PCN Psychiatry and Clinical Neurosciences

REGULAR ARTICLE

Disrupted-in-schizophrenia 1 protein aggregates in cerebrospinal fluid are elevated in patients with first-episode psychosis

Marlene Pils, MSc [1,2] Julia Rutsch, MSc,[3] Feride Eren, PhD,[4] Göran Engberg, MD,[4] Fredrik Piehl, MD,[5,6] Simon Cervenka, MD, PhD,[7,8] Carl Sellgren, MD, PhD,[4,7] Svenja Troßbach, PhD,[3] Dieter Willbold, PhD,[1,2,9] Sophie Erhardt, PhD,[4] Oliver Bannach, PhD[1,2]* and Carsten Korth, MD, PhD [3]*

Aim: The disrupted-in-schizophrenia 1 (DISC1) protein is a key regulator at the intersection of major signaling pathways relevant for adaptive behavior. It is prone to posttranslational changes such as misassembly and aggregation but the significance of such transformations for human mental illness has remained unclear. We aimed to demonstrate the occurrence of DISC1 protein aggregates in patients with first-episode psychosis (FEP).

Method: Cerebrospinal fluid samples of patients with FEP ($n = 50$) and matched healthy controls (HCs; $n = 47$) were measured by the highly sensitive surface-based fluorescence intensity distribution analysis technology that enables single aggregate detection.

Results: We demonstrate that DISC1 protein aggregates are increased in cerebrospinal fluid samples of patients with FEP versus HCs. The concentration was in the low femtomolar range. No correlations were found with specific symptom levels, but the difference was particularly significant in the subset of patients with the diagnoses schizophrenia, unspecified (DSM-IV 295.9) or schizoaffective disorder (DSM-IV 295.70) at 18-month follow-up. DISC1 protein aggregate levels did not significantly change within the 18-month observation interval and were on average higher for individuals carrying the major DISC1 rs821577 allele, before correction.

Conclusion: The occurrence of protein aggregates *in vivo* in patients with psychotic disorders has not been previously reported. It underscores the significance of post-translational modifications of proteins both as pathogenetic mechanisms and as potential diagnostic markers in these disorders.

Keywords: *biomarker, cerebrospinal fluid, disrupted-in-schizophrenia 1 protein, pathomechanisms, schizophrenia.*

http://onlinelibrary.wiley.com/doi/10.1111/pcn.13594/full

Molecular markers for major psychiatric diseases such as schizophrenia and recurrent affective disorders that can aid in the diagnostic workup and/or therapeutic monitoring are urgently needed. The current lack of such markers in part reflects an incomplete understanding of underlying disease mechanisms. Initial hopes of genetic markers to fulfill such a role have not materialized so far. Although schizophrenia shows a high degree of heritability, currently known common genetic variants together account for less than 10% of variance in disease risk.[1]

Proteins are the ultimate executors of cellular functions and are involved in signaling pathways governing adaptive behavior. They are also the most recognized molecular markers in medical routine diagnostics, usually detected by specific immunological assays. Posttranslationally modified proteins play a key role in neurodegenerative diseases,[2] where the presumed causally responsible protein aggregates such as Aβ, tau, α-synuclein, or others can be measured in *post mortem* brain, and *in vivo* by using positron emission tomography, cerebrospinal fluid (CSF) or blood.

The establishment of a role of posttranslationally modified proteins, such as misassembled or aggregated proteins, and particularly the identification of candidate proteins in major psychiatric diseases is still in its infancy.[3,4] The disrupted-in-schizophrenia 1 *(DISC1)* gene was identified as a familial gene in a large Scottish pedigree[5] but was subsequently not found to feature in larger studies of common gene variants.[1,6] The protein, however, is subject to posttranslational modifications such as phosphorylation[7] and multimerization.[8]

[1] Institute of Biological Information Processing (Structural Biochemistry: IBI-7), Forschungszentrum Jülich, Jülich, Germany
[2] attyloid GmbH, Düsseldorf, Germany
[3] Department of Neuropathology, Heinrich Heine University of Düsseldorf, Düsseldorf, Germany
[4] Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden
[5] Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden
[6] Department of Neurology, Karolinska University Hospital, Stockholm, Sweden
[7] Centre for Psychiatry Research, Department of Clinical Neuroscience, Karolinska Institutet, and Stockholm Health Care Services, Region Stockholm, Stockholm, Sweden
[8] Department of Medical Sciences, Psychiatry, Uppsala University, Uppsala, Sweden
[9] Institut für Physikalische Biologie, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany
* Correspondence: Email: ckorth@hhu.de; o.bannach@fz-juelich.de

Furthermore, it is highly aggregation-prone, both *in vitro* and *in vivo*.[8,9] In *post mortem* brains, DISC1 aggregates have been identified biochemically in patients with schizophrenia and with recurrent affective disorders, but were not found in healthy controls (HCs) or patients with classical neurodegenerative diseases.[8,10] An animal model displaying DISC1 aggregation showed dysregulated dopamine homeostasis,[11] consistent with a role for DISC1 aggregates in non-adaptive behavior.

We set out to identify DISC1 aggregates in patients with first-episode psychosis (FEP) using a highly sensitive surface-based fluorescence intensity distribution analysis (sFIDA). We previously introduced sFIDA as a protein oligomer-specific quantitation method for counting single proteinaceous particles. sFIDA features a combination of an enzyme-linked immunosorbent assay–like biochemical setup and a microscopy-based readout with subfemtomolar sensitivity (Fig. 1).[12,13] By using the same or overlapping epitopes for capture and detection, specificity for aggregates versus monomers is obtained. The method has been applied more recently to quantify α-synuclein and Tau oligomers in CSF of patients diagnosed with tauopathies and synucleinopathies.[14] Furthermore, sFIDA has been employed to determine levels of α-synuclein aggregates in stool samples obtained from patients with idiopathic rapid eye movement sleep disorder and Parkinson disease,[15] as well as Aβ aggregate levels in *ex vivo* brain samples from patients with Alzheimer disease.[16]

The main aim of our study was to use sFIDA to quantitatively compare DISC1 protein aggregates in CSF between patients with FEP versus HCs and to explore the presence and the potential diagnostic significance of DISC1 aggregate detection for clinical use. A second aim was to compare DISC1 aggregate levels with the Positive and Negative Syndrome Scale (PANSS), as well as cognitive functions. Finally, DISC1 aggregates levels were compared between subsets of patients depending on their DSM-IV diagnosis at follow-up to explore the use of DISC1 as a potential biomarker for clinical progression.

Methods

Patients

The present study was approved by Stockholm's regional ethics committee (Dnr 2010/879–31/1). Prior to participating in the trial, all patients and HCs completed a written informed consent form in line with the Declaration of Helsinki. The Karolinska Schizophrenia Project recruited all participants in partnership with four psychiatric institutes in Stockholm, Sweden: PRIMA Vuxenpsykiatri, Södra Stockholms Psykiatri, Norra Stockholms Psykiatri, and Psykiatri Nordväst. The study's participants were recruited between March 2011 and March 2019. The presence of neurologic disorders or severe somatic sickness, a history of illicit substance addiction, and the existence neurodevelopmental abnormalities such as autism spectrum disorder were all exclusion factors for the study. Urine testing was used to assess drug usage. To rule out macroscopic brain abnormalities, magnetic resonance imaging (MRI) was employed. To assess clinical characteristics of the patients, the Global Assessment of Functioning (GAF; where symptom and functioning dimensions were assessed separately), PANSS, Clinical Global Impression (CGI), Alcohol Use Disorders Identification Tests, and Drug Use Disorders Identification Tests were used. A clinical interview using DSM-IV or a consensus diagnostic process was employed to establish the diagnosis. Patients were contacted for reassessment after roughly 18 months, and the corresponding DSM-IV diagnosis was determined for 50 patients: paranoid schizophrenia (PS; DSM-IV 295.30) ($n = 23$); schizophrenia, unspecified (SU; DSM-IV 295.9) ($n = 9$); other schizophrenia (OS; DSM-IV 295.40) ($n = 1$); hebephrenic schizophrenia (HS; DSM-IV 295.10) ($n = 3$); residual schizophrenia (RS; DSM-IV 295.60) ($n = 1$); delusional disorders (DD; DSM-IV 297.1) ($n = 3$); acute and transient psychotic disorder, unspecified (ATPDU; DSM-IV 298.8) ($n = 1$); unspecified nonorganic psychosis (UNO; DSM-IV 298.9) ($n = 4$); schizoaffective disorder (SAD; DSM-IV 295.70) ($n = 4$); and major depressive disorder (MD; DSM-IV 296.21) ($n = 1$). Tobacco products were permitted to be used by patients, and 13 of the patients (26%) used nicotine derivatives (smoking or snuff), with information on nicotine missing in one patient. During their participation in the study, several patients needed sedatives and anxiolytics. Benzodiazepines were administered to 21 of the 50 patients during plasma and CSF sampling. At the time of sampling, 29 of 50 (58%) patients were taking antipsychotic medications. For these individuals, the highest number of days on antipsychotic therapy was 25, and the mean number±SEM of days was 14.2 ± 2.2 ($n = 14$). The remaining 21 patients had not used antipsychotics before or during plasma and CSF collection. The following antipsychotics were used by patients: olanzapine, aripiprazole, risperidone, quetiapine, paroxetine, or haloperidol. Patients' close relatives supplied knowledge available on the duration of untreated psychosis.

Healthy controls

The study enlisted 47 healthy individuals through advertising. A physical examination, brain MRI, blood and urine collection, and other procedures were performed to assess the HC participants. The Mini International Neuropsychiatric Interview was used to screen patients for past psychiatric illness. The study excluded participants who used illicit substances, were not taking medicine, or had first-degree relatives with a mental disorder. During the study, any current drug usage was assessed by the Alcohol Use Disorders Identification Test/Drug Use Disorders Identification Test. An expert

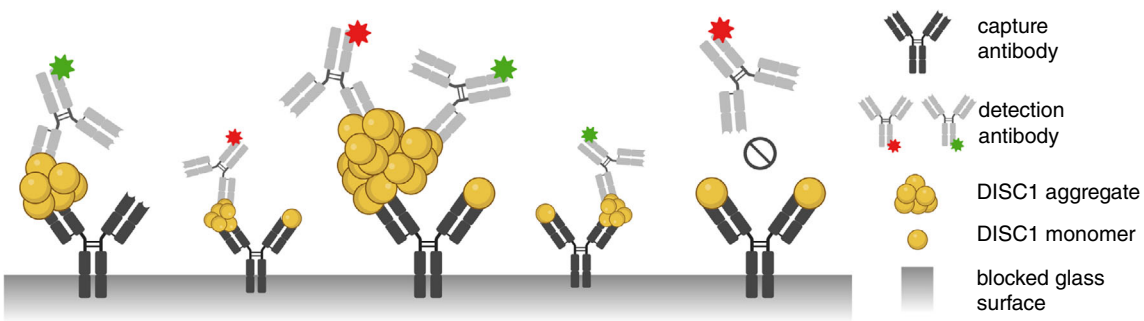

Fig. 1 Scheme of the surface-based fluorescence intensity distribution analysis (sFIDA) assay for the quantitation of disrupted-in-schizophrenia 1 (DISC1) aggregates. The glass surface of a microtiter plate is coated with 14F2 antibodies directed against linear epitopes of DISC1 (aa 747–768). Afterwards, a human cerebrospinal fluid sample is incubated on the assay surface, with both monomeric and aggregated DISC1 immobilized by the capture antibodies. However, the fluorescently labeled detection antibodies (14F2 CF633 and 14F2 CF488A) can only detect aggregated DISC1 species because all assay antibodies bind to the same epitope. In case of monomeric DISC1, the epitope is occupied by the capture antibody, preventing binding of the probe antibody. Finally, the assay surface is imaged by dual-color total internal reflection fluorescence microscopy and only single particles with colocalized fluorescence signals on the well surface are counted by image-data analysis. *Created with BioRender.com*.

neuroradiologist at Karolinska University Hospital, Solna reviewed the MRI data at an MRI center, and just one participant had a structural anomaly on the MRI. This individual exhibited early demyelination, but it was inadequate for a multiple sclerosis diagnosis because no neurological symptoms had earlier been reported, and a clinical neurological test indicated normal results. Lumbar puncture and cognitive tests for HCs were performed within 37.4 ± 5.7 days (mean ± SEM).

CSF collection

For CSF collection, standard lumbar puncture methods were used. A disposable atraumatic needle (22G Sprotte, Pajunk GmbH Medizintechnologie) was placed at the L4–L5 level in all patients who were in the right decubitus position. CSF (18 mL) was allowed to drop into a plastic test tube protected from light. CSF supernatant from all patients was separated into 10 aliquots and frozen at −80°C within 1 h of collection after centrifugation (rotor 5810R, Eppendorf SE) at 3500 rpm (1438 g) for 10 min to separate cells and supernatant. The lumbar puncture was performed on the majority of individuals ($n = 76$; 33 patients with FEP and 43 HCs) between 7:45 AM and 10 AM following a night's sleep. Morning sampling was not achievable for the rest of the patients with FEP (n = 17) due to clinical routines. To account for this confounding effect, four healthy individuals likewise underwent lumbar puncture at the same time window (i.e. 10:30 AM and 1:15 PM). Participants were asked not to participate in physical activity for the prior 8 h, although their activity could not be monitored.

sFIDA analysis

Assay development and analytical validation were performed using artificial DISC1 aggregates, which were either DISC1-coated silica nanoparticles (SiNaPs) or synthetic DISC1 (598–785) aggregates[17] spiked into buffer showing femtomolar detection limits (6.08 fM LoD

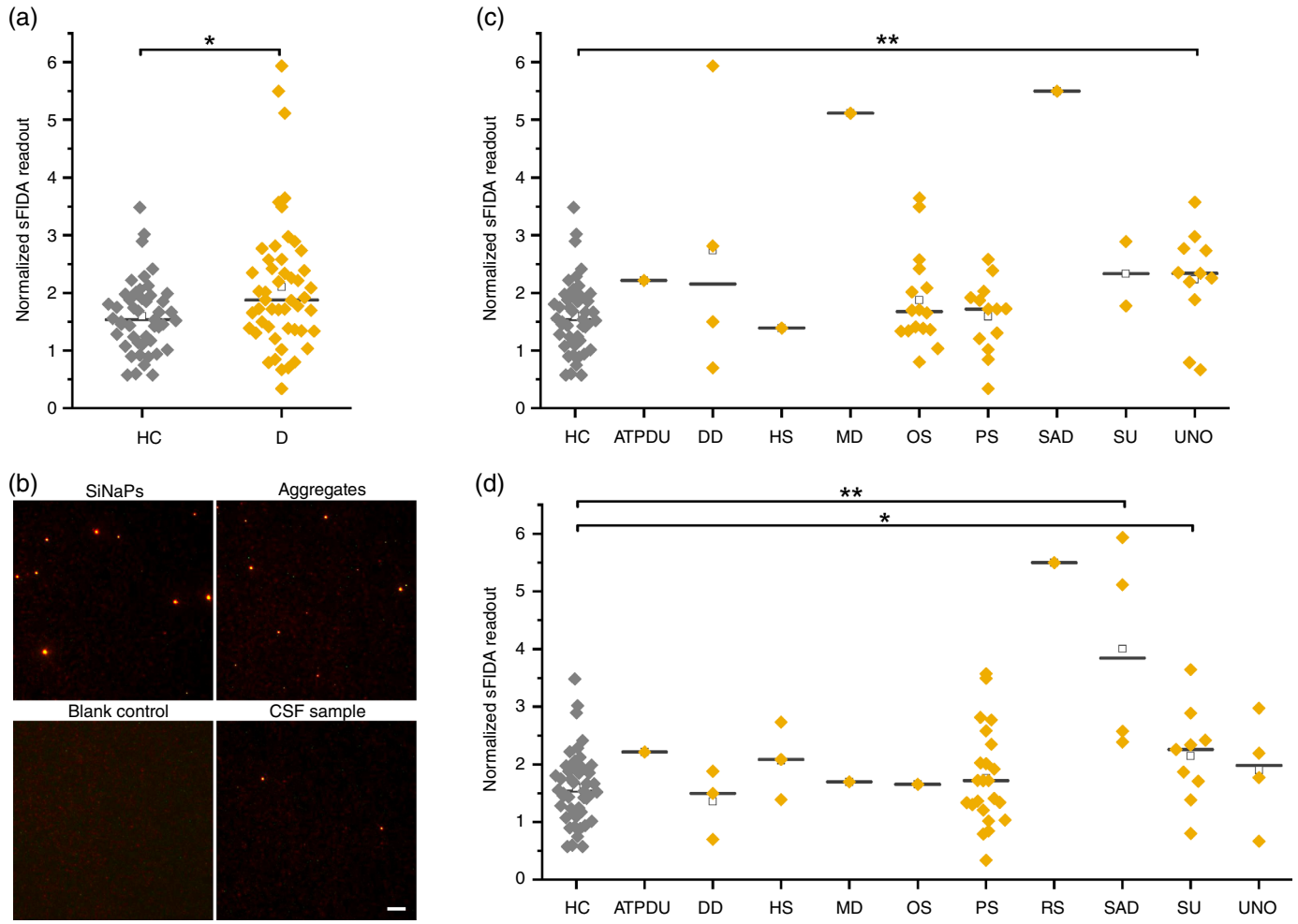

Fig. 2 Quantitation of disrupted-in-schizophrenia 1 (DISC1) aggregates in cerebrospinal fluid (CSF) samples of patients with first-episode psychosis (FEP). (a) Scatter plot of normalized surface-based fluorescence intensity distribution analysis readouts for healthy controls (HCs; $n = 47$) and patients with FEP ($n = 50$). Statistical analysis was performed using two-sided Mann-Whitney *U* test (confidence interval [CI], 0.05), and significantly elevated DISC1 levels in FEP patient samples were determined, indicated by a *P*-value of 0.027. (b) Representative total internal reflection fluorescence microscopy images as a composition of channel 0 and channel 1 (with an excitation: 633 nm, emission: 705 nm [channel 0] and an excitation: 488 nm, emission: 525 nm [channel 1], both with an exposure time of 500 ms and gain of 800) of DISC1-coated SiNaPs (160 fM), synthetic DISC1 aggregates (32 fM, subunit concentration), blank control (blocking solution only), and human CSF sample (FEP patient sample). Scale bar: 20 μm. Samples were divided according to their diagnosis at baseline (c) or at follow-up (d) and examined for significant differences from HC samples using one-sided Mann-Whitney *U* test (CI, 0.05). Using the baseline diagnosis for grouping, significantly higher DISC1 levels were determined for unspecified nonorganic psychosis (UNO; 298.9; P=0.005) compared with the HC group. In contrast, using the follow-up diagnosis for grouping revealed significantly higher DISC1 levels for schizoaffective disorder (SAD; 295.70) (P=0.001) and schizophrenia, unspecified (SU; 295.9); (P=0.024) compared with the HC group. The line indicates the median and the square indicates the mean. Significant differences between cohorts were signed with *(*P = 0.01–0.05; **P = 0.001–0.01). ATPDU, acute and transient psychotic disorders, unspecified (all diagnoses are DSM-IV 298.8); DD, delusional disorders (297.1); HS, hebephrenic schizophrenia (295.10), MD, major depressive disorder (296.21); OS, other schizophrenia (295.40); PS, paranoid schizophrenia (295.30); RS, residual schizophrenia (295.60); sFIDA, surface-based fluorescence intensity distribution analysis; UNO, unspecified nonorganic psychosis (298.9).

and 9.54 fM LLoQ) and low intra-assay variability (DISC1-coated SiNaPs 15.8%, synthetic DISC1 aggregates 7.9%) (Fig. S1), high assay comparability (differences <10%) (Fig. S2), and complete selectivity (~100%) (Fig. S3).

DISC1-coated SiNaPs, synthetic DISC1 aggregates, and a set of 97 CSF samples from patients with FEP ($n = 50$, 31 male, 19 females) and HCs ($n = 47$; 20 male, 27 females) enrolled in the Karolinska Schizophrenia Project were subjected to sFIDA analysis (Fig. 1). Patient and control samples were mixed and analyzed blinded to group allocation and to exclude any human bias. Patients who on follow-up did not receive a clinical diagnosis were excluded from the overall analysis. Due to the high number of assay points, the measurements were performed on three 384-well microtiter plates, and each sample was applied in quadruplicate. The assay surface was imaged *via* total internal reflection fluorescence microscopy as described above and for analysis a cutoff of 0.005% of blank control values was used to determine the sFIDA readouts. For assay calibration and as positive control, dilution series (see above) of DISC1-coated SiNaPs and synthetic DISC1 aggregates, respectively, were applied on each plate. However, since the sFIDA readouts of DISC1 aggregates in human CSF samples were close to buffer control readouts, not enough calibration points of SiNaPs were available to obtain a robust calibration and consequently only the noncalibrated sFIDA readout could be provided for the CSF samples. To account for measurement bias on multiple plates, samples were normalized using individual plate specific normalization factors based on the readouts of the HC group. For a detailed description of sFIDA development, normalizations, and further statistics, see Supplemental Methods and Table S1. Since normalized data showed non-normal distribution (Table S2), we used the two-sided Mann-Whitney *U* test for statistical computation

Table 1. Demographic information on CSF samples

Variable	All cohorts	HCs	Patients with FEP	Kruskal-Wallis ANOVA
Number	97	47	50	
Female, No. (%)	58 (59.8)	27 (57.4)	31 (62.0)	0.05652
Age (mean ± SEM)	28.52 ± 0.74	26.85 ± 0.78	30.08 ± 1.19	0.07229
BMI (mean ± SEM)	23.84 ± 0.38	24.23 ± 0.55	23.47 ± 0.53	0.44859
Nicotine, yes, No. (%)	20 (20.6)	7 (14.9)	13 (22.8)	0.16267
Medication, yes, No. (%)	29 (29.9)	0 (0)	29 (58)	5.52×10^{-10}

Note: Kruskal-Wallis analysis of variance (ANOVA): confidence interval, 0.05. Significant differences between both cohorts are in italics.

Table 2. Correlations of DISC1 aggregate levels with symptom levels and cognitive function

Test	Baseline		Follow-up	
	Spearman r	*P*-value	Spearman r	*P*-value
PANSS positive	−0.08	0.72	0.08	0.72
PANSS negative	−0.49	0.023	0.02	0.93
PANSS general	−0.01	0.95	0.34	0.15
PANSS total	−0.2	0.37	0.21	0.37
GAF-Symp	0.07	0.75	−0.17	0.49
GAF-Func	0.19	0.4	−0.02	0.93
CGI	−0.1	0.66	−0.08	0.73
TMT	−0.05	0.81	0.23	0.34
BACS-SC	0.56	0.008	0.06	0.81
HVLT-R	0.29	0.21	0.08	0.75
WMS-II-SS	0.09	0.69	0.31	0.18
LNS	0.29	0.21	0.11	0.64
NAB-Mazes	0.31	0.17	0.07	0.78
BVMT-R	0.29	0.19	0.13	0.58
Fluency	0.33	0.14	0.04	0.86
MSCEIT-ME	−0.13	0.58	0.09	0.69
CPT-IP	0.27	0.24	0.23	0.35

Note: No significant correlations (after false discovery rate corrections, not included in the table) between disrupted-in-schizophrenia 1 (DISC1) levels in cerebrospinal fluid and the following variables[11–14] were observed. BACS-SC, Brief Assessment of Cognition in Schizophrenia–Symbol Coding Test; BVMT-R, Brief Visuospatial Memory Test – Revised; CGI, Clinical Global Impression; CPT-IP, Continuous Performance Test–Identical Pairs; GAF-Symp, Global Assessment of Symptoms; GAF-Func, Global Assessment of Functioning; HVLT-R, Hopkins Verbal Learning Test—Revised; LNS, letter number sequencing; MSCEIT-ME, Mayer-Salovey-Caruso Emotional Intelligence Test's Managing Emotions Component; NAB-Mazes, Neuropsychological Assessment Battery–Mazes; PANSS, Positive and Negative Syndrome Scale; TMT, Trail Making Test; WMS-II-SS-R, Wechsler Memory Scale III Spatial Span.

Results

sFIDA analysis revealed that concentrations of aggregated DISC1 species were significantly increased in CSF samples of patients with FEP compared with HCs (P=0.026) (Fig. 2a). Representative total internal reflection fluorescence microscopy images of DISC1-coated SiNaPs, synthetic DISC1 aggregates, blank control, and a human CSF sample from a patient with FEP are shown in Fig. 2b.

Sex, body mass index, and nicotine usage were not significantly different between HCs and patients with FEP (demographic information, Table 1). The mean age ± SEM for HCs was 26.9 ± 0.8 years and for patients with FEP 30.1 ± 1.2 years. No significant correlations were evident between CSF DISC1 aggregate levels and age, body mass index, sex, smoking status, or medication (Tables S3 and S4).

We then looked at correlations of DISC1 protein aggregate levels with PANSS scores, GAF scale, CGI scale, as well as cognitive tests from the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) battery, but no significant result was obtained after false discovery rate correction (Table 2).

When we separated different subsets of patients, based on the clinical diagnosis at follow-up, assuming that DISC1 aggregate levels might be more elevated in some subsets rather than in others. A one-sided Mann-Whitney U test (confidence interval, 0.05) was used to reveal differences between the respective subsets and HCs (Fig. 2d, Table 3). Significantly higher CSF levels of aggregated DISC1 compared with controls were observed in SAD (DSM-IV 295.70 [P=0.001]) and SU (DSM-IV 295.9 [P=0.024]).

Next, we performed receiver operating characteristic analysis to quantify the diagnostic accuracy of the sFIDA assay for the detection of DISC1 aggregates in CSF samples (Fig. 3). While HC could be discriminated from all FEP samples with a specificity of 83.0% and a sensitivity of 46.0% with an area under the curve of 0.632 using sFIDA, the diagnostic validity, i.e. the diagnostic sensitivity, was improved using SAD and SU cases as a specific subset of FEP (specificity: 89.4%, sensitivity: 69.2% [area under the curve: 0.789]).

When we investigated both the segregation of a limited number of single-nucleotide polymorphisms (SNPs) within the *DISC1* gene

Table 3. P-values of Mann-Whitney U test for comparison of observed sFIDA readouts of HC and FEP groups or subgroups according to their final clinical diagnosis after 18 months

HC vs	P-value
FEP groups	*0.0257*
ATPDU	-
DD	0.7094
HS	0.1181
MD	-
OS	-
PS	0.2911
RS	-
SAD	*0.0012*
SU	*0.0235*
UNO	0.1953

Note: Significant differences (P<0.05) between the first-episode psychosis (FEP) (sub)group and healthy controls (HCs) are in italics. For the comparison of HCs vs patients with FEP, two-sided Mann-Whitney U test was performed, and for the comparison of HCs vs each subgroup, one-sided Mann-Whitney U test was performed. No Mann-Whitney U test was performed for acute and transient psychotic disorder unspecified (ATPDU; DSM-IV 298.8), major depressive disorder (MD; DSM-IV 296.21), other schizophrenia (OS; DSM-IV 295.40), and residual schizophrenia (RS; DSM-IV 295.60) as only one value was available.
Abbreviations: DD, delusional disorders (DSM-IV 297.1); HS, hebephrenic schizophrenia (DSM-IV 295.10); PS, paranoid schizophrenia (DSM-IV 295.30); SAD, schizoaffective disorder (DSM-IV 295.70); sFIDA, surface-based fluorescence intensity distribution analysis; SU, schizophrenia, unspecified (DSM-IV 295.9); UNO, unspecified nonorganic psychosis (DSM-IV 298.9).

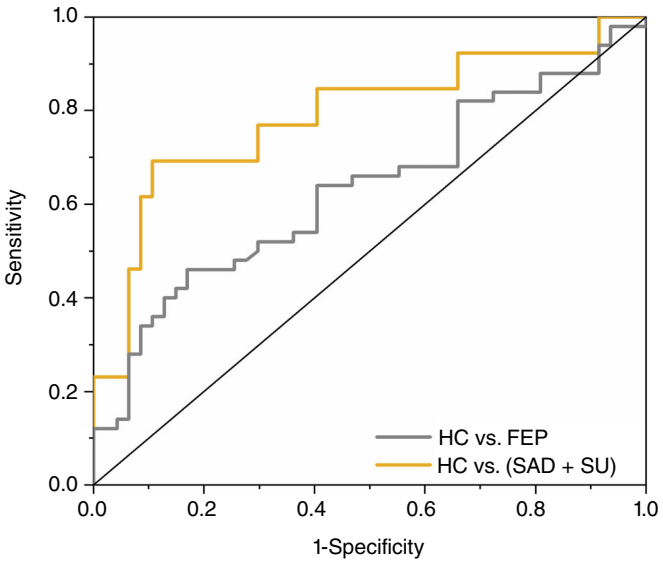

Fig. 3 Receiver operating characteristic analysis for the detection of disrupted-in-schizophrenia 1 aggregates in human cerebrospinal fluid samples. Discrimination of healthy controls (HCs) and patients with first-episode psychosis (FEP) showed a diagnostic specificity of 83% and a sensitivity of 46%, with an area under the curve of 0.632 and a respective P-value of 0.026 (gray line). In comparison, diagnostic validity was improved by including only patients with schizoaffective disorder (SAD; DSM-IV 295.70) and schizophrenia, unspecified (SU; DSM-IV 295.9) as a specific subset of FEP in the analysis. Patients with SAD and SU *versus* HCs showed a diagnostic specificity of 89.4% and a sensitivity of 69.2%, with an area under the curve of 0.789 and a respective P-value of 0.002 (yellow line).

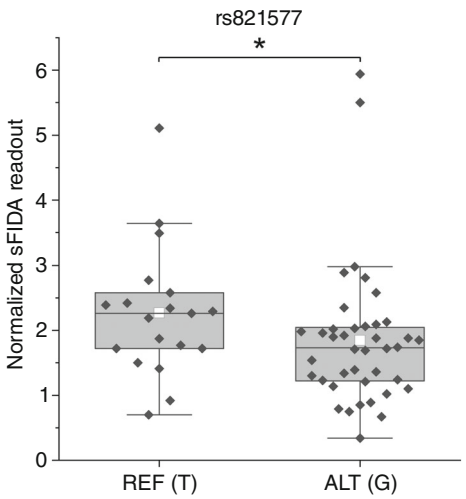

Fig. 4 Comparison of disrupted-in-schizophrenia 1 (DISC1) oligomer levels for DISC1 single-nucleotide polymorphism rs821577 for all samples using two-tailed Mann-Whitney U test. ALT, alternative nucleotide; G, guanine; REF, reference nucleotide; sFIDA, surface-based fluorescence intensity distribution analysis; T, thymine. Confidence interval, 0.05 (*$P \leq 0.05$).

PCN Psychiatry and Clinical Neurosciences

with FEP (Table S5), as well as the DISC1 oligomer levels for DISC1 SNPs in all individuals (Table S6, Fig. 4), we noted that rs821577[18] stood out in being associated with both the FEP groups and higher DISC1 oligomer levels. Statistical significance of this result, however, was lost after correcting for repetitive analysis.

Discussion

We report the presence of DISC1 protein aggregates, or oligomers, in CSF of patients with FEP and controls and their elevation in patients with FEP compared with controls. To this end, such DISC1 aggregates were shown only in *post mortem* brains of patients with chronic mental illnesses, and as coaggregates in patients with Huntington disease[19] and frontotemporal dementia.[20] A limitation is that these protein aggregates were in the femtomolar range and require highly sensitive methods such as sFIDA which are able to count single protein aggregates. sFIDA has been previously developed to exploit pathological oligomers and aggregates as a biomarker for neurodegeneration.[12,14–16] sFIDA has the capacity to measure single particles through fluorescence imaging, thereby making it possible to determine the size of aggregates larger than ~200 nm. However, due to the constraints posed by diffraction-limited optics, it is not feasible to directly analyze the size distribution of subresolution particles, including small protein aggregates and oligomers. The scope of future work will be to introduce subresolution size standards, which can be used for particle sizing based on pixel intensity. Furthermore, at this point we cannot make statements regarding measuring truncated versus full-length DISC1 protein. The small amounts of protein present, as measured with our highly sensitive method, makes proteomic investigations in this direction difficult.

Since it is likely that different biological causes are summarized in the clinical diagnosis of psychosis, DISC1 aggregates may have higher diagnostic validity for specific subsets of FEP as shown in receiver operating characteristic analyses (Fig. 3). However, there was only a low number of cases of SAD, thus making the present results preliminary. Moreover, schizophrenia subtypes were shown to have low diagnostic reliability and validity, resulting in their removal in DSM-5. Nevertheless, the present results suggest that DISC1 aggregate-positive cases may be clinically rather atypical and could overlap with symptoms from affective disorders. Supporting DISC1 aggregate-positive cases as a possible diagnostic category not matching current clinical boundaries is also the absence of solid correlations to cognitive symptom measures (Table 2). Interestingly, phenotypic variability spanning from schizophrenia to affective disorders has also been reported in the Scottish pedigree with DISC1 mutations where the *DISC1* gene was discovered.[5] In that context it is noteworthy that *DISC1* SNP rs821577 has been associated with bipolar disorders,[18] has been reported to be in complex epistasis with other DISC1 SNPs,[18,21] and was significantly associated with the FEP groups as well as elevated DISC1 oligomers, even though statistical significance did not survive correction for multiple testing. The low number of cases limits the significance of genetic studies, and the low number of SAD cases within the FEP group limits our ability to draw robust conclusions regarding genetics from the present data set.

It is also noteworthy that from the initial diagnosis at baseline, only one clinical subset of patients featured significantly elevated DISC1 aggregate levels (UNO) (Fig. 2c, Table S7), but that in the more solid clinical diagnosis after 18 months, the clinical subsets of SAD and SU turned out to be significantly associated with elevated DISC1 aggregate levels. This was mainly due to some individuals who at baseline were diagnosed as having OS (DSM-IV 295.40), on follow-up instead fulfilled criteria for PS (DSM-IV 295.30, seven cases) and SU (DSM-IV 295.9, five cases), and SAD (DSM-IV 295.70, one case).

For 21 patients with FEP (11 male, 10 female) and 15 HCs (three male, 12 females) in the present study, CSF samples were obtained again after 18 months, allowing us to investigate longitudinal differences. However, for both HCs (P=0.074) and patients with FEP (P=0.487), no significant changes in DISC1 aggregate levels were detected by Wilcoxon rank test (Fig. 5), indicating that DISC1 aggregate levels seem to be stable over the medium term and thus representing a more stable phenotype.

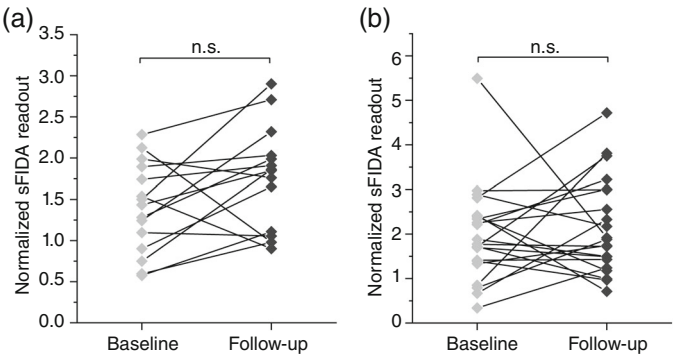

Fig. 5 Changes of disrupted-in-schizophrenia 1 (DISC1) aggregate levels in cerebrospinal fluid (CSF) samples over time. Baseline (inpatient admission) and follow-up levels (reassessment) of DISC1 aggregate in CSF of 15 healthy controls (HCs; a) and 21 patients with first-episode psychosis (FEP; b) were investigated for significant changes by Wilcoxon rank sum test (confidence interval, 0.05). For both HC and diseased samples, no significant changes in biomarker levels were observed, as indicated by *P*-values of 0.074 (HC) and 0.487 (FEP). sFIDA, surface-based fluorescence intensity distribution analysis.

In summary, we demonstrate, to our knowledge for the first time, that DISC1 protein aggregates can be detected in CSF and that levels are increased in patients with FEP. Increases were seen specifically in the subsets of SU or SAD, warranting more extensive, further studies, in conjunction with comprehensive genetic studies. The existence of a subset of patients with psychosis featuring high DISC1 aggregate levels, previously termed DISC1opathies,[22] strengthens the notion that this brain proteinopathy represents a novel pathogenic mechanism independent of familial or common genetic traits, and highlights DISC1 protein aggregates as a candidate biomarker for phenotypic characterization in FEP.

Author contributions

CK conceived the project. MP, ST, and JR developed the assay. MP designed and performed the experiments and validation studies and analyzed the sFIDA data. CSM and SC coordinated recruitment and clinical characterization of the patients. FP coordinated and performed the lumbar punctures. MP wrote the manuscript in consultation with OB, SE, FE, and CK. OB, SE, GE, SC, CS, DW, and CK supervised the project. All authors discussed the results and provided critical feedback.

Acknowledgments

We thank Volker Nischwitz (Central Institute for Engineering, Electronics and Analytics, Analytics (ZEA-3), Forschungszentrum Jülich, 52428, Jülich, Germany) for determination of silicon concentration of the used DISC1-coated SiNaPs by ICP-MS. CK was supported by a grant from the DFG 1679/14-1. The study was further supported by the Swedish Research Council (grant No. 523-2014-3467 (SC), Erling Persson Foundation (CMS), Vetenskapsrådet 2021-02251_VR (SE), 523-2014-3467 (SE), 2019-01452_VR (GE), Karolinska Institutet and Stockholm County Council (20160328, 20180487 [SC], (20190175) [SE], 20190447 [CMS]). Development of the sFIDA technology was supported by the programs Biomarkers Across Neurodegenerative Diseases I + II of The Alzheimer's Association, Alzheimer's Research UK, and the Weston Brain Institute (11084 and BAND-19-614337). We are also grateful for support from The Michael J. Fox Foundation for Parkinson's Research (14977, 009889), the ALS Association, and the Packard Center (19-SI-476). Open Access funding enabled and organized by Projekt DEAL.

Disclosure statement

The authors declare no competing nonfinancial interests but the following competing financial interests: DW and OB are shareholders of attyloid GmbH. FP has received gratuities for expertise testimony from Novartis, and has advised Chugai, Lundbeck and Roche. All other authors declare no competing financial interests related to this work.

Ethics statement

Ethical approval for the study was granted by the regional ethics committee in Stockholm (2010/879–31-1 with amendments).

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions. For image data analysis, we used the sFIDAta software tool, which can be made available on request from the corresponding author.

References

1. Trubetskoy VPA, Qi T, Pardiñas AF *et al*. Mapping genomic loci implicates genes and synaptic biology in schizophrenia. *Nature* 2022; **604**: 502–508.
2. Taylor JP, Hardy J, Fischbeck KH. Toxic proteins in neurodegenerative disease. *Science* 2002; **296**: 1991–1995.
3. Bradshaw NJ, Korth C. Protein misassembly and aggregation as potential convergence points for non-genetic causes of chronic mental illness. *Mol. Psychiatry* 2019; **24**: 936–951.
4. Hui KK, Endo R, Sawa A, Tanaka M. A perspective on the potential involvement of impaired Proteostasis in neuropsychiatric disorders. *Biol. Psychiatry* 2022; **91**: 335–345.
5. Millar JK, Wilson-Annan JC, Anderson S *et al*. Disruption of two novel genes by a translocation co-segregating with schizophrenia. *Hum. Mol. Genet.* 2000; **9**: 1415–1423.
6. Mathieson I, Munafo MR, Flint J. Meta-analysis indicates that common variants at the DISC1 locus are not associated with schizophrenia. *Mol. Psychiatry* 2011; **16**: 1–8.
7. Ishizuka K, Kamiya A, Oh EC *et al*. DISC1-dependent switch from progenitor proliferation to migration in the developing cortex. *Nature* 2011; **473**: 92–96.
8. Leliveld SR, Bader V, Hendriks P *et al*. Insolubility of disrupted-in-schizophrenia 1 disrupts oligomer-dependent interactions with nuclear distribution element 1 and is associated with sporadic mental disease. *J. Neurosci.* 2008; **28**: 3839–3845.
9. Cukkemane A, Becker N, Zielinski M *et al*. Conformational heterogeneity coupled with β-fibril formation of a scaffold protein involved in chronic mental illnesses. *Transl. Psychiatry* 2021; **11**: 639.
10. Ottis P, Bader V, Trossbach S *et al*. Convergence of two independent mental disease genes on the protein level: Recruitment of dysbindin to cell invasive DISC1 aggresomes. *Biol. Psychiatry* 2011; **70**: 604–610.
11. Trossbach SV, Bader V, Hecher L *et al*. Misassembly of full-length disrupted-in-schizophrenia 1 protein is linked to altered dopamine homeostasis and behavioral deficits. *Mol. Psychiatry* 2016; **21**: 1561–1572.
12. Kulawik A, Heise H, Zafiu C, Willbold D. Advancements of the sFIDA method for oligomer-based diagnostics of neurodegenerative diseases. *FEBS Lett.* 2018; **592**: 516–534.
13. Herrmann Y, Kulawik A, Kühbach K *et al*. sFIDA automation yields sub-femtomolar limit of detection for Aβ aggregates in body fluids. *Clin. Biochem.* 2017; **50**: 244–247.
14. Blömeke L, Pils M, Kraemer-Schulien V *et al*. Quantitative detection of α-Synuclein and tau oligomers and other aggregates by digital single particle counting. *NPJ Parkinsons Dis.* 2022; **8**: 68.
15. Schaffrath A, Schleyken S, Seger A *et al*. Patients with isolated REM-sleep behavior disorder have elevated levels of alpha-synuclein aggregates in stool. *NPJ Parkinsons Dis* 2023; **9**: 14.
16. Kass B, Schemmert S, Zafiu C *et al*. Aβ oligomer concentration in mouse and human brain and its drug-induced reduction ex vivo. *Cell Rep Med* 2022; **3**: 3.
17. Leliveld SR, Hendriks P, Michel M *et al*. Oligomer assembly of the C-terminal DISC1 domain (640-854) is controlled by self-association motifs and disease-associated polymorphism S704C. *Biochemistry* 2009; **48**: 7746–7755.
18. Hennah W, Thomson P, McQuillin A *et al*. DISC1 association, heterogeneity and interplay in schizophrenia and bipolar disorder. *Mol. Psychiatry* 2009; **14**: 865–873.
19. Tanaka M, Ishizuka K, Nekooki-Machida Y *et al*. Aggregation of scaffolding protein DISC1 dysregulates phosphodiesterase 4 in Huntington's disease. *J. Clin. Invest.* 2017; **127**: 1438–1450.
20. Endo R, Takashima N, Nekooki-Machida Y *et al*. TAR DNA-binding protein 43 and disrupted in schizophrenia 1 Coaggregation disrupts dendritic local translation and mental function in frontotemporal lobar degeneration. *Biol. Psychiatry* 2018; **84**: 509–521.
21. Schumacher J, Laje G, Jamra RA *et al*. The DISC locus and schizophrenia: Evidence from an association study in a central European sample and from a meta-analysis across different European populations. *Hum. Mol. Genet.* 2009; **18**: 2719–2727.
22. Korth C. Aggregated proteins in schizophrenia and other chronic mental diseases: DISC1opathies. *Prion* 2012; **6**: 134–141.

Supporting Information

Additional supporting information can be found online in the Supporting Information section at the end of this article.