Understanding diabetic neuropathy: from subclinical nerve lesions to severe nerve fiber deficits. A cross-sectional study in patients with type 2 diabetes and healthy controls

Short running title: Diabetic neuropathy is a continuum

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Diabetes Publish Ahead of Print, published online December 11, 2019

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Abstract

Studies on magnetic resonance neurography (MRN) in diabetic polyneuropathy (DPN) have found proximal sciatic nerve lesions. The aim of this study was to evaluate the functional relevance of sciatic nerve lesions in DPN, expecting correlations with the impairment of large fiber function. 61 patients with diabetes mellitus type 2 (48 with, 13 without DPN) and 12 controls were enrolled, undergoing MRN, quantitative sensory testing, and electrophysiological examinations. There were differences in mechanical detection (Aβ fibers) and mechanical pain (Aδ fibers), but not in thermal pain and thermal detection clusters (C fibers) between the groups. Lesion load correlated with lower Aα, Aβ, and Aδ fiber, but not C fiber function in all participants. Patients with lower function showed a higher load of nerve lesions than patients with elevated function or no measurable deficit despite apparent DPN. Longer diabetes duration was

associated with higher lesion load in patients with DPN, suggesting that nerve lesions in DPN may accumulate over time and become clinically relevant once a critical amount of nerve fascicles is affected. Moreover, MRN is an objective method for determining lower function mainly in medium and large fibers in DPN.

Introduction

Distal symmetric diabetic polyneuropathy (DPN) is an important diabetic complication, significantly increasing morbidity and mortality in affected patients (1). In some patients, DPN is painful, causing tingling, spontaneous pain or burning sensations, hyperalgesia, allodynia, or overt sensitivity to temperature changes, while other patients predominantly suffer from painless DPN, often associated with numbness (2). For the central nervous system, several structural and functional differences between patients with painful and painless diabetic neuropathy have been described (3-6). However, no specific distinguishing features of the peripheral nervous system between painful and painless diabetic neuropathy have been determined so far (7). Moreover, the pathophysiology and the natural course of DPN are poorly understood (8). It is assumed that both pre-diabetes and diabetes lead to microstructural alterations in affected nerves, which seem to begin in small unmyelinated C fibers (9), consequently leading to a loss of C fibers (10). Later findings over the course of the disease appear to be demyelination accompanied by axonal degeneration of myelinated fibers (Aβ, Aδ) (11). Studies of the underlying pathophysiological aspects of DPN in humans are limited since obtaining nerve tissue is both difficult and risky. Furthermore, nerve biopsies are restricted to distal nerves, which do not allow for an evaluation of proximal fibers. Therefore, it is of great importance to establish non-invasive, objective in-vivo methods that allow for the detection and exact localization of nerve damage of DPN at an early stage in order to get a better insight into the pathophysiology and to evaluate potential therapeutic options. In recent studies, high-resolution magnetic resonance

neurography (MRN) at 3 Tesla (3T) has proven effective for the detection and exact localization of peripheral nerve lesions in DPN (12; 13). Nerve lesions that appear hyperintense in a T2-weighed (T2w), fat suppressed sequence have been shown to be negatively associated with parameters of nerve conduction (13). The exact clinical impact and relation to nerve fiber types of the lesions detected by MRN, however, has not been studied in detail, since to date lesions have only been correlated with basic clinical scores and electrophysiological testing (EPT) (12; 13). Therefore, it is uncertain which types of nerve fibers are affected by T2w-lesions. Moreover, detecting early stages of diabetic neuropathy would be beneficial for evaluation of potential therapeutic methods in future clinical studies. The most sensitive clinical method for the characterization of neuronal impairment is complete quantitative sensory testing (QST), which includes mechanical testing for large fiber function (2; 14-17) and has not been used to evaluate the clinical relevance of MRN yet.

We therefore performed a cross-sectional study in patients with diabetes mellitus type 2 with and without diabetic polyneuropathy as well as in control subjects without diabetes or neuropathy by combining MRN, EPT, and QST as the potentially most sensitive and specific non-invasive clinical methods for evaluation of both the exact clinical and neurophysiological status and the load of structural nerve lesions. We hypothesized that MRN mainly detects lesions in larger fibers due to its resolution. Therefore, we expected stronger correlations with clinical large and medium fiber functions than with small fiber functions.

Materials and methods

Patients

Participants aged between 18 and 85 years were recruited at the University Hospital Heidelberg (UHH) between 2016 and 2018. Clinical examinations were performed at the Clinical Study Center for Diabetes Research of the UHH. MRN studies were performed at the Department of Neuroradiology of the UHH. The study was approved by the local ethics committee (ethics numbers S-146/2015 and S-383/2016, ClinicalTrials.gov Identifier NCT03022721), and all participants gave written informed consent. Patients with diabetes mellitus type 2 with and without DPN as well as subjects without diabetes and neuropathy were included. In order to diagnose DPN, all participants were asked for symptoms associated with polyneuropathy for evaluating the Neuropathy Symptom Score (NSS) according to the national German guidelines (18; 19). Moreover, they underwent routine clinical examination including TipTherm® (TipTherm GmbH, Dorsten, Germany), vibration using a 64 Hz tuning fork, and reflex testing as well as basic testing for pain sensations using a 512 mN von Frey filament (Marstock Nervtest OptiHair, Schriesheim, Germany) in order to calculate Neuropathy Deficit Score (NDS) as described before and recommended in the national German guidelines (18; 19). Patients with diabetes mellitus and an NSS and/or NDS of ≥3 were considered to be affected by DPN (13; 18). For patients with diabetes without DPN as well as subjects without diabetes, both NSS and NDS had to be 0. Subjects with scores of 1 and 2 were not included in the study in order to avoid inconclusive findings.

Specifically trained personnel performed all clinical tests. It was strictly monitored by the medical personnel performing the study that symptoms were typical for distal symmetric polyneuropathy in order to rule out other causes for elevated NSS scores like cramps due to magnesium deficit, neuropathic symptoms due to disc herniation, or others common causes. Patients with any contraindications for MRN examination and patients with other known potential causes for neuropathy, such as chronic alcoholism, end-stage renal disease, Parkinson's disease, rheumatic autoimmune diseases, malignant tumors, or spinal lesions were excluded from the study.

Fasting blood draw was performed for evaluation of HbA1c, creatinine, as well as serum lipids, and urinary albumin/creatinine ratio (uACR) was determined. In subjects without diabetes mellitus, oral glucose tolerance tests were performed to rule out disorders of glucose metabolism according to standard protocol (20). Blood and urine analysis was performed in the accredited Central Laboratory of the UHH according to standard protocols. eGFR was calculated according to CKD-EPI (21).

Quantitative sensory testing (QST)

Full QST was performed on one dorsal foot, including cold detection threshold (CDT), warm detection threshold (WDT), cold pain threshold (CPT), heat pain threshold (HPT), thermal sensory limen (TSL), and paradoxical heat sensations (PHS) by use of a thermode (Medoc Ltd. TSA 2001-II, Israel) as described before (14). Additionally,

mechanical testing was performed using von Frey filaments (Marstock Nervtest OptiHair, Schriesheim, Germany) for mechanical detection threshold (MDT) and using a PinPrick® set (MRC Systems GmbH, Heidelberg, Germany) for mechanical pain threshold (MPT), mechanical pain sensitivity (MPS), and wind up ratio (WUR). For determining dynamic mechanic allodynia (DMA), a regular Q-tip, a cotton ball, and a brush (Somedic SenseLab AB Brush-05, Sösdala, Sweden) were used. Moreover, vibration detection threshold (VDT) was evaluated using a 64 Hz tuning fork, and pressure pain threshold (PPT) by using a pressure algesiometer (Wagner Instruments FDN 200 with Rubber Tip 1 cm^2, Greenwich, CT, USA). CDT, WDT, and TSL represent thermosensory functions of small fibers (Aδ und C fibers), whereas CPT, HPT, PPT, and especially MPS and MPT reflect nociceptive functions of small fibers (Aδ and C fibers) (14). MDT and VDT represent tactile functions of larger Aβ fibers (14). All tests have been described before in detail (14; 16).

Electrophysiological testing (EPT)

Nerve-conduction velocities, amplitudes of compound-motor and sensory-action potentials, as well as distal-motor latencies have been evaluated for tibial and peroneal nerves on the same leg as QST using the Viking Quest system (VIASYS Healthcare GmbH, Höchberg, Germany).

MRN imaging protocol

In all participants, high-resolution MRN of the right thigh was performed in a 3 Tesla MR-scanner (Magnetom TIM-TRIO, Siemens Healthcare, Erlangen, Germany). A 15-channel transmit-receive extremity coil was used and an axial high-resolution T2-weighted turbo spin echo (TSE) 2D sequence with spectral fat saturation was applied. Sequence parameters were: relaxation time (TR) 5970 ms, echo time (TE) 55 ms, field of view (FOV) 160 x 160 mm^2, matrix size 512 x 512, slice thickness 4 mm, interslice gap 0.35 mm, voxel size 0.5 x 0.3 x 4.0 mm^3, 24 slices, visualizing a total nerve length of about 10 cm. The sequence was centered on the sciatic nerve bifurcation in every patient. In order to avoid artificial alteration of the acquired T2w signal we did not apply any prior image filters.

Image post-processing and statistical analysis

A total number of 73x24=1752 images were recorded. All images were pseudonymized and analyzed in a semi-automatic approach using ImageJ® and custom-written code in Matlab® v7.14.0.0739 (R2012a) (22; 23). Anatomical segmentation of sciatic nerve fascicles was performed manually by two trained neuroradiologists (JMEJ, FTK) blinded to clinical data. In accordance with previous studies (12), a T2w-hyperintense lesion was defined as a nerve fascicle with an elevated T2w signal intensity of at least 25% above that of adjacent muscle tissue, a T2w-hypointense lesion was defined as a nerve fascicle with a decreased T2w signal intensity of at least 25% below that of adjacent muscle tissue. Thresholds for lesions were adapted to the surrounding muscle

signal on each of the 24 images per patient in order to avoid effects caused by image artifacts due to field and coil inhomogeneities as well as magic angle artifacts. The exact process of nerve segmentation has been described before (12). Afterwards, binarized images of lesions and healthy nerve tissue were analyzed in Matlab and the lesion load in percent of the full nerve volume was calculated. The process of image segmentation and binarization is illustrated in Fig.1.

Statistical analysis

Absolute values were used for analysis of EPT results (representing motor Aα fiber functions). For QST results, each individual parameter was normalized to a published cohort of the same age, sex, and test region by subtracting the mean and dividing by the standard deviation of the healthy subject control cohort (24). Resulting z-scores have an expected zero mean and unity variance in healthy subjects. Z-scores outside the +/- 1.96 standard deviations (SD) range are outside the 95% CI and can be considered abnormal findings (14; 16; 24). Negative z-scores (<-1.96 SD) indicate lower thermo-receptive, nociceptive, or tactile functions. Positive z-scores (>1.96 SD) indicate elevated function (hyperalgesia or allodynia). Z values of the single QST tests were clustered as follows to create compound z scores: thermal detection (average values of CDT, WDT, and TSL, representing sensory c fiber function), thermal pain (average values of CPT and HPT, representing sensory Aδ fiber function), mechanical pain (MPT and MPS, representing sensory Aδ fiber function), and mechanical detection (VDT and MDT, representing sensory Aβ fiber function). For all absolute parameters, median and range are given.

Both Kolmogorov-Smirnov and Shapiro Wilk tests showed non-Gaussian distributions of T2w lesions and all absolute results for QST parameters except WDT. Therefore, non-parametric analyses were performed for absolute values (Kruskal-Wallis-test and Mann-Whitney-U-test for group comparisons of absolute values, Spearman correlation coefficient rSp was calculated for correlation analyses), whereas ANOVA was used for group comparisons of compound z scores of QST parameters.

Additionally, due to the wide distribution of lesion load in patients with diabetes mellitus and DPN, quartiles for the lesion load were calculated and patients were divided into three groups accordingly to verify the relation between the severity of lesions in MRN and the clinical impairment observed: Patients with low lesion load below the 25th percentile (<4 % of nerve tissue affected), a second group consisted of patients with moderate lesion load within the 25th percentile and the 75th percentile (4 % - 11 % of nerve tissue affected), and a third group of patients with high lesion load above the 75th percentile (>11 % of nerve tissue affected).

For another statistical analysis patients were categorized by their QST-profiles in three different groups: patients with gain of function, patients with loss of function, and patients without deficit (no deficit). Gain of function was defined as pathological hyperalgesia (thermal or mechanical pain thresholds) with preserved nerve fiber function in detection thresholds. Loss of function was defined as pathological decrease of detection thresholds in small, medium and large fibers. Cut-off values for pathological test results of z-scores have been set at +1.96 SD for gain-of-function and -

1.96 SD for loss-of-function compared to a standardized control group as described before (14; 16).

Linear regression analysis was performed for evaluation of potential confounders. SPSS® Statistics Version 23.0 (IBM Deutschland, Ehningen, Germany) was used for all statistical analyses, whereas GraphPad Prism 7 (GraphPad Software, San Diego, CA, USA) was used for all figures.

Results

In total, 73 participants were included, 48 patients with diabetes mellitus type 2 and an NSS and/or NDS of ≥3 (representing at least incipient diabetic polyneuropathy (18)), 13 patients with diabetes mellitus type 2 and an NSS and NDS of 0, as well as 12 participants without diabetes and an NSS and NDS of 0. All participants underwent MRN. The calculated T2w hyperintense lesion load ranged from 1 % to 49 %. Full patients' characteristics are given in Table 1.

Age did not differ between the three groups (p=0.241), but gender differed, with the control group mainly consisting of women, whereas there were more men than women in the diabetes groups (Table 1). No significant difference in lesion load in MRN between the three groups (p= 0.238) could be found, although the median percentage of lesions/healthy nerve tissue was 8.07 % in patients with diabetes and DPN compared to 4.75 % in control subjects (Table 1, Figure 2A). Moreover, HbA1c, GFR (CKD-EPI),

Creatinine, uACR, and diabetes duration did not differ between the two diabetes groups.

No correlations between clinical symptoms and T2w hyperintense lesion load in MRN could be found. However, tingling correlated significantly with both MDT ($p=0.049$, r_{Sp} 0.306) and VDT ($p=0.034$, r_{Sp} -0.327).

In a linear regression analysis including all study participants, gender was the only significant parameter, with male sex being associated with a higher T2w hyperintense lesion load ($p=0.041$, $R^2=0.674$), which remained when only including patients with diabetes mellitus and polyneuropathy in the analysis ($p=0.031$, $R^2=0.259$). Moreover, diabetes duration was a predictor for lesion load in patients with diabetes and polyneuropathy ($p=0.022$, $R^2=0.483$). In both analyses, age, BMI, HbA1c, uACR, insulin use, alcohol consumption, smoking, coronary heart disease, and peripheral artery disease were not associated with lesion load.

T2w-hypertintense lesion load and large fiber function

EPT was performed for evaluating function of large Aα motor fibers. In the comparison between controls, patients with diabetes without polyneuropathy, and patients with polyneuropathy, conduction velocity of tibial nerve did not differ significantly ($p=0.126$), while conduction velocity of peroneal nerve was significantly different ($p=0.02$) (Figure 2B and C). In all participants, moderate negative correlations between lesion load and nerve conduction velocities of both tibial ($p=0.005$, r_{Sp} -0.362) and peroneal ($p<0.001$, r_{Sp} -0.554) nerves (Figure 3A and B), as well as amplitudes of

peroneal (p=0.014, r_{Sp} -0.317) and tibial (p=0.035, r_{Sp} -0.276) nerves and DML of peroneal nerve (p=0.037, r_{Sp} 0.275) could be found. These correlations remained when only analyzing patients with diabetes and polyneuropathy (peroneal NCV p=0.002, r_{Sp} -0.497; tibial NCV p=0.002, r_{Sp} -0.518; peroneal amplitude p=0.048, r_{Sp} -0.337, tibial amplitude p=0.002, r_{Sp} -0.518). In subjects without diabetes, only the amplitude of the tibial nerve correlated with lesion load in MRN (p=0.029, r_{Sp} 0.654), whereas the other parameters did not show any correlations.

Patients with diabetes type 2 with DPN and high lesion load of more than 11 % (above the 75th percentile) showed significant reductions in tibial (p=0.024) and peroneal (p=0.032) nerve conduction velocity (Figure 4A and B) as well as in amplitudes of tibial (p=0.016) and peroneal (p=0.048) nerves compared to patients with moderate or low lesion load.

T2w-hyperintense lesion load and nociceptive medium and small fiber function in all participants

To evaluate the clinical relevance of MRN, associations between lesion load in MRN and clinical characteristics concerning sensory nerve functions evaluated by QST were studied.

In the comparison between controls and patients with diabetes mellitus with and without polyneuropathy, significant differences could be found for the compound z scores for mechanical detection (p<0.001, lower function in patients with DPN) and mechanical pain (p=0.02, higher function in patients without DPN), whereas no

significant differences for thermal pain or thermal detection could be found (ANOVA Table 2, Figure 2D-G).

In all study participants, irrespective of diabetes or polyneuropathy, moderate correlations between lesion load and composite z score clusters for mechanical detection (p=0.007, r_{Sp} -0.312) and mechanical pain (p=0.036, r_{Sp} -0.246) could be found, whereas thermal pain (p=0.202, r_{Sp} -0.152) and thermal detection (p=0.488, r_{Sp} -0.083) did not show correlations with lesion load (Figure 3C-F).

T2w-hyperintense lesion load and nociceptive medium and small fiber function in patients with diabetes mellitus type 2 and diabetic polyneuropathy

In the group comparison of patients with diabetes type 2 and DPN between low, moderate, and high lesion load, patients with high lesion load showed the most severe clinical impairments in mechanical pain (p=0.008) and thermal pain clusters (p=0.009), whereas no differences in mechanical detection or thermal detection clusters could be found (ANOVA Table 3 and Figure 4C-F).

When categorizing patients with polyneuropathy according to QST results into three groups, namely subjects without any clinical deficits, subjects with relative gain of function, and participants with relative loss of function, a significant difference in lesion load (p=0.033) could be found between patients with loss of function and the two other patient groups without deficit or with gain of function in QST. These results are shown in Figure 5.

T2w-hypointense lesions

T2w-hypointense lesions were also investigated in 70 subjects (10 controls, 12 with diabetes type 2 without polyneuropathy, and 48 with diabetes type 2 and polyneuropathy). There was a mild positive correlation between T2w-hyperintense and hypointense lesions (r_{Sp} 0.275, p=0.021). T2w-hypointense lesions showed similar results as T2w-hyperintense lesions, with no significant differences between the groups (p=0.77), but with a moderate negative correlation between hypointense lesion load and the z-scores of the mechanical detection cluster (r_{Sp} -0.343, p=0.004), whereas no correlations with the other QST clusters could be found. In electrophysiological testing, there were moderate correlations between T2w-hypointense lesion load and peroneal nerve function (nerve conduction velocity: r_{Sp} -0.378, p=0.004; amplitude: r_{Sp} -0.355, p=0.007; distal motoric latency: r_{Sp} 0.318, p=0.018), but not with tibial nerve function (nerve conduction velocity: r_{Sp} -0.211, p=0.122; amplitude: r_{Sp} -0.118, p=0.385; distal motoric latency: r_{Sp} 0.174, p=0.199).

Discussion

This study confirmed our hypothesis that the load of T2w-lesions in MRN is mainly associated with lower medium and large fiber function in patients with diabetes mellitus type 2 and DPN, since clinical parameters reflecting functions of sensory Aδ and Aβ fibers as well as Aα motor fibers were affected in patients with high lesion load. Since MRN itself does not allow for exact differentiation of nerve fiber types, these

results lead to a better understanding of the lesions visualized, with the degree of clinical alterations in medium and large fiber function being related to the lesion load determined by MRN-morphological alterations. This is of clinical importance since large fiber dysfunction is more commonly associated with ulcerations, amputations, and cardiovascular mortality than small-fiber dysfunction (10; 16). T2w-hypointense lesions showed similar results to hyperintense lesions. Patients with higher hypointense lesion load showed lower function of Aβ-Fibers and peroneal motor fibers, whereas no associations with smaller sensory fiber functions could be found.

Every participant enrolled in this study, irrespective of diabetes mellitus or polyneuropathy, showed a certain amount of T2w-hyperintense lesions in MRN in peripheral nerves. Therefore, one potential explanation might be that nerve lesions occur physiologically as part of the natural ageing process. This idea is supported by findings from former studies on diffusion tensor imaging in peripheral nerve, showing that a nerve's fractional anisotropy as a parameter for its structural integrity shows an age-dependent decline (25), indicating that nerve microstructure deteriorates with age. Due to cross sectional design of this study, however, this assumption remains hypothetical. Males showed a higher lesion load than women since male sex was associated with higher lesion load in a linear regression analysis. This is in line with reference values for QST parameters, which are also age- and gender-dependent, with older people and men physiologically showing inferior results (14). This further renders possible that vascular damage has an impact on the development of T2w-lesions, since vascular damage and atherosclerosis are usually more prominent and more frequent in

males than females (26). Also, changes in the relation of lipid material and water in the myelin microstructure may lead to significant changes in MR signal characteristics (27), however, the definitive pathophysiological mechanisms underlying the nerve lesions visualized by MRN remain unclear to this point. A critical aspect in the detection of T2w-lesions is the echo time (TE) of the sequences applied. MR neurography sequences typically use strongly weighted T2 sequences with long echo times (28). Our sequence used an (optimized) echo time of 55 ms to generate an optimal contrast between healthy nerve and nerve lesions. While an alteration of echo time towards longer echo times may increase this contrast, lesion location will not change. Only lower echo times may reduce this contrast, and therefore lesion detection (27). Other factors that may influence T2 contrast in nerve fibers are fiber orientation, myelin water ratio, neuronal g-factors, or diffusion effects (27). Another critical aspect is the anatomical region of lesion detection, since it is known from previous studies that the amount of nerve lesions differs between proximal and distal parts of the sciatic nerve (12). Therefore, scans were centered to the sciatic nerve's bifurcation in order to guarantee that the anatomical region examined was identical in all participants.

Lesions did not seem to be directly linked to hyperglycemia, since neither blood glucose nor HbA1c were associated with lesion load. One has to consider, however, that the history of HbA1c values was unknown which is why HbA1c values in this study only represented momentary glucose control. Moreover, several interventional studies only showed limited effects of glucose control on the course DPN, indicating that other risk factors like dyslipidemia and microvascular damage might play an equal or even more

important role (29). As for the progression of nerve damage, diabetes duration was independently associated with lesion load. Due to the cross-sectional nature of our data, further longitudinal studies on the effect of age, disease duration, and other risk factors for the formation of nerve lesions in patients with diabetic polyneuropathy are required.

Interestingly, patients without clinically apparent diabetic polyneuropathy according to NSS and NDS scores showed relative hyperalgesia compared to controls and to patients with apparent diabetic polyneuropathy according to NSS and NDS, who showed relative hypoalgesia. Moreover, when only considering patients with diabetes mellitus type 2 and polyneuropathy, patients with lower function in QST showed significantly higher lesion load compared to subjects without any deficit or with elevated function. These aspects could support the hypothesis that elevated function usually occurs at the early stages, whereas lower function signals later stages of DPN, and that hyperalgesia requires relatively intact fibers as visualized by low lesion load.

No significant differences in lesion load between patients with DPN and controls without diabetes could be found despite the median lesion load being almost twice as high in patients with diabetes and DPN compared to control subjects without diabetes. The main reason for this finding could be the large range of lesion load in patients with DPN and the variability of patients with diabetes mellitus with regards to glucose control, phenotype, and other clinical parameters. Therefore, large cohorts would be required to achieve statistical significance. Also, one has to keep in mind that the diagnosis of DPN was based on the NSS and NDS, two scores that have been validated

for the diagnosis of DPN in clinical settings but that do not allow for precise conclusions on axonal function.

Remarkably, electrophysiological testing showed correlations between lesion load in MRN and both segmental demyelination and axonal damage in patients with diabetes mellitus type 2 and DPN. Both types of nerve damage have been described in DPN before (30). In controls without diabetes in contrast, only the tibial nerve amplitude correlated with the lesion load. This is supposedly due to the fact that only the lesion load of the tibial compartment of the sciatic nerve was evaluated in MRN, since the curving natural course of the peroneal compartment does not allow for precise binarization of nerve lesions. Moreover, it is possible that T2w-lesions are associated with subclinical axonal damage, which might also occur with age in healthy subjects without neuropathy. Subclinical neuronal loss or axonal damage are well established findings in the course of degenerative or inflammatory disorders of the central nervous system like Parkinson's disease or multiple sclerosis (31; 32). The findings of this study therefore indicate that subclinical axonal damage may also occur in the peripheral nervous system.

The fact that no correlations between clinical symptoms and lesion load in MRN could be found in patients with DPN is not unusual since a discrepancy between clinical symptoms and functional impairment is a common finding in studies addressing diabetic polyneuropathy (33). However, to our knowledge, this is the first study showing a correlation between clinical symptoms and QST parameters, with VDT and MDT being associated with tingling. Potentially, longitudinal studies on larger cohorts

might be necessary to detect further connections between symptoms and objective clinical impairment.

Since there was a strong correlation between the objectively detectable nerve lesions in MRN and most QST parameters reflecting a broad range of sensory functions and nerve fibers, this study shows the validity and sensitivity of QST even within physiological ranges and likewise a potential functional relevance of these lesions, even in subjects without diabetes or neuropathy. This is an important finding since QST parameters are subjective and depend on a patient's cooperation during testing.

There are some limitations to this study. One may argue, for instance, that the in-plane resolution of MRN-imaging was limited to 300 x 500 um, which only allows the visualization of fascicular structures but precludes imaging of different fiber types (such as Aα-fibers with a diameter of 20um or C-fibers with a diameter of about 5um) directly. It has to be acknowledged, however, that even preclinical MRI scanners at 9.4 or 14.4 Tesla do not allow for the visualization of single nerve fibers in rodents or in vitro samples of human nerves. This is why, in this study, in vivo MRN lesions at 3 Tesla were correlated with QST parameters as an established method for the differentiation of nerve fiber types. One could further argue that the control group mainly consisted of women, whereas patients with diabetes were predominantly male. For quantitative sensory testing, calculation of z-scores accounted for any gender differences. Male gender was associated with higher lesion load. This effect remained when only considering patients with diabetes mellitus and polyneuropathy. However, since the relevant results of the study were within the groups and the study was not

powered for group comparison, a significant influence on the outcome is unlikely. Some of the correlations found in this study were only moderate, which is a common finding in studies combining clinical and imaging data (34). Moreover, sample size was rather small and the study was mono-centric. The study was not powered to show differences in T2w-lesion load between the groups, primary outcome was to show the impact of T2w-lesions on nerve function. Therefore, sample size seems valid since an association between T2w lesion load and nerve function in clinical testing could be shown. One may of course argue that the main limitation of our study is the cross-sectional design, precluding any definite conclusions regarding the effect of age and disease duration, since no longitudinal MRI and QST data of the patients examined are available. While this is true, the finding of an association between T2D duration and lesion load, indicating that neuropathy worsens with disease duration, is in line with the findings of several previous clinical studies. Long-term studies with larger sample sizes are needed to study the impact of these lesions on the natural course on diabetic neuropathy. For these studies, patients with subclinical diabetic polyneuropathy (low NSS and NDS scores) should be included as well.

This study exclusively included patients with diabetic neuropathy in type 2 diabetes, which precludes conclusions about the effects of MRN lesions in patients with type 1 diabetes. Since it has recently been shown that both hyperintense and hypointense nerve lesions also occur in type 1 diabetes but that the distribution of nerve lesions differs between diabetes types, it seems very likely that associations of lesions with QST parameters will also be found in type 1 diabetes (12). Therefore, further studies are

required to investigate the impact of MRN lesions on QST parameters in type 1 diabetes.

In conclusion, the study suggests that lesions in T2-weighted MRN are a physiological finding in peripheral nerves, potentially as a consequence of ageing. They are mainly associated with lower medium and large fiber nerve function. Since the sciatic nerve's lesion load correlates with diabetes duration as well as with sensory and motoric nerve function in DPN patients and controls, it is very likely that lesions are of pathophysiological relevance. Therefore, future studies should focus on the composition of these lesions and their development or progression over time in a longitudinal design in both subjects with and without diabetes.

Data availability statement

The dataset and sub-datasets generated and/or analysed in the current study are not publicly available because they contain patient data from the University Hospital Heidelberg; data can be made available after anonymization from the corresponding author upon reasonable request for research purpose after approval by the local Ethics Committee.

Acknowledgements

JBG was involved in the study concept, enrolled patients, performed clinical analyses, performed statistical analyses, wrote the manuscript, designed figures and tables. JMEJ

was involved in the study concept, performed radiological imaging analyses, wrote the manuscript, designed figures. FTK performed radiological imaging analyses, reviewed manuscript. ZK enrolled patients, performed clinical analyses, reviewed the manuscript. R-DT gave expertise in quantitative sensory testing, gave advice in statistical analyses, contributed to the discussion. SS-H gave expertise in quantitative sensory testing, contributed to the discussion. PPN was involved in the study concept, reviewed the manuscript. MB gave expertise in radiological imaging analyses, reviewed the manuscript. SK is the head of the study, was involved in study concept, reviewed and edited the manuscript. Jan Benedikt Groener is taking responsibility for the contents of this article. This study was initiated and carried out under support of the Deutsche Forschungsgemeinschaft (DFG) within Collaborative Research Center 1158 (CRC 1158; subprojects A03 and S01). This work has been also funded by the Deutsches Zentrum für Diabetesforschung (DZD) e.V. and by Collaborative Research Center 1118 (CRC 1118; subproject B05). The funding sources were not involved in any aspects of the study design, interpretation of the results, or manuscript preparation. All authors report no conflicts of interest.

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Tables

Table 1 Patients' characteristics.

	Patients with type 2 with DPN	Patients with type 2 without DPN	Control persons
Age [years]	65 (38-78)	65 (49-83)	61 (48-70)
Gender [M / F]	n = 33 / 15	n = 8 / 5	n = 3 / 9
DM duration [years]	10 (0-30)	3 (1-20)	0
Patients with >10 years diabetes duration	n = 20	n = 4	n = 0
NSS [total score, range 0-9]	6 (0-9)	0	0
NDS [total score, range 0-10]	5 (0-10)	0	0
Retinopathy [yes / no]	n = 6 / 42	n = 0 / 13	n = 0 / 12
Nephropathy [yes / no]	n = 13 / 35	n = 1 / 12	n = 0 / 12
Stroke [yes / no / unknown]	n = 1 / 40 / 7	n = 0 / 12 / 1	n = 0 / 12 / 0
TIA [yes / no / unknown]	n = 0 / 41 / 7	n = 0 / 12 / 1	n = 0 / 12 / 0
CHD [yes / no / unknown]	n = 7 / 36 / 5	n = 0 / 12 / 1	n = 0 / 12 / 0
Myocardial infarction [yes / no / unknown]	n = 1 / 42 / 5	n = 0 / 12 / 1	n = 0 / 12 / 0
PAD [yes / no / unknown]	n = 3 / 45 / 0	n = 2 / 11 / 0	n = 0 / 11 / 1
Arterial hypertension [yes / no]	n = 40 / 8	n = 4 / 9	n = 2 / 10
Smoking [yes / no]	n = 6 / 42	n = 1 / 12	n = 0 / 12
Insulin therapy [yes / no / unknown]	n = 10 / 36 / 2	n = 0 / 12 / 1	n = 0 / 12 / 0
BMI [kg/m^2] / unknown	29.7 (21.5-45.3) / n = 1	30.5 (24.6-45.3) / n = 0	27.2 (24.8-39.8) / n = 0
eGFR [ml/min]	94.6 (38.3-125.6)	71.1 (34-104.8)	85.1 (79.7-98.3)
Patients with eGFR <60 ml/min	*n = 1*	*n = 3*	*n = 0*
uACR [mg/mmolCrea]	9.76 (2.42-653.94)	12.74 (4.07-129.33)	6.73 (3.77-67.5)
Patients with uACR 30-300 mg/mmolCrea	*n = 8*	*n = 1*	*n = 1*
Patients with uACR >300 mg/mmolCrea	*n = 2*	*n = 0*	*n = 0*
HbA1c [%]	6.75 (5.4-10.8)	7.3 (5.2-7.9)	5.2 (4.7-5.8)
HbA1c (IFCC) [mmol/mol]	50.5 (35.5-94.5)	56 (33-63)	33 (28-40)
Total cholesterol [mg/dl]	179 (113-280)	177 (151-223)	231 (158-300)
TG [mg/dl]	181 (63-391)	108 (75-308)	120 (45-234)
HDL [mg/dl]	44 (32-111)	47 (36-70)	76 (46-93)
LDL [mg/dl]	95 (46-173)	100 (68-144)	140 (69-202)

Total body water [%]	43.9 (38.6-50.5)	45.7 (41.2-50.2)	51.2 (39.9-58.2)
T2w-lesions / healthy nerve [%]	8.07 (1-49)	6.13 (3-14)	4.75 (2-12)
Tibial NCV [m / sec]	39 (25-47)	41 (34-54)	44 (36-55)
Peroneal NCV [m / sec]	38 (27-47)	40 (21-52)	45 (39-49)
Compound z score mechanical detection [SD]	-1.74 (1.81)	0.89 (0.83)	0.69 (0.50)
Compound z score mechanical pain [SD]	0.63 (1.93)	2.08 (1.15)	1.39 (0.88)
Compound z score thermal pain [SD]	-0.51 (0.87)	-0.68 (0.90)	-0.34 (1.14)
Compound z score thermal detection [SD]	-1.15 (1.11)	-0.69 (1.05)	-0.33 (1.13)

Given are median and range, n = absolute number of patients. Only for compound z scores, mean values and standard deviations are given since these scores are normally distributed. One patient only had known diabetes for 2 months, therefore, diabetes duration was defined as 0 years in this case. Distribution of NSS amongst patients with DPN was as follows: No symptoms (NSS 0): n=10, mild symptoms (NSS 3-4): n=7, moderate symptoms (NSS 5-6): n=13, severe symptoms (NSS 7-9): n=18. Distribution of NDS amongst patients with DPN was as follows: No neuropathic deficits (NDS 0-2): n=12, mild neuropathic deficits (NDS 3-5): n=16, moderate neuropathic deficits (NDS 6-8): n=16, severe neuropathic deficits (NDS 9-10): n=4. Patients with DPN and an NSS of 0 had a pathological NDS and vice versa, since inclusion criteria were NSS and/or NDS ≥3 points. Total body water was measured by body impedance analysis.

Table 2 ANOVA table for comparison between overall groups.

			Sum of Squares	df	Mean Square	F	Significance
z Mechanical Detection * Overall Group	Between Groups	(Combined)	75.537	2	37.769	16.005	<0.001
	Within Groups		165.190	70	2.360		
	Total		240.727	72			
z Mechanical Pain * Overall Group	Between Groups	(Combined)	23.514	2	11.757	4.122	0.020
	Within Groups		199.649	70	2.852		
	Total		223.163	72			
z Thermal Pain * Overall Group	Between Groups	(Combined)	0.730	2	0.365	0.427	0.654
	Within Groups		58.988	69	0.855		
	Total		59.717	71			
z Thermal Detection * Overall Group	Between Groups	(Combined)	7.408	2	3.704	3.059	0.053
	Within Groups		84.759	70	1.211		
	Total		92.167	72			

Comparison of QST parameters between controls, patients with diabetes type 2 without polyneuropathy, and patients with diabetes type 2 with polyneuropathy. Compound z scores were used.

Table 3 ANOVA table for comparison between patients with diabetes type 2 separated by lesion load.

			Sum of Squares	df	Mean Square	F	Significance
z Mechanical Detection * Lesion Load Groups	Between Groups	(Combined)	8.981	2	4.491	1.392	0.259
	Within Groups		145.185	45	3.226		
	Total		154.166	47			
z Mechanical Pain * Lesion Load Groups	Between Groups	(Combined)	33.587	2	16.794	5.330	0.008
	Within Groups		141.778	45	3.151		
	Total		175.365	47			
z Thermal Pain * Lesion Load Groups	Between Groups	(Combined)	6.695	2	3.347	5.189	0.009
	Within Groups		29.030	45	0.645		
	Total		35.725	47			
z Thermal Detection * Lesion Load Groups	Between Groups	(Combined)	1.900	2	0.950	0.766	0.471
	Within Groups		55.794	45	1.240		
	Total		57.694	47			

Comparison of QST parameters between patients with diabetes type 2 and polyneuropathy with high, moderate, and low lesion load. Compound z scores were used.

Figure legends

Figure 1

Nerve segmentation and binarization of lesions.

Multislice-segmentation of the sciatic nerve, binarization of nerve lesions, and statistical analysis of lesion load and vital nerve tissue.

Figure 2

Comparison between groups of patients with diabetes mellitus type 2 with polyneuropathy, diabetes mellitus type 2 without polyneuropathy, and controls concerning T2w lesion load, electrophysiological testing, and compound z scores.

A) Group comparison concerning T2w lesion load in magnetic resonance neurography. B) Group comparison concerning conduction velocity of tibial nerve. C) Group comparison concerning conduction velocity of peroneal nerve. D) Group comparison concerning compound z score for mechanical detection (comprising vibration and mechanical detection thresholds) in quantitative sensory testing. E) Group comparison concerning compound z score for mechanical pain (comprising mechanical pain threshold and mechanical pain sensitivity) in quantitative sensory testing. F) Group comparison concerning compound z score for thermal pain (comprising cold and heat pain thresholds) in quantitative sensory testing. G) Group comparison concerning compound z score for thermal detection (comprising cold and warm detection thresholds and thermal sensory limen) in quantitative sensory testing. with DPN = patients with diabetes mellitus type 2 with diabetic polyneuropathy according to NSS and NDS scores; w/o DPN = patients with diabetes mellitus type 2 without polyneuropathy according to NSS and NDS scores; Controls = participants without diabetes and without polyneuropathy according to NSS and NDS scores; NCV = nerve conduction velocity; m = meters; sec = second; SD = standard deviation.

Figure 3

Correlation analysis of T2w lesion load with electrophysiological testing and compound z scores in all participants.

A) Correlation between conduction velocity of tibial nerve and T2w lesion load; B) Correlation between conduction velocity of peroneal nerve and T2w lesion load; C) Correlation between compound z score for mechanical detection (comprising vibration and mechanical detection thresholds) in quantitative sensory testing and T2w lesion load; D) Correlation between compound z score for mechanical pain (comprising mechanical pain threshold and mechanical pain sensitivity) in quantitative sensory testing and T2w lesion load. E) Correlation between compound z score for thermal pain (comprising cold and heat pain thresholds) in quantitative sensory testing and T2w lesion load. F) Correlation between compound z score for thermal detection (comprising cold and warm detection thresholds and thermal sensory limen) in quantitative sensory testing and T2w lesion load. with DPN = patients with diabetes mellitus type 2 with diabetic polyneuropathy according to NSS and NDS scores; w/o DPN = patients with diabetes mellitus type 2 without polyneuropathy according to NSS and NDS scores; Controls = participants without diabetes and without polyneuropathy according to NSS and NDS scores; T2w = T2-weighted magnetic resonance neurography. NCV = nerve conduction velocity; m = meters; sec = second; SD = standard deviation.

Figure 4

Comparison between groups of patients with diabetes mellitus type 2 and diabetic polyneuropathy divided by the lesion load in magnetic resonance neurography (MRN).

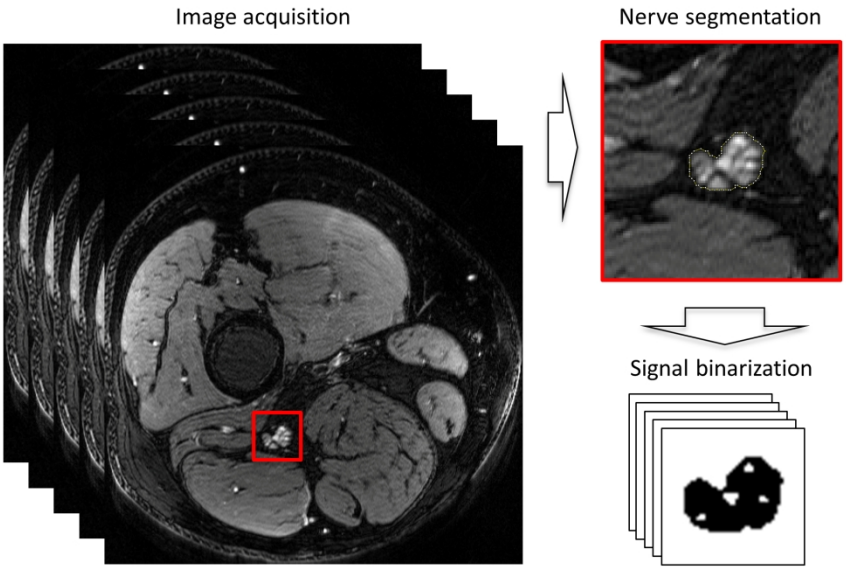
Group “low” consists of patients with a lesion load below the 25th percentile, which accounts for an absolute load of hyperintense lesions of less than 4%. Group “intermediate” consists of patients with moderate lesion load between the 25th and 75th

percentile, which accounts for an absolute load of hyperintense lesions of 4-11%. Group "high" consists of patients with a high lesion load in MRN above the 75th percentile, which accounts for an absolute load of hyperintense lesions of more than 11%. A) shows the comparison of conduction velocity of tibial nerve. B) shows the comparison of conduction velocity of peroneal nerve. C) shows the comparison of compound z score for mechanical detection (comprising vibration and mechanical detection thresholds) in quantitative sensory testing. D) shows the comparison of compound z score for mechanical pain (comprising mechanical pain threshold and mechanical pain sensitivity) in quantitative sensory testing. E) shows the comparison of compound z score for thermal pain (comprising cold and heat pain thresholds) in quantitative sensory testing. F) shows the comparison of compound z score for thermal detection (comprising cold and warm detection thresholds and thermal sensory limen) in quantitative sensory testing. NCV = nerve conduction velocity; m = meters; sec = second; SD = standard deviation.

Figure 5

Comparison of lesion load between patients without deficits, with relative gain of function, and with relative loss of function in quantitative sensory testing.

Shown is the comparison of lesion load in patients with diabetes mellitus type 2 and diabetic polyneuropathy. In this case due to the inherently low number of patients with polyneuropathy according to NSS but without deficit in quantitative sensory testing (neuropathic symptoms without objective neural deficits in clinical testing), Kruskal-Wallis-Test was not significant. Therefore, Mann-Whitney-U Test was used to compare patients without deficit or with gain of function to patients with loss of function.



Nerve segmentation and binarization of lesions.Multislice-segmentation of the sciatic nerve, binarization of nerve lesions, and statistical analysis of lesion load and vital nerve tissue.

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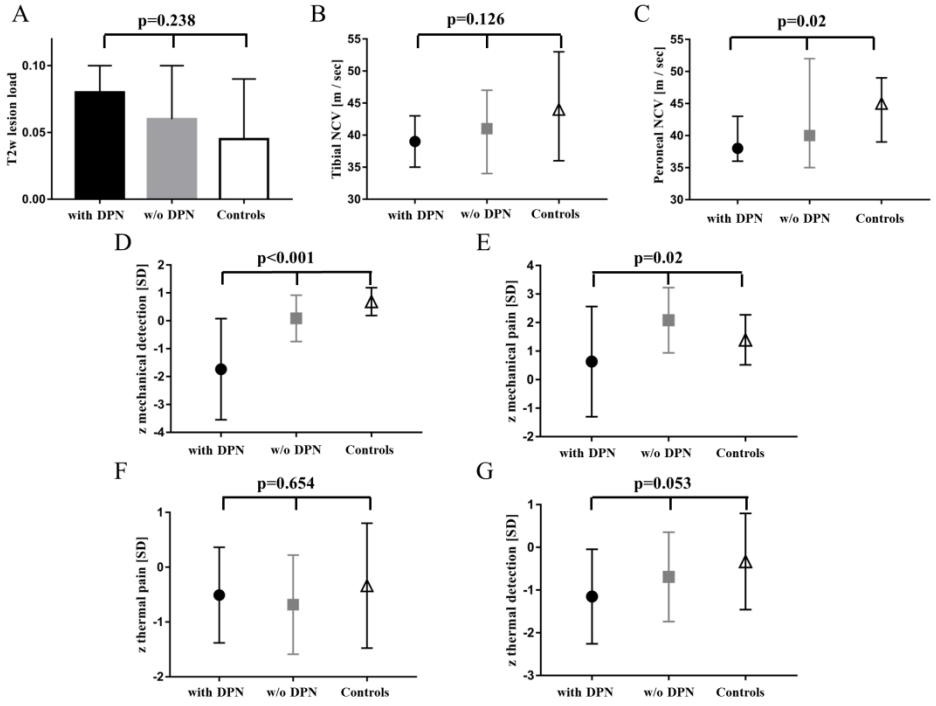

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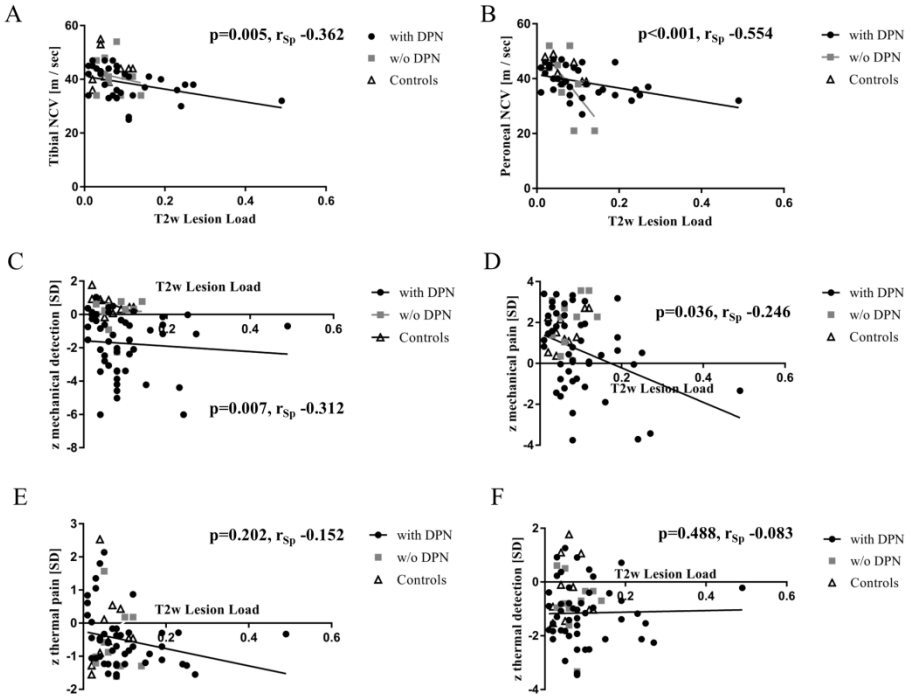

Correlation analysis of T2w lesion load with electrophysiological testing and compound z scores in all participants.

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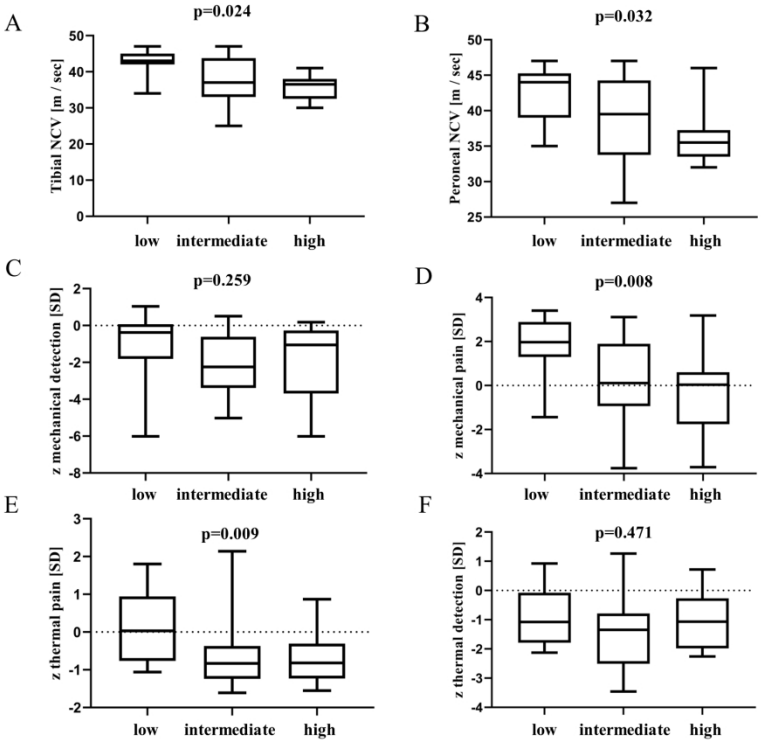

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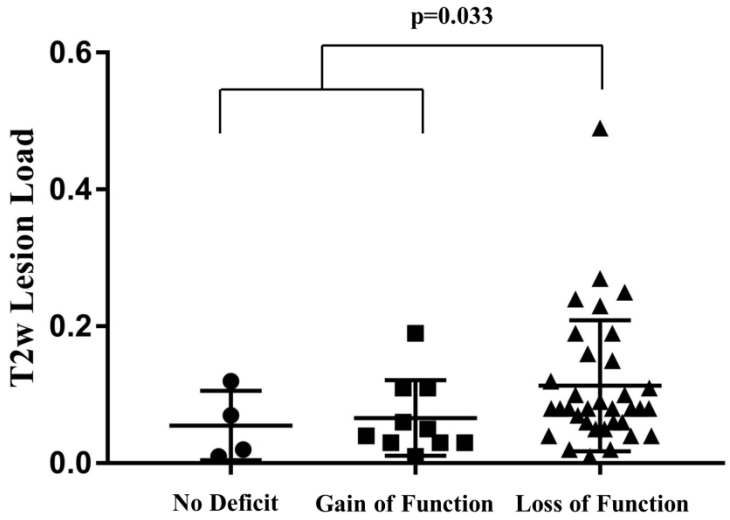

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