White matter brain changes in chronic pancreatitis

A 7-year longitudinal follow-up study

Muthulingam, Janusiya Anajan; Olesen, Søren Schou; Hansen, Tine Maria; Drewes, Asbjørn Mohr; Frøkjær, Jens Brøndum

Published in:
Pancreatology

DOI (link to publication from Publisher):
10.1016/j.pan.2022.08.008

Publication date:
2022

Document Version
Publisher's PDF, also known as Version of record

Pancreatology 22 (2022) 871–879

Contents lists available at ScienceDirect

Pancreatology

journal homepage: www.elsevier.com/locate/pan

White matter brain changes in chronic pancreatitis: A 7-year longitudinal follow-up study

Janusiya Anajan Muthulingam [a, b], Søren Schou Olesen [b, c, d], Tine Maria Hansen [a, b], Asbjørn Mohr Drewes [b, c], Jens Brøndum Frøkjær [a, b, *]

[a] Mech-Sense, Department of Radiology, Aalborg University Hospital, Aalborg, Denmark
[b] Department of Clinical Medicine, Aalborg University, Aalborg, Denmark
[c] Centre for Pancreatic Diseases, Department of Gastroenterology & Hepatology, Aalborg University Hospital, Aalborg, Denmark
[d] Mech-Sense, Department of Gastroenterology and Hepatology, Aalborg University Hospital, Aalborg, Denmark

ARTICLE INFO

Article history:
Received 13 January 2022
Received in revised form
9 August 2022
Accepted 12 August 2022
Available online 22 August 2022

Keywords:
Chronic pancreatitis
Diffusion tensor imaging (DTI)
Magnetic resonance imaging (MRI)
Microstructural abnormalities white matter
Tobacco

ABSTRACT

Background/objectives: The progression of cerebral white matter changes over time has not been explored in chronic pancreatitis (CP). We aimed to characterize such alterations in individuals with CP at baseline and after 7-years as compared with controls and to explore associations to risk factors and clinical parameters.

Methods: Diffusion tensor imaging was used to evaluate 20 individuals with CP and 13 healthy controls at baseline and after 7-years (CP: n = 9, controls: n = 11). Tract-based spatial statistics were used to assess whole-brain white matter structure, extract significant fractional anisotropy (FA) clusters between groups, mean FA skeleton, mean FA and mean diffusivity (MD). FA of the extracted significant clusters between groups were used for regression analyses with risk factors and clinical parameters, including duration of CP, smoking, and diabetes.

Results: At baseline, widespread reductions in FA were found in CP compared to controls involving corpus callosum, the anterior, posterior thalamic radiation, and superior and posterior corona radiata (cluster volume: 49,431 mm^3, all $P < 0.05$). At baseline, also the mean FA ($P = 0.004$) and FA skeleton ($P = 0.002$) were reduced in CP compared to controls. FA of the extracted significant cluster was associated with the daily tobacco use ($P = 0.001$) and duration of CP ($P = 0.010$). At follow-up, the whole-brain FA skeleton was reduced by 1.7% for both CP individuals and controls ($P = 0.878$).

Conclusion: Individuals with CP had widespread cerebral white matter alterations at baseline that can likely be explained by the CP disease and exposure to toxic substances. Otherwise, further progression resembles that in healthy controls.

1. Introduction

Chronic pancreatitis (CP) is a chronic inflammatory and fibrotic disease of the pancreas, which often results in abdominal pain and reduced quality of life [1,2]. Both environmental factors such as excessive alcohol consumption and smoking as well as genetic risk factors are strongly associated with CP [3]. Accumulating evidence demonstrates that nicotine has the potential to facilitate pancreatic fibrosis in CP by promoting the activation of pancreatic stellate cells [4]. In addition to alcohol, smoking also seems to be associated with altered brain structure, particularly in the white matter [5,6]. Although it is well-known that the environmental factors (especially alcohol and smoking) are independent etiological risk factors for CP, the microstructural white matter brain changes in CP and the influence of these environmental factors remain unexplored. Particularly, CP has been associated with abnormal brain structure and function [7,8]. Some of these findings may be associated with pain (including central sensitization), malabsorption, diabetes, and toxic exposures that all characterize the disease [7–10]. Using magnetic resonance imaging (MRI), accumulating evidence points at widespread gray matter volume loss and cortical thinning, altered cerebral metabolites, and functional brain networks in individuals with CP with chronic pain [7–10]. However, it still

* Corresponding author. Mech-Sense, Department of Radiology, Aalborg University Hospital, P.O. Box 365, DK-9100, Aalborg, Denmark.
E-mail address: jebf@rn.dk (J.B. Frøkjær).

https://doi.org/10.1016/j.pan.2022.08.008

remains unclear whether the environmental factors i.e., alcohol, smoking and/or clinical outcomes, i.e., pain, contribute towards microstructural white matter changes in individuals with CP.

Prospective neuroimaging studies have only sparsely been conducted in CP. However, our recent 7-years follow-up study provided evidence of a reduction of gray matter volume in the precentral gyrus, putamen, and thalamus in individuals with CP compared with the baseline [9]. In addition to gray matter changes, diffusion tensor imaging (DTI) has shown white matter reductions in fractional anisotropy (FA) and high mean diffusivity (MD) in CP [11] and other diseases such as irritable bowel syndrome [12]. This reflects diffuse white matter changes with increased mobility of water molecules and disruption of tissue architecture [13,14]. Particularly, FA is an index of water diffusion directionality reflecting the structure of axonal cell membranes and myelin sheaths tracts [15]. To date, no study has examined white matter changes using DTI in CP over a longer follow-up period and its association with environmental risk factors as well as clinical outcomes.

We hypothesized that 1) individuals with CP with abdominal pain would have reduced FA in widespread brain regions as compared with healthy controls, 2) clusters of brain areas with reduced FA in comparison to controls would be associated with risk factors and clinical parameters, and 3) individuals with CP would exhibit more pronounced white matter changes over time than healthy controls. Therefore, the current longitudinal study aimed to investigate MRI diffusion parameters using the whole-brain tract-based spatial statistics (TBSS) approach to 1) determine microstructural white matter changes in individuals with CP as compared with healthy controls both at the regional and whole-brain level, 2) explore the associations between observed regional microstructural white matter changes and various clinical parameters as well as risk factors characteristics such as diabetes, CP duration, pain, smoking, and 3) characterize white matter alterations longitudinally over 7 years between individuals with CP and healthy controls.

2. Methods

2.1. Participants and study design

Twenty individuals with CP and 13 age- and sex-matched healthy controls were enrolled in the current study conducted at the Centre for Pancreatic Disease, Departments of Gastroenterology and Radiology, Aalborg University Hospital, Denmark. Individuals with CP were recruited from our outpatient clinic as a part of another trial assessing the effect of pregabalin on CP pain [16]. Subjects were seen on two occasions: 1) baseline visit (from 2009 to 2010) and 2) 7-year follow-up visit (from 2016 to 2017).

For individuals with CP, the inclusion criteria were as follows: 1) diagnosis of CP based on the Mayo Clinic diagnostic criteria [17], 2) chronic abdominal pain ($\geq$3 days per week for at least 3 months), and individuals with stable opioid and nonopioid medication, meaning that the individuals did not have any major adjustment on the medicine within one month prior to the inclusion of the study. Exclusion criteria for the CP group included 1) inability to undergo MRI, 2) major illness, i.e., cancer, 3) pain syndromes other than CP, 4) ongoing alcohol or drug abuse, and 5) history of major depression or psychiatric illness. Healthy controls were recruited among hospital staff at Aalborg University hospital and were age and sex-matched with the individuals with CP [18]. The healthy controls did not have any pain complaints and did not have any gastrointestinal symptoms. They were allowed to take their ordinary medicine, such as blood pressure medication but were excluded if they used central nervous system (CNS) active medication. Inclusion and exclusion criteria were identical for both the baseline visit and 7-year follow-up visit, and only participants who still maintained these criteria were re-scanned.

All subjects gave written informed consent before participating in the study. The local ethics committee approved the study of Northern Jutland (reference numbers N-20080028MCH and N-20090008). The study was conducted in accordance with the Declaration of Helsinki.

2.2. Clinical data: clinical parameters and risk factors

All individuals with CP were asked to fulfill a one-week pain diary before the baseline and follow-up visits using a visual analogue scale (VAS). The VAS was graded on a scale of 0–10, in which they rated the average and maximal daily pain intensities. They also completed the short form brief pain inventory (BPI) questionnaire at both visits [19]. The BPI questionnaire assessed the severity of pain and its interference with daily activities with two subscales (BPI pain and BPI interference). The pain subscale consisted of four items that measured pain score over the past 24 h, and each item was scored from 0 (no pain) to 10 (worst pain). The interference subscale indicated the extent of pain that disrupted daily activities with 0 (without interference) and 10 (maximum interference). Finally, we reviewed the electronic medical records for demographic and clinical characteristics, including etiology of CP based on the TIGAR-O system [20], duration of CP, the status of diabetes, and daily tobacco use (g/day), and pharmacological treatment, including use of opioids (morphine equivalent) and use of analgesics categorized according to the WHO-classification (none, weak and strong analgesics).

2.3. Magnetic resonance imaging data acquisition

Imaging data were collected on a 3 T MRI scanner (Signa HDxt; General Electrics, Milwaukee, WI, USA) equipped with an 8-channel phased-array head coil. For each participant, a high resolution 3-dimensional T1-weighted structural image was acquired, lasting 5.5 min. Following parameters were used for the structural scan: echo time 3.6 ms, repetition time 9.0 ms, 150 slices, a field of view 250 mm, flip angle 14°, resolution 0.78 $\times$ 0.78 mm, matrix size 320 $\times$ 320 mm, slice thickness 1 mm, full head coverage, no gap.

A DTI scan was conducted with the following parameters: repetition time 9000, and the minimum echo was used to achieve the highest image quality with the shortest time [range: 90.2–109.8 ms], 36 contiguous slices; FOV: 307 $\times$ 307 mm, matrix: 128 x 128, spatial resolution: 1.88 x 1.88 $\times$ 2.6 mm, directions: 32.

2.4. Diffusion tensor imaging – whole-brain tract-based spatial statistics (baseline visit)

DTI data processing and analysis were carried out using FMRIB Software Library (FSL) software, version 5.0.6 (fsl.fmrib.ox.ac.uk/fsl/fslwiki). Firstly, the DTI images were visually inspected before FSL's brain extraction tool (BET) was used for brain extraction with a threshold of 0.2 [21], and then the masks were quality controlled. Secondly, the DTI images were corrected for distortions and motion artifacts using FSL's eddy current correction. Thirdly, the FMRIB diffusion toolbox (FDT; version 5.0) was applied to fit a diffusion tensor model to the data for each voxel using DTIfit. Finally, FA values were calculated and fed into the voxel-wise whole-brain TBSS analysis [22,23].

Whole-brain TBSS analysis was performed between individuals with CP (n = 20) and healthy controls (n = 13) in FSL [22,23]. The FMRIB58 FA 1 mm standard space template image was used as the registration target for each subject's FA image. Each subject's FA

images were then aligned into standard 1 x 1 × 1 mm Montreal Neurological Institute 152 space using the nonlinear registration tool FNIRT, merged into a single 4D image. Next, the mean FA volume was estimated and thinned to generate the mean FA skeleton. The mean FA skeleton was thresholded at 0.2. Individual subjects aligned FA images were projected onto this skeleton, and the resulting skeletonized FA data were fed into the voxel-wise nonparametric permutation tests [24].

Whole-brain TBSS analysis was performed to investigate group differences in FA using randomize, a nonparametric permutation-based inference tool for thresholding statistical maps. Potential differences in age and gender could have an influence on DTI indices and were therefore included as covariates in the randomize step. The number of permutations was set to 5,000, and the threshold-free cluster enhancement option to correct for multiple comparisons was chosen. Voxel-wise statistics on skeletonized FA were conducted between groups using a series of unpaired two-sample t-tests. The resulting significant TFCE-corrected p-value statistical maps were thresholded at 0.95 (corresponding to $P = 0.05$), and a cluster extent threshold of 10 was applied. Fig. 1 shows the DTI image data processing flow chart.

2.5. Diffusion tensor imaging – whole-brain tract-based spatial statistics (follow-up visit)

For the follow-up visit, we quantified longitudinal changes in white matter microstructure using FSL software. The preprocessing and TBSS pipeline were the same as described for the baseline analysis pipeline. Two separate whole-brain TBSS analyses were performed: 1) for individuals with CP (n = 9) and 2) for healthy controls (n = 11) who completed both visits. The tool randomize in FSL was used to investigate group differences in FA between follow-up and baseline. Thus, age and gender were not included as covariates in the randomize step. The voxel-wise statistics on skeletonized FA were conducted between follow-up and baseline utilizing a series of paired two-sample t-tests for CP and healthy controls, respectively. The resulting significant TFCE-corrected p-value statistical maps were thresholded at 0.95 (corresponding to $P = 0.05$), and a cluster extent threshold of 10 was applied.

2.6. Extraction of FA values (baseline and follow-up visit)

For extraction of FA of significant clusters from the voxel-wise statistics on skeletonized FA (see above), fslmeants tool incorporated in FSL software was used with the 4D skeletonized FA image specified as the input, and the extracted FA values were imported to SPSS statistical software, version 27 (IBM Corp., Armonk, NY, USA) for further analysis. This was done for FA values of significant clusters at both baseline and follow-up.

2.7. Statistical analyses

All statistical analyses for demographical data, pain diary, and questionnaires were performed in SPSS statistical software. An unpaired *t*-test was performed at both baselines and at 7-year follow-up to examine whether groups differed in age. A Fisher's exact test of independence was also conducted to determine whether groups differed in sex.

At baseline, unpaired t-tests were performed to determine significant differences in DTI parameters (extracted mean FA skeleton, mean FA, and mean MD) between CP and healthy individuals. Moreover, putative associations between FA of significant clusters

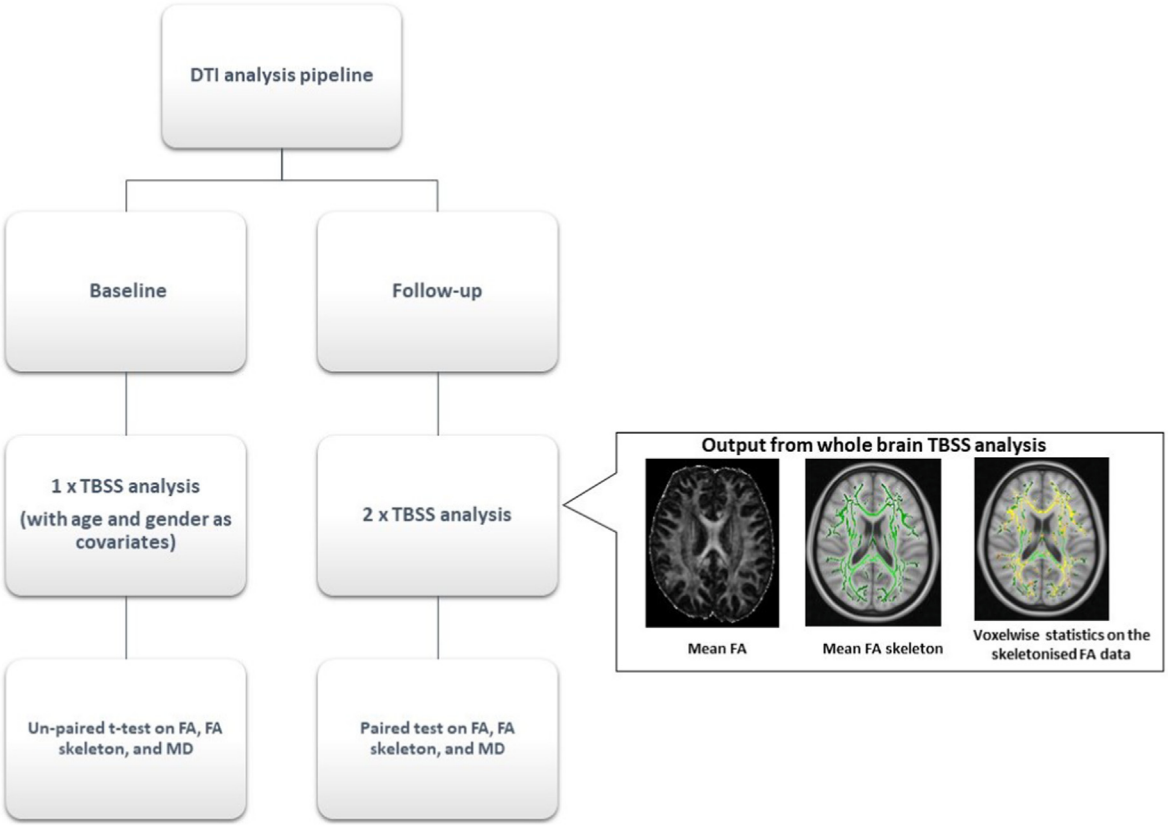

Fig. 1. DTI image data analysis pipeline. TBSS: Tract-based spatial statistic. FA: Fractional anisotropy. MD: Mean diffusivity.

and 1) duration of CP, 2) use of analgesics, 3) diabetes, 4) toxic-metabolic etiology of CP, 5) BPI pain score, 6) BPI interference, 7) daily tobacco use, and 8) average pain (VAS score) were analyzed using univariate regression analyses for individuals with CP only. Then, multivariate regression analysis with backward stepwise elimination (cutoff value of P = 0.1) was performed to evaluate independent predictors. No regression analyses were performed at follow-up due to the low sample size.

Paired t-tests were performed to compare the extracted mean FA skeleton, mean FA, and mean MD between baseline and 7-year follow-up for CP (n = 9) and healthy controls (n = 11).

Finally, the absolute differences (Δ) between both visits were calculated for FA skeleton, FA, and MD for both individuals with CP and healthy controls. Then, unpaired t-tests were performed for all three variables to assess any significant changes on Δ FA skeleton, Δ FA, and Δ MD between individuals with CP and healthy controls.

3. Results

3.1. Demographic and clinical characteristics

Demographic and clinical data are presented in Table 1. Twenty individuals with CP with a mean age of 52.2 (standard deviation (SD) 11.9) years and 13 healthy controls with a mean age of 47.3 (SD 10.9) years underwent brain MRI at baseline. Both groups were comparable in age and sex at baseline. Based on the medical records, only four CP individuals used CNS active drugs, see Supplementary material 1 for more information on medication of CP individuals at baseline. Nine individuals with CP and 11 healthy controls completed the follow-up visit, in which a significant age difference was present (P = 0.017). None of the individuals with CP had any psychiatric disorders during the follow-up period. Drop-out for follow-up was 55% (n = 11) for individuals with CP and 15% (n = 2) for healthy controls. Drop-out reasons for individuals with CP were the following: Moved to another place (n = 2), declining to participate (n = 4), death (n = 3), and continued excessive alcohol drinking (n = 2). The drop-out reason for healthy control was due to poor data quality (n = 2). No significant differences were found in FA skeleton, FA, and MD between individuals with CP who completed the study (n = 9) and drop-out individuals with CP (n = 11) based on the baseline scan (all P > 0.05), see Supplementary material 2.

3.2. Baseline

3.2.1. Whole-brain white matter microstructure

3.2.1.1. Whole-brain TBSS analysis. Whole-brain TBSS revealed that individuals with CP, as compared to healthy controls, had

Table 1
Demographic and clinical characteristics of individuals with chronic pancreatitis (CP) and healthy controls.

BASELINE	Individuals with CP (n = 20)	Healthy controls (n = 13)	*P-value*
Age, mean (SD), years	52.2 (11.9)	47.3 (10.9)	0.239
Sex, n (%)			0.503
Males	12 (60)	7 (54)	
Females	8 (40)	6 (46)	
Etiology of CP, n (%)			
Toxic-metabolic	11 (55)		
Idiopathic	5 [25]		
Genetic	2 [10]		
Recurrent and severe acute pancreatitis	1 [5]		
Obstructive	1 [5]		
Duration of CP, mean (SD), years	8.3 (7.3)		
Diary pain score, mean (SD), (VAS 0–10)			
Average pain	3.6 (1.9)		
Maximal pain	4.8 (2.1)		
Brief Pain Inventory short form, mean (SD)			
Pain score:	3.1 (1.7)		
Interference:	3.7 (2.1)		
Analgesic group, n (%):			
None	4 [20]		
Weak analgesics	5 [25]		
Opioids	11 (55)		
Use of opioids, mean (SD), morphine equivalent	62.2 (72.7)		
Diabetes mellitus, n (%)	6 (18.2)	0 (0)	
Daily tobacco use, mean (SD), g/day	14.25 (12.1)	N/A	

FOLLOW-UP	**Individuals with CP baseline (n = 9)**	**Individuals with CP follow-up (n = 9)**	**Healthy controls baseline (n = 11)**	**Healthy controls follow-up (n = 11)**	***P-value***
Age mean (SD), years	58.5 (7.3)	65.4 (7.2)	47.2 (11.5)	54.1 (11.0)	0.017*
Duration of CP, mean (SD), years	11.8 (9.1)	18.9 (9.0)			
Diary pain score, mean (SD), (VAS 0–10)					
Average pain	3.3 (2.1)	3.2 (2.9)			
Maximal pain	4.8 (2.1)	4.8 (3.3)			
Brief Pain Inventory short form, mean (SD)					
Pain score:	3.5 (1.7)	2.9 (3.1)			
Interference:	3.1 (2.2)	2.5 (2.6)			
Opioids. mean (SD), morphine equivalents	52.7 (83.3)	41.1 (84.9)			
Diabetes mellitus, n (%)	2 (22.2)	4 (44.4)	0 (0)	1 (9.1)	0.127

Descriptive statistical values are represented as percentages (%) or mean (standard deviation (SD)). n: numbers. Baseline comparisons included 20 CP and 13 controls, while follow-up comparisons included 9 CP and 11 controls. * Between CP and healthy controls at the follow-up visit. VAS: Visual analogue scale. CP: chronic pancreatitis.

significantly lower mean FA in one large cluster consisting of several white matter regions throughout the corpus callosum (genu, body, and splenium) and in the anterior, posterior thalamic radiation, and superior and posterior corona radiata (TFCE corrected P = 0.001, MNI coordinates (41, −16, −24), cluster volume: 49,431 mm^3), see Fig. 2. There were no regions with significantly increased mean FA as compared with healthy controls.

3.2.1.2. Group differences in the whole-brain mean FA skeleton, mean FA, and mean MD. As compared with healthy controls, individuals with CP showed a reduction in terms of whole-brain mean FA skeleton (P = 0.002), see Table 2. Similarly, declines were also found for individuals with CP in mean FA (P = 0.004), but not for mean MD (P = 0.884), see Table 2.

3.2.2. Association between microstructural white matter changes and clinical variables/risk factors

A significant association was observed at baseline between FA of the extracted significant cluster (from the voxel-wise statistics on skeletonized FA of whole-brain TBSS analysis, see above) and daily tobacco use (g/day) (coefficient: -0.00130 per g/day, P = 0.019), see Table 3. No relationship was found between FA of the extracted significant cluster and 1) duration of CP, 2) pain VAS score, 3) BPI pain score, 4) BPI interference score, 5) quality of life score, 6) diabetes, and 7) toxic-metabolic etiology of CP. In the multivariate model with backward stepwise elimination, an independent association to FA of the extracted significant cluster was found for daily tobacco use (coefficient: -0.00215 per g/day, P = 0.001) and duration of CP (coefficient: -0.000222 per year, P = 0.010), and with R^2 for the multivariate model of 0.45.

3.3. Follow-up after 7 years

3.3.1. Whole-brain white matter microstructural changes

3.3.1.1. Whole-brain TBSS analysis. TBSS analysis revealed no significant clusters of regional changes in whole-brain white matter over time for individuals with CP and healthy controls (both P > 0.05, TFCE corrected).

3.3.1.2. Group differences in whole-brain mean FA skeleton, mean FA, and mean MD. Compared with baseline, the whole-brain mean FA was reduced for individuals with CP (P = 0.043) at the follow-up visit, see Table 4. No significant changes were found for the mean FA skeleton and mean MD. Compared to baseline, healthy controls had reduced whole-brain FA skeleton (P < 0.001) and mean FA (P = 0.005) at the follow-up visit. No changes were observed for mean MD between baseline and follow-up, see Table 4. Individual data on mean FA skeleton is shown in Fig. 3.

At the follow-up visit, both individuals with CP and healthy controls showed a 1.7% reduction in mean FA of skeletonized white matter. No differences in the 7-year changes (Δ FA skeleton, Δ mean FA, and Δ mean MD) were observed between the individuals with CP and healthy controls (all P > 0.4), see Table 4.

4. Discussion

4.1. Summary

To the best of our knowledge, this is the first study to examine whole-brain white matter changes using TBSS analysis between individuals with CP and healthy controls at baseline and over a 7-year follow-up period. This investigation yielded two major findings. The first was that microstructural organization, as assessed by FA, was decreased at baseline in widespread regions of the brain in individuals with CP as compared to healthy volunteers. Moreover, the duration of CP and daily tobacco use were associated with these altered microstructural white matter measures. The second finding was that individuals with CP observed after 7 years had no significant further reduction of white matter changes compared to controls, both assessed with whole-brain and regional analysis. Our data suggest that the cerebral white matter alterations probably already had occurred in the earlier stages of CP, likely due to the CP disease itself and including toxic substances, i.e., smoking.

4.2. White matter changes in CP individuals at baseline

Given that FA reflects the structure of axonal cell membranes and myelin sheaths, a reduced FA may result from several conditions such as demyelination, axonal loss, gliosis, and inflammation [25]; hence our data implicate loss of white matter integrity and axonal injury [26]. Our observation of reduced white matter FA in CP as compared with healthy controls is concordant with other DTI studies [26–29] on individuals with CP [28], chronic pain patients with irritable bowel syndrome (IBS) [12], participants with exposure to smoking [29], and participants with exposure to alcohol [30]. Ellingson et al. found that IBS patients had reduced FA in thalamic regions, the basal ganglia, and sensory/motor regions as compared with healthy controls, suggesting that IBS patients with

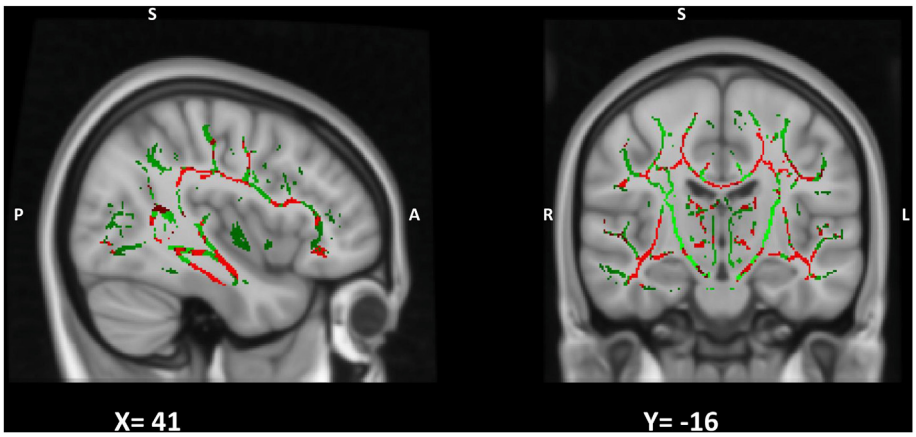

Fig. 2. Microstructural white matter changes between individuals with chronic pancreatitis and healthy controls at the baseline visit. Representative slices (X and Y coordinates) from tract-based spatial statistics analysis, showing the main white matter tract map with green. Red represented the white matter fiber bundle skeleton area with decreased fractional anisotropy value between individuals with chronic pancreatitis and healthy controls. P: Posterior. S: Superior. A: Anterior. R: Right. L: Left.

Table 2
Group differences in the whole-brain mean FA skeleton, mean FA, and mean MD between individuals with chronic pancreatitis (CP) and healthy controls.

	DTI parameter	Individuals with CP (n = 20)	Healthy controls (n = 13)	
Whole-brain TBSS analysis				
Whole-brain		**(mean±SD)**	**(mean±SD)**	**P-value**
	FA skeleton	0.414 ± 0.029	0.443 ± 0.013	0.002*
	FA	0.253 ± 0.019	0.272 ± 0.011	0.004*
	MD (x 10^{-4} mm^2/s)	1.82 ± 0.22	1.83 ± 0.39	0.884

SD: standard deviation. FA: fractional anisotropy. MD: mean diffusivity. * Significant difference.

Table 3
Univariate and multivariate analysis of disease characteristics and risk factors possible associated with decreased FA (based on the extracted significant cluster between CP individuals and healthy controls). All individuals with CP (n = 20) from baseline visits were included in the models.

Model	Variables	Coefficient (95% confidence interval)	P-value
Significant FA cluster			
Univariate model	Duration of CP	−.0000131 (−.0001902 .000164)	0.878
	Analgesic use	.00316 (−.01560 .02190)	0.727
	Diabetes	−.0174 (−.0482 .0134)	0.249
	Toxic-metabolic etiology	.000092 (−.029472 .029656)	0.995
	BPI pain	−.00271 (−.01126 .00583)	0.512
	BPI interference	−.000916 (−.008507 .006675)	0.802
	Tobacco	−.00130 (−.00236 -.00024)	0.019*
	VAS mean	.00147 (−.00671 .00964)	0.710
Backward stepwise model	Tobacco	−.00215 (−.00331 -.00099)	0.001*
	Duration of CP	−.000221 (−.000383 -.000060)	0.010*

FA: Fractional anisotropy. CP: Chronic pancreatitis. VAS: Visual analogue scale. BPI: brief pain inventory. * Significant difference.

Table 4
Group changes in whole-brain mean FA skeleton, mean FA, and mean MD at follow-up compared to baseline.

	DTI parameter	CP individuals			Healthy controls		
Whole brain TBSS							
Whole brain		**Baseline (n = 9)**	**Follow-up (n = 9)**	**P-value**	**Baseline (n = 11)**	**Follow-up (n = 11)**	**P-value**
	FA skeleton	0.477 ± 0.02	0.4693 ± 0.022	0.079	0.497 ± 0.01	0.489 ± 0.017	<0.001*
	FA	0.254 ± 0.013	0.249 ± 0.014	0.043*	0.271 ± 0.012	0.264 ± 0.012	0.005*
	MD (x 10^{-4} mm^2/s)	1.80 ± 0.20	1.76 ± 0.21	0.231	1.88 ± 0.37	1.77 ± 0.23	0.215
Δ (change over 7 years)	Δ FA skeleton	−0.0079 ± 0.0118			−0.0086 ± 0.007		0.878
	Δ FA	−0.0054 ± 0.0067			−0.0082 ± 0.0075		0.408
	Δ MD	0.0000 ± 0.00001			0.0000 ± 0.00003		0.417

CP: Chronic pancreatitis. Data are provided as mean ± SD. n: numbers. FA: fractional anisotropy. MD: mean diffusivity. Δ: delta. * Significant difference.

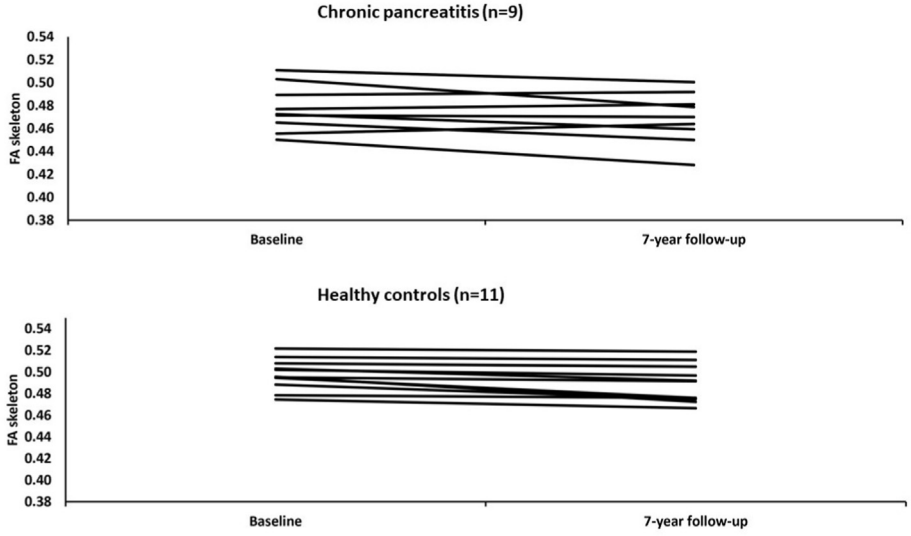

Fig. 3. Individual data on whole-brain mean FA skeleton changes over time in individuals with chronic pancreatitis and healthy controls. FA: Fractional anisotropy.

chronically recurring visceral pain have long-term microstructural changes in regions associated with the integration of sensory information and cortico-thalamic modulation [12]. The finding of lower FA in the posterior thalamic radiation in individuals with CP at baseline could also be indicative of long-term neural reorganization of chronic pain pathways and regions associated with sensory integration. Reduced white matter FA in the widespread areas of frontal and temporal regions has also been observed in depression, indicating loss of integrity within frontal and temporal white matter tracts [31]. A possible explanation of reduced FA in individuals with CP could also be related to cognitive disturbances in individuals with CP [32], but this should be investigated in future studies.

In the present study, the reduction of FA in the extracted significant cluster was associated with daily use of tobacco. This finding is consistent with DTI studies conducted in participants with exposure to smoking [5], suggesting that nicotine and other toxic substances can impact the microstructure of white matter in a specific area of the brain [5]. Previous DTI studies on subjects with exposure to alcohol provide strong evidence of widespread reduction of FA in tracts of the corpus callosum, internal and external capsule, cingulate, fornix, longitudinal fasciculi, fronto-occipital, and cortico-striatal regions [33]. Often, individuals with CP with alcohol use also have a high prevalence of smoking [34]. Thus, it can be speculated that the relationship between smoking and the development/progression of FA changes in CP is biased since it is difficult to separate the effects of toxic exposures [34]. Since we did not collect any quantitative data on alcohol use and given that tobacco and alcohol use is typically linked, we can in our study only conclude on a general potential toxic effect on the brain.

In contrast to the previous study by Frøkjær et al. [11], demonstrating a negative correlation between pain and FA in individuals with CP, we found no such association. Lack of association between brain changes and pain has also been demonstrated in previous structural and functional MRI studies with individuals with CP [7,9]. These studies suggest that the alcoholic etiology of CP is likely the primary driver of the brain alterations, potentially obscuring and hindering the association between brain structure and pain from being discovered. This might also explain the lack of association between FA changes and pain in the current study, given that toxic factors, i.e., smoking, were associated with white matter alteration.

4.3. Microstructural white matter changes over time

In our previous neuroimaging study with the same cohort of individuals with CP, we have reported reduced gray matter volume and reduced cortical thickness over a 7-year follow-up period as compared to the healthy controls [9]. Given that individuals with CP have gray matter changes, reflecting alterations in neurons and glia cells, it is also expected that the corresponding white matter density connected to the neurons should be affected [9,27]. However, our results did not reveal significant progression in white matter changes over time compared with healthy controls. Hence, our data could suggest that gray matter and white matter changes may represent different pathophysiological processes in individuals with CP. Thus, the pathophysiological features of CP in the early disease stages may be characterized by white matter reduction.

In contrast, the gray matter changes probably evolve slowly over time during the later stages of CP. Furthermore, the lack of further FA reduction in white matter over time in individuals with CP with longer-lasting disease indicates that white matter has a less influential role than gray matter for the further progression of established CP. Similar findings have also been observed in other studies, such as migraine [12,35].

The lack of further progression of whole-brain white matter changes as compared to healthy controls could likely be explained by the neurotoxic effects of both alcohol and smoking. Hudkins et al. proposed a model whereby FA would rise during the early years of smoking and then decline with continued smoking in later years [29]. We found reduced FA in widespread regions for individuals with CP as compared with healthy controls at baseline, which was furthermore negatively correlated with the use of tobacco. This indicates that the individuals with CP in our study might have been exposed to smoking (and likely also alcohol) for a long period before they were included in the study. Although we do not have systematic data regarding smoking at follow-up, it is presumed that the individuals with CP during the 7-year follow-up may have quit smoking as this is highly recommended as part of the treatment, and are well-treated, which could explain the lack of accelerated white matter decline at 7-year follow-up. Also, the CP individuals included were not allowed to have ongoing alcohol misuse, meaning that the individuals with CP might also mimic the healthy controls in terms of lifestyle at the time point of the follow-up investigation for this study. However, this should be confirmed by future studies collecting lifestyle data systematically. Overall, our data suggest minimal white matter decline over time that mimics the normal aging seen in healthy controls. Due to the lack of systematic data regarding lifestyle, it is not possible to comprehensively explain the potential factors normalizing the further decline in FA compared to healthy controls. We are confident that FA changes observed at baseline can be explained by CP accompanied by risk factors and poor lifestyle. However, there is substantial uncertainty regarding the lifestyle change during the follow-up period. Thus, it is challenging to uniquely identify the factors driving the microstructural white matter changes. Given that the individuals with CP and healthy controls developed comparably regarding FA at follow-up, some of the individuals with CP may have gone through lifestyle changes. To confirm this, future studies need to systematically collect lifestyle changes during the follow-up period.

4.4. Limitations

There are several limitations of this study. Firstly, it should be noted that systematic data regarding daily alcohol use was not collected for both individuals with CP and healthy controls, and similarly, data on how long individuals with CP have been abstinence before being included in the study was not collected either; thus, our findings should be interpreted cautiously, and our results, should be confirmed by future studies collecting lifestyle data systematically. Secondly, the sample size for the follow-up study was relatively small due to a high drop-out rate (55% for individuals with CP and 15% for healthy controls). The fact that about half of the CP individuals discontinued may have contributed to the DTI results at the 7-year follow-up, as these could potentially have been those where microstructural white matters progressed the most, leading to selection bias with potential under-estimation of FA during the follow-up period. However, speculations can be raised about whether CP individuals who dropped out were more sick than the individuals who completed both visits, since we found no differences on all the DTI parameters between drop-out group and non-drop-out group. Thus, it remains unknown if drop-out of individuals with CP are the reason for not detecting progression in white matter abnormalities. Thirdly, some individuals with CP were taking medications with effects on the brain, including opioids, which may have impacted our findings. However, in the current study, only four of the twenty CP individuals used CNS active drugs, and we consider that our findings are not driven by those four individuals. Similarly, other confounding factors may potentially be relevant to the main findings, i.e., minor psychiatric conditions like

anxiety. Fourthly, to assess whether smoking uniquely is associated with reduced FA in CP, a control group of individuals with CP who are non-smokers should be included in future studies. Fifthly, the TBSS approach is primarily suitable for detecting large fiber bundles; thus, small fibers may be underestimated. Finally, newer DTI protocols would be more optimal, i.e., an increased number of directions and additional measures such as radial and axial diffusivity may provide more specific data than a composite measure of FA.

4.5. Conclusion

Our findings in individuals with longstanding CP and chronic abdominal pain showed significant widespread alterations in white matter integrity compared to healthy controls. In addition, these white matter changes were associated with tobacco use and the duration of CP. Progression of whole-brain FA was seen for both healthy controls and individuals with CP after 7 years, and a tendency of FA skeleton decline in individuals with CP. Taken together, the cerebral white matter alterations probably already had occurred in the earlier stages of CP, likely explained by the neurotoxic effects of smoking and previous alcohol misuse, even though the impact of chronic pain cannot be excluded. The results provide a basis for understanding the longitudinal natural history of microstructural brain changes in individuals with CP. However, future research is needed to confirm our findings in larger cohorts.

Grant support

The study was conducted as a sponsor-investigator-initiated study with financial support from Independent Research Fund Denmark (DFF-7016-00073), Axel-Muusfeldts Foundation, and Doctor Sofus Carl Emil Friis and wife Hustru Olga Doris Friis' Grant.

Authorship statement

JBF, JAM, SSO, TMH, and AMD conceived and designed the study and participated in the planning of the study. JAM collected the data. JAM analyzed the data and drafted the initial version of the manuscript. All authors made significant contributions to the development and conceptualization of the protocol. All authors reviewed the draft versions of the manuscript and have read and approved the final manuscript.

Declaration of competing interest

The authors declare no conflicts of interest.

Acknowledgment

We thank Dr. Emily Steinkohl for feedback on the manuscript and fruitful discussions. We would also like to thank Kenneth Krogh Jensen for his help with data collection. Finally, we would like to thank all our funding sources. The authors declare no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pan.2022.08.008.

References

[1] Singh VK, Yadav D, Garg PK. Diagnosis and management of chronic pancreatitis: a review. JAMA, J Am Med Assoc 2019;322(24):2422–34.
[2] Drewes AM, Bellin MD, Besselink MG, Bouwense SA, Olesen SS, van Santvoort H, et al. Assessment of pain associated with chronic pancreatitis: an international consensus guideline. Pancreatology 2021;21(7):1256–84. https://doi.org/10.1016/j.pan.2021.07.004.
[3] Singh VK, Yadav D, Garg PK. Diagnosis and management of chronic pancreatitis: a review. JAMA, J Am Med Assoc 2019;322(24):2422–34.
[4] Li Z, Lu D, Jin T, Liu X, Hao J. Nicotine facilitates pancreatic fibrosis by promoting activation of pancreatic stellate cells via α7nAChR-mediated JAK2/STAT3 signaling pathway in rats. Toxicol Lett 2021;349(8):84–91.
[5] Huang H, Zhang Y, Cheng J, Wang W, Wen M. Evaluating the changes of white matter microstructures in tobacco addicts based on diffusion tensor imaging. Med Sci Monit 2020;26:e919105.
[6] Karoly HC, Skrzynski CJ, Moe EN, Bryan AD, Hutchison KE. Exploring relationships between alcohol consumption, inflammation and brain structure in a heavy drinking sample. Alcohol Clin Exp Res 2021;45(11):2256–70.
[7] Muthulingam JA, Hansen TM, Olesen SS, Drewes AM, Frokjaer JB. Altered brain morphology in chronic pancreatitis patients and its association with pain and other disease characteristics. Eur J Gastroenterol Hepatol 2019;31(9):1092–8.
[8] Muthulingam JA, Hansen TM, Drewes AM, Olesen SS, Frøkjær JB. Disrupted functional connectivity of default mode and salience networks in chronic pancreatitis patients. Clin Neurophysiol 2020;131(5):1021–9.
[9] Muthulingam J, Olesen SS, Hansen TM, Seminowicz DA, Burrowes S, Drewes AM, et al. Progression of structural brain changes in patients with chronic pancreatitis and its association to chronic pain: a 7-year longitudinal follow-up study. Pancreas 2018;47(10):1267–76.
[10] Hansen TM, Muthulingam JA, Drewes AM, Olesen SS, Frøkjær JB. Cingulate glutamate levels associate with pain in chronic pancreatitis patients. NeuroImage Clin 2019:101925. https://doi.org/10.1016/j.nicl.2019.101925.
[11] Frøkjær JB, Olesen SS, Gram M, Yavarian Y, Bouwense SAW, Wilder-Smith OHG, et al. Altered brain microstructure assessed by diffusion tensor imaging in patients with chronic pancreatitis. Gut 2011;60(11):1554–62.
[12] Ellingson BM, Mayer E, Harris RJ, Ashe-McNally C, Naliboff BD, Labus JS, et al. Diffusion tensor imaging detects microstructural reorganization in the brain associated with chronic irritable bowel syndrome. Pain 2013 Sep;154(9):1528–41.
[13] Hubbard CS, Becerra L, Heinz N, Ludwick A, Rasooly T, Yendiki A, et al. Microstructural white matter abnormalities in the dorsal cingulum of adolescents with IBS. eNeuro 2018;5(4). ENEURO.0354-17.2018.
[14] Schneider R, Genç E, Ahlborn C, Gold R, Lukas C, Bellenberg B. Temporal dynamics of diffusion metrics in early multiple sclerosis and clinically isolated syndrome: a 2-year follow-up tract-based spatial statistics study. Front Neurol 2019;10:1165.
[15] Gan J, Zhong M, Fan J, Liu W, Niu C, Cai S, et al. Abnormal white matter structural connectivity in adults with obsessive-compulsive disorder. Transl Psychiatry 2017;7(3):e1062–7. https://doi.org/10.1038/tp.2017.22.
[16] Olesen SS, Bouwense SAW, Wildersmith OHG, Van Goor H, Drewes AM. Pregabalin reduces pain in patients with chronic pancreatitis in a randomized, controlled trial. Gastroenterology 2011;141(2):536–43.
[17] Layer P, Yamamoto H, Kalthoff L, Clain JE, Bakken LJ, DiMagno EP. The different courses of early- and late-onset idiopathic and alcoholic chronic pancreatitis. Gastroenterology 1994;107(5):1481–7.
[18] Bouwense SAW, Olesen SS, Drewes AM, Poley JW, van Goor H, Wilder-Smith OHG. Effects of pregabalin on central sensitization in patients with chronic pancreatitis in a randomized, controlled trial. PLoS One 2012;7(8):e42096..
[19] Cleeland CS. Brief pain inventory (BPI). Cleeland CS MD. Anderson Cancer Cent 1982;1100:6.
[20] Etemad B, Whitcomb DC. Chronic pancreatitis: diagnosis, classification, and new genetic developments [Internet] Gastroenterology 2001;120(3):682–707. http://www.ncbi.nlm.nih.gov/pubmed/11179244.
[21] Smith SM. Fast robust automated brain extraction. Hum Brain Mapp 2002;17(3):143–55.
[22] Smith SM, Smith SM, Johansen-berg H, Johansen-berg H, Jenkinson M, Jenkinson M, et al. Acquisition and voxelwise analysis of multi-subject diffusion data with tract-based spatial statistics. Nat Protoc 2007;2(3):499–503. http://www.ncbi.nlm.nih.gov/pubmed/17406613.
[23] Smith SM, Jenkinson M, Johansen-Berg H, Rueckert D, Nichols TE, Mackay CE, et al. Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. Neuroimage 2006;31(4):1487–505.
[24] Winkler AM, Ridgway GR, Webster MA, Smith SM, Nichols TE. Permutation inference for the general linear model [Internet] Neuroimage 2014;92:381–97. https://doi.org/10.1016/j.neuroimage.2014.01.060.
[25] Yu D, Yuan K, Qin W, Zhao L, Dong M, Liu P, et al. Axonal loss of white matter in migraine without aura: a tract-based spatial statistics study. Cephalalgia 2013;33(1):34–42.
[26] Fang J, Li S, Li M, Chan Q, Ma X, Su H, et al. Altered white matter microstructure identified with tract-based spatial statistics in irritable bowel syndrome: a diffusion tensor imaging study. Brain Imaging Behav 2017;11(4):1110–6.
[27] Taylor FR. Migrainerelated gray matter and white matter changes at a 1-year follow-up evaluation, vol. 54. Headache; 2014. p. 926.
[28] Frokjaer JB, Olesen SS, Gram M, Yavarian Y, Bouwense SaW, Wilder-Smith OHG, et al. Altered brain microstructure assessed by diffusion tensor imaging in patients with chronic pancreatitis. Gut 2011;60(11):1554–62.
[29] Hudkins M, O'Neill J, Tobias MC, Bartzokis G, London ED. Cigarette smoking and white matter microstructure. Psychopharmacology (Berl) 2012;221(2):

285–95.
[30] Pfefferbaum A, Sullivan EV. Disruption of brain white matter microstructure by excessive intracellular and extracellular fluid in alcoholism: evidence from diffusion tensor imaging. Neuropsychopharmacology 2005;30(2):423–32.
[31] Nobuhara K, Okugawa G, Sugimoto T, Minami T, Tamagaki C, Takase K, et al. Frontal white matter anisotropy and symptom severity of late-life depression: a magnetic resonance diffusion tensor imaging study. J Neurol Neurosurg Psychiatry 2006;77(1):120–2.
[32] Jongsma MLA, Postma SAE, Souren P, Arns M, Gordon E, Vissers K, et al. Neurodegenerative properties of chronic pain: cognitive decline in patients with chronic pancreatitis. PLoS One 2011;6(8):e23363.
[33] Zahr NM, Pfefferbaum A. Alcohol's effects on the brain: neuroimaging results in humans and animal models. Alcohol Res 2017;38(2):183–206.
[34] Singhvi Ajay, Yadav Dhiraj. Myths and Realities about alcohol and smoking in chronic pancreatitis HHS Public Access. Physiol Behav 2016;176(1):139–48.
[35] Liu J, Lan L, Li G, Yan X, Nan J, Xiong S, et al. Migraine-related gray matter and white matter changes at a 1-year follow-up evaluation. J Pain 2013 Dec;14(12):1703–8.