by evaluating the link between disease-related functional alterations and the spatial distribution of specific neurotransmitter systems and their underlying gene expression levels.

Methods: Maps of fractional amplitude of low-frequency fluctuations (fALFF) were derived as a measure of local activity from resting-state functional magnetic resonance imaging for 52 bvFTD patients (mean age = 61.5 ± 10.0 years; 14 females) and 22 healthy controls (HC) (mean age = 63.6 ± 11.9 years; 13 females). We tested if alterations of fALFF in patients co-localize with the non-pathological distribution of specific neurotransmitter systems and their coding mRNA gene expression. Furthermore, we evaluated if the strength of co-localization is associated with the observed clinical symptoms.

Results: Patients displayed significantly reduced fALFF in frontotemporal and frontoparietal regions. These alterations co-localized with the distribution of serotonin (5-HT1b and 5-HT2a) and γ-aminobutyric acid type A (GABAa) receptors, the norepinephrine transporter (NET), and their encoding mRNA gene expression. The strength of co-localization with NET was associated with cognitive symptoms and disease severity of bvFTD.

Conclusions: Local brain functional activity reductions in bvFTD followed the distribution of specific neurotransmitter systems indicating a selective vulnerability. These findings provide novel insight into the disease mechanisms underlying functional alterations. Our data-driven method opens the road to generate new hypotheses for pharmacological interventions in neurodegenerative diseases even beyond bvFTD.

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Editor's evaluation

This study presents important findings linking structural and functional changes in frontotemporal dementia to underlying neurotransmitter systems. The evidence to support the claims is solid, however, relationships are relatively modest. This study will appeal to clinicians and neuroscientists who are interested in the potential effects of certain neurotransmitter systems on clinical features of frontotemporal dementia.

Introduction

Frontotemporal lobar degeneration is the second most common type of early-onset dementia under the age of 65 years (***Harvey et al., 2003***). Its most common subtype, behavioral variant frontotemporal dementia (bvFTD), is characterized by detrimental changes in personality and behavior (***Pressman and Miller, 2014***). Patients can display both apathy and disinhibition, often combined with a lack of insight, and executive and socioemotional deficits (***Schroeter et al., 2011***; ***Schroeter et al., 2012***). Despite striking and early symptoms, bvFTD patients are often (i.e. up to 50%) misdiagnosed as having a psychiatric illness rather than a neurodegenerative disease (***Woolley et al., 2011***).

In addition to the presence of symptoms, the diagnosis requires consideration of family history due to its frequent heritable component and examination of different neuroimaging modalities (***Pressman and Miller, 2014***; ***Bang et al., 2015***; ***Schroeter et al., 2014***; ***Schroeter et al., 2008***). Whereas atrophy in frontoinsular areas only occurs in later disease stages, glucose hypometabolism in frontal, anterior cingulate, and anterior temporal regions visible with fluorodeoxyglucose positron emission tomography (FDG-PET) is already detectable from an early stage onwards (***Bang et al., 2015***; ***Diehl-Schmid et al., 2007***). The fractional amplitude of low-frequency fluctuations (fALFF) is a resting-state functional magnetic resonance imaging (rsfMRI) derived measure with good test–retest reliability that closely correlates with FDG-PET (***Aiello et al., 2015***; ***Holiga et al., 2018***; ***Deng et al., 2022***). In frontotemporal dementia (FTD) patients, fALFF was reduced in inferior parietal, frontal lobes, and posterior cingulate cortex and holds great potential as MRI biomarker (***Premi et al., 2014***; ***Borroni et al., 2018***). Low local fALFF activity in the left insula was linked to symptom deterioration (***Day et al., 2013***).

On a molecular level, frontotemporal lobar degeneration can be differentiated into three different subtypes based on abnormal protein deposition: tau (tau protein), transactive response DNA-binding protein with molecular weight 43 kDa (TDP-43), and FET (fused-in-sarcoma [FUS] and Ewing sarcoma [EWS] proteins, and TATA-binding protein-associated factor 15 [TAF15]) (***Bang et al., 2015***; ***Haass and***

Neumann, 2016). Whereas tau and TDP pathologies each occur in half of the bvFTD patients, FUS pathology is very rare (*Whitwell et al., 2011*). Several possible mechanisms are discussed in the literature for the spread of these proteins throughout the brain, from a selective neuronal vulnerability (i.e. specific neurons being inherently more susceptible to the underlying disease-related mechanisms) to prion-like propagation of the respective proteins (*Walsh and Selkoe, 2016*; *Hock and Polymenidou, 2016*). The latter entails that misfolded proteins accumulate and induce a self-perpetuating process so that protein aggregates can spread and amplify, leading to gradual dysfunction and eventually death of neurons and glial cells (*Hock and Polymenidou, 2016*). For example, tau can cause presynaptic dysfunction prior to loss of function or cell death (*Zhou et al., 2017*), whereas overexpression of TDP-43 leads to impairment of presynaptic integrity (*Heyburn and Moussa, 2016*). The role of FET proteins is not fully understood, although their involvement in gene expression suggests a mechanism of altered RNA processing (*Svetoni et al., 2016*).

Neuronal connectivity plays a key role in the spread of pathology as it is thought to transmit along neural networks. Supporting the notion, previous studies also found an association between tau levels and functional connectivity in functionally connected brain regions, for example across normal aging and Alzheimer's disease (*Franzmeier et al., 2019*). Thereby, dopaminergic, serotonergic, glutamatergic, and GABAergic neurotransmission is affected. More specifically, current research indicates a deficit of neurons and receptors in these neurotransmitter systems (*Hock and Polymenidou, 2016*; *Huey et al., 2006*; *Murley and Rowe, 2018*). Furthermore, these deficits have been associated with clinical symptoms. For example, whereas GABAergic deficits have been associated with disinhibition, increased dopaminergic neurotransmission and altered serotonergic modulation of dopaminergic neurotransmission have been associated with agitated and aggressive behavior (*Engelborghs et al., 2008*; *Murley et al., 2020*). Another study related apathy to glucose hypometabolism in the ventral tegmental area, a hub of the dopaminergic network (*Schroeter et al., 2011*). Despite this compelling evidence of disease-related impairment at functional and molecular levels, the relationship between both remains poorly understood. It also remains unknown if the above neurotransmitter alterations reflect a disease-specific vulnerability of specific neuron populations or merely reflect a consequence of the ongoing neurodegeneration.

Based on the above findings, we hypothesize that the spatial distribution of fALFF and gray matter (GM) pathology in FTD will be related to the distribution of dopaminergic, serotonergic, and GABAergic neurotransmission. The aim of the current study was to gain novel insight into the disease mechanisms underlying functional and structural alterations in bvFTD by examining if there is a selective vulnerability of specific neurotransmitter systems. We evaluated the link between disease-related functional alterations and the spatial distribution of specific neurotransmitter systems and their underlying gene expression levels. In addition, we tested if these associations are linked to specific symptoms observed in this clinical population.

Materials and methods

Subjects

We included 52 Caucasian patients with bvFTD (mean age = 61.5 ± 10.0 years; 14 females) and 22 Caucasian age-matched healthy controls (HC) (mean age = 63.6 ± 11.9 years; 13 females) examined in nine centers of the German Consortium for Frontotemporal Lobar Degeneration (http://www.ftld.de; *Otto et al., 2011*) into this study. Details regarding the distribution of demographic characteristics across centers are reported in *Supplementary file 1a*. Diagnosis was based on established international diagnostic criteria (*Rascovsky et al., 2011*). Written informed consent was collected from each participant. The study was approved by the ethics committees of all universities involved in the German Consortium for Frontotemporal Lobar Degeneration (Ethics Committee University of Ulm approval number 20/10) and was in accordance with the latest version of the Declaration of Helsinki. The clinical and neuropsychological test data included the Mini Mental State Exam (MMSE), Verbal Fluency (VF; animals), Boston Naming Test (BNT), Trail Making Test B (TMT-B), Apathy Evaluation Scale (AES) (companion-rated) (*Glenn, 2005*), Frontal Systems Behavior Scale (FrSBe) (companion-rated) incl. subscales (executive function [EF], inhibition, and apathy) (*Grace and Malloy, 2001*), and Clinical Dementia Rating-Frontotemporal Lobar Degeneration scale-modified (CDR-FTLD) (*Knopman et al., 2008*). Demographic and neuropsychological test information for both groups is displayed in *Table 1*.

Table 1. Demographic and clinical information for bvFTD patients and HC.

	bvFTD		HC		Group comparison	
Age (years)	61.5 ± 10.0	*N* = 52	63.6 ± 11.9	*N* = 22	*t* = −0.78	p = 0.44
Sex (male/female)	38/14	*N* = 52	9/13	*N* = 22	X^2 = 6.90	p = 0.009*
Education (years)	13.7 ± 3.19	*N* = 50	13.5 ± 2.56	*N* = 22	*t* = 0.21	p = 0.84
Disease duration (years)	3.98 ± 5.22	*N* = 49	–	–	–	–
Verbal Fluency (number of animals)	12.2 ± 6.48	*N* = 49	27.5 ± 4.77	*N* = 19	*t* = −9.30	p < 0.001*
Boston Naming Test (total score)	12.9 ± 2.79	*N* = 49	15.0 ± 0.22	*N* = 20	*t* = −3.28	p = 0.002*
Mini Mental State Exam (total score)	25.2 ± 4.48	*N* = 50	29.3 ± 0.64	*N* = 20	*t* = −4.03	p < 0.001*
Trail Making Test B (s)	179 ± 84.4	*N* = 36	78.5 ± 22.0	*N* = 19	*t* = 5.09	p < 0.001*
Apathy Evaluation Scale (total score)	32.7 ± 11.0	*N* = 35	9.50 ± 5.26	*N* = 4	*t* = 4.13	p < 0.001*
Frontal Systems Behavior Scale (companion-rated, total frequency)	72.7 ± 16.1	*N* = 34	38.8 ± 12.3	*N* = 5	*t* = 4.49	p < 0.001*
Frontal Systems Behavior Scale (companion-rated, total distress)	66.9 ± 21.0	*N* = 29	32 ± 9.56	*N* = 4	*t* = 3.25	p = 0.003*
Frontal Systems Behavior Scale: Executive Function (companion-rated, total distress)	23.6 ± 7.39	*N* = 34	11.8 ± 4.50	*N* = 4	*t* = 3.11	p = 0.004*
Clinical Dementia Rating-Frontotemporal Lobar Degeneration (total score)	8.06 ± 3.92	*N* = 45	0.05 ± 0.16	*N* = 19	*t* = 5.07	p < 0.001*

bvFTD – behavioral variant frontotemporal dementia, HC – healthy controls.
*Significant at p < 0.05.

MRI acquisition and preprocessing of imaging data

Structural T1-weighted magnetization-prepared rapid gradient-echo MRI and rsfMRI (TR = 2000 ms, TE = 30 ms, FOV = 64 × 64 × 30, voxel size = 3 × 3 × 5 mm, 300 volumes) were acquired on 3T devices. ***Table 2*** reports center-specific imaging parameters confirming a high level of harmonization.

All initial preprocessing of imaging data was performed using SPM12 (***Penny et al., 2011***). To calculate voxel-wise GM volume (GMV), structural images were segmented, spatially normalized to MNI space, modulated, and smoothed by a Gaussian convolution kernel with 6 mm full-width at half maximum (FWHM). RsfMRI images were realigned, unwarped, co-registered to the structural image, spatially normalized to MNI space, and smoothed with a Gaussian convolution kernel with 6 mm FWHM. A GM mask was applied to reduce all analyses to GM tissue. Images were further processed in the REST toolbox (***Song et al., 2011***) version 1.8. Mean white matter and cerebrospinal fluid signals as wells as 24 motion parameters (Friston-24) were regressed out before computing voxel-based measures of interest. fALFF was calculated at each voxel as the root mean square of the blood oxygen level-dependent signal amplitude in the analysis frequency band (here: 0.01–0.08 Hz) divided by the amplitude in the entire frequency band (***Song et al., 2011***). fALFF is closely linked to FDG-PET and other measures of local metabolic activity as has been shown in healthy participants but also for example in Alzheimer's disease (***Deng et al., 2022***; ***Marchitelli et al., 2018***).

Contrast analyses of fALFF and GMV

To test for fALFF alterations, group comparisons were performed in SPM12 using a flexible-factorial design with group (bvFTD or HC) as a factor and age, sex, and site (i.e. one dummy variable per site) as covariates (***Huotari et al., 2019***). To test for group differences in GMV, the same design with addition of total intracranial volume (TIV) was used. Pairwise group *t*-contrasts (i.e. HC > bvFTD, bvFTD > HC) were evaluated for significance using an exact permutation-based cluster threshold (1000 permutations permuting group labels, p < 0.05) to control for multiple comparisons combined with an uncorrected voxel-threshold of p < 0.001. A permutation-based cluster threshold combined with an uncorrected voxel-threshold was used since standard correction methods such as a family wise error rate of 5% may lead to elevated false-positive rates (***Eklund et al., 2016***).

Table 2. Center-specific imaging parameters for structural and functional imaging.

Center	rsfMRI					Structural MRI			
	TE (ms)	TR (ms)	FOV (*X, Y, Z*)	Voxel size (mm)	Volumes	TE (ms)	TR (ms)	FOV (*X, Y, Z*)	Voxel size (mm)
Bonn	30	2000	64 × 64 × 30	3 × 3 × 5	300	3.06	2300	240 × 256 × 176	1 × 1 × 1
Erlangen	34	3000	64 × 64 × 30	3 × 3 × 5	300	2.98	2300	240 × 256 × 176	1 × 1 × 1
Göttingen	30	2000	64 × 64 × 30	3 × 3 × 6	300	2.96	2300	256 × 256 × 176	1 × 1 × 1
Homburg	30	2000	64 × 64 × 30	3 × 3 × 5	300	2.98	2300	240 × 256 × 176	1 × 1 × 1
Leipzig	30	2000	64 × 64 × 30	3 × 3 × 5	300	2.98	2300	240 × 256 × 176	1 × 1 × 1
München (TU)	30	2000	64 × 64 × 30	3 x 3 × 5	300	2.98	2300	240 × 256 × 176	1 × 1 × 1
Rostock	30	2200	64 × 64 × 34	3.5 × 3.5 × 3.5	300	4.82	2500	256 × 256 × 192	1 × 1 × 1
Tübingen	30	2000	64 × 64 × 30	3 × 3 × 5	300	2.96	2300	240 × 256 × 176	1 × 1 × 1
Ulm	30	2000	64 × 64 × 30	3 × 3 × 5	300	2.05	2300	240 × 256 × 192	1 × 1 × 1

rsfMRI – resting-state functional magnetic resonance imaging, MRI – magnetic resonance imaging, TE – echo time, TR – repetition time, FOV – field of view.
Bonn – University of Bonn, German Center for Neurodegenerative Diseases (DZNE), University Hospital Bonn.
Erlangen – University Hospital Erlangen.
Göttingen – Medical University Göttingen.
Homburg – Saarland University Hospital.
Leipzig – Max-Planck-Institute for Human Cognitive and Brain Sciences.
TU München – Technical University of Munich.
Rostock – University Hospital Rostock, German Center for Neurodegenerative Diseases (DZNE).
Tübingen – University Hospital Tübingen, Centre for Neurology, Hertie-Institute for Clinical Brain Research.
Ulm – Ulm University.

Spatial correlation with neurotransmitter density maps

Confounding effects of age, sex, and site were regressed out from all images prior to further spatial correlation analyses. To test if fALFF alterations in bvFTD patients (relative to HC) are correlated with specific neurotransmitter systems, the JuSpace toolbox (***Dukart et al., 2021***) was used. The JuSpace toolbox allows for cross-modal spatial correlations of different neuroimaging modalities with nuclear imaging derived information about the relative density distribution of various neurotransmitter systems. All neurotransmitter maps were derived as averages from an independent healthy volunteer population and processed as described in the JuSpace publication including rescaling and normalization into the Montreal Neurological Institute space. More specifically, we wanted to test if the spatial structure of fALFF maps in patients relative to HC is similar to the distribution of nuclear imaging derived neurotransmitter maps from independent healthy volunteer populations included in the toolbox (5-HT1a receptor [***Savli et al., 2012***], 5-HT1b receptor [***Savli et al., 2012***], 5-HT2a receptor [***Savli et al., 2012***], serotonin transporter [5-HTT; ***Savli et al., 2012***], D1 receptor [***Kaller et al., 2017***], D2 receptor [***Sandiego et al., 2015***], dopamine transporter [DAT; ***Dukart et al., 2018***], Fluorodopa [FDOPA; ***García Gómez et al., 2018***], γ-aminobutyric acid type A [GABAa] receptors [***Dukart et al., 2018***; ***Myers et al., 2012***], μ-opioid [MU] receptors [***Aghourian et al., 2017***], and norepinephrine transporter [NET; ***Hesse et al., 2017***]). Detailed information about the publicly available neurotransmitter maps is provided in ***Supplementary file 1c***. In contrast to standard analyses of fMRI data, this analysis might provide novel insight into potential neurophysiological mechanisms underlying the observed correlations (***Dukart et al., 2021***). Using the toolbox, mean values were extracted from both neurotransmitter and fALFF maps using GM regions from the Neuromorphometrics atlas. Extracted mean regional values of the patients' fALFF maps were *z*-transformed relative to HC. Spearman correlation coefficients (Fisher's *z*-transformed) were calculated between these *z*-transformed fALFF maps of the patients and the spatial distribution of the respective neurotransmitter maps. Exact permutation-based p-values as implemented in JuSpace (10,000 permutations randomly assigning group labels using orthogonal permutations) were computed to test if the distribution of

the observed Fisher's *z*-transformed individual correlation coefficients significantly deviated from zero. Furthermore, adjustment for spatial autocorrelation was performed by computing partial correlation coefficients between fALFF and neurotransmitter maps adjusted for local GM probabilities estimated from the SPM12-provided TPM.nii (***Dukart et al., 2021***). All analyses were false discovery rate (FDR) corrected for the number of tests (i.e. the number of neurotransmitter maps). To further test if and how the observed fALFF co-localization patterns are explained by the underlying global atrophy, we repeated the co-localization analysis ($p < 0.05$) for the significant fALFF–neurotransmitter associations after controlling for total GMV. Additionally, the receiver operating characteristic (ROC) curves and corresponding areas under the curve (AUC) were calculated for patients (Fisher's *z*-transformed Spearman correlations) vs. HC (leave-one-out *Z*-score maps) to examine discriminability of the resulting fALFF–neurotransmitter correlations.

Correlation with structural data

To test if the significant correlations observed between fALFF and neurotransmitter maps were driven by structural alterations (i.e. partial volume effects), the JuSpace analysis using the same parameters was repeated with local GMV incl. a correction for confounding effects of age, sex, site, and TIV. For further exploration, fALFF and GMV Fisher's *z*-transformed Spearman correlations as computed by the JuSpace toolbox were correlated with each other for each patient over all neurotransmitters. The median of those correlation coefficients was squared to calculate the variance in fALFF explained by GMV.

Correlation with clinical data

To test if fALFF–neurotransmitter correlations are related to symptoms of bvFTD, we calculated Spearman correlation coefficients between significant fALFF–neurotransmitter correlations (Fisher's *z*-transformed Spearman correlation coefficients from JuSpace toolbox output) and clinical scales and neuropsychological test data (see ***Table 1***). All analyses were FDR corrected for the number of tests. In addition, to test for the specificity of these associations we examined the direct associations between fALFF and the neuropsychological tests by computing Spearman correlations with the Eigenvariates extracted from the largest cluster of the HC > bvFTD SPM contrast.

Association with gene expression profile maps

Furthermore, to test if fALFF alterations in bvFTD patients associated with specific neurotransmitter systems in the JuSpace analysis were also spatially correlated with their underlying mRNA gene expression profile maps, the MENGA toolbox (***Rizzo et al., 2016***; ***Rizzo et al., 2014***) was used. *Z*-scores were calculated for the patients relative to HC using the confound-corrected images. The analyses were performed using 169 regions of interest and genes corresponding to each significantly associated neurotransmitter from the JuSpace analysis (5-HT1b: *HTR1B*; 5-HT2a: *HTR2A*; GABAa (19 subunits): *GABRA1–6*, *GABRB1–3*, *GABRG1–3*, *GABRR1–3*, *GABRD*, *GABRE*, *GABRP*, *GABRQ*; NET: *SLC6A2*). More specifically, Spearman correlation coefficients were calculated between the genomic values and re-sampled image values in the regions of interest for each patient and for each mRNA donor from the Allen Brain Atlas (***Hawrylycz et al., 2012***) separately. The Fisher's *z*-transformed correlation coefficients were averaged over the six mRNA donors. Bonferroni-corrected one-sample *t*-tests were performed for each neurotransmitter to examine, whether the correlation coefficient differed significantly from zero.

Neurotransmitter-genomic correlations and gene differential stability

To further examine the association of fALFF–neurotransmitter correlations and mRNA gene expression profile maps, we explored the relationship between neurotransmitter maps included in the JuSpace toolbox and mRNA maps provided in the MENGA toolbox. The MENGA analysis was repeated using the same parameters to obtain Fisher's *z*-transformed Spearman correlation coefficients between the neurotransmitter maps and the mRNA gene expression profile maps.

To evaluate the robustness of the mRNA maps between donors, gene differential stability was estimated by computing the Fisher's *z*-transformed Spearman correlation coefficients between the genomic values of each of the six mRNA donors, which were then averaged (***Hawrylycz et al., 2012***).

Results

Contrast analysis of fALFF and GMV

First, we tested for group differences in fALFF between HC and patients. Compared to HC, bvFTD patients showed a significantly reduced fALFF signal in frontoparietal and frontotemporal regions (***Figure 1A***). Furthermore, patients also showed reduced GMV in medial and lateral prefrontal, insular, temporal, anterior caudate, and thalamic regions in comparison to HC (***Figure 1B***). For a detailed representation of the thresholded fALFF and GMV t-maps, see ***Figure 1—figure supplement 1***. Cluster size, peak-level MNI coordinates, and corresponding anatomical regions incl. the additional fALFF analysis with correction for total GMV are reported in ***Supplementary file 1d***. For the distribution of Eigenvariates for the two groups in both modalities, see ***Figure 1—figure supplement 2***.

Spatial correlation with neurotransmitter maps

We performed correlation analyses to test if fALFF alterations in bvFTD significantly co-localize with the spatial distribution of specific neurotransmitter systems. fALFF alterations in bvFTD as compared to HC were significantly associated with the spatial distribution of 5-HT1b (mean $r = -0.21$, $p < 0.001$), 5-HT2a (mean $r = -0.16$, $p = 0.0014$), GABAa (mean $r = -0.12$, $p = 0.0149$), and NET (mean $r = -0.13$, $p = 0.0157$) ($p_{FDR} = 0.0157$; ***Figure 2A***). The directionality of these findings (i.e. a negative correlation) suggest bvFTD displayed stronger reductions in fALFF relative to HC in areas which are associated with a higher non-pathological density of respective receptors and transporters. When controlling for total GMV, the co-localization findings remained significant except for the co-localization with GABAa. The AUC resulting from the ROC curves between Spearman correlation coefficients of patients and controls revealed a good discrimination for 5-HT1b (AUC = 0.74) and 5-HT2a (AUC = 0.71) and a fair discrimination for GABAa (AUC = 0.68) and NET (AUC = 0.67) (***Figure 3A***).

Next, we tested if similar co-localization patterns are observed with GMV. GMV alterations in bvFTD were not significantly associated with any of the neurotransmitter systems (***Figure 2B***). fALFF–neurotransmitter and GMV–neurotransmitter correlations displayed a positive yet weak association with structural alterations explaining only 10% of variance in the fALFF alterations (***Figure 3B***). All correlations and their corresponding permutation-based p-values incl. the analysis utilizing fALFF images additionally corrected for total GMV are provided in ***Supplementary file 1c***. To exclude a potential bias caused by the collection of imaging data at different sites, we performed a Kruskal–Wallis test to examine differences on the Fisher's *z*-transformed correlations coefficients across sites. No significant differences ($X^2 = 6.34$, $p = 0.50$, $df = 7$) were found among the sites.

Relationship to clinical symptoms

Furthermore, we tested if the significant fALFF–neurotransmitter correlation coefficients are also associated with symptoms or test results of bvFTD. After FDR correction ($p = 0.0085$), the strength of fALFF co-localization with NET distribution was significantly associated with VF (mean $r = 0.37$, $p = 0.0086$; $N = 49$; ***Figure 2C***) and MMSE (mean $r = 0.40$, $p = 0.0039$; $N = 50$; ***Figure 2D***). The positive correlation coefficients suggest that more negative correlations between fALFF and neurotransmitter maps were associated with lower test performance, that is the higher/more fALFF reductions in areas with high neurotransmitter density, the lower the test performance. Associations with other neuropsychological tests were not significant (***Supplementary file 1c***). We also tested if Eigenvariates extracted from the largest cluster of the HC > bvFTD contrast correlated with the specific symptoms of bvFTD (***Supplementary file 1f***). None of the correlations remained significant after correction for multiple comparisons.

Association with gene expression profile maps

Next, we evaluated if co-localization of fALFF is also observed with mRNA gene expression underlying the significantly associated neurotransmitter systems. For genes encoding the 19 GABAa subunits, we first evaluated the variability between the subunits regarding their fALFF–mRNA correlations, their correlation with GABAa density and their mRNA autocorrelations (see ***Figure 2—figure supplement 1*** and ***Figure 3—figure supplement 1***). As the variability between the genes was high, we limited the analyses to genes encoding the three main subunits (GABRA1, GABRB1, and GABRG1).

Correlations of fALFF alterations with mRNA gene expression profile maps in bvFTD relative to HC differed significantly from zero for *HTR1B* (encoding the 5-HT1b receptor; mean $r = -0.02$, p

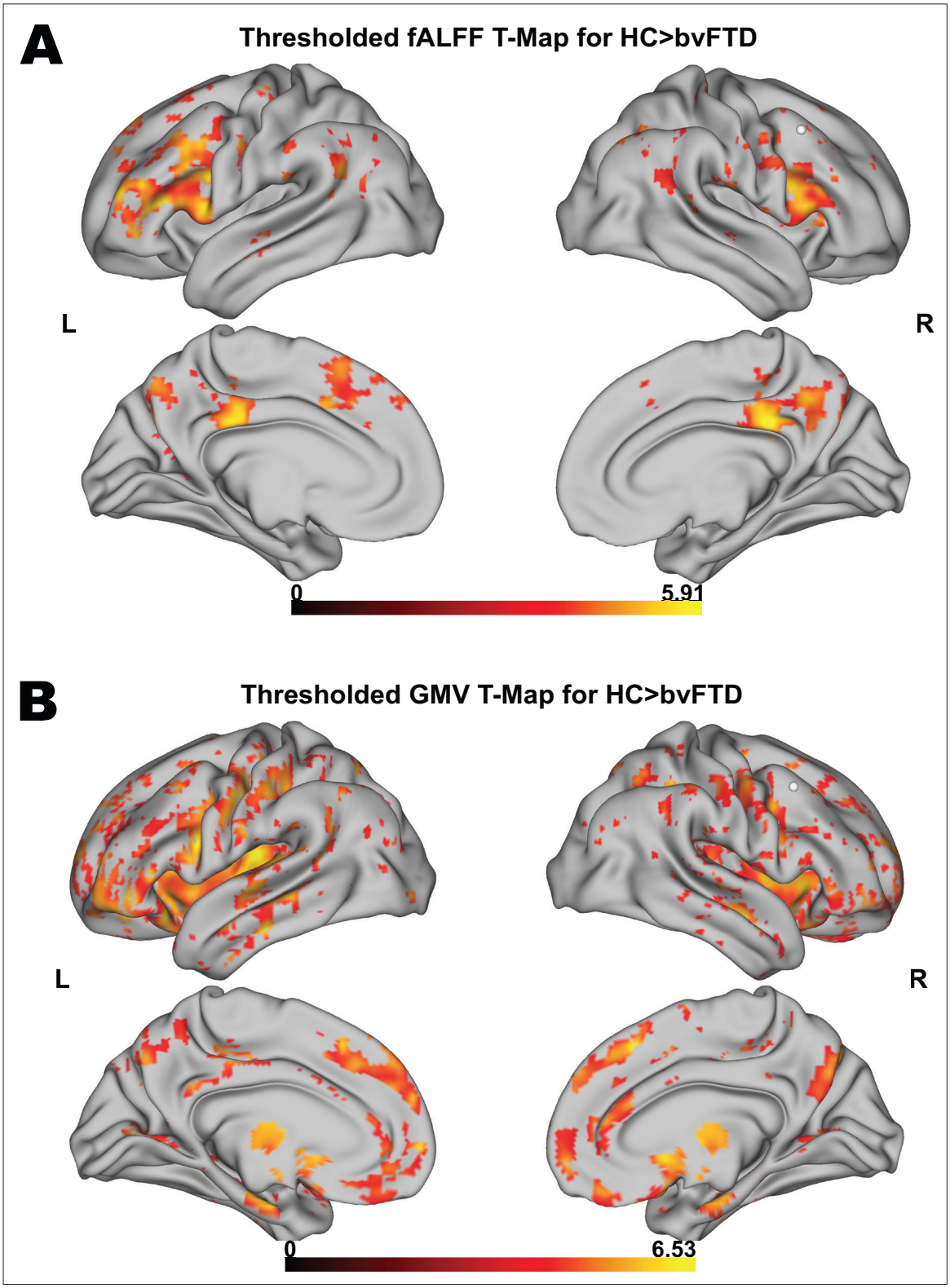

Figure 1. Voxel-wise results for fractional amplitude of low-frequency fluctuation (fALFF) and gray matter volume (GMV) group comparisons. Thresholded fALFF t-map (**A**) and thresholded GMV t-map (**B**) for healthy control (HC; N = 22) > behavioral variant frontotemporal dementia (bvFTD; N = 52) using a permutation-based threshold (1000 permutations permuting group labels) at cluster-level $p < 0.05$ and voxel-level $p < 0.001$.

Figure 1 continued on next page

Figure 1 continued

The online version of this article includes the following source data and figure supplement(s) for figure 1:

Figure supplement 1. Detailed voxel-wise results for fractional amplitude of low-frequency fluctuation (fALFF) and gray matter volume (GMV) group comparisons.

Figure supplement 2. Eigenvariates from fractional amplitude of low-frequency fluctuation (fALFF) and gray matter volume (GMV) for behavioral variant frontotemporal dementia (bvFTD) patients and controls.

Figure supplement 2—source data 1. Eigenvariates of fractional amplitude of low-frequency fluctuation (fALFF) and gray matter volume (GMV) for largest clusters of healthy control (HC) > behavioral variant frontotemporal dementia (bvFTD) *t*-contrasts shown in ***Figure 1—figure supplement 2***.

= 0.0144), *HTR2A* (encoding the 5-HT2a receptor; mean $r = -0.04$, $p < 0.001$), *GABRB1* (encoding subunit of the GABAa receptor; mean $r = -0.08$, $p < 0.001$) and *SLC6A2* (encoding NET; mean $r = 0.06$, $p < 0.001$), but not for GABRA1 (encoding subunit of the GABAa receptor; mean $r = 0.02$, $p = 0.1414$) and GABRG1 (encoding subunit of the GABAa receptor; mean $r = -0.03$, $p = 0.0730$) (***Figure 2G***). Thereby, correlations were negative for *HTR1B*, *HTR2A*, and *GABRB1*, that is fALFF was reduced in areas with higher expression of respective genes, and positive for *SLC6A2*.

Furthermore, we tested if there was an association between the neurotransmitter maps included in the JuSpace toolbox and the mRNA gene expression profile maps provided in the MENGA toolbox that were both derived from independent healthy volunteer populations. The correlations between spatial distributions of 5-HT1b, 5-HT2a, GABAa, and NET, and corresponding mRNA gene expression profile maps were positive (5-HT1b/*HTR1B*: mean $r = 0.12$; 5-HT2a/*HTR2A*: mean $r = 0.20$; GABAa/*GABRA1*: mean $r = 0.14$; GABAa/*GABRB1*: mean $r = 0.14$; NET/*SLC6A2*: mean $r = 0.02$) with exception of the GABRG1 gene (GABAa/*GABRG1*: mean $r = -0.13$) (***Figure 3C***). Positive correlation coefficients suggest that higher neurotransmitter density was associated with higher expression of those neurotransmitters.

Lastly, to evaluate the robustness of the mRNA analyses (i.e. gene differential stability), genomic autocorrelations were calculated. The genomic autocorrelation was high for *GABRB1* (mean $r = 0.92$) and *GABRG1* (mean $r = 0.64$), small for *HTR1B* (mean $r = 0.23$), *SLC6A2* (mean $r = 0.22$), and *GABRA1* (mean $r = 0.21$), and very small for *HTR2A* (mean $r = 0.05$) (***Figure 3D***).

Discussion

In the current study, we examined if there is a selective vulnerability of specific neurotransmitter systems in bvFTD to gain novel insight into the disease mechanisms underlying functional and structural alterations. More specifically, we evaluated if fALFF alterations in bvFTD co-localize with specific neurotransmitter systems. We found a significant spatial co-localization between fALFF alterations in patients and the in vivo derived distribution of specific receptors and transporters covering serotonergic, norepinephrinergic, and GABAergic neurotransmission. These fALFF–neurotransmitter associations were also observed at the mRNA expression level and their strength correlated with specific clinical symptoms. All of the observed co-localizations with in vivo derived neurotransmitter estimates were negative with lower fALFF values in bvFTD being associated with a higher density of the respective receptors and transporters in health. The directionality of these findings supports the notion of higher vulnerability of respective networks to disease-related alterations. These findings are also largely in line with previous research concerning FTD showing alterations in all of the respective neurotransmitter systems (***Huey et al., 2006***; ***Murley and Rowe, 2018***).

The in vivo co-localization findings might also support the notion that propagation of proteins involved in bvFTD may align with specific neurotransmitter systems (***Hock and Polymenidou, 2016***). With regard to other brain disorders, linking functional connectivity with receptor density and expression, recent studies found an association between functional connectivity and receptor availability in schizophrenia, and an association between structural–functional decoupling and receptor gene expression in Parkinson's disease (***Zarkali et al., 2021***; ***Horga et al., 2016***). A potential mechanism for the selective vulnerability of specific neurotransmitter systems is the propagation of proteins along functionally connected networks that has been previously demonstrated for various neurodegenerative diseases (***Zhou et al., 2012***; ***Seeley et al., 2009***). For example, in Alzheimer's disease and normal aging, tau levels closely correlated with functional connectivity (***Franzmeier et al., 2019***). We found moderate to large AUC when using the strength of the identified co-localizations for differentiation

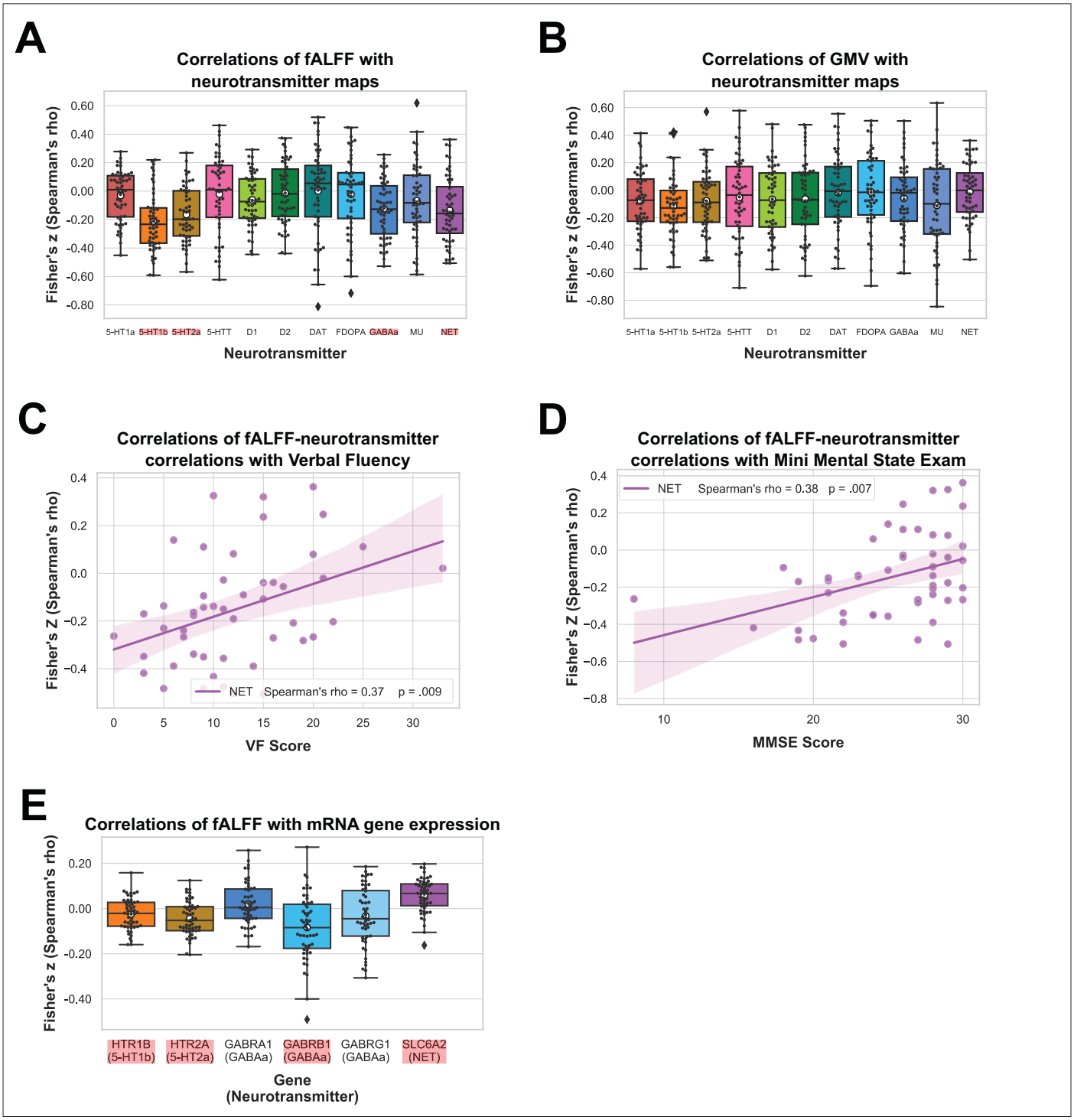

Figure 2. Results of spatial correlation analyses with in vivo and mRNA data. Correlation of fractional amplitude of low-frequency fluctuation (fALFF) (**A**) and gray matter volume (GMV) (**B**) with spatial distribution of neurotransmitter systems incl. 95% confidence intervals. Correlations of Verbal Fluency (N = 49) (**C**) and Mini Mental State Exam (N = 50) (**D**) with fALFF–neurotransmitter strength of association incl. bootstrapped 95% confidence intervals. Correlations of fALFF with mRNA gene expression maps (N = 52) (**E**). Statistically significant correlations in (**A**), (**B**), and (**E**) are marked in red and means are represented by white circles. Black circles in (**A**), (**B**), and (**E**) represent individual Fisher's z-transformed Spearman correlation coefficients for each patient (N = 52) relative to controls with each neurotransmitter map. Colored circles in (**C**) and (**D**) represent individual Fisher's z-transformed Spearman correlation coefficients between fALFF–neurotransmitter correlations and each neuropsychological scale. The statistical significance of all correlation coefficients was evaluated at $p < 0.05$ including FDR correction for (**A**), (**B**), and (**E**).

Figure 2 continued on next page

Figure 2 continued

The online version of this article includes the following source data and figure supplement(s) for figure 2:

Source data 1. Fisher's *z*-transformed Spearman correlation coefficients shown in ***Figure 2A–E***.

Figure supplement 1. Results of spatial correlation of fractional amplitude of low-frequency fluctuation (fALFF) with mRNA gene expression maps of all γ-aminobutyric acid type A (GABAa) subunits.

Figure supplement 1—source data 1. Fisher's *z*-transformed Spearman correlation coefficients of fractional amplitude of low-frequency fluctuation (fALFF) with mRNA gene expression of all γ-aminobutyric acid type A (GABAa) subunits shown in ***Figure 2—figure supplement 1***.

between patients and HC suggesting that these findings may represent a measure of the affectedness of respective neurotransmitter systems. In bvFTD, neurodegeneration is thought to progress through the salience network involved in socioemotional tasks, which comprises the anterior cingulate and frontoinsular cortex, as well as the amygdala and the striatum (***Bang et al., 2015***; ***Hock and Polymenidou, 2016***). The three neurotransmitter systems found to be deficient in our sample are relevant for the functioning of these structures (anterior cingulate cortex: e.g. serotonin and norepinephrine, ***Tian et al., 2017***; ***Koga et al., 2020***; amygdala: e.g. GABA and serotonin, ***Castro-Sierra et al., 2005***; striatum: e.g. GABA, ***Semba et al., 1987***). Although spread of misfolded proteins through the salience network provides a potential disease mechanism, further research of the exact mechanisms involved is needed.

For GMV, we did not find any significant co-localization with specific neurotransmitter systems. As the correlations with GMV showed a distinct pattern to fALFF and the variance explained by GMV in the observed fALFF–neurotransmitter associations was small, the observed associations with fALFF seem to be driven indeed by functional alterations and not by the underlying atrophy of respective regions. As propagation of misfolded proteins leads to a gradual dysfunction and eventually cell death (***Hock and Polymenidou, 2016***), some regions displaying high density of a specific neurotransmitter might suffer dysfunction (i.e. functional alterations), whereas others might already be exposed to cell death (i.e. structural alterations/atrophy). An interesting future direction might compose integration of structural connectivity as measured by diffusion tensor imaging. A study by ***Dopper et al., 2014*** showed reduced fractional anisotropy in healthy individuals carrying mutations compared to non-carriers (***Dopper et al., 2014***). Given that there were structural connectivity differences even before disease onset, it would be of interest to re-examine structural connectivity differences between HC and patients (i.e. after disease onset). Repeating the neurotransmitter analyses might facilitate understanding of the underlying disease mechanism.

The strength of co-localization of fALFF with NET was correlated with VF and MMSE, both being impaired in patients with bvFTD (***Schroeter et al., 2012***; ***Diehl and Kurz, 2002***; ***Schroeter et al., 2018***). Thereby, a stronger negative co-localization (i.e. lower fALFF in patients in high-density regions in health) was moderately associated with decreased test performance. Similarly, a correlation between MMSE and NE plasma concentration has been previously reported in Alzheimer's disease (***Pillet et al., 2020***). Combined, these findings point to a potentially more general role of norepinephrinergic neurotransmission in cognitive decline observed across different dementia syndromes. This interpretation is in line with the recently proposed role of the locus coeruleus, the source of norepinephrine in the brain, in regulating processes of learning, memory, and attention (***Tsukahara and Engle, 2021***). In contrast to the study by ***Murley et al., 2020*** who reported an association of GABA concentrations in the inferior frontal gyrus in FTD with disinhibition, we did not find this association. Beside the use of different methodology, a potential explanation may constitute the use of different inhibition measures. Whereas we measured disinhibition using the FrSBe, ***Murley et al., 2020*** used a stop-signal task.

Although, except for α1 and γ1 GABAa subunits, all of the co-localizations with fALFF identified with in vivo estimates were also significant at the respective mRNA gene expression level, we found correlation coefficients of both directionalities. Interestingly, whereas these correlations were solely negative for the in vivo derived maps, the correlations with gene expression profile maps were positive for NET, and negative for 5-HT1b, 5-HT2a, and β1 GABAa subunit. Thus, for NET, we observed higher fALFF values in bvFTD patients in areas with high mRNA gene expression in health, whereas for 5-HT1b, 5-HT2a, and β1 GABAa subunit we observed lower fALFF values in bvFTD patients in areas with high mRNA gene expression in health. One explanation for these seemingly contradictory

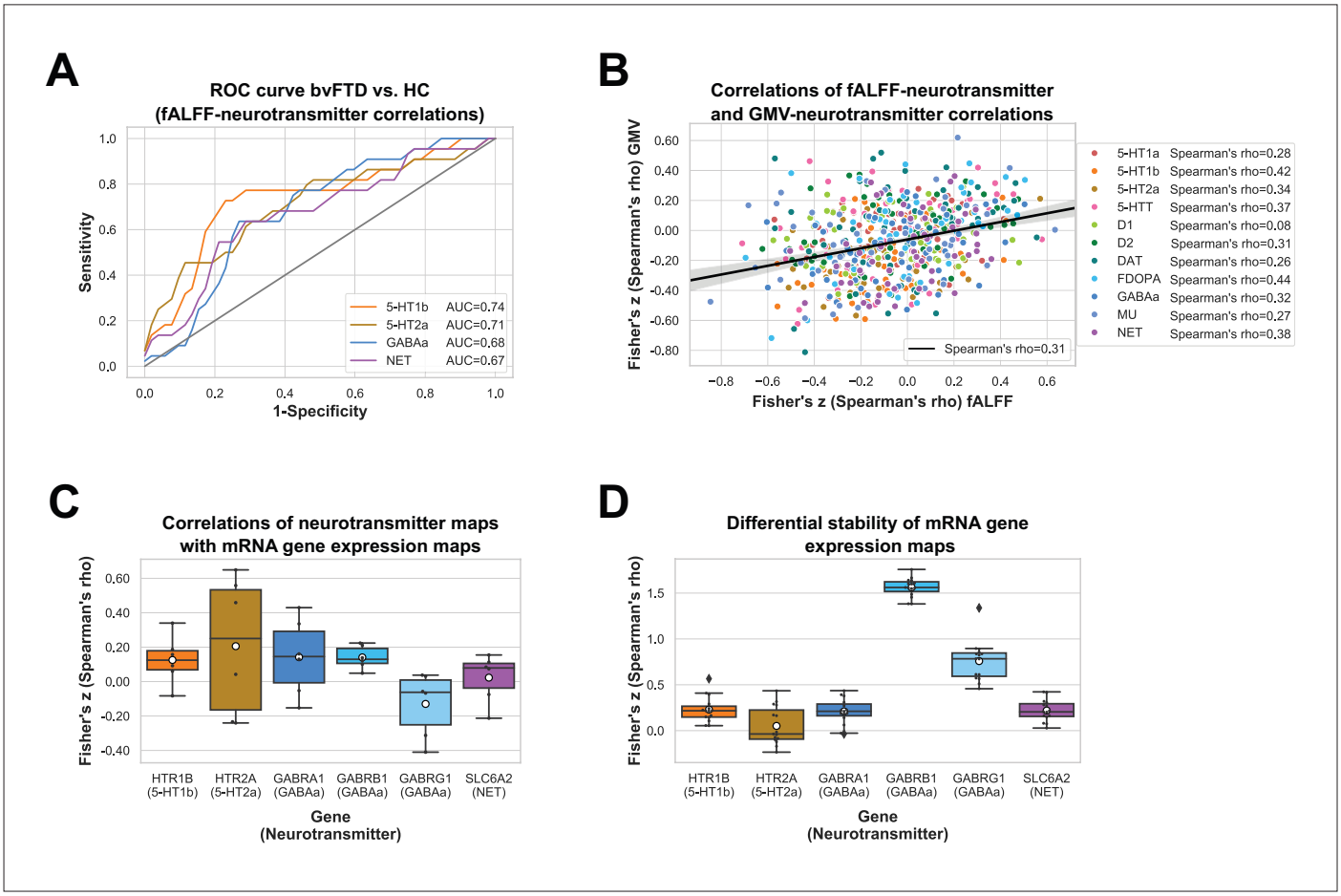

Figure 3. Results for fractional amplitude of low-frequency fluctuation (fALFF)–neurotransmitter receiver operating characteristic (ROC) curve, correlations of fALFF–neurotransmitter and gray matter volume (GMV)–neurotransmitter correlations, correlations of neurotransmitter and mRNA gene expression maps, and autocorrelations of mRNA gene expression maps. ROC curves for healthy controls (HC) vs. behavioral variant frontotemporal dementia (bvFTD) patients are displayed for significant fALFF–neurotransmitter correlations (N_{bvFTD} = 52, N_{HC} = 22) (**A**). Spearman correlation coefficients of fALFF–neurotransmitter and GMV–neurotransmitter correlations are displayed for each patient and each significant neurotransmitter (N = 52) (**B**). Spearman correlation coefficients of neurotransmitter and mRNA gene expression maps (**C**) and autocorrelations of mRNA gene expression maps averaged across mRNA donors (N = 6) (**D**) are displayed for significant fALFF–neurotransmitter associations incl. 95% confidence intervals.

The online version of this article includes the following source data and figure supplement(s) for figure 3:

Source data 1. Sensitivity and 1 − specificity shown in ***Figure 3A***, fractional amplitude of low-frequency fluctuation (fALFF)–neurotransmitter and gray matter volume (GMV)–neurotransmitter Fisher's *z*-transformed Spearman correlation coefficients shown in ***Figure 3B***, and Fisher's *z*-transformed Spearman correlation coefficients of neurotransmitter and mRNA gene expression maps shown in ***Figure 3C ,D***.

Figure supplement 1—source data 1. Fisher's *z*-transformed Spearman correlation coefficients of neurotransmitter and mRNA gene expression maps for all γ-aminobutyric acid type A (GABAa) subunits shown in ***Figure 3—figure supplement 1***.

Figure supplement 1. Results for correlations of neurotransmitter and mRNA gene expression mapsof all γ-aminobutyric acid type A (GABAa) subunits.

Spearman correlation coefficients of mRNA gene expression maps with the GABAa neurotransmitter map (N = 6) (**A**) and their mRNA autocorrelations (N = 6) (**B**). The genes encoding the 19 GABAa subunits include GABRA1–6, GABRB1–3, GABRG1–3, GABRR1–3, GABRD, GABRE, GABRP, and GABRQ. Means are represented by white circles.

findings is that mRNA gene expression seems to vary strongly between individuals. In our mRNA gene expression profile maps, the autocorrelation between mRNA donors was low for 5-HT1b, 5-HT2a, and α1 GABAa subunit, and NET, limiting the confidence in some of these findings. Additionally, the association of mRNA expression with protein products may also vary greatly between genes, being not associated at all or even negatively associated for some, and strongly correlated for others (***Kousounadis et al., 2015***; ***Moritz et al., 2019***). Similarly, a previous study found the correspondence between receptor density and mRNA expression to be low (***Hansen et al., 2022***). Potential reasons

for the lack of or even negative correlations may be a decoupling in time as well as that other levels of regulation overrode the transcriptional level (***Koussounadis et al., 2015***). We observed a similar phenomenon in our data with the correlation of neurotransmitter density maps with their underlying mRNA gene expression being weak for all neurotransmitters except β1 and γ1 GABAa subunits.

Our findings support the notion of fALFF as useful marker for assessing bvFTD-related decline in brain function. In line with previous literature in bvFTD, we observe fALFF reductions mainly in frontal and temporal lobes, but also in the parietal lobe (***Premi et al., 2014***; ***Borroni et al., 2018***). These findings support the notion of fALFF being a useful marker of metabolic impairment (***Bang et al., 2015***; ***Diehl-Schmid et al., 2007***). Moreover, we found a clear association of fALFF with several neurotransmitter systems pointing to a selective neurotransmitter vulnerability in bvFTD, as suggested in previous research (***Huey et al., 2006***; ***Murley and Rowe, 2018***). In particular, the co-localization of fALFF with NET was associated with VF and MMSE, suggesting the sensitivity of fALFF to reflect modality-specific cognitive decline.

The current study was limited by the unavailability of medication information. Therefore, we were not able to control for its potential confounding effects. However, as bvFTD medication is typically restricted to serotonin reuptake inhibitors its effects should be primarily associated with availability of 5-HTT and directionally negate the effects of the disease. Furthermore, as the included PET maps were derived from healthy subjects, the applied approach only tests for co-localization of imaging changes with the non-pathological distribution of the respective neurotransmitter systems. Similarly, the reliability of the co-localization analyses is partly limited by the number of healthy volunteers used to derive the respective neurotransmitter average maps. Finally, the current study was limited by the availability of neurotransmitter maps included in the JuSpace toolbox.

To summarize, we found fALFF reductions in bvFTD to co-localize with the in vivo and ex vivo derived distribution of serotonergic, GABAergic, and norepinephrinergic neurotransmitter systems, pointing to a crucial vulnerability of these neurotransmitters. The strength of these associations was linked to some of the neuropsychological deficits observed in this disease. We propose a combination of spread of pathology through neuronal connectivity and more specifically, through the salience network, as a disease mechanism. Thereby, these findings provide novel insight into the mechanisms underlying the spatial constraints observed in progressive functional and structural alterations in bvFTD. Our data-driven method might even be used to generate new hypotheses for pharmacological intervention in neuropsychiatric diseases beyond this disorder.

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Additional information

Competing interests

Henryk Barthel: received payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Life Molecular Imaging and Novartis/AAA. The author has no other competing interests to declare. Matthis Synofzik: has received consulting fees from, and currently act as a consultant for Aviado Bio, Prevail, Servier, Reata and Orphazyme. They have received payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from GenOrph. The author has no other competing interests to declare. Jens Wiltfang: has received consulting fees from Boehringer-Ingelheim, F. Hoffmann-La Roche, Biogen, Immungenetics,

Roboscreen and Abbott. They currently act as a consultant for Boehringer-Ingelheim, F. Hoffmann-La Roche, Biogen and Immungenetics, and hold a Leadership or fiduciary role at CSF Society, AGNP and DGLN. The author has received payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Pfizer, Janssen, MSD SHARP & DOHME, Amgen, Roche Pharma, Actelion Pharmaceutical, Guangzhou Glorylen Medicial Technology Co. (China), Bejing Yibai Science and Technology Ltd. The author has been issued the following patents; EP2095128B1 and EP3105589A1. The author has no other competing interests to declare. Janine Diehl-Schmid: has received a speaker fee from Jansen and Roche. The author has no other competing interests to declare. Markus Otto: has received grants from BMBF - FTLD consortium, moodmarker, ALS association and EU - MIRIADE. The author has received consulting fees from, and currently acts as a consultant for, BIOGEN, Axon and Roche. The author has been issued a patent for Foundation state Baden-Wuerttemberg, Beta Syn as Biomarker for neurodegenerative diseases. The author holds an unpaid leadership or fiduciary role at the German Society for CSF diagnostics and neurochemistry and the Society for CSF diagnostics and neurochemistry, and as a speaker at the FTLD consortium. The author is co-inventor of a patent application (PCT/EP2020/072559) for using beta-synuclein measurement in blood.The author has no other competing interests to declare. Juergen Dukart: former employee of and current consultant for F.Hoffmann-La Roche. The other authors declare that no competing interests exist.

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Author contributions

Lisa Hahn, Formal analysis, Investigation, Visualization, Writing - original draft; Simon B Eickhoff, Conceptualization, Resources, Writing – review and editing; Karsten Mueller, Henryk Barthel, Klaus Fassbender, Klaus Fliessbach, Johannes Kornhuber, Johannes Prudlo, Matthis Synofzik, Jens Wiltfang, Janine Diehl-Schmid, Markus Otto, Matthias L Schroeter, Conceptualization, Data curation, Funding acquisition, Writing – review and editing; Leonhard Schilbach, Conceptualization, Supervision, Writing – review and editing; FTLD Consortium, Conceptualization, Data curation, Funding acquisition; Juergen Dukart, Conceptualization, Supervision, Methodology, Writing – review and editing

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Ethics

Written informed consent was collected from each participant. The study was approved by the ethics committees of all universities involved in the German Consortium for Frontotemporal Lobar Degeneration (Ethics Committee University of Ulm approval number 20/10) and was in accordance with the latest version of the Declaration of Helsinki.

Decision letter and Author response
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Author response https://doi.org/10.7554/eLife.86085.sa2

Additional files

Supplementary files

- Supplementary file 1. Supplementary tables including information about the subject distribution across centers, detailed information about the neurotransmitter maps, contrast peak voxel information for the t-contrasts, Spearman correlation coefficients and corresponding p-values from the fALFF- and GMV-neurotransmitter analyses and their relationship to clinical symptoms.
- MDAR checklist

Data availability

The original data and their derivatives cannot be made publicly available as the study includes sensitive patient data and public data sharing was not covered in the informed consent. The original data supporting the findings of this study are available from the senior author (Matthias L. Schroeter) upon reasonable request. All derived statistical measures used here are available from the first author upon request. The software applied is publicly available at https://github.com/juryxy/JuSpace (JuSpace, ***Juryxy, 2023***) and https://github.com/FAIR-CNS/MENGA (MENGA, ***Rizzo, 2016***). The code for the main analyses is publicly available at https://github.com/liha-coding/Neurotransmitter-vulnerability-in-bvFTD (copy archived at ***Hahn, 2023***). Processed data used for the creation of the figures are available as supplementary material.

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5 Discussion

The three studies evaluated different biomarker approaches for PPD, AjD, and bvFTD. While the first two studies examined behavioral and neuroimaging biomarkers for PPD, the third study focused on utilizing neuroimaging to assess neurotransmitter vulnerability in bvFTD. More specifically, the first study demonstrated the value of remote EPDS and MPAS scores in addition to remote bi-daily mood assessments for early recognition of PPD and AjD. However, the second study did not find robust early structural and functional brain alterations in PPD and AjD, suggesting that neuroimaging is not a suitable early biomarker. The third study highlighted the potential of utilizing spatial correlations between fALFF and neurotransmitter density maps from a healthy volunteer population as biomarker for neurotransmitter vulnerability regarding the GABAergic, norepinephrinergic, and serotonergic neurotransmitter system in bvFTD.

5.1 Potentials and pitfalls of neuroimaging and behavioral biomarkers for postpartum depression

In study 1, we found differences regarding the distribution of risk factors between women with either PPD or AjD and controls (87). These results were in line with previous studies, which similarly found moderate to strong associations with a history of depression, postpartum blues, and stressful life events in women with PPD (46–49). However, umbrella reviews demonstrated that highly suggestive to convincing evidence only exists for antenatal anxiety, physical (partner) and psychological violence, smoking during pregnancy, history of PMS, primiparity, and unwanted pregnancy (47,48). Only PMS and the total number of children were included in study 1, showing a higher number of women with PMS in the patient groups. While active participation of the husband in maternal healthcare was previously identified as protective factor (47,48), there were no differences between patients and controls regarding the subjective quality of support at home in study 1. Additionally, there were no differences regarding the distribution of risk factors between women with PPD and AjD. Moreover, EPDS and MPAS scores differed between the two patient groups only after six and 12 weeks, respectively. Similarly, there was significant overlap between PPD and AjD regarding remote mood and stress assessments, only showing distinct developments after four to five weeks postpartum. These differences in symptom development fall around the official diagnostic timeframe for PPD (i.e., four weeks postpartum), making the early differentiation of PPD and AjD difficult.

Regarding the discrimination of PPD, AjD, and HC, high BACs of 80% to 90% were achieved in both exploration and independent validation cohort using remote mood, EPDS and MPAS scores, providing evidence that these remote assessments can be used as DP biomarker for PPD and AjD. Interestingly, the model using sociodemographic-anamnestic information alone performed considerably worse with a BAC of around 70% for patient-control discrimination. In line with the univariate results, the sociodemographic-anamnestic model performed at chance-level for the differentiation of women with PPD and AjD. Generally, studies utilizing DP in PPD are still rare (90). Currently, an ongoing study is collecting passive data (e.g., social media activity, geographical movement patterns, smartphone activity) in addition to active data (e.g., social circumstances, mental and physical health, background information) in a large sample of Swedish women (i.e., 5000 planned participants) via the Mom2B app during pregnancy and after childbirth (91). For other mood disorders such as MDD, more research involving DP has been conducted. For example, smartphone-based assessments showed clear patterns such as decrease in the number of calls and SMS messages, decrease in temperature and heart rate variability, and decrease in activities and walking (92). Therefore, passive data might even further improve early recognition and differentiation of PPD and AjD.

DP is a powerful tool, which enables early recognition of PPD and AjD in the postpartum period even before an official diagnosis is possible. The utilized features can easily be implemented into clinical practice through active data collection via smartphone-based assessments such as the Mom2B app (91). Additionally, automatic evaluation of the collected data is possible, but ethical considerations must be taken into account such as where the data are stored (e.g., secure server or patient system) and who can access the information (e.g., general practitioner, gynecologist). Given the potential of early intervention and treatment, attempts at overcoming those barriers should be made to reduce the suffering of mothers and their offspring in addition to lowering the economic burden.

The similarities between PPD and AjD regarding risk factors, mood and stress assessments, and MPAS and EPDS scores in the early postpartum period raise the question of whether PPD and AjD in the postpartum period are distinct phenomena or whether they share an underlying pathological mechanism leading to similar clinical manifestations. Similar to women with PPD, women with AjD might be vulnerable to hormonal shifts across the lifespan (35,54). Therefore, research is needed into either what causes women with PPD to continue suffering or what allows women with AjD to recover. Finding differentiating biomarkers and/or underlying pathological mechanisms

can reduce the number of women to be monitored at risk for PPD, which in turn reduces the total cost of monitoring women at risk.

In study 2, we did not find group differences in GMV, fALFF, LCor, and GCor within a week after childbirth. However, we found a positive correlation of LCor and EPDS score at 12 weeks postpartum in the left superior medial frontal gyrus, pointing to GMV increases in women with higher depressive symptoms. Generally, the results of previous studies have been widely variable (65,66). While we did not find early structural and functional brain changes within one week after childbirth, previous studies considered time points up to a year postpartum, with the shortest time frame of one month postpartum (66). Therefore, the differing measurement time points might explain the inconsistency of results. Broadening the time frame beyond four weeks postpartum allows the inclusion of cases with late-onset PPD, which is thought to have a separate etiology from early-onset PPD cases such as psychosocial factors (93,94). Additionally, a later measurement time point might allow symptoms to deteriorate in uncaught PPD cases. Given that we did not find early brain alterations in contrast to studies with measurements taken at later time points, it is likely that brain changes in PPD co-occur with symptoms instead of preceding them. For AjD, a similar explanation is likely with symptoms improving before brain structure and/or function change. Independent of the results, the acceptability of undergoing brain scans might be low in the first (often stressful) weeks after childbirth.

To conclude, given the inconsistent results and low number of studies on neuroimaging in PPD (66), there is more research with larger sample sizes needed to clarify inconsistent results across studies. Additionally, different measurement time points should be considered to discriminate between early- and late-onset PPD in future studies. Therefore, early structural and functional brain alterations currently cannot be utilized as early diagnostic or prognostic biomarkers for PPD and AjD.

5.1.2 The future of biomarkers for postpartum depression

While we demonstrated that DP using remote postnatal depression, attachment, and mood scores is a robust biomarker for early recognition of PPD, this was not the case for early brain alterations. In the last decade, PPD research started to focus on ML approaches. While most studies utilized sociodemographic and psychiatric diagnoses as features, followed by gynecological and obstetric factors, fewer studies considered pediatric, medical and psychosocial factors with even less including medication use (95–101). Similarly, several peripheral or omics-based variables were only considered

separately (102–104). Some studies utilized a new set of features such as content characteristics and linguistic style extracted from social media data (99,105,106) or acoustic features such as the infant's cry (101) to predict PPD. Interestingly, no study utilized neuroimaging features and only a few studies used a multimodal ML approach (102–104).

Looking at the combined results, several systematic reviews found that sociodemographic features (i.e., age, education, marital status, income and ethnicity), obstetric and pediatric factors (i.e., mode of delivery, parity, gestational age at delivery, APGAR score, and sex of the newborn), psychosocial and medical factors (i.e., depression history, anxiety or depression during pregnancy, stress or stressful life events, smoking, thyroid dysfunction, BMI, and sleep status) were the most important predictors (95,96,107). However, no study included a combination of remote mood and stress assessments, EPDS and MPAS scores. While using different algorithms ranging from logistic regression over support vector machines to more complex sequences of decision trees and neural networks, most of the studies reached an area under the curve above 0.7, indicating that ML approaches are well-suited for the classification of PPD (95). However, the defined onset period of the disorder varied widely between studies from six weeks to up to a year postpartum, posing a similar problem to neuroimaging studies.

Therefore, future studies need to establish differences between early- and late-onset PPD. Whereas late-onset PPD may be more similar to MDD in general, the differing heritability, epigenetic information and symptom severity point to early-onset PPD being a distinct phenomenon (108). For example, MDD shows a considerably lower heritability of 32% in comparison to a heritability of 50% in perinatal depression (46,50). Since we specifically focused on early recognition within the diagnostic time frame of four weeks postpartum, ML classifiers developed for MDD might be more useful to predict late-onset PPD. Additionally, collection of biomarkers during pregnancy might improve the current prediction model, since many hormonal differences between women with PPD and HC are already detectable during pregnancy.

An additional important future consideration is the early recognition of PPD in fathers. With a prevalence of 8% to 10%, a substantial number of fathers suffer from PPD in the postpartum period (109). Given the highest prevalence within three to six months postpartum, most PPD cases in men develop more slowly, rendering the standard DSM-5 diagnostic criteria questionable (109,110). Similar to maternal depression, paternal depression is also affected by hormonal changes. For example, fathers with PPD display lower levels of testosterone, estrogen, cortisol, vasopressin, and prolactin (110). In

addition to maternal depression, other risk factors for paternal depression include a history of mental illness and psychosocial factors such as financial instability and unemployment, relationship dissatisfaction, low education level, lack of parenting self-efficacy, lack of social support, work-related stress, and perceived stress (110,111). The body of research on PPD in fathers is still small, requiring more studies examining neuroimaging, genetics, and other peripheral biomarkers. Additionally, separate diagnostic criteria should be developed, since many PPD cases in fathers will not fall within the DSM-5 diagnostic time frame of four weeks postpartum. The current ML classifier might be useful for early-onset PPD in fathers, but the observation period of future studies should be extended to additionally include later-onset cases.

Since considering interactions of genetic, epigenetic, hormonal, and environmental factors might increase the screening accuracy (88), future studies should utilize a multimodal approach. Due to the considerable heritability and the involvement of reproductive hormones in addition to alterations in other peripheral biomarkers, a combination of genetic information and peripheral biomarkers including hormone levels should be considered together with remote mood and stress assessments in addition to postnatal depression and maternal attachment scores. Furthermore, future studies should concentrate on disentangling the similarities and differences of early- and late-onset PPD, PPD in fathers, MDD, and AjD in the postpartum period.

5.2 Potentials and pitfalls of utilizing spatial correlation of neuroimaging with neurotransmitter maps as neurotransmitter vulnerability biomarker for frontotemporal dementia

In study 3, we demonstrated that spatial correlations between fALFF, a FC measure closely correlated to glucose metabolism (112–114), and neurotransmitter maps containing information about receptor and transporter density as derived from PET in a healthy volunteer population gives a close estimation of neurotransmitter vulnerability in bvFTD (89). More specifically, fALFF was negatively correlated with the density of the serotonin 1b receptor (5-HT1b), serotonin 2a receptor (5-HT2a), GABA type A receptor (GABAa), and the norepinephrine transporter (NET), pointing to reduced fALFF in areas with high density of these neurotransmitters in healthy volunteers. Additionally, the strength of co-localization was associated with Verbal Fluency and the Mini Mental State Examination. Our results were in line with previous studies identifying deficits in dopaminergic, serotonergic, and GABAergic neurotransmission in FTD patients (115). However, previous studies additionally found glutamatergic deficits (115), which we could

not investigate at the time of analysis conduction due to unavailability of the respective neurotransmitter maps.

Moreover, the correlation of fALFF with gene expression profile maps underlying the previously identified receptors and transporters was negative for serotonergic 5-HT1b and 5-HT2a receptors and the β1 GABAa receptor subunit, and positive for NET (89). However, the results must be interpreted with caution, since the correlation of gene expression profile maps between mRNA donors was highly variable. A previous study by Hansen et al. (116) made similar observations, indicating that gene expression may differ between cortical and subcortical structures. Therefore, association of neuroimaging with gene expression profile maps should be considered carefully and might not constitute a practical biomarker for FTD.

A study by Premi and colleagues (117) took a similar approach correlating GMV with several neurotransmitter maps in genetic FTD patients relative to healthy non-mutation carriers from the GENFI cohort. They found that GMV in C9orf72, GRN, and MAPT mutation carriers was negatively correlated with serotonin 1a receptor (5-HT1a), dopamine D1 receptor (D1), dopamine transporter (DAT), fluorodopa (FDOPA), and the Vesicular acetylcholine transporter (VAChT). While negative correlations with 5HT1b and 5-HTT were only found in MAPT mutation carriers, a negative association of GMV with the metabotropic glutamate receptor type 5 (mGLUR5) was found in C9orf72 and GRN mutation carriers. Moreover, the strength of co-localization for D1 and 5-HT1a receptors, and DAT correlated with neuropsychological measures for C9orf72 and MAPT mutation carriers. Comparing our results to the study by Premi and colleagues (117), there is no overlap except for a negative association with the 5HT1b receptor. However, the studies are fundamentally different. Our study focused on rsfMRI, performing correlations with GMV as control analysis to exclude that this correlation was driven by underlying structural alterations in addition to focusing on clinical subtypes. In contrast, the study by Premi et al. concentrated on correlations with GMV in genetic subtypes of FTD. While we did not find significant associations for GMV, our sample largely consisted of sporadic cases of bvFTD only including four C9orf72 mutation carriers and no MAPT or GRN mutation carriers. Future studies should examine differences between genetic vs. sporadic FTD, clinical manifestations, and proteinopathies to gain more insight into whether neurotransmitter vulnerability is subtype specific. In the case of differential associations of neuropsychological assessments with the strength of co-localization for subtypes, this approach might shed light on the underlying pathological mechanisms.

In our study the correlations between GMV with neurotransmitter maps were not significant, indicating that the association of fALFF with neurotransmitter density were not

driven by underlying atrophy. This observation provided insight into the underlying pathological mechanism, suggesting that functional impairments of neurotransmission in specific neurotransmitter systems occur before structural impairments (i.e., cell death). This is in line with the hypothesis that prion-like propagation of misfolded proteins gradually affects neurons (67,70,74–78). While fALFF is closely correlated with glucose metabolism (114), other related FC measures such as regional homogeneity (114) and different imaging modalities such as white matter volume or structural connectivity should be considered in future studies.

The approach of utilizing spatial correlations of fALFF with publicly available neurotransmitter density maps from a healthy volunteer population provided insight into specific neurotransmitter vulnerability in patients with bvFTD and shed light onto the underlying pathological mechanism. Future studies should further examine neurotransmitter vulnerability comparing several imaging modalities including other FC measures in genetic, proteinopathological, and clinical subtypes.

5.2.2 The future of biomarkers for frontotemporal dementia

We demonstrated the potential of spatial correlations between sample-dependent neuroimaging and publicly available neurotransmitter maps from a healthy volunteer population as neurotransmitter vulnerability biomarker. However, this approach might hold additional potential. For example, in a longitudinal study with repeated MRI scans, the methodology could serve as disease progression marker comparing affected neurotransmitter systems in presymptomatic, early symptomatic, and progressed FTD. Moreover, targets for pharmacological treatments could be identified to counteract neurotransmitter deficiencies.

One major concern regarding the diagnosis of FTD is the differentiation from psychiatric diseases, especially in the early stages of the diseases. Here, the value of neurotransmitter vulnerability biomarkers needs to be critically examined for overlap with other psychiatric diseases. For example, serotonergic, dopaminergic, glutamatergic, and GABAergic neurotransmitter systems are similarly affected in schizophrenia (118). The question of a shared cause between FTD and schizophrenia has previously been raised due to the phenotypic overlap (79,119). Therefore, differentiation of FTD from other psychiatric diseases may be easier compared to the differentiation from schizophrenia.

Similar to other areas of research in the field of neuropsychiatry, dementia research started to discover the potential of ML approaches. Interestingly, many studies focus on AD and the discrimination between dementias in contrast to differentiating FTD subtypes

(120–122). For example, De Francesco et al. (121) developed a classifier using sociodemographic, clinical, and MRI-based variables, to discriminate patients with AD, FTD, and dementia with Lewy bodies from cognitively normal controls with a precision and recall of 88%. In contrast, Kim and colleagues (123) utilized a hierarchical classification approach for discriminating AD from the subtypes of FTD using cortical thickness with an overall accuracy of 75.8%. In the first step, controls are discriminated from patients, which is followed by a differentiation of FTD and AD. Next, bvFTD is differentiated from PPA and in the final step svPPA and nfvPPA are discriminated (123). In addition to classification frameworks, ML can be used to identify clusters or subtypes of a disease. Combining clustering with the current approach might be utilized to discover subtypes of neurotransmitter vulnerability and to examine if the resulting clusters coincide with genetic subtypes, proteinopathies, and/or clinical manifestations.

Next to ML, DP shows promises as biomarker for FTD, since many neuropsychological tests including speech sampling can easily be implemented into smartphone apps (124,125). For example, Taylor et al. (124) implemented a total of 17 tests including several executive function, memory, speech and language tests in addition to assessments for gait, balance, and finger tapping into their ALLFTD mobile app. Next to the commitment being perceived as acceptable by patients, disease severity was associated with poorer performance on several tests. However, more research is needed into whether DP could serve a diagnostic biomarker and/or discriminate between subtypes.

In summary, early recognition of FTD and differentiation from other neuropsychiatric diseases is crucial to enable early patient and symptom management. Promising ML classifiers have been developed, which need external validation and confirmation in clinical practice. Generally, a multimodal approach should be utilized, potentially including genetic testing and peripheral biomarkers. However, genetic testing is costly and is only informative for genetic FTD. Despite the availability of promising CSF biomarkers, the patient's well-being should be considered, since the CSF extraction may be perceived as uncomfortable. Finally, the value of neurotransmitter vulnerability biomarkers regarding subtype-clustering and disease progression should be examined in future studies.

5.3 Implications and future directions for biomarker development in the field of neuropsychiatry

Regarding the different types of biomarkers we examined, the classifier from the first study utilizing DP including postnatal depression, attachment, and mood scores can be seen as risk or susceptibility biomarker, since its primary purpose is the early recognition of PPD and AjD (87). However, one could argue that it can be viewed as diagnostic biomarker, since the time frame for the differentiation of AjD and PPD from HC overlaps with the diagnosis time frame. This is especially the case for the differentiation of AjD and PPD, since it exceeds the diagnostic time frame of four weeks postpartum. Moreover, postnatal depression, attachment, and mood scores constitute a surrogate endpoint biomarker, since they also "substitute for how a patient feels, functions, or survives" (18,20). However, this does not lower the diagnostic potential of the biomarker, since it facilitates the early recognition of PPD and differentiation from women with AjD. While study 2 did not reveal a potential for early structural and functional brain alterations (88), it might be a suitable biomarker for late-onset PPD, as previous studies found alterations in brain structure and function at later measurement time points (65,66).

Moreover, study 3 demonstrated the promises of spatial correlations between fALFF and neurotransmitter maps from a healthy volunteer population as biomarker for neurotransmitter vulnerability (89). The categorization of this biomarker is less straightforward, since its full potential has not been examined. If neurotransmitter vulnerability can discriminate between clinical, genetic and/or proteinopathological subtypes in addition to differentiation from psychiatric disorders, it could function as diagnostic biomarker. If the affected neurotransmitter systems show progression in longitudinal studies with repeated measurements either by magnitude of the correlation coefficients or by involvement of more neurotransmitter systems, it could also prove useful as a monitoring biomarker. Although the neurotransmitter vulnerability approach is largely dependent on the availability of neurotransmitter maps, the methodology still holds advantages over individual PET imaging requiring repeated scans with different tracers to assess multiple neurotransmitters.

Taking together the results of the three studies, the type of biomarker which is beneficial for chronic vs. non-chronic diseases strongly depends on the disease. For example, monitoring biomarkers might not be meaningful for non-chronic, treatable diseases such as PPD, since a course of progression of the disorder is not expected after treatment. In contrast, monitoring biomarkers are crucial for neurodegenerative diseases to adjust

patient management and symptom treatment to the needs of the patient. However, the usefulness of specific biomarkers should be evaluated for the diseases individually.

Our approaches to biomarker development in PPD, AjD, and FTD also show promises for the field of neuropsychiatry. While the classifier for PPD and AjD is likely specific for the two disorders, the combination of DP and machine learning holds potential for other diseases (126,127). For example, DP in the form of smartphone-based assessments already shows promises for FTD (124,125). While structural and functional alterations were not proven to be useful for PPD and AjD, neuroimaging biomarkers still hold great (predictive) value for other neuropsychiatric diseases such as schizophrenia (128,129). Similarly, neurotransmitter vulnerability estimation through spatial correlation of neuroimaging and neurotransmitter maps has also been applied to other diseases such as schizophrenia, bipolar disorder, Parkinson's disease, and ADHD (130). Thus, the approaches utilized in the three studies show promises for the field of neuropsychiatry.

Considering the integration into clinical practice, the PPD classifier could be easily implemented using smartphone-based assessments with automatic application of the classifier to the collected data. In contrast, the neurotransmitter vulnerability biomarker still needs further research to be implemented into clinical practice. While brain scans are typically obtained for a probable diagnosis of FTD, a classifier for discrimination of subtypes and differentiation from psychiatric diseases, which can automatically be applied to the images, is currently missing. However, there are two major issues with many existing ML models. First, while many promising ML classifiers exist, only a few are externally validated. Therefore, the risk that accuracies were caused by overfitting to the training sample is high (131). Second, despite the possibility of integrating ML classifiers into clinical practice, this is rarely the case. In future studies, more efforts should be made to test the performance in clinical practice. For example, the Computer-Assisted Risk Evaluation (CARE) study evaluates previously validated ML classifiers in clinical practice to optimize and individualize the treatment (132).

While this thesis provided insight into the potentials and pitfalls of the utilized neuroimaging and behavioral biomarkers for the neuropsychiatric field, it is crucial to acknowledge several limitations. First, the sample sizes of the PPD and bvFTD cohorts were considerably small in the era of 'Big Data', in which many datasets contain more than 1,000 participants. This might limit the generalizability of the current results. A similar concern arises from the PPD cohort being collected at one site (i.e., a university hospital), which might not generalize to cases at other and/or smaller, less specialized hospitals. However, the ML classifier was validated internally (i.e., at the same site), in a second, independent cohort. Since the first approach was specifically designed for early

recognition of PPD and AjD in the postpartum period, this strongly limits the generalizability to other neuropsychiatric diseases including MDD. However, the neurotransmitter vulnerability approach utilized in bvFTD patients was previously successfully applied to other neuropsychiatric diseases (130). Regardless, the potential of the current biomarkers should be validated in larger and external datasets. Second, the current focus on neuroimaging and behavioral biomarkers restricted the evaluation of other biomarker modalities. For example, the current biomarkers for PPD considered one modality at a time, not utilizing the potential of multimodal analyses. Since we did not find robust early structural and functional brain alterations in PPD and AjD, the ML approach utilized in the first study was limited by the availability of other modalities in the dataset (e.g., genetic information). However, the potential of the general approach of combining DP and ML was discussed within the scope of the current literature. In contrast, the approach utilized for the bvFTD cohort considered multiple modalities including neuroimaging-neurotransmitter correlations, neuroimaging-mRNA correlations, and association of strength of co-localization with clinical variables. Finally, despite efforts to evaluate the current biomarkers for PPD, AjD, and bvFTD in a broader context (e.g., other neuropsychiatric diseases), this evaluation is solely based on the available literature. It is important that the current approaches of combining DP with ML and utilizing structural and functional brain alterations to assess neurotransmitter vulnerability need to be examined in other diseases to draw conclusions. However, the neurotransmitter vulnerability approach has been evaluated in other neuropsychiatric diseases (130), showing promises for the whole field. Similarly, DP und ML were previously already successfully utilized in other neuropsychiatric diseases such as FTD (124,125).

Recognizing the limitations, the current biomarkers should be validated in larger and specifically external samples. Moreover, future studies should consider additional modalities to the current behavioral and neuroimaging approaches for biomarker development. Especially for ML analyses, multimodal approaches should be taken into account to benefit from the ensemble learning principle. Generally, the field of neuropsychiatry should focus on biomarker development for early recognition to reduce the suffering of patients and their caretakers and lower the economic burden.

5.5 Conclusion

Biomarkers for neuropsychiatric diseases can facilitate early recognition and diagnosis to reduce the number of under- and misdiagnosed cases. The utilized approaches of

combining DP with ML, examining early structural and functional brain alterations, and utilizing spatial correlations of fALFF with neurotransmitter maps from a healthy volunteer population to assess neurotransmitter vulnerability showed promises for the field of neuropsychiatric. More specifically, a combination of DP and ML allowed early recognition of PPD and AjD. While there were no differences regarding early structural and functional brain alterations in PPD, AjD, and HC, this approach might still hold potential for late-onset PPD and other neuropsychiatric diseases. Finally, identifying neurotransmitter vulnerability using the current spatial correlation methodology proved useful in bvFTD and might even hold unused potential requiring more research for subtyping and disease monitoring of FTD.

Moreover, the methodologies have already proven their value for other diseases. While DP has been successfully utilized in FTD patients (124,125), structural and functional brain alterations were predictive of other diseases such as schizophrenia (128,129). Additionally, neurotransmitter vulnerability approaches have shown similar results in other neuropsychiatric diseases and dementias (130). Future studies should consider the combination of clinical-anamnestic information, neuropsychological testing, DP, and peripheral, CSF, and omics markers within ML frameworks.

Taken together, the utility of several behavioral and neuroimaging biomarkers was evaluated for PPD, AjD, and FTD, showing potential for the facilitation of the diagnosis of these diseases. Additionally, the current approaches show promises for the field of neuropsychiatry, requiring more research in other diseases. As a major concern of the field, early recognition may be facilitated by the utilization of the current biomarker approaches to reduce the suffering of patients and lowering the economic burden.

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