

Tracking Pain in Resting State Networks in Patients with Hereditary and Diabetic Neuropathy

Aslıhan TAŞKIRAN SAĞ¹, Arzu Ceylan HAS², Neşe ÖZTEKİN¹, Çağrı Mesut TEMUÇİN³, Kader KARLI OĞUZ⁴

¹Department of Neurology, Ankara Numune Training and Research Hospital, Ankara, Turkey

²National Magnetic Resonance Research Center, Bilkent University, Ankara, Turkey

³Department of Neurology, Hacettepe University Faculty of Medicine, Ankara, Turkey

⁴Department of Radiology, Hacettepe University Faculty of Medicine, Ankara, Turkey

ABSTRACT

Introduction: Chronic pain is associated with maladaptive plastic changes in the brain. It is usually more prominent in acquired pathologies of nerve fibers as in diabetic neuropathy despite less severe degeneration than hereditary neuropathies. Based on clinical differences concerning pain perception, we hypothesized that functional connectivity analysis would reveal distinct patterns in resting-state networks in these groups.

Methods: Ten diabetic patients with painful neuropathy (5F/5M; mean age=50.10±6.05 years), 10 patients with hereditary neuropathy (5F/5M; mean age=37.80±14.01 years), 18 age- and gender-matched healthy controls (eight for painful diabetic neuropathy and 10 for hereditary neuropathy) and seven diabetic controls without painful neuropathy were enrolled in the study. All subjects (n=45) underwent a 5-min resting-state scan in a 3T magnetic resonance scanner. The images were analyzed with seed-based functional connectivity method. The group-level maps of the default mode network and insula-cingulate network were identified for each group.

Results: Patients with hereditary neuropathy displayed increased connectivity between left insula and left anterior cingulate cortex and inversely correlated activity between left insula and left inferior parietal lobule compared to their controls. In patients with painful diabetic neuropathy, the major findings were the increased connectivity between left anterior cingulate cortex and posterior cingulate cortex/precuneus, and the increased connectivity between medial prefrontal cortex and left medial temporal region compared to their controls.

Conclusion: This study revealed that hereditary and diabetic painful neuropathy patients exhibit different patterns of functional connectivity. The clinical differences in these groups regarding the presence of neuropathic pain may relate to this difference in cortical organization.

Keywords: Default-mode network, functional connectivity, insula, cingulate, neuropathic pain, Charcot-Marie-Tooth

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INTRODUCTION

Neuropathic pain is caused by a lesion or a disease of the somatosensory system itself (1). Any disruption of the pain-signaling pathway along its course from periphery to brain leads to chronic neuropathic pain.

It is striking that neuropathic pain is quite common and usually moderate to severe in acquired pathologies of nerve fiber (2) despite less severe degeneration compared to hereditary neuropathies (HNP). Most of the patients with hereditary motor and sensory neuropathy (HMSN) 1 or 2 do not complain of severe neuropathic pain but motor and sensorial deficits (3). On the basis of clinical differences concerning pain perception, we hypothesized that resting state functional connectivity analysis would reveal distinct patterns in patients with HNP and painful diabetic neuropathy (DNP).

The insula, cingulate cortex, primary and secondary somatosensory cortices, posterior parietal regions, anterior frontal and supplementary motor areas are commonly considered as the pain-processing circuit

and have shown activation with painful stimuli in functional imaging studies (4). Yet, it is questionable whether pain-sensing areas are different from pain generating and propagating sites in the brain. Many studies have addressed functional connectivity alterations in various chronic pain diseases. Cauda *et al* investigated the spontaneous component of neuropathic pain with resting state functional connectivity analysis and determined two different patterns in default mode network (DMN) (5). Since pain has somatosensory, attentional, affective and self-monitoring aspects, we decided to focus on two resting state networks that include nodes with the aforementioned functions of interest, i. e. default mode and insula-cingulate networks. DMN is mainly composed of medial prefrontal cortex, cingulate cortex, inferior parietal lobules and medial temporal structures (6). It is a baseline-organized network of the brain at rest, which is suspended during specific mental tasks (7). It functions during introspection, mind-wandering and self-referential processes (8). Insula-cingulate network anchoring bilateral anterior cingulate cortices and insula functions as salience network (9, 10). This study aimed to give a

Correspondence Address: Aslıhan Taşkıran Sağ, Hacettepe Üniversitesi Nörolojik Bilimler ve Psikiyatri Enstitüsü, Ankara, Turkey

E-mail: aslihantaskiran@gmail.com

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descriptive report of connectivity patterns of these pain-related networks in hereditary and painful diabetic neuropathy patients compared to healthy control subjects.

METHODS

Patient Selection

Ten right-handed diabetic patients with painful neuropathy (5F/5M; mean age=50.10±6.05 years) and 10 right-handed patients with hereditary neuropathy (5F/5M; mean age=37.80±14.01 years) were enrolled in the study (Table 1). All the patients were met consecutively in the outpatient clinics. Exclusion criteria included current or lifetime diagnosis of stroke, epilepsy, depression, anxiety disorder, somatization disorder, or any other psychiatric disease that can affect pain threshold, chronic painful states (e.g. migraine, lumbalgia, fibromyalgia), alcohol/drug abuse, medication use for neuropathic pain during the last month, chronic analgesic/antidepressant use, and any contraindication for MRI. All patients underwent complete neurological examination, EMG study, standardized mini mental test and pain scales (visual analogue scale-VAS, painDETECT-PD-Q) (11, 12). All the subjects with pain had the complaint for longer than six months.

Eighteen age- and gender-matched healthy controls (eight for painful diabetic neuropathy and 10 for hereditary neuropathy) and seven diabetic controls without painful neuropathy meeting the same criteria were enrolled.

All subjects gave their informed consent, in line with the Declaration of Helsinki. The study was approved by the Local Ethics Committee.

Imaging Procedure

Data Acquisition

All imaging data were obtained with a 3T MR scanner (Magnetom, Trio TIM system, Siemens, Germany) equipped with an 8-channel phase-array head coil. The whole brain was scanned using a T2* weighted gradient

echo spiral pulse sequence (repetition time [TR] 2000 ms, time to echo [TE] 35 ms, flip angle (FA) 75° and 1 interleaved, slice number: 28, slice thickness: 3.4 mm, interslice gap: 20%). The field of view was 220×220 mm², and the matrix size was 64×64, giving an in-plane spatial resolution of 3.4 mm. All subjects underwent a 150 dynamic resting-state series during a 5-min scan. They were given no specific instruction except to keep their eyes closed and hold still. Imaging protocol also included T1-weighted 3D high resolution images with 0.9 mm isotropic voxels (magnetization prepared rapid gradient echo-MPRAGE) (TR/TE: 2600/3.4 ms, FA: 90, FOV: 256 mm; matrix: 224×256; distance factor: 50%).

The resting state-fMRI scans were preprocessed using SPM8 (<http://www.fil.ion.ucl.ac.uk/spm/software/spm8/>). Preprocessing of the resting state-fMRI data included motion correction, realignment, slice-timing correction, coregistration and normalization, and spatial smoothing. Each subject's functional volume was realigned to the first volume and ordered with slice timing and then co-registered with the structural image. All images were normalized to the SPM8 Montreal Neurological Institute template (MNI), and functional images were smoothed with a 6 mm³ isotropic Gaussian kernel.

Functional Connectivity Analysis (Group analysis)

Using the CONN toolbox in MATLAB R2008a (www.nitrc.org/projects/conn/), we identified principal components associated with segmented white matter (WM) and cerebrospinal fluid (CSF) for each subject and entered WM, CSF and realignment parameters as confounds in a first-level analysis (13). The data were band-pass filtered to 0.008 Hz-0.09 Hz.

We then used a seed-voxel analysis and specified connectivity patterns separately for twelve seed regions as spheres with a 6-mm radius using the CONN and Montreal Neurological Institute (MNI) atlas. These regions were located in bilateral cingulate cortex, dorsolateral prefrontal cortex, insular cortex, parahippocampal cortex, anterior cingulate regions and midline medial prefrontal cortex, and posterior cingulate cortex/precuneus (Table 2). These seeds were selected because they were

Table 1. Clinical properties of the patients

| Patient | Age, sex | DNP | HNP | Pain duration (months) | VAS (0–100 mm) | PD-Q (0–38) | HbA1 c | SMMT |
|---------|----------|-----|----------|------------------------|----------------|-------------|--------|------|
| 1 | 51, F | + | | 6 | 60 | 18 | 8.4 | 30 |
| 2 | 52, M | + | | 12 | 50 | 21 | 6.8 | 29 |
| 3 | 50, M | + | | 120 | 90 | 19 | 11.4 | 29 |
| 4 | 54, F | + | | 30 | 80 | 22 | 8.9 | 24 |
| 5 | 37, M | + | | 15 | 70 | 23 | 6.3 | 30 |
| 6 | 46, M | + | | 18 | 70 | 20 | 6.8 | 30 |
| 7 | 58, F | + | | 24 | 90 | 21 | 5.7 | 26 |
| 8 | 38, M | | +(HMSN1) | 0 | 0 | 6 | | 29 |
| 9 | 36, F | | +(HMSN1) | 48 | 40 | 11 | | 28 |
| 10 | 19, M | | +(HMSN1) | 0 | 0 | 6 | | 28 |
| 11 | 31, F | | +(HMSN2) | 0 | 0 | 9 | | 26 |
| 12 | 47, M | | +(HMSN1) | 12 | 30 | 11 | | 30 |
| 13 | 19, M | | +(HSN) | 42 | 30 | 13 | | 30 |
| 14 | 40, F | | +(HMSN1) | 0 | 0 | 0 | | 30 |
| 15 | 64, M | | +(HMSN1) | 0 | 0 | 0 | | 29 |
| 16 | 45, F | + | | 48 | 70 | 19 | 6.3 | 30 |
| 17 | 53, F | + | | 6 | 60 | 23 | 5.8 | 29 |
| 18 | 52, F | | +(HMSN1) | 12 | 60 | . | | . |
| 19 | 32, M | | +(HMSN1) | 0 | 0 | 0 | | . |
| 20 | 55, M | + | | 12 | 50 | 18 | 6.5 | 29 |

DNP, diabetic neuropathy; HNP, hereditary neuropathy; HMSN, hereditary motor and sensory neuropathy; SMMT, standardized mini-mental test; HSN, hereditary sensory neuropathy; VAS, visual analogue scale; PD-Q, painDETECT questionnaire; F, female M, male

Table 2. Coordinates of selected seed regions according to Montreal Neurological Institute atlas

| Names of Seed Regions | x | y | z |
|--------------------------------------|-----|-----|-----|
| Left cingulate cortex | -13 | -55 | 9 |
| Right cingulate cortex | 17 | -55 | 8 |
| Left anterior cingulate cortex | -4 | 19 | 18 |
| Right anterior cingulate cortex | 7 | 19 | 18 |
| Left parahippocampal cortex | -32 | -27 | -24 |
| Right parahippocampal cortex | 33 | -27 | -25 |
| Left dorsolateral prefrontal cortex | -49 | 41 | 10 |
| Right dorsolateral prefrontal cortex | 54 | 42 | 8 |
| Medial prefrontal cortex | 0 | 48 | -4 |
| Posterior cingulate cortex/precuneus | -6 | -52 | 40 |
| Left insular cortex | -40 | -5 | 7 |
| Right insular cortex | 43 | -5 | 5 |

either parts of the networks of interest or assumed to be related to pain processing. Using the specified twelve seed ROI, temporal correlations were computed between these seeds and all other voxels in the brain. This procedure was applied to all groups.

Correlation coefficient images between twelve specified seeds and all other voxels were z-transformed, with one and two sample t-tests examining within-and between connectivity. Seed-to-voxel results were reported as significant at a cluster-level threshold of $p < 0.05$ FDR corrected. The group-level maps of the DMN and insula-cingulate network were identified and isolated for each group. Only significant connectivity changes in regions that are points of interest are given as results and discussed.

Anatomical localizations were defined based on the coordinates obtained from MNI (Montreal Neurological Institute) Anatomical Atlas.

Assessment of Peripheral Nerve Damage

Nerve conduction studies were performed using surface silver/silver chloride disc recording electrodes. Motor nerve conduction studies were performed in

the median, ulnar, tibial, and peroneal nerves with conventional techniques. Sensory nerve conduction studies were performed in the median, ulnar, and sural nerves using orthodromic conventional techniques. Motor and sensory nerve conduction velocities, compound muscle action potential (CMAP) and sensory nerve action potential amplitudes were measured.

Statistics

Statistical analyses of clinical measures were performed using PASW Statistics 18 (SPSS, Inc., 2009, Chicago, IL, USA). Descriptive analyses of variables which were normally distributed (SMMT, PD-Q score) are presented using means and standard deviations, while the ones related to variables without normal distribution (posterior tibial nerve CMAP amplitude, VAS score) are given as medians and min-max ranges. We used independent samples t-test and Mann-Whitney U test to compare the means and medians of the neuropathy groups with their own controls pairwise, respectively. A p-value of less than 0.05 was considered to show statistical significance.

RESULTS

Clinical and Electrophysiological Data

The demographics of the patients are summarized in Table 1. Patients with painful DNP (mean age \pm SD: 50.10 \pm 6.05, median age: 51.50) were significantly older than the patients with HNP (mean age \pm SD: 37.80 \pm 14.01, median age: 37.00) ($p=0.02$). This urged us to compare each patient group separately with age- and sex-matched controls instead of comparing with each other.

Since sural sensory nerve action potentials were not obtained in most patients, we used amplitude of posterior tibial nerve CMAP as an index of peripheral nerve damage for statistical analyses. Patients with HNP had significantly lower CMAPs (median: 0.55 mV; min: 0.10 max: 11.0) than patients with painful DNP (median: 6.40 mV; min: 2.90 max: 18.0) ($p=0.003$). In contrast, pain scores were significantly lower in the hereditary group (VAS median: 0 min: 0 max: 40 for HNP; and median: 70 min: 50 max: 90 for painful DNP) ($p < 0.001$) (painDETECT mean \pm SD: 6.22 \pm 5.19 for HNP; and mean \pm SD: 20.40 \pm 1.90 for painful DNP) ($p < 0.001$).

Serum levels of sodium, vitamin B12, thyroid hormones, urea, creatinine and liver enzymes of all participants were in normal range. Mean

Table 3. Clinical and biochemical profiles of DNP, diabetic control and healthy control groups

| | DNP n=10 | Diabetic controls n=7 | Healthy controls of DNP n=8 | |
|--------------------------------------|----------------------|-------------------------------|--------------------------------|----------------------------|
| Gender (F/M) | 5/5 | 4/3 | 4/4 | |
| Age, mean \pm SD (years) | 50.10 \pm 6.05*§ | 51.00 \pm 6.30* | 48.13 \pm 7.22§ | * $p=0.770$ § $p=0.536$ |
| Duration of diabetes, median (years) | 5.0 | 4.0 | - | $p=0.588$ |
| Serum glucose, mean \pm SD (mg/dl) | 152.20 \pm 43.77*§ | 146.14 \pm 51.73* | 95.13 \pm 4.82§ | * $p=0.660$ § $p=0.003$ |
| HbA1c %, median | 6.65 | 6.20 | - | $p=0.525$ |
| SMMT, mean \pm SD | 28.60 \pm 2.01*§ | 28.71 \pm 1.11* | 29.50 \pm 0.76§ | * $p=0.610$ § $p=0.332$ |
| Oral antidiabetic use | 7/10 | 4/7 | - | $p=0.644$ |
| Insulin use | 1/10 | 2/7 | - | $p=0.537$ |
| CMAP amplitude, median (mV) | 6.40 | 8.30 (5 out of 7 patients) | - | $p=0.297$ |

p values in bold represent statistical significance.

DNP, painful diabetic neuropathy; SMMT, standardized mini-mental test; CMAP, compound muscle action potential; SD, standard deviation; F, female M, male

serum glucose levels were 95.22 ± 13.13 mg/dl in hereditary group and 152.20 ± 43.77 mg/dl in painful diabetic group; and the difference was significant ($p=0.002$). SMMT scores were similar between the two groups ($p=0.860$).

The clinical and biochemical features of painful DNP patients, their diabetic controls, and healthy controls are shown in Table 3. Mean serum glucose level of the painful DNP group was higher than the healthy controls but similar to the diabetic controls. CMAP amplitudes were higher in diabetic controls than painful DNP patients; however, the difference was not significant ($p=0.297$).

Imaging Data

HNP vs. healthy controls: In comparison with the age- and sex-matched healthy controls, HNP patients displayed increased connectivity between left insula and left anterior cingulate cortex. There was an inversely correlated activity between left insula and left inferior parietal lobule ($T=-4.20$) (Table 4). The connectivities between right and left insula, between left cingulate and left inferior parietal lobule, and the connectivity between posterior cingulate cortex/precuneus and left inferior parietal lobule were also increased. There was also a slight increase in the connectivity of medial prefrontal cortex and right cingulate (Table 4).

DNP vs. healthy controls: In patients with painful DNP, the distinct alterations were increased connectivity between left anterior cingulate cortex and posterior cingulate cortex/precuneus, and increased connectivity between medial prefrontal cortex and left medial temporal region. They also displayed increased connectivity between left cingulate and left inferior parietal lobule, between posterior cingulate cortex/

precuneus and left inferior parietal lobule, between right cingulate and medial prefrontal cortex, and between right and left insula. There was an inverse connectivity between medial prefrontal cortex and right dorsolateral prefrontal cortex ($T=-3.43$) (Table 4).

Diabetic controls vs. healthy controls: We checked the findings of DNP against that of controls suffering diabetes for similar durations but not neuropathic pain, to see whether some of these findings were related to diabetes itself rather than neuropathic pain. The only alteration we identified that was common to both diabetic groups was the inverse connectivity between medial prefrontal cortex and right dorsolateral prefrontal cortex. Interestingly, the connectivity of some regions was increased in all comparisons including DNP vs. healthy controls, diabetic control group vs. healthy controls, and HNP vs. healthy controls. These regions were left cingulate-left medial temporal, right insula-right cingulate, medial prefrontal cortex-bilateral inferior parietal lobules and medial prefrontal cortex-posterior cingulate/precuneus. It is noteworthy that the increase in connectivity between medial prefrontal cortex and posterior cingulate/precuneus was the strongest in DNP vs. healthy subjects, moderate in diabetic controls vs. healthy subjects and weakest in HNP vs. matched healthy controls (Table 4).

DISCUSSION

The main finding of this study is that the two different types of neuropathies exhibited different patterns of alterations in default mode and insula-cingulate network maps. There was increased connectivity in the left insula-cingulate network in patients with HNP and within the left nodes of DMN in patients with painful DNP.

Table 4. Significant connectivity changes and their T values showing the strength of connectivity in our study groups

| DMN | | | | | | | |
|--|--|--------------------------|--------|--------------------------|--------|-------------------------|--------|
| Seed | Regions | DNP vs. healthy controls | | HNP vs. healthy controls | | DC vs. healthy controls | |
| | | T value | p* | T value | p* | T value | p* |
| MPFC | R cingulate | 13.59 | 0.0026 | 3.28 | 0.036 | insignificant | |
| | R inferior parietal lobule | 4.39 | 0.0235 | 4.48 | 0.0122 | 5.16 | 0.0174 |
| | L inferior parietal lobule | 7.90 | 0.004 | 4.84 | 0.0102 | 6.11 | 0.0115 |
| | L medial temporal | 4.19 | 0.026 | insignificant | | insignificant | |
| R DLPFC | MPFC | -3.43 | 0.047 | insignificant | | -4.47 | 0.0443 |
| L Medial temporal | L cingulate | 9.61 | 0.0105 | 7.06 | 0.0043 | 11.73 | 0.0048 |
| Posterior cingulate cortex/ Precuneus | L inferior parietal lobule | 5.50 | 0.0189 | 4.00 | 0.0435 | insignificant | |
| | MPFC | 10.32 | 0.0053 | 3.88 | 0.0435 | 7.01 | 0.0185 |
| L cingulate | L inferior parietal lobule | 5.32 | 0.0274 | 3.47 | 0.0355 | insignificant | |
| Insula-cingulate network | | | | | | | |
| Seed | Regions | DNP vs. healthy controls | | HNP vs. healthy controls | | DC vs. healthy controls | |
| | | T value | p* | T value | p* | T value | p* |
| R insula | L insula | 7.78 | 0.0225 | 12.82 | 0.0004 | 7.11 | 0.0179 |
| | R cingulate | 5.41 | 0.0362 | 6.61 | 0.0031 | 4.61 | 0.047 |
| L insula | L inferior parietal lobule | insignificant | | -4.20 | 0.0203 | insignificant | |
| | L anterior cingulate cortex | insignificant | | 3.84 | 0.0258 | insignificant | |
| L anterior cingulate cortex | Posterior cingulate cortex/ Precuneus | 4.94 | 0.0358 | insignificant | | insignificant | |

*All p values are corrected for multicomparisons.

DMN, default mode network; MPFC, medial prefrontal cortex; R, right; L, left; DLPFC, dorsolateral prefrontal cortex; DNP, painful diabetic neuropathy; HNP, hereditary neuropathy; DC, diabetic controls.

We found that right and left insula of neuropathic patients were more strongly connected to each other compared to the matched healthy subjects. This effect was more prominent in hereditary group. The connectivities between left cingulate and left inferior parietal lobule, between posterior cingulate/precuneus and left inferior parietal lobule and the connectivity between medial prefrontal cortex and right cingulate were all increased in DNP and to a lesser extent also in HNP patients implying that these changes may be related to chronic deafferentation. The inferior parietal lobule is involved in sensory processing and has sensory and limbic connections (14). Our findings suggest increased information flow to this structure from other limbic structures such as cingulate cortex with the loss of normal sensory afferent input as a compensatory mechanism during the process of sensory attention in any kind of neuropathy. Enhanced interconnection of bilateral insula especially in HNP patients may reflect a similar mechanism in these sensory limbic structures trying to maintain self-awareness and homeostasis (15). This is in accordance with a previous study reporting that patients with peripheral neuropathy, both hereditary and acquired, exhibited multiple resting state functional connectivity abnormalities involving the sensorimotor, auditory and visual networks in comparison to healthy controls (16). Analogous to our results, the authors demonstrated increased resting state bold signal in posterior cingulate cortex/precuneus and suggested these modifications might reflect an adaptive mechanism.

Interestingly, some nodes displayed increased connectivity in the comparisons of all three groups; DNP, HNP and diabetic controls vs. matched healthy subjects (Table 4). Since these subjects share nothing in common other than suffering from a chronic disease that is different from matched healthy controls, we failed to explain what these alterations mean.

The main purpose of this study was to seek the cortical connectivity correlates of chronic pain based on the differences between hereditary and diabetic neuropathies. Many anatomical regions are involved in physiological pain processing and they are supposed to interact at three levels of functioning (17). First-order processing occurs in a nociceptive cortical matrix (posterior insula, parietal operculum, mid-cingulate and primary sensorimotor cortex). The second-order perceptual matrix (mainly anterior and mid-insula, anterior cingulate) mediates transition to conscious pain. Vegetative reactions, cognitive control, and attentional modulation of pain take place within this network. Lastly, the subjective value of pain and pain memory is formed by the third order reappraisal-affective matrix (perigenual cingulate, orbitofrontal cortex and anterolateral prefrontal cortex). According to this model of pain processing elaborated by Garcia-Larrea and Peyron from previous notions, we hypothesized that there may be alterations in first and second-order pain processing since hereditary neuropathy is a genetic form of disease and the patients are born with the pathophysiology already set in the maturing nervous system. The results of our study may support this idea. In HNP patients, the connectivity between left insula and left anterior cingulate cortex, components of second order processing, was increased and this alteration was not present in DNP compared to matched controls.

Another finding in HNP was the inverse correlation between left insula and left inferior parietal lobule. In general, decreased connectivity strength or loss of connectivity may be regarded as functional isolation or decorrelation in the cortical circuits and Brambilla et al. proposed that such fragmentation of network may be blamed for neuropathic pain (18). The inverse correlation that emerged in HNP patients between left insula and left inferior parietal lobule might be interpreted as a control mechanism working reciprocally between the sensory association

center-inferior parietal lobule-and one of the major components of the pain matrix-insula. The importance of laterality here is unknown.

The major alteration in patients with DNP in the present study was in the default mode network. The connectivities between medial prefrontal cortex-left medial temporal lobe and left anterior cingulate cortex-posterior cingulate cortex/precuneus were increased in our DNP patients. These alterations were not present in HNP compared to matched controls. Previously, Baliki et al. studied DMN in a group of chronic back pain patients and found that medial prefrontal cortex, amygdala and posterior cingulate cortex showed lower deactivation than expected during a visual attention fMRI task compared to controls (19). Among chronic pain syndromes like migraine, visceral pain, osteoarthritis etc., neuropathic pain is unique as it results from damage of the somatosensory system itself and central mechanisms accompanying neuropathic pain, in fact, may differ from other chronic painful states. However, our findings were very similar to Baliki and collaborators' findings, though with a difference in MRI data acquisition method; i. e. resting state vs. task based. Cauda et al. compared the DMN of diabetic neuropathic pain patients with that of normal subjects and reported increased connectivity in left precuneus, bilateral dorsolateral prefrontal and frontopolar cortices, both insulae, inferior parietal lobules, and also decreased connectivity in the bilateral dorsal anterior cingulate cortices, relating these changes to the cognitive-emotional components of pain (5). Some of those connectivity changes such as increase in bilateral insula, left posterior cingulate/precuneus and left inferior parietal lobule were also present in our results. Since we identified them in both hereditary and diabetic neuropathy groups, we interpret these alterations as the implication of chronic deafferentation.

The role of posterior cingulate cortex/precuneus in chronic pain is less obvious. It was proposed that posterior cingulate cortex/precuneus mediates phasic pain signals, conveying painful stimuli to the anterior portion of the cingulum (20). Increased communication between posterior cingulate cortex/precuneus and left anterior cingulate cortex that we recognized in DNP patients might reflect the reinforced central processing of faulty sensory input arising from damaged peripheral nerves. Posterior cingulate cortex/precuneus may be projecting this sensory information anteriorly and the anterior cingulate cortex may be adding the unpleasant, affective tone to it resulting in neuropathic pain.

Diabetes itself is known to cause structural and metabolic alterations in the brain (21-23). In our study, we enrolled age- and sex-matched diabetic controls, with diabetes for similar durations but not neuropathic pain, to distinguish the effects of pain from the effects of diabetes alone on the resting state brain activity. The sole common alteration seen in diabetic controls and DNP patients (but not in HNP) was inverse connectivity between medial prefrontal cortex and right dorsolateral prefrontal cortex. The changes in prefrontal regions that emerged in both of our diabetic groups and several other functional connectivity studies performed in diabetic patients (5, 24-26) seem to result from the direct influence of diabetes on the cerebral cortex.

In our study, the main changes were seen in the dominant hemisphere. However, in terms of laterality, the spontaneous component of neuropathic pain could be related more to the non-dominant hemisphere, which is associated with the subjective awareness of the "feeling self". Thus, this finding needs further verification.

Limitations

One of the restraints of this study is that diabetic neuropathy is an acquired neuropathy developing under metabolic derangement and many central effects on the nervous system can be observed irrespective

of peripheral nerve damage. However, we believe that we minimized this systematic error by introducing diabetic controls without painful neuropathy into our study.

Small group sizes and the lack of any electrophysiological correspondence with connectivity changes are the other weaknesses of this study. The need for two age and sex-matched control groups for the DNP group was one reason for the relatively small groups.

Some studies draw attention to the central sensitization dynamics in the spinal cord and the differences at the cord level between hereditary and diabetic neuropathic conditions (27). Descending supraspinal modulation of dorsal horn neurons includes both inhibitory and excitatory control (28). Just as the loss of function in descending inhibitory pathways contributes to central sensitization and chronic pain states (29), loss of function in descending excitatory modulation of the dorsal horn may lead to blunted neuropathic pain. This might contribute to the mechanisms underlying the painless state in hereditary neuropathies. What determines descending supraspinal facilitation or inhibition to predominate and at which time point of life this change occurs is yet to be discovered (30). Future studies of spinal cord fMRI might delineate these issues.

After all, the answer to our question may not be in the connectome but at cellular level. Schwann cell-neuron interactions may interfere with the genesis of neuropathic pain (peripheral sensitization, dorsal root ganglia changes etc.) at the periphery. This might also affect central processes in the brain that induce neuropathic pain such as the neuroglial interactions. Additionally, Charcot-Marie-Tooth disease is mostly considered a large-fiber disease and unmyelinated fibers were usually intact in nerve biopsies (31, 32) despite some studies indicating impairment in small fiber functions (33, 34). These peripheral factors may be associated with the inconsistent neuropathic pain in hereditary neuropathy patients.

In conclusion, we herein documented the distinction between the imprint of chronic pain and the effect of deafferentation on cortical functional connectivity in two candidate resting state networks of the brain. Resting state functional connectivity analysis in such a study design constitutes a good way of investigating chronic neuropathic pain and its cortical neural correlates.

Whatever the mechanism, once the changes that prevent hereditary neuropathy patients from suffering severe chronic pain are elucidated, new therapeutic strategies may be put forward for the benefit of millions of patients with pain.

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Committee Approval: This study was done in the direction of the Declaration of Helsinki and approved by the Local Ethics Committee.

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Peer-review: Externally peer-reviewed

Author Contributions: Concept – ATS, ACH, NÖ, CMT, KKO; Design – ATS, ACH, NÖ, CMT, KKO; Supervision – ATS, ACH, NÖ, CMT, KKO; Resources – ATS, NÖ, CMT, KKO; Materials – ATS, NÖ, CMT, KKO; Data Collection and/or Processing – ATS, ACH, CMT, KKO; Analysis and/or Interpretation –ATS, KKO; Literature Search –ATS, ACH, NÖ, CMT, KKO; Writing Manuscript – ATS; Critical Review – ATS, ACH, NÖ, CMT, KKO.

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