Identification of Factors Associated With Sural Nerve Regeneration and Degeneration in Diabetic Neuropathy

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OBJECTIVE—Patients with diabetic neuropathy (DN) demonstrate variable degrees of nerve regeneration and degeneration. Our aim was to identify risk factors associated with sural nerve regeneration in patients with DN.

RESEARCH DESIGN AND METHODS—Demographic, anthropometric, biochemical, and anatomical data of subjects with DN from a 52-week trial of acetyl-L-carnitine were retrospectively examined. Based on the change in sural nerve myelinated fiber density (ΔMFD%), subjects were divided into three groups: regenerator (top 16 percentiles, n = 67), degenerator (bottom 16 percentiles, n = 67), and intermediate (n = 290), with dramatically increased, decreased, and steady ΔMFD%, respectively. ANOVA, Fisher exact test, and multifactorial logistic regression were used to evaluate statistical significance.

RESULTS—ΔMFD% was 35.6 ± 17.4 (regenerator), −4.8 ± 12.1 (intermediate), and −39.8 ± 11.0 (degenerator). HbA1c at baseline was the only factor significantly different across the three groups (P = 0.01). In multifactorial logistic regression, HbA1c at baseline was also the only risk factor significantly different between regenerator (8.5 ± 1.6%) and degenerator (9.2 ± 1.8%) (odds ratio 0.68 [95% CI 0.54–0.85]; P < 0.01). Support Vector Machine classifier using HbA1c demonstrated 62.4% accuracy of classifying subjects into regenerator or degenerator. A preliminary microarray experiment revealed that upregulated genes in the regenerator group are enriched with cell cycle and myelin sheath functions, while downregulated genes are enriched in immune/inflammatory responses.

CONCLUSIONS—These data, based on the largest cohort with ΔMFD% information, suggest that HbA1c levels predict myelinated nerve fiber regeneration and degeneration in patients with DN. Therefore, maintaining optimal blood glucose control is likely essential in patients with DN to prevent continued nerve injury.

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duration of diabetes tend to have different levels of baseline MFD (4), we opted to investigate MFD percent change (ΔMFD%), rather than an absolute change in MFD to identify the clinical factors closely associated with MFD change over the specific duration of the disease course. With use of this classification approach, biomarkers and differential gene expression distinguishing patients exhibiting regeneration were examined and identified.

### RESEARCH DESIGN AND METHODS

For all subjects, demographic, anthropometric, biochemical, and anatomical data included in the current study were reported previously (6,7). Briefly, human sural nerve biopsies were obtained during a double-blind, placebo-controlled, 52-week trial of ALC. The trial included both type 1 and 2 diabetic patients—all with existing neuropathy. A sural nerve biopsy (week 0, baseline) and a blood sample were collected at the time of patient enrollment, and the following measures were recorded: HbA1c, hematocrit, serum triglycerides, cholesterol, and albumin. After 52 weeks of treatment, measures of DN were reassessed and a second sural nerve biopsy was harvested (week 52) from the opposite leg. All the harvested biopsies were processed at the Nerve Biopsy Laboratory, University of Michigan, according to the published protocols (10). No blood sample was collected at the end of the trial. Among the 748 participants in the trial, 427 participants had two sural nerve biopsies and complete blood chemistry analyses.

### Table 1—Characteristics of subjects and statistical evaluation results

<table>
<thead>
<tr>
<th></th>
<th>Degen</th>
<th>Inter</th>
<th>Regen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total subjects</td>
<td>67</td>
<td>290</td>
<td>67</td>
</tr>
<tr>
<td>ALC treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>24</td>
<td>89</td>
<td>20</td>
</tr>
<tr>
<td>ALC</td>
<td>43</td>
<td>201</td>
<td>47</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>18</td>
<td>94</td>
<td>29</td>
</tr>
<tr>
<td>Male</td>
<td>49</td>
<td>196</td>
<td>38</td>
</tr>
<tr>
<td>Insulin treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>32</td>
<td>181</td>
<td>36</td>
</tr>
<tr>
<td>No</td>
<td>35</td>
<td>109</td>
<td>31</td>
</tr>
<tr>
<td>Diabetes type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 1</td>
<td>10</td>
<td>68</td>
<td>16</td>
</tr>
<tr>
<td>Type 2</td>
<td>57</td>
<td>222</td>
<td>51</td>
</tr>
<tr>
<td>Age (years)</td>
<td>55.0±9.7</td>
<td>52.7±10.8</td>
<td>53.6±10.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.2±5.7</td>
<td>29.5±5.8</td>
<td>29.5±5.1</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>10.5±8.1</td>
<td>12.7±8.1</td>
<td>12.6±9.5</td>
</tr>
<tr>
<td>HbA1c, % (mmol/mol)</td>
<td>9.2±1.8, (76.7±20.0)</td>
<td>8.8±1.7, (73.1±18.9)</td>
<td>8.3±1.6, (66.9±17.7)</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>3.6±6.6</td>
<td>2.5±2.7</td>
<td>2.6±2.1</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>5.7±1.7</td>
<td>5.6±1.3</td>
<td>5.6±1.3</td>
</tr>
<tr>
<td>Albumin (mmol/L)</td>
<td>42.6±2.6</td>
<td>42.2±2.8</td>
<td>42.5±3.3</td>
</tr>
<tr>
<td>Hematocrit (fraction)</td>
<td>0.5±0.0</td>
<td>0.5±0.0</td>
<td>0.5±0.0</td>
</tr>
<tr>
<td>O'Brien neuropathy score</td>
<td>2,839.8±1,167.5</td>
<td>3,563.4±1,119.7</td>
<td>3,427.6±1,132.7</td>
</tr>
<tr>
<td>MFD baseline (fibers/mm²)</td>
<td>2,949.2±1,504.5</td>
<td>4,057.4±1,762.8</td>
<td>3,100.0±1,634.9</td>
</tr>
<tr>
<td>MFD 52 weeks (fibers/mm²)</td>
<td>1,792.4±971.0</td>
<td>3,850.0±1,700.7</td>
<td>4,054.6±1,950.6</td>
</tr>
<tr>
<td>MFD change (fibers/mm²)</td>
<td>−1,156.8±670.1</td>
<td>−207.4±539.4</td>
<td>954.6±442.9</td>
</tr>
<tr>
<td>MFD percent change (%)</td>
<td>−39.8±11.0</td>
<td>−4.8±12.1</td>
<td>35.6±17.4</td>
</tr>
</tbody>
</table>

Degen, degenerator; Inter, intermediate; Regen, regenerator. *Significant, P < 0.05.

### Outcome measures

The primary outcome measure was ΔMFD% at week 52. Three outlier subjects with >200% increase in MFD were excluded from any further analyses. In the remaining 424 subjects (ΔMFD% range −78.6 to 87.7%), 183 subjects (42.9%) demonstrated positive ΔMFD%. Based on ΔMFD%, the subjects were divided into three groups: regenerator (top 16 percentiles equivalent to beyond 1 SD from the mean); degenerator (bottom 16 percentiles), and intermediate (remaining subjects).

### Neuropathy evaluations

Electrophysiological measurements, including bilateral sural NCV and amplitude, peroneal NCV, and amplitude on the dominant side and median motor and
sensory NCV and amplitude on the non-dominant side, were performed the baseline and completion of the trial to generate an O’Brian neuropathy score (6). These measurements were done in triplicate, and the median value was used.

**Computational classifier for regenerator and degenerator**
Computational classifiers of regenerator and degenerator were generated and evaluated using ORANGE (http://orangibiolab.slu), an open-source, component-based data-mining and machine learning software suite (11). Seven classification algorithms (Naive Bayes, Logistic Regression, K Nearest Neighbors, Classification Tree, CN2 rules, Support Vector Machine [SVM], and Random Forest) available for binary class prediction were used with 20-fold cross-validation sampling to classify the subjects as regenerator or degenerator based on the demographic, anthropometric, and biochemical data.

**Microarray data analysis**
Based on the new grouping, we reanalyzed the previously published microarray data set (8) and an additional batch of unpublished microarray data (n = 35 and n = 33, respectively). These 68 microarrays included samples from 14 degenerators, 7 regenerators, and 45 intermediates. Microarrays were normalized using Robust Multiarray Average (12), and the batch effect was corrected using the distance-weighted discrimination method (13). Intensity-based moderated T statistics (14) was used to determine the DEGs among the groups. Owing to the small number of available microarrays (7 regenerators), a nominal P value of 0.05 without multiple testing corrections was used as the cutoff for DEGs.

The identified DEGs were further analyzed with the Database for Annotation, Visualization and Integrated Discovery (DAVID) (http://david.abcc.ncifcrf.gov/) (15,16), to determine overrepresented biological functions in terms of Gene Ontology (http://www.geneontology.org/) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (http://www.genome.jp/kegg/). LRpath (http://lrpath.ncbi.org/), a logistic regression-based gene set enrichment testing tool, was also used in our analysis. LRpath accepts statistical significance values from all genes on the tested array and does not require a predefined DEG set (17).

**Correlation between regeneration cluster density and MFD change**
Electron microscopy (EM) was performed on the baseline and/or 52-week biopsies of approximately half of the subjects (n = 219) immediately after the termination of the 52-week trial. The number of regenerating nerve clusters were counted, and the density of regenerating clusters was calculated (6). In the current study, we correlated the ΔMFD% over 52 weeks with changes in the density of regenerating clusters for subjects with both baseline and 52-week biopsies examined by EM (n = 168).

**Table 2—Factor analysis using multivariate logistic regression**

<table>
<thead>
<tr>
<th>Factor</th>
<th>P</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALC treatment (treated)</td>
<td>0.58</td>
<td>1.23 (0.58–2.61)</td>
</tr>
<tr>
<td>Sex (female)</td>
<td>0.08</td>
<td>2.01 (0.92–4.36)</td>
</tr>
<tr>
<td>Insulin treatment (no)</td>
<td>0.90</td>
<td>0.95 (0.41–2.21)</td>
</tr>
<tr>
<td>Diabetes type (type 1)</td>
<td>0.97</td>
<td>0.97 (0.26–3.68)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.32</td>
<td>0.98 (0.94–1.02)</td>
</tr>
<tr>
<td>BMI</td>
<td>0.67</td>
<td>0.99 (0.92–1.06)</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>0.25</td>
<td>1.03 (0.98–1.09)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>0.00**</td>
<td>0.68 (0.54–0.85)</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>0.30</td>
<td>0.94 (0.82–1.06)</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>0.22</td>
<td>1.25 (0.87–1.78)</td>
</tr>
<tr>
<td>Albumin (mmol/L)</td>
<td>0.74</td>
<td>0.98 (0.86–1.12)</td>
</tr>
<tr>
<td>Hematocrit (fraction)</td>
<td>0.79</td>
<td>0.29 (0.00–2.506)</td>
</tr>
</tbody>
</table>

**RESULTS**

**Group classification**
Based on the ΔMFD%, subjects were divided into three groups: regenerator (n = 67), degenerator (n = 67), and intermediate (n = 290). The mean ΔMFD% ± SD were 35.6 ± 17.4 (regenerator), -4.8 ± 12.1 (intermediate), and -39.8 ± 11.0 (degenerator). Table 1 summarizes the demographic, anthropometric, and biochemical characteristics for all subjects, comparing subjects from regenerator, degenerator, and intermediate groups by ANOVA and Fisher exact tests.

At baseline, there were no differences between groups in age, sex, diabetes duration or type, BMI, triglyceride, or total cholesterol. There were also no significant differences between groups in the number of subjects randomized to ALC or subjects treated with insulin. In addition, there were no differences between MFD at baseline between degenerator and regenerator, but MFD was significantly higher at baseline in the intermediate group (P = 8.34E-08). The groups differed in the O’Brien neuropathy rank-sum score, based on the electrophysiological measurements, with the lowest score observed in the regenerator group (2,839.8 ± 1,167.5) and the highest score in the regenerator (3,427.6 ± 1,132.7) and intermediate (3,563.4 ± 1,119.7) groups (P = 1.88E-05). Among the other evaluated risk factors shown in Table 1, HbA1c was the only risk factor significantly different across the three groups: regenerator (8.3 ± 1.6%), degenerator (9.2 ± 1.8%), and intermediate (8.8 ± 1.7%) (P = 0.01).

To further understand potential reasons that would drive regeneration or degeneration, we next compared the two extreme groups (degenerator vs. regenerator). Again, HbA1c level was the only significantly different biochemical factor (Bonferroni-corrected P = 0.01) between the two groups, while all other variables listed were not
HbA1c predicts peripheral nerve change in DN

Figure 1 — Receiver operating characteristic of the classifiers. The seven machine learning classification algorithms available in ORANGE were evaluated for classifying DN patient regenerator and degenerator groups. Classifiers were trained on the HbA1c levels of the subjects from these two groups, and testing was performed in 20-fold cross-validation. CN2, Clark Niblett 2; FP, false positive; kNN, k-nearest neighbor; TP, true positive.

significantly different (Table 1). The multivariable logistic regression analysis also confirmed that the HbA1c level at baseline was the only significantly different factor (odd ratio [OR] 0.68 [95% CI 0.54–0.85]; P < 0.01) with other variables adjusted (Table 2). The regenerator group included more females and more patients with type 1 diabetes; however, the differences were not statistically significant. Interestingly, although these two groups had similar baseline MFD (2,949.2 ± 1,504.5 [degenerator] and 3,100.0 ± 1,634.9 [regenerator]; P = 1), the baseline O’Brien score was significantly lower in the degenerator group compared with the regenerator group (Table 1) (2,839.8 ± 1,167.5 vs. 3,427.6 ± 1,132.7 respectively, P = 0.01).

Computational classifier for regenerator and degenerator

A machine-learning approach was used to test whether risk factors may predict the classification category outcome of participants with DN. Among the seven machine learning algorithms using a 20-fold cross-validation, two algorithms (SVM and logistic regression) achieved a classification accuracy (CA) >60%, with logistic regression being the best classifier (CA = 62.7%). Figure 1 illustrates receiver operating characteristic curves of the evaluated classifiers and indicates that SVM and logistic regression are the best classifiers. The addition of other factors resulted in degraded classification performance; however, O’Brien neuropathy score slightly improved the classifiers, with SVM achieving the highest CA of 64.2%.

Microarray data analysis

To examine the gene expression profiles that are significantly different between the two extreme groups, two batches of human sural nerve microarray datasets were combined (one published [8]). Intensity-based moderated T statistics identified a total of 490 DEGs between regenerator (n = 7) and degenerator (n = 15) at a nominal P value of 0.05 without multiple testing corrections. Supplementary Table 1 lists the 10 most upregulated and 10 most downregulated DEGs. Multiple immune-related genes such as CD177 molecule (CD177) (19), human leukocyte antigen (HLA) complex group 4 (HCG4), and chemokine (C-X-C motif) ligand 10 (CXCL10) (20) are upregulated in regenerator, indicating possible activation of multiple immune cell types such as neutrophils (19) and natural killer cells (20).

Table 3 lists the top 20 concepts (gene sets defined by biological functional terms such as Gene Ontology terms) identified by LRpath that have a significantly lower false discovery rate (FDR) for differential gene expression. Although some immune activation gene markers (CD177 and CXCL10) were markedly upregulated in regenerator, LRpath suggests that genes associated with immune response (FDR = 2.23E-08), defense response (FDR = 3.73E-07), and inflammatory response (FDR = 6.16E-04) were generally downregulated in regenerator. The top concepts upregulated in regenerator included condensed chromosome (FDR = 4.47E-04) and transmission of nerve impulse (FDR = 5.44E-04). Supplementary Table 2 lists all the significant genes in these 20 top concepts. DAVID, another gene set enrichment analysis tool, identified biological functional terms significantly overrepresented in the 490 DEG set. A heat map (Supplementary Fig. 1) was generated to summarize the most significant functions and indicates that the genes upregulated in regenerators were highly enriched with cell cycle, suggesting active regeneration.

Correlation with regeneration clusters

Approximately one-third (n = 168) of the study subjects had their baseline and 52-week biopsies examined by EM, and the Pearson correlation coefficient between ΔMFD% and the regeneration fiber density change was 0.33 (P < 0.001). When the analysis is limited only to the regenerator and degenerator groups, the correlation coefficient was 0.35 (P = 0.014 with n = 48). Although the correlation is not strong, the data suggest that the change in MFD is partially reflected in the decreased level of nerve regeneration. We also
examined whether HbA1c level is correlated with the absolute density and the change of the regenerating clusters. The correlation between baseline HbA1c and the changes in regenerating clustering density was 0.02 for all 168 subjects; however, this correlation became −0.15 with the analysis limited to the regenerator and degenerator groups (n = 48). Although this does not reach the statistical significance cutoff, the negative correlation values suggest that there is a trend for decreased regeneration cluster density over 52 weeks with a higher baseline HbA1c level. However, no linear correlation was observed between the baseline HbA1c level and the baseline regeneration cluster density (correlation coefficient = 0.01 in both sets using all subjects and the two extreme sets). These results suggest that the HbA1c level at a certain time point may not be predictive of the absolute level of the regenerating cluster density but may partially predict the changes in the regenerating cluster and MFD over time.

**CONCLUSIONS**—Previous analyses by our group of human sural nerve biopsies harvested as part of a double-blind, placebo-controlled, 52-week trial of ALC for DN (6,7) revealed that elevated serum triglycerides measured at trial onset correlate with DN progression (7) and that the alterations in immune response and lipid metabolism genes are also associated with progressive DN (8). Further examination of these data, however, revealed that MFD improved in ~43% of the subjects, and although modest regeneration has been documented in DN (9), no study has investigated critical factors affecting nerve regeneration in DN. In the current study, we examined demographic, anthropometric, and biochemical data of these subjects to identify the potential risk factors that correlate with myelinated nerve fiber regeneration and degeneration. We found that HbA1c was the only factor significantly associated with regeneration and degeneration. In fact, the baseline HbA1c level alone was able to correctly classify 62.7% of the subjects as a regenerator or degenerator.

This study is an extension of the previously published analysis of the same cohort and was pursued in an attempt to better understand factors contributing to nerve fiber degeneration associated with DN. The previous study used the absolute loss of MFD over the course of the 52-week clinical trial as the classifier, resulting in three groups of patients (progressor group with ≥500 fibers/mm² of MFD loss, nonprogressor group with ≤100 fibers/mm² of MFD loss, and intermediate group for the remainders) (7,8). Therefore, the nonprogressor group in the previous study comprised all of the regenerator subjects from the current study, as well as a large portion of the intermediate subjects. Furthermore, the present subject groups were selected using the ΔMFD% as the classifier rather than absolute MFD change, as patients at different ages and with different durations of diabetes tend to have substantially variable levels of baseline MFD. Thus, the percent change, rather than absolute change of MFD, was used to evaluate the effects of several important clinical factors shown to contribute to DN progression.

Peripheral nerves undergo spontaneous regeneration upon injury; however, the risk factors in diabetes affecting nerve regeneration and degeneration are not clearly understood. Although the overall MFD decreases with age, the nerve itself may regenerate either spontaneously or in response to external stimuli (21). Axonal regeneration actively takes place as a natural compensatory response to damage caused by diabetes, but incomplete or unsuccessful regeneration may constitute a critical component of DN progression (5). We anticipated that genes related to axonal regeneration or cell growth would be more actively expressed in the

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**Table 3—Top 20 most differential biological functions between degenerator and regenerator identified by LRpath**

<table>
<thead>
<tr>
<th>Name</th>
<th>Concept type</th>
<th>#Genes</th>
<th>#SigGenes</th>
<th>OR</th>
<th>FDR</th>
<th>Direction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune response</td>
<td>GO BP</td>
<td>518</td>
<td>12</td>
<td>0.21</td>
<td>2.23E-08</td>
<td>Down</td>
</tr>
<tr>
<td>Regulation of immune system process</td>
<td>GO BP</td>
<td>348</td>
<td>12</td>
<td>0.17</td>
<td>5.62E-08</td>
<td>Down</td>
</tr>
<tr>
<td>Defense response</td>
<td>GO BP</td>
<td>500</td>
<td>11</td>
<td>0.24</td>
<td>3.73E-07</td>
<td>Down</td>
</tr>
<tr>
<td>Myeloid cell differentiation</td>
<td>GO BP</td>
<td>135</td>
<td>8</td>
<td>0.10</td>
<td>8.52E-06</td>
<td>Down</td>
</tr>
<tr>
<td>Regulation of immune response</td>
<td>GO BP</td>
<td>195</td>
<td>7</td>
<td>0.14</td>
<td>1.13E-05</td>
<td>Down</td>
</tr>
<tr>
<td>External side of plasma membrane</td>
<td>GO CC</td>
<td>111</td>
<td>7</td>
<td>0.09</td>
<td>2.24E-05</td>
<td>Down</td>
</tr>
<tr>
<td>Ribonucleoprotein complex</td>
<td>GO CC</td>
<td>418</td>
<td>13</td>
<td>0.28</td>
<td>5.04E-05</td>
<td>Down</td>
</tr>
<tr>
<td>Regulation of response to stimulus</td>
<td>GO BP</td>
<td>428</td>
<td>10</td>
<td>0.27</td>
<td>8.71E-05</td>
<td>Down</td>
</tr>
<tr>
<td>Hematopoietic cell lineage</td>
<td>KEGG</td>
<td>62</td>
<td>4</td>
<td>0.05</td>
<td>1.34E-04</td>
<td>Down</td>
</tr>
<tr>
<td>Mitochondrial part</td>
<td>GO CC</td>
<td>530</td>
<td>12</td>
<td>0.34</td>
<td>1.69E-04</td>
<td>Down</td>
</tr>
<tr>
<td>Condensed chromosome</td>
<td>GO CC</td>
<td>113</td>
<td>5</td>
<td>6.16</td>
<td>3.36E-04</td>
<td>Up</td>
</tr>
<tr>
<td>Condensed chromosome kinetochore</td>
<td>GO CC</td>
<td>61</td>
<td>4</td>
<td>10.99</td>
<td>4.47E-04</td>
<td>Up</td>
</tr>
<tr>
<td>Condensed chromosome, centromeric region</td>
<td>GO CC</td>
<td>66</td>
<td>4</td>
<td>9.95</td>
<td>4.47E-04</td>
<td>Up</td>
</tr>
<tr>
<td>Outer kinetochore of condensed chromosome</td>
<td>GO CC</td>
<td>10</td>
<td>1</td>
<td>94.73</td>
<td>4.47E-04</td>
<td>Up</td>
</tr>
<tr>
<td>Mitochondrial lumen</td>
<td>GO CC</td>
<td>202</td>
<td>7</td>
<td>0.21</td>
<td>4.47E-04</td>
<td>Down</td>
</tr>
<tr>
<td>Mitochondrial matrix</td>
<td>GO CC</td>
<td>202</td>
<td>7</td>
<td>0.21</td>
<td>4.47E-04</td>
<td>Down</td>
</tr>
<tr>
<td>Positive regulation of immune system process</td>
<td>GO BP</td>
<td>222</td>
<td>6</td>
<td>0.20</td>
<td>5.44E-04</td>
<td>Down</td>
</tr>
<tr>
<td>Myeloid leukocyte differentiation</td>
<td>GO BP</td>
<td>64</td>
<td>4</td>
<td>0.06</td>
<td>5.44E-04</td>
<td>Down</td>
</tr>
<tr>
<td>Transmission of nerve impulse</td>
<td>GO BP</td>
<td>286</td>
<td>6</td>
<td>0.30</td>
<td>5.44E-04</td>
<td>Up</td>
</tr>
<tr>
<td>Inflammatory response</td>
<td>GO BP</td>
<td>295</td>
<td>6</td>
<td>0.25</td>
<td>6.16E-04</td>
<td>Down</td>
</tr>
</tbody>
</table>

Direction indicates that the significant genes are upregulated (up) or downregulated (down) in regenerator. BP, Biological Process; CC, Cellular Component; GO, Gene Ontology; MF, Molecular Function; #Genes, number of genes associated with the given concept; #SigGenes, number of significantly DEGs associated with the given concept.
HbA1c predicts peripheral nerve change in DN

regenerator compared with the degenera-
tor group.

Microarray analyses confirmed that those genes involved in cell cycle func-
tions are highly upregulated in regen-
erator compared with degenerator. Biologi-
cal functions associated with neu-
ron projection and myelin sheath were
highly enriched in those genes upregu-
lated in regenerator compared with the
intermediate group. These functions were
not significantly overrepresented in the
DEGs between degenerator and regener-
ator, which is probably due to the low
power of detecting differential expression
with a limited number of samples. The
results should be only considered as pre-
liminary, and more samples need to be
processed to increase the statistical power.
It should also be noted that Schwann cells
are major contributors to the mRNA in the
sural nerve biopsies, with a small contribu-
tion coming from axons, epineural fi-
broblasts, adipocytes, vascular endothelial
cells, and immune cells such as macro-
phages. Therefore, the gene expression
changes observed by microarray are most
likely to represent the changes in Schwann
cells in response to diabetes.

Another interesting finding is the fact
that in spite of similar MFD at baseline in
the degenerator and regenerator groups,
the degenerator group had a much lower
O’Brien neuropathy score. The O’Brien
scores are accepted methods to quantify
multiple electrophysiological measure-
ments obtained from nerve conduction
studies (NCSs). NCSs assess mostly large
myelinated nerve fiber function and are still
considered by most as the gold standard
end point for DN in clinical trials (22). Our
findings suggest that nerve fiber func-
tion as assessed by NCS may be decreased
before an anatomical loss of myelinated
fibers and can be used in combination with
other factors (such as HbA1c) to predict
degeneration.

Fiber regeneration delays have been
observed in both the tibial (largely motor)
and sural (sensory) distal sciatic branches
after both sciatic nerve crush injury and
complete sciatic nerve transection in streptozocin-treated mice, a type 1 di-
abetes animal model (23). Interestingly,
macrophage invasion was associated with
the delay in this model, supporting a po-
tential mechanism for impaired regenera-
tion due to abnormal macrophage partici-
pation in nerve repair (23); how-
ever, the role of macrophages in nerve re-
pair is still controversial (24–26). According to our microarray data,
macrophage differentiation had a FDR of
0.018. Although only one gene, THO
complex 5 (THOC5), was deemed a signif-
icant gene according to this concept,
the overall changes of genes related to mac-
rophage differentiation and other similar
terms, such as macrophage activation,
were found to be downregulated in regen-
erator by LRpath. More studies on the po-
tential role of macrophages will be necessary
to elucidate the exact mechanisms.

Study limitations include that blood
chemistry data were only available at
baseline and no subsequent measuring
was done during or at the end of the trial.
Therefore, controlling for in-trial changes
in the covariates analyzed was not possi-
ble. It is possible that the overall HbA1c
levels changed during the trial and data
regarding lifestyle or diet changes were
not available. In addition, even though
the study cohort is the largest one avail-
able to date with ΔMFD% information, we
may have lacked sufficient power to
detect meaningful effects for all risk fac-
tors. Although diabetes type did not
have a statistically significant effect on
regenerator and degenerator classification
in the current study, future separate anal-
yses of type 1 and 2 diabetic subjects may
be informative and will be pursued.

In the current study, we evaluated
potential biomarkers and gene expression
profiles of the sural nerve biopsies from the
largest available DN patient cohort with
ΔMFD% information in order to as-
certain factors associated with nerve re-
generation and degeneration in DN. The
data suggest that HbA1c levels are signifi-
cantly associated with the nerve regener-
ation and degeneration and may be pre-
dictive of future sural peripheral nerve
regeneration. The microarray data suggest
that immune and inflammatory responses
may play a crucial role in nerve regenera-
tion and degeneration. Although the ex-
act mechanisms must still be elucidated,
these data indicate that optimal blood
sugar control in patients with DN is
likely to impact sural nerve regeneration
and that the immune response may play
an important role in this process.

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