known that diabetic neuropathies are frequently asymptomatic. Kim et al[12] reported that 6.8% of their diabetic patients had asymptomatic electrophysiologic CTS. However, the lack of reliable information on electrodiagnostic discriminators of CTS from DPN therefore has major implications in both clinical and research contexts.

The aim of this study was to evaluate whether the sonographic findings in the median nerve corresponded to the results of motor NCS in diabetic patients. Although NCS have also been widely used for the diagnosis of CTS, they are not perfect reference standards. Clearly, there have been patients with clinically defined CTS without any abnormality on NCS. Our data showed that the CSA in the carpal tunnel was correlated with a reduced MCV and also with delayed latency. Moreover, the CSA in this study was significantly larger in the diabetic patients with DPN than in the control participants and diabetic patients without DPN at the carpal tunnel level. These findings suggest that asymptomatic CTS exists in diabetic patients, and both entrapment and other factors such as metabolic and vascular causes affect diabetic polyneuropathy. We presume that the combination of NCS and sonography may be able to estimate asymptomatic CTS with diabetes mellitus more accurately.

It has been reported that histologic abnormalities in diabetic neuropathy include axonal degeneration in nerve fibers and primary demyelination resulting from Schwann cell dysfunction. Early morphologic changes include minimal alteration of myelinated and unmyelinated fibers and axonal regeneration.[28,29] Suzuki et al[30] reported that sorbitol itself and secondary sodium accumulation caused by an increase in sorbitol may have been major contributors to the increase in intracellular hydration in a hydrogen 1 nuclear magnetic resonance study. It has further been hypothesized that in individuals with diabetes mellitus, the peripheral nerve is swollen because of increased water content related to increased aldose reductase conversion of glucose to sorbitol.[31] It is suggested that histologic findings may reflect the internal echo in the peripheral nerve. In our study, the correlation coefficients were relatively low, even though the size of the CSA correlated with NCS. We hypothesize that an enlarged median nerve in diabetic patients may occur because of increased water content rather than entrapment. Our findings suggest that the nerve swelling may partly contribute to slowing of nerve conduction.

Finally, a study with a much larger number of participants might yield different results because the small number of participants might have limited this study. In addition, our study had some other limitations that warrant discussion. For example, advanced sonographic techniques such as color Doppler and power Doppler imaging were not used in this study. We also did not attempt histologic evaluation, only sonographic size measurement; therefore, our study design certainly led to less evidence for discussion. However, sonography is a noninvasive method that can be used to evaluate detailed nerve structures, and measurement of the nerve CSA is an easy method that gives important additional information in patients with suspected neuropathy. Further studies are needed to confirm the findings in larger groups of diabetic patients.

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Sonographic Evaluation of the Peripheral Nerve in Diabetic Patients

The Relationship Between Nerve Conduction Studies, Echo Intensity, and Cross-sectional Area

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Objective. Early detection of nerve dysfunction is important to provide appropriate care for patients with diabetic polyneuropathy. The aim of this study was to assess the echo intensity of the peripheral nerve and to evaluate the relationship between nerve conduction study results and sonographic findings in patients with type 2 diabetes mellitus. ***Methods.*** Thirty patients with type 2 diabetes (mean ± SD, 59.8 ± 10.2 years) and 32 healthy volunteers (mean, 53.7 ± 13.9 years) were enrolled in this study. The cross-sectional area (CSA) and echo intensity of the peripheral nerve were evaluated at the carpal tunnel and proximal to the wrist (wrist) of the median nerve and in the tibial nerve at the ankle. ***Results.*** There was a significant increase in the CSA and hypoechoic area of the nerve in diabetic patients compared with controls (wrist, 7.1 ± 2.0 mm^2, 62.3% ± 3.0%; ankle, 8.9 ± 2.8 mm^2, 57.6% ± 3.9%; and wrist, 9.8 ± 3.7 mm^2, 72.3% ± 6.6%; ankle, 15.0 ± 6.1 mm^2, 61.4% ± 5.3% in controls and diabetic patients, respectively; $P < .05$). Cross-sectional areas were negatively correlated with reduced motor nerve conduction velocity and delayed latency. ***Conclusions.*** These results suggest that sonographic examinations are useful for the diagnosis of diabetic neuropathy. ***Key words:*** cross-sectional area; diabetic neuropathy; echo intensity; median nerve; sonography; tibial nerve.

Abbreviations
BMI, body mass index; BSA, body surface area; CMAP, compound muscle action potential; CSA, cross-sectional area; CTS, carpal tunnel syndrome; DPN, diabetic polyneuropathy; MCV, motor nerve conduction velocity; MMCV, median motor nerve conduction velocity; NCS, nerve conduction study; ROC, receiver operating characteristic; TMCV, tibial motor nerve conduction velocity; TTS, tarsal tunnel syndrome

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The World Health Organization estimates that more than 180 million people worldwide have diabetes mellitus. This figure is estimated to more than double by 2030.[1] In Japan, the number of diabetic patients has increased and reached 8 million, and it is assumed that 35% to 45% of diabetic patients have symmetric diabetic polyneuropathy (DPN). Advanced DPN causes serious complications such as diabetic foot ulcers, gangrene, and Charcot joint, all of which worsen the quality of life of diabetic patients. Therefore, early detection of nerve dysfunction is important to provide appropriate care for patients with DPN.[3]

The diagnosis of diabetic neuropathy is based primarily on characteristic symptoms and is confirmed with a nerve conduction study (NCS). Although imaging analyses for neuropathy have not been used for diagnosis, high-resolution diagnostic ultrasound equipment has improved greatly, making revelation of minute peripheral nerves by sonographic evaluation possible.[4] Recent

studies using sonography for entrapment neuropathy such as carpal tunnel syndrome (CTS)[4–9] have been presented. However, there are few reports of DPN diagnosis using sonography. We showed previously that the cross-sectional area (CSA) of the median nerve in the carpal tunnel of patients with DPN is greater than that of controls and correlates with NCS.[10] The aim of this study was to assess the echo intensity of the peripheral nerve and to evaluate the relationship between NCS results and sonographic findings in diabetic patients.

Materials and Methods

Participants

Thirty patients with type 2 diabetes were enrolled in this study at the Gifu University Hospital from October 2007 to January 2009 (17 men and 13 women; age range, 36–83 years; mean ± SD, 59.8 ± 10.2 years). Our control group consisted of 32 healthy volunteers without diabetes mellitus or CTS (25 men and 7 women; age range, 24–72 years; mean, 53.7 ± 13.9 years). Patients' wrists with symptoms of CTS were not included in the study; those that were included had negative Phalen test results. Every participant was able to walk unaided, and none had received hemodialysis.

We studied a total of 95 peripheral nerves (including 62 median nerves and 33 tibial nerves) of 62 participants who received both sonography and NCS. This study was approved by the Institutional Review Board of Gifu University Hospital, and informed consent was obtained from all participants.

Sonographic Examinations

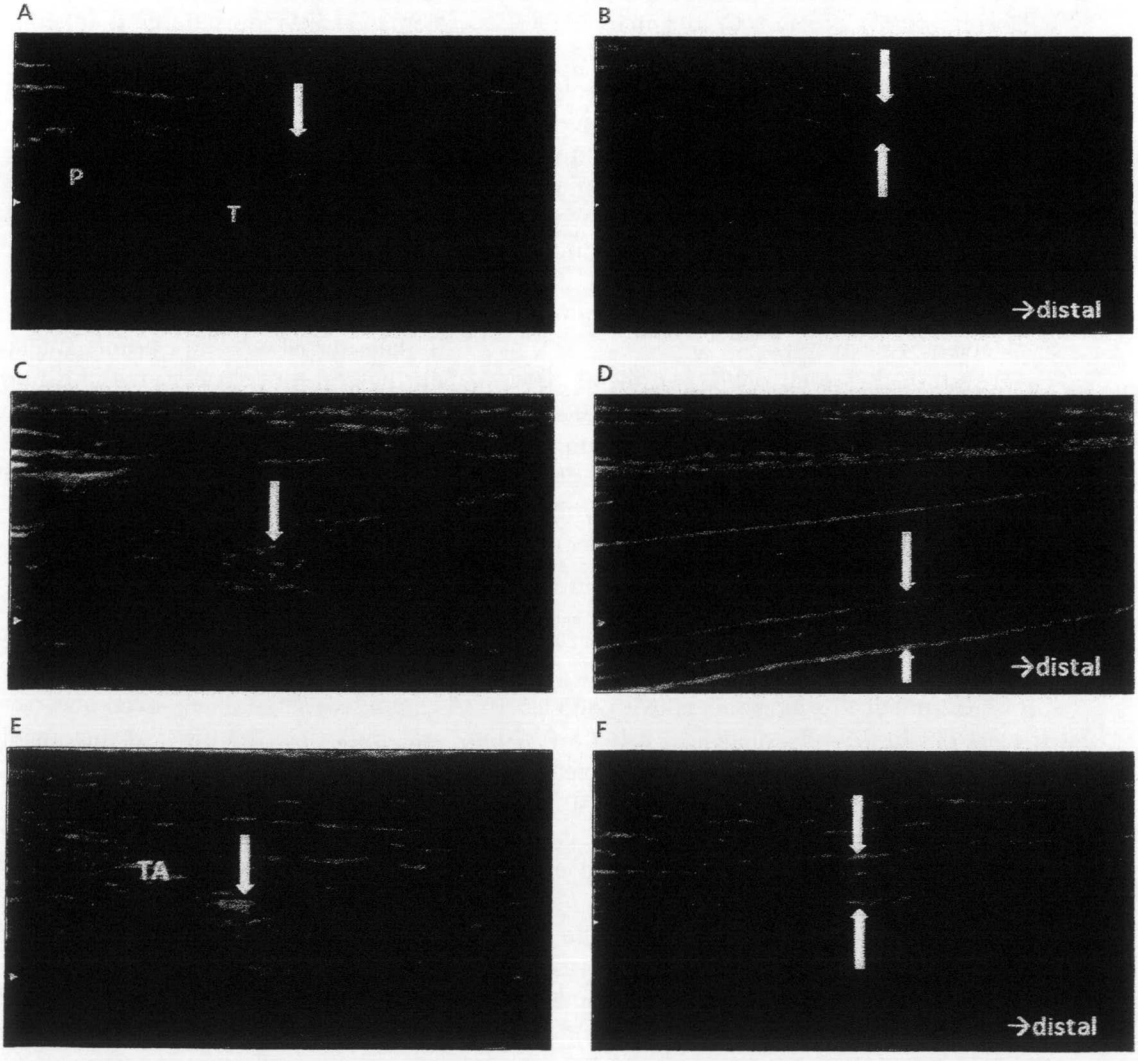
Sonographic examinations were performed by 1 of 2 experienced sonographers (with at least 10 years of ultrasound experience) using a 6.0- to 14.0-MHz linear array probe (portable real-time apparatus: EUB-7500; Hitachi Corporation, Tokyo, Japan; or ProSound Alpha 10; Aloka Co, Ltd, Tokyo, Japan). Sonograms were quantitatively analyzed using ImageJ software (National Institutes of Health, Bethesda, MD), and only the image obtained by a specific zoom setting was used. Computer analyses were performed by 2 other sonographers who did not have any knowledge of the electrodiagnostic results (observer A had 15 years of experience, and observer B had 6 years of experience). All participants were in the supine position on a table with fingers semiextended during examination of the median nerve and in the prone position during examination of the tibial nerve. The major axis, minor axis, and CSA of the median nerve were measured at the carpal tunnel and at 5 cm proximal to the wrist (wrist). The major axis, minor axis, and CSA of the tibial nerve were measured at the posterior medial malleolus (ankle). The CSA was calculated by the indirect method using the formula major axis × minor axis × π × 1/4 (square millimeters). There are 2 sonographic measurement methods of the nerve CSA: the indirect method (ellipsoid formula) and the direct method (tracing). Recently, Alemán et al[11] reported that median nerve CSA measurements are reproducible by either the direct or indirect method when a standardized sonographic examination protocol is applied. In addition, Sernik et al[12] also reported a high correlation ($r = 0.99$) between the areas calculated by the indirect and direct methods; consequently, we used the easier indirect method in our study. The volar wrist crease and pisiform bone or medial malleolus were used as initial external reference points and landmarks during scanning. Transverse and longitudinal sonograms of the nerve at each position were recorded (Figure 1). The peripheral nerve's speckled pattern on sonography enabled us to assess its size and echo intensity.

Of the 62 participants, 26 (42%; 13 diabetic patients and 13 controls) were recorded as specific zoom images on the Hitachi EUB-7500 ultrasound equipment. The stored images were further analyzed by 2 sonographers on a personal computer using ImageJ. Each observer received training specific to this study before initiation of the data collection. The images were saved as JPEG files and transferred to a personal computer for analysis. The monochrome sonogram was quantized to 8 bits (ie, 256 gray levels). Histogram analysis on sonography has been expected to offer an objective index for estimating echo intensity such as in diagnosis of fatty liver. The region of interest was set to cover the entire nerve, excluding its hyperechoic rim. The bright-

ness of the pixels ranged from 0 (black) to 255 (white). We used the percentage of the hypoechoic area as the index after the effects of the gain shift on the echo intensity in the median nerve were confirmed (Figure 2).

The normal appearance of the peripheral nerve should be readily recognized. The nerve consists of multiple hypoechoic bands corresponding to neuronal fascicles, which are separated by hyperechoic lines that correspond to the epineurium. Thus, the mean echogenicity was used as a threshold level for the analysis of the percentage of the hypoechoic area because the echogenicity of the peripheral nerve was obtained as a graded echo density from black to white. The percentage of the hypoechoic area was studied using computer analysis. Using ImageJ, the amount of the hypoechoic area falling below the threshold echo intensity level was calculated (Figure 3). Each measurement was taken 5 times, and the mean value was used in the analysis.

Figure 1. Transverse and longitudinal sonograms of the median and tibial nerves at each level. **A**, **C**, and **E**, Transverse sonograms showing the median nerve in the carpal tunnel (**A**) and 5 cm proximal to the wrist (**C**) and the tibial nerve in the posterior medial malleolus (**E**). The nerve (arrows) shows speckled pattern. **B**, **D**, and **F**, Longitudinal sonograms showing the median nerve in the carpal tunnel (**B**) and 5 cm proximal to the wrist (**D**) and the tibial nerve in the posterior medial malleolus (**F**). P indicates pisiform bone; T, tendon; and TA, tibial artery.



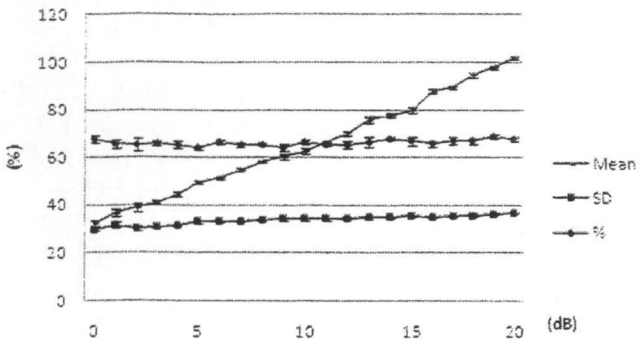


Figure 2. Effects of gain shift on the echo intensity in the median nerve. The line connecting rectangles shows a change of the mean; the line connecting squares shows a change of the SD; and the line connecting circles shows a change of the percentage according to the gain shift.

Intraobserver reproducibility was expressed as the difference between 2 repeated measurement results from the same observer. Interobserver reproducibility was expressed as the difference between 2 measurements obtained by 2 observers. Intraobserver reproducibility and interobserver reproducibility were estimated according to intraclass and interclass correlation coefficients.

Electrophysiologic Examinations

Routine NCS was performed using conventional procedures and standard electromyography (Neuropack MEB-2200; Nihon Kohden Corporation, Tokyo, Japan). All examinations were performed in a room with an ambient temperature of 25°C. The skin surface temperature in all cases was 31°C to 33°C. Motor nerve conduction velocity (MCV) was calculated from the distance of 2 stimulating points and the difference in each response time, and the compound muscle action potential (CMAP) was recorded from the abductor pollicis brevis muscle of the median nerve and the extensor digitorum brevis of the tibial nerve.

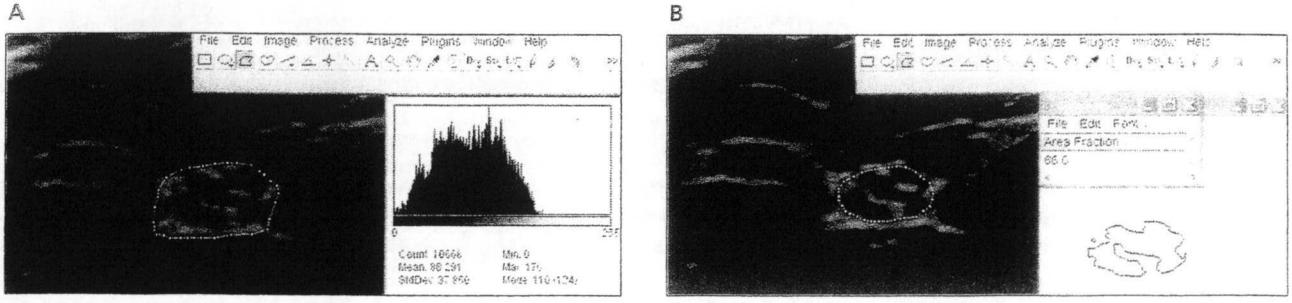
The rough reference ranges for the MCVs from the Gifu Laboratory are 50 m/s for the median motor nerve conduction velocity (MMCV) and 40 m/s for the tibial motor nerve conduction velocity (TMCV); therefore, we divided the patients into 4 groups (median nerve >50 and <50 m/s [high- and low-MMCV groups] and tibial nerve >40 and <40 m/s [high- and low-TMCV groups]) and compared them with the controls.

Statistical Analysis

The Mann-Whitney *U* test was used to compare the data between 2 groups. Pearson correlation coefficients were used to investigate the correlation of the CSA and percentage of the hypoechoic area with clinical parameters. Results are given as mean ± SD, and statistical significance was assessed as $P < .05$.

A receiver operating characteristic (ROC) curve was fitted to sonographic measurements using clinical DPN criteria as the reference standards to determine the optimum cutoff point and to evaluate the diagnostic accuracy of sonographic measurements. Receiver operating characteristic curves are plots of the true-positive rate (sensitivity) against the false-positive rate (1.0 – specificity) for the different possible cutoff points of a diagnostic test. The cutoff value at the Younden index point indicates the optimum threshold. To see the change of diagnostic accuracy according

Figure 3. Method of quantitative analysis for echo intensity using ImageJ software. **A**, The region of interest was set to cover the entire nerve, including the hyperechoic rim surrounding the nerve. The mean pixel brightness value was used for further analysis as a threshold. **B**, The percentage was calculated using the Analyze Particles function on the hypoechoic area in the nerve after threshold input.



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to reference standards, another ROC curve was fitted using MCV results, and the sensitivity and specificity of sonography and NCS were calculated and compared between 2 examinations.

Results

Detailed demographic data for the diabetic patients and controls are shown in Table 1. There were no significant differences in age, height, weight, or body mass index (BMI) between controls and diabetic patients. Intraobserver reproducibility was high for both observers (observer A, intraclass correlation coefficient = 0.998; observer B: SD = intraclass correlation coefficient = 0.987); interobserver reproducibility was also high (interclass correlation coefficient = 0.995). Results of the sonographic examinations and NCS of the diabetic patients and controls are shown in Table 2. Cross-sectional areas in the high-MMCV group were 8.8 ± 2.1 mm^2 in the carpal tunnel and 6.7 ± 1.4 mm^2 in the wrist. Cross-sectional areas in the low-MMCV group were 14.0 ± 6.1 mm^2 in the carpal tunnel and 9.8 ± 3.7 mm^2 in the wrist. Cross-sectional areas in the controls were 8.3 ± 1.8 mm^2 in the carpal tunnel and 7.1 ± 2.0 mm^2 in the wrist. There was a significant increase in the median nerve CSA in the low-MMCV group compared with that in the controls (carpal tunnel, $P < .001$; wrist, $P < .05$) and the high-MMCV group (carpal tunnel, $P < .001$; wrist, $P < .01$). Cross-sectional areas in the ankle were 15.0 ± 6.1 mm^2 in the low-TMCV group, 8.8 ± 2.9 mm^2 in the high-TMCV group; and 8.9 ± 2.8 mm^2 in the controls. There was a significant increase in the tibial nerve CSA in the low-TMCV group compared with that in the controls ($P < .01$) and the high-TMCV group ($P < .05$).

The percentage of the hypoechoic area was significantly increased in the low-MMCV group compared with that in the controls ($P < .01$) and the high-MMCV group ($P < .05$). Sonograms and histograms of the diabetic patients and controls are shown in Figure 4.

The MCV and CMAP of both the median and tibial nerves in the low-MCV group showed a significant decrease compared with those in the controls and the high-MCV group. On the other hand, latency was significantly slower in the low-MCV group than in the controls and the high-MCV group.

Table 3 shows the correlation results between several sonographic findings and characteristics in the median nerve. The CSA in the carpal tunnel showed a significant correlation with age ($r = 0.306$; $P < .05$), MCV ($r = -0.655$; $P < .001$), latency ($r = 0.552$; $P < .001$), and CMAP ($r = -0.311$; $P < .05$). In the wrist, the CSA was also significantly correlated with age ($r = 0.266$; $P < .05$), body surface area (BSA; $r = 0.271$; $P < .05$), MCV ($r = -0.502$; $P < .001$), latency ($r = 0.296$; $P < .05$), and CMAP ($r = -0.285$; $P < .05$). The percentage of the hypoechoic area showed a significant correlation with MCV ($r = -0.624$; $P < .001$) and latency ($r = 0.595$; $P < .01$).

Table 4 shows the correlation results between several sonographic findings and characteristics in the tibial nerve. The CSA in the ankle showed a significant correlation with age ($r = 0.493$; $P < .01$), weight ($r = 0.359$; $P < .05$), BMI ($r = 0.454$; $P < .05$), MCV ($r = -0.532$; $P < .01$), latency ($r = 0.525$; $P < .01$), and CMAP ($r = -0.414$; $P < .05$). The percentage of the hypoechoic area showed a significant correlation with weight ($r = 0.432$; $P < .05$), BMI ($r = 0.432$; $P < .05$), BSA ($r = 0.435$; $P < .05$), MCV ($r = -0.565$; $P < .01$), and latency ($r = 0.466$; $P < .05$).

The latency period of the median nerve was divided into 3 groups: latency of less than 3.5 milliseconds, latency of 3.5 to 4.0 milliseconds, and latency of greater than 4.0 milliseconds. The MCV of the median nerve was also divided into 3 groups: MCV of less than 50 m/s, MCV of 50 to 55 m/s, and MCV of greater than 55 m/s. Categorizing of participants into tertiles of laten-

Table 1. Characteristics of All Participants

Parameter	Controls	Diabetic Patients
n	32	30
Male/female	25/7	17/13
Age, y	53.7 ± 13.9	59.8 ± 10.2
Height, cm	164.9 ± 6.9	161.2 ± 7.7
Weight, kg	62.6 ± 9.5	62.6 ± 10.2
BMI, %	22.6 ± 2.8	24.1 ± 2.9
Duration, y	NA	15.4 ± 10.7
HbA1c, %	NA	8.9 ± 1.9
CVRR, %	NA	1.96 ± 1.19
Presence of sensory symptoms, n (%)	NA	47 (14/30)
Bilaterally decreased or absent Achilles tendon, n (%)	NA	66.7 (20/30)
Decreased vibratory sensation, n (%)	NA	66.7 (20/30)

CVRR indicates coefficient of variation of R-R intervals; HbA1c, hemoglobin A1c; and NA, not applicable.

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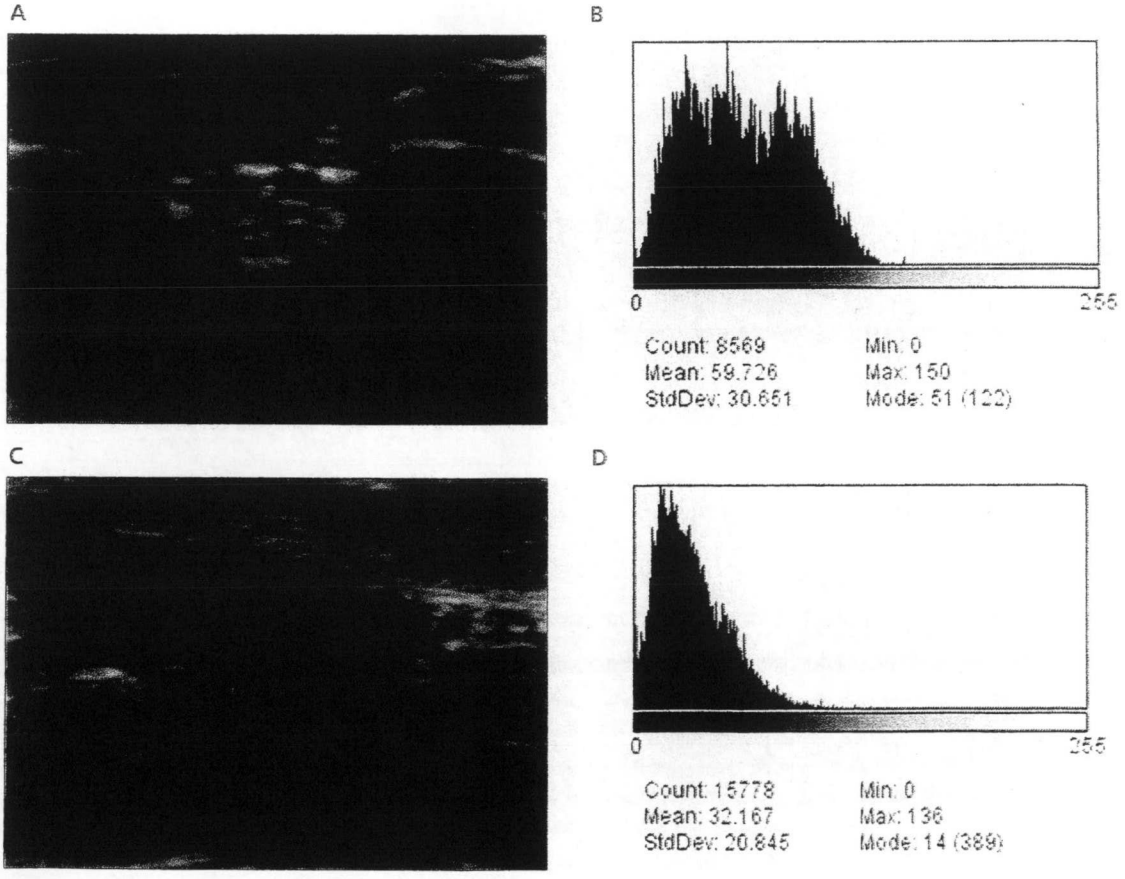
Table 2. Sonographic and Electrophysiologic Measurements of Patients With Diabetes Mellitus and Controls

Parameter	Median Nerve			Tibial Nerve		
		Diabetic Patients			Diabetic Patients	
	Controls (n = 32)	High MMCV (n = 16)	Low MMCV (n = 14)	Controls (n = 14)	High TMCV (n = 5)	Low TMCV (n = 14)
Sonographic measurements (CSA)						
Level of carpal tunnel, mm^2	8.3 ± 1.8	8.8 ± 2.1	14.0 ± 6.1[a,b]			
Level of 5 cm proximal to the wrist, mm^2	7.1 ± 2.0	6.7 ± 1.4	9.8 ± 3.7[c,d]			
Level of posterior medial malleolus, mm^2				8.9 ± 2.8	8.8 ± 2.9	15.0 ± 6.1[e,f]
Sonographic measurements (echo intensity)						
SD	31.3 ± 3.2	31.3 ± 4.5	26.0 ± 5.1[c,g]	28.3 ± 3.4	27.3 ± 1.2	26.5 ± 3.5
Hypoechoic area, %	62.3 ± 3.0	61.8 ± 5.0	72.3 ± 6.6[e,g]	57.6 ± 3.9	60.3 ± 2.7	61.4 ± 5.3
Electrophysiologic measurements						
MCV, m/s	54.9 ± 4.3	53.1 ± 2.8	45.3 ± 3.5[a,b]	50.1 ± 3.3	42.1 ± 1.9[e]	35.8 ± 2.4[a,h]
Latency, ms	3.7 ± 0.6	3.8 ± 0.5	4.7 ± 1.3[e,g]	4.1 ± 0.5	4.5 ± 0.8	5.6 ± 1.0[a,f]
CMAP, mV	13.7 ± 4.9	6.4 ± 3.2[a]	5.8 ± 3.2[a]	18.7 ± 6.8	10.3 ± 4.1[c]	5.9 ± 5.1[a]

Mann-Whitney *U* test: [a]$P < .001$ versus controls; [b]$P < .001$ versus high MMCV; [c]$P < .05$ versus controls; [d]$P < .01$ versus high MMCV; [e]$P < .01$ versus controls; [f]$P < .05$ versus high TMCV; [g]$P < .05$ versus high MMCV; [h]$P < .01$ versus high TMCV.

Figure 4. Transverse sonograms and histograms of the nerve at each level. **A**, Sonogram of the median nerve in a control participant's wrist. **B**, Histogram of the median nerve in a control participant's wrist. **C**, Sonogram of the median nerve in a diabetic patient's wrist. **D**, Histogram of the median nerve in a diabetic patient's wrist (continued).



cy yielded 3 separate groups. Compared with the first tertile, CSAs of the median nerve increased significantly with each tertile. Moreover, after combining tertiles of latency and the MCV, even more comprehensive CSA stratification was possible, with all CSAs ranging from 7.1 mm^2 in participants in the lowest tertile of both parameters to 14.6 mm^2 in participants in the highest tertile (Figure 5). The latency period of the tibial nerve was divided into 3 groups: latency of less than 4.5 milliseconds, latency of 4.5 to 5.0 milliseconds, and latency of greater than 5.0 milliseconds. The MCV of the tibial nerve was also divided into 3 groups: MCV of less than 40 m/s, MCV of 40 to 50 m/s, and MCV of greater than 50 m/s. The CSA of the tibial nerve stratification was 16.3 mm^2 in participants in the highest tertile for both parameters (Figure 6).

In the ROC analysis (Figure 7), the following cutoff values corresponded to the highest diagnostic accuracy: 11 mm^2 for the sonographic CSA of the carpal tunnel, 8 mm^2 for the sonographic CSA of the wrist, and 10 mm^2 for the sonographic CSA of the ankle; 62% for the hypoechoic area of the wrist and 66% for the hypoechoic area of the ankle; 52 m/s for the NCS MCV of the median nerve and 42 m/s for the NCS MCV of the tibial nerve; and 4.0 milliseconds for latency in the median nerve and 4.4 milliseconds for latency in the tibial nerve. According to the cutoff values derived from ROC analysis, the most effective sonographic parameter was the CSA of the carpal tunnel (68.2% sensitivity and 85% specificity), whereas the most effective NCS parameter was the tibial nerve MCV (87.5% sensitivity and 93.5% specificity). The sensitivity was lower with sonog-

Figure 4. (continued) **E**, Sonogram of the tibial nerve in a control participant's ankle. **F**, Histogram of the tibial nerve in a control participant's ankle. **G**, Sonogram of the tibial nerve in a diabetic patient's ankle. **H**, Histogram of the tibial nerve in a diabetic patient's ankle.

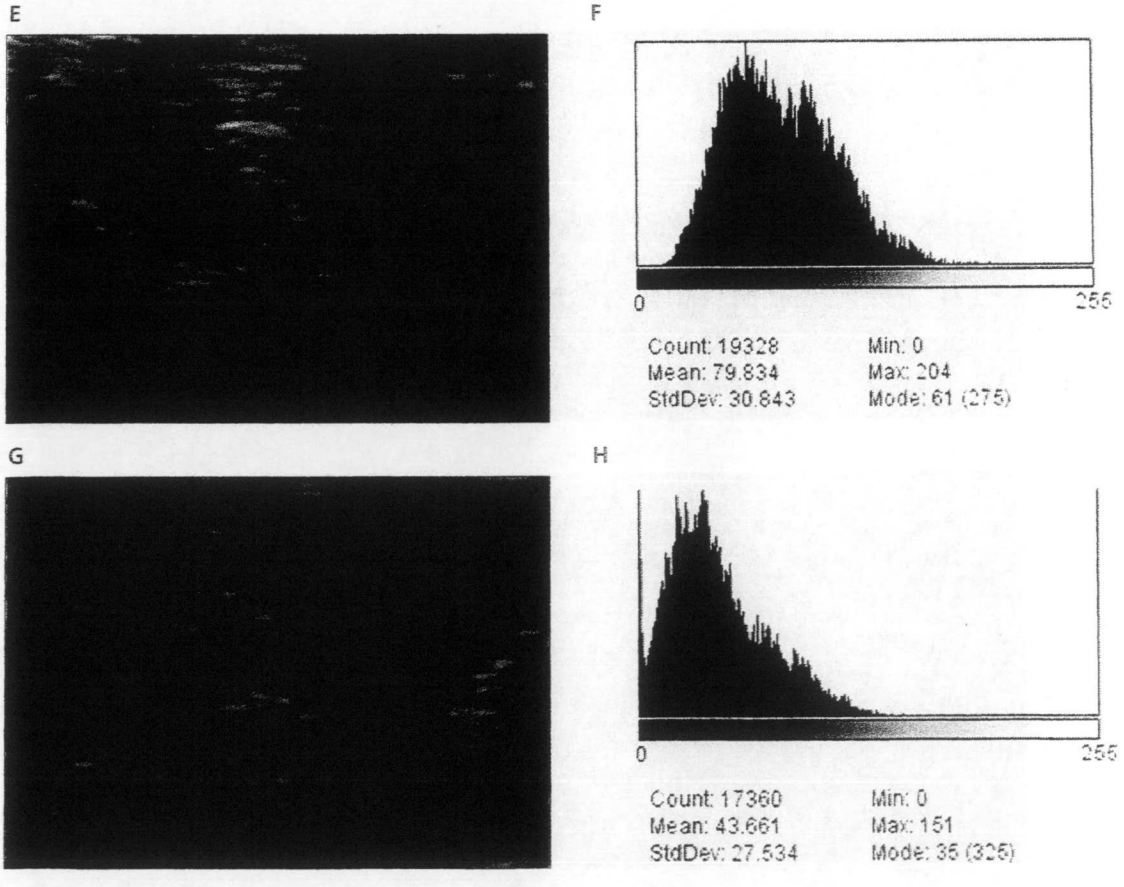


Table 3. Correlation Between Several Sonographic Findings and Characteristics in the Median Nerve

	Correlation Coefficient		
	CSA		Percentage of
Parameter	Carpal Tunnel	Wrist	Hypoechoic Area
Age	0.306[a]	0.266[a]	0.358
Height	−0.041	0.187	−0.020
Weight	0.131	0.217	0.232
BMI	0.186	0.189	0.328
BSA	0.123	0.271[a]	0.182
CVRR	−0.050	0.125	0.101
HbA1c	−0.251	−0.098	0.301
Duration	0.298	0.173	−0.219
MCV	−0.655[b]	−0.502[b]	−0.624[b]
Latency	0.552[b]	0.296[a]	0.595[c]
CMAP	−0.311[a]	−0.285[a]	−0.336

The CSA and percentage of the hypoechoic area were compared with the participant's age, physical parameters, coefficient of variation of R-R intervals (CVRR), hemoglobin A1c (HbA1c) level, duration, and nerve conduction study by Pearson correlation coefficients.
[a]$P < .05$; [b]$P < .001$; [c]$P < .01$.

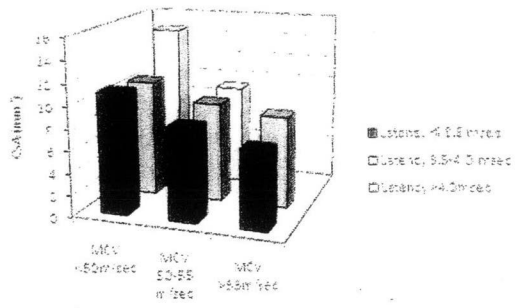


Figure 5. Cross-sectional area stratification in the median nerve by combining tertiles of latency and MCV. The CSA increased from 7.1 mm^2 in participants in the first tertiles of both nerve conduction study parameters to 14.6 mm^2 in participants in the third tertile of latency and MCV. The latency was divided into 3 groups: latency of less than 3.5 milliseconds, latency of 3.5 to 4.0 milliseconds, and latency of greater than 4.0 milliseconds. The MCV was also divided into 3 groups: MCV of less than 50 m/s, MCV of 50 to 55 m/s, and MCV of greater than 55 m/s.

raphy than NCS, but the specificity was similar between sonography and NCS (Table 5).

Discussion

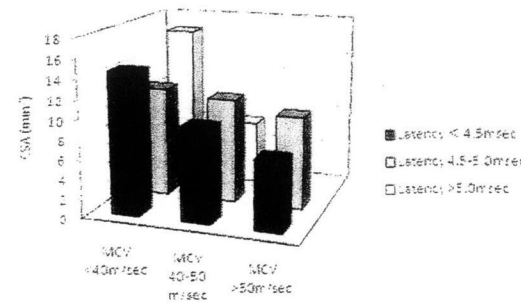
Diabetes mellitus is becoming a major cause of premature disability in Japan, and peripheral neuropathy is a common complication of diabetes.[13] The diagnosis of diabetic neuropathy is based on its characteristic symptoms and can be confirmed with NCS.[13–16] However, NCS is time-consuming, slightly invasive, and generally not well tolerated for repeated evaluations.[17] In contrast, sonographic examinations can be performed to assess peripheral nerves with less discomfort and have already been used for the evaluation of disorders of the peripheral nervous system.[4–10]

Table 4. Correlation Between Several Sonographic Findings and Characteristics in the Tibial Nerve

	Correlation Coefficient	
Parameter	CSA	Percentage of Hypoechoic Area
Age	0.493[a]	0.062
Height	−0.079	0.182
Weight	0.359[b]	0.432[b]
BMI	0.454[b]	0.432[b]
BSA	0.229	0.435[b]
CVRR	0.115	−0.038
HbA1c	0.241	0.156
Duration	0.018	−0.109
MCV	−0.532[a]	−0.565[a]
Latency	0.525[a]	0.466[b]
CMAP	−0.414[b]	−0.321

The CSA and percentage of the hypoechoic area were compared with the participant's age, physical parameters, coefficient of variation of R-R intervals (CVRR), hemoglobin A1c (HbA1c) level, duration, and nerve conduction study by Pearson correlation coefficients.
[a]$P < .01$; [b]$P < .05$.

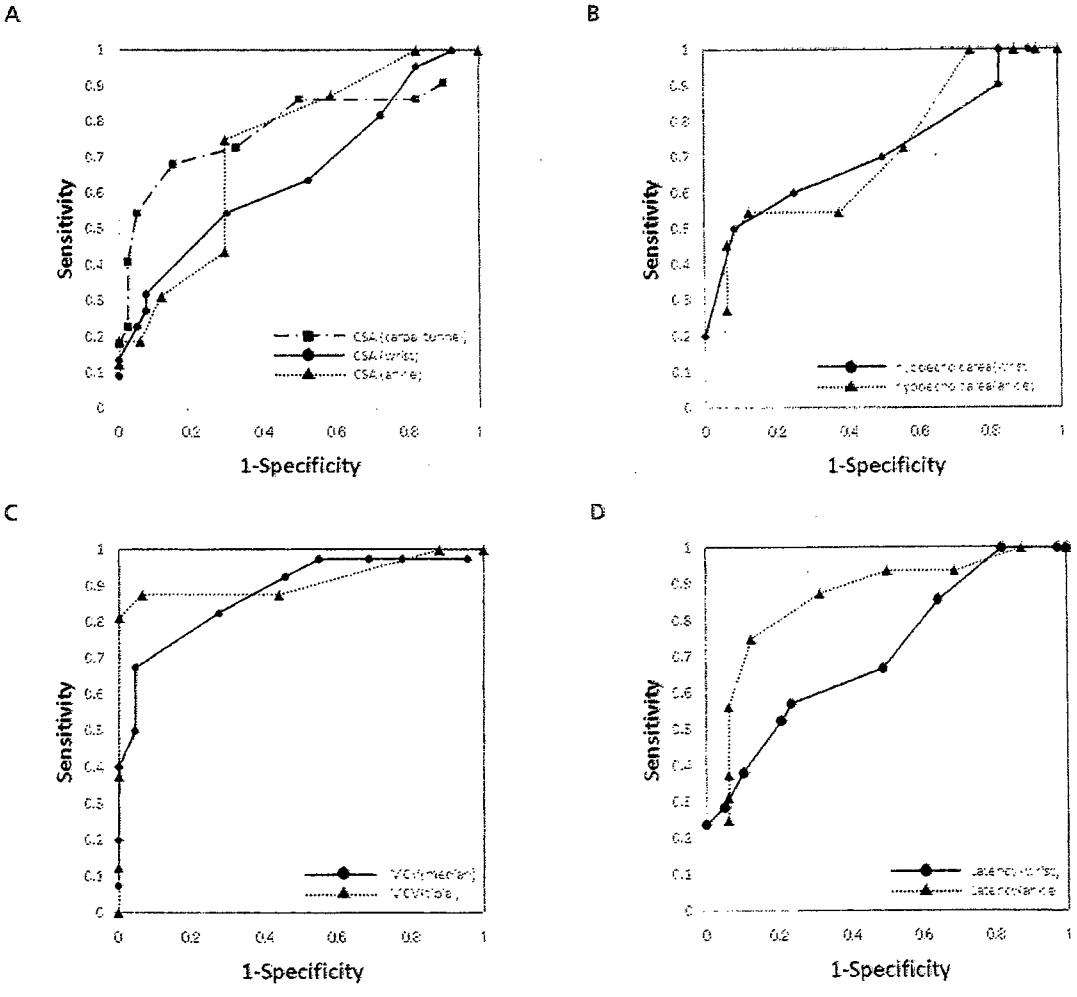
Figure 6. Cross-sectional area stratification in the tibial nerve by combining tertiles of latency and MCV. The CSA was 16.3 mm^2 in participants in the highest tertile of both latency and MCV. The latency was divided into 3 groups: latency of less than 4.5 milliseconds, latency of 4.5 to 5.0 milliseconds, and latency of greater than 5.0 milliseconds. The MCV was also divided into 3 groups: MCV of less than 40 m/s, MCV of 40 to 50 m/s, and MCV of greater than 50 m/s.



Conventional motor and sensory NCS has been widely used to diagnose DPN.[18–24] Symptoms of DPN appear bilaterally from the toes or the soles of the feet. Thus, NCS in the lower limbs should be more suitable to assess DPN severity. However, NCS in the lower limbs is time-consuming, and the action potential in the lower limbs sometimes cannot be evoked in cases of patients with advanced DPN. Some previous studies have reported that nerve conduction velocity slowing in the upper limbs is similar to that in the lower limbs of diabetic patients.[25,26] Skin temperature and humidity, however, easily affect the sensory nerve conduction velocity at the time of measurement. Mizumoto et al[27] reported that they chose instead to look at distal motor latency and the MCV because the sensory nerve conduction velocity was not measurable in many patients and appeared to be an unsuitable parameter. For the reasons above, we performed only the motor nerve conduction study.

Sonographic criteria for the diagnosis of neuropathy have been proposed by several studies. Cartwright et al[28] evaluated the CSA reference value studied for nerve sonography. In their study, the mean area of the tibial nerve at the

Figure 7. Receiver operating characteristic curves fitted for difference modality. **A**, When the ROC curve was fitted using sonographic CSA results, the CSA of the carpal tunnel was most effective. **B**, When the ROC curve was fitted using the sonographic hypoechoic area, the area of the tibial nerve was most effective. **C**, When the ROC curve was fitted using NCS MCV results, the MCV of the tibial nerve was most effective. **D**, When the ROC curve was fitted using NCS latency results, the latency of the tibial nerve was most effective.



ankle was 13.7 mm^2, which was greater than the value of 8.9 mm^2 that we obtained. They also reported that age and height showed weak correlations with the nerve CSA, whereas weight and BMI showed stronger correlations with the nerve CSA. Their study participants' mean BMI and weight were 26.5 and 74.5 kg, respectively, which were markedly greater than those of our participants, explaining the discrepancy with our findings. Mean normal median nerve CSA values cited in the literature vary between 6.1 and 10.4 mm^2; the difference between these normal values constitutes 51% of the normal median nerve CSA (8.4 mm^2), which is similar to our result of 8.3 mm^2.[29] The aim of our study was to evaluate whether the sonographic findings in the median and tibial nerves corresponded to the results of motor NCS in diabetic patients. We found that the CSA of both median and tibial nerves in diabetic patients were significantly larger than those in controls. Carpal tunnel syndrome and tarsal tunnel syndrome (TTS) are the most common entrapment neuropathies. A cross-sectional study of diabetic neuropathy reported by Dyck et al[16] found that polyneuropathy was the most common form of diabetic neuropathy, followed by CTS. It is well known that diabetic neuropathies are frequently asymptomatic. Kim et al[15] reported that 6.8% of diabetic patients had asymptomatic electrophysiologic CTS. The most common etiologies of TTS are mass lesions in the tunnel such as lipomas, ganglions, osteochondromas, varicosities, and synovitis because of rheumatoid arthritis or chronic uremia; however, to our knowledge, TTS is a rare complication of diabetes mellitus. Using sonography in the median nerve, Sernik et al[12] showed decreased echogenicity of the median nerve in symptomatic CTS wrists. Although sonography provides excellent detail of peripheral nerve parenchymal changes, there is currently a lack of quantitative examinations of peripheral nerve echogenicity. We therefore attempted to create a standardized quantitative analysis of sonographic images to allow a more objective assessment of nerve echogenicity in diabetic patients. This study was not designed to compare sonographic findings with histological changes; rather, the intent of this initial study was to develop a method of computer quantitation of echogenicity changes and to assess for a correlation with NCS. In our data, it appears that the percentage of the hypoechoic area of the peripheral nerve was significantly greater in lower-MCV patients with diabetes mellitus compared with controls and higher-MCV patients with diabetes mellitus. It is likely that these findings reflect pathologic changes, although the pathogenesis of nerve enlargement and an increasing percentage of the hypoechoic area in peripheral nerves are uncertain because affected median or tibial nerves have rarely been biopsied in patients with diabetes mellitus.

Table 5. Comparison of Sensitivity and Specificity Between Sonography and NCS

Parameter	Sensitivity, %	Specificity, %
Sonography		
CSA (median nerve, carpal tunnel)	68.2	85.0
CSA (median nerve, wrist)	54.5	70.0
CSA (tibial nerve, ankle)	75.0	70.6
Hypoechoic area (median nerve, wrist)	50.0	91.7
Hypoechoic area (tibial nerve, ankle)	54.5	87.5
NCS		
MCV (median nerve)	67.5	95.5
MCV (tibial nerve)	87.5	93.8
Latency (median nerve)	57.1	76.9
Latency (tibial nerve)	87.5	68.8

Diabetic neuropathy is characterized by axonal loss combined with demyelination, which is reflected in typical neuropathophysiologic findings, including reduced CMAP amplitudes combined with slowed nerve conduction. With regard to the relationship between sonography and NCS in this study, we found an increased CSA of the peripheral nerve in diabetic patients, especially those with a low MCV, compared with healthy controls. Moreover, the percentage of the hypoechoic area in the median nerve increased significantly compared with the controls and diabetic patients with a high MCV. According to ROC curve analysis, to investigate whether using sonography and NCS could judge the diagnostic accuracy for DPN, we compared the sensitivity and specificity of different modalities. Both the sensitivity and specificity were higher for NCS than sonography. These results are consistent with current widely accepted status of NCS as being more sensitive than sonography in the evaluation of polyneuropathy and CTS. However, although sonographic measurement had insuffi-

cient sensitivity, its specificity was similar to that of NCS. In addition, sonography was able to directly show morphologic change in the peripheral nerve. Compared with NCS, sonography caused less discomfort to patients and took less time. For these reasons, we have insisted on the possibility of using sonography to diagnose DPN.

Severinsen and Andersen[30] reported that the nerve conduction velocity may be reduced not only because of loss of the fastest conducting axons but also demyelination and acute metabolic dysregulation, which may cause lower nerve conduction velocity. Suzuki et al[31] reported that sorbitol itself and secondary sodium accumulation caused by an increase in sorbitol may be major contributors to the increase in intracellular hydration using a ^{1}H-nuclear magnetic resonance study. It has further been hypothesized that the peripheral nerve is swollen in individuals with diabetes mellitus because of increased water content related to increased aldose reductase conversion of glucose to sorbitol.[32] We hypothesize that an increased hypoechoic area of the peripheral nerve in diabetic patients may occur because of increased water content, which is also a cause of an enlarged peripheral nerve. Furthermore, our data showed that CSAs were negatively correlated with both a reduced MCV and delayed latency. Our findings may reveal that asymptomatic CTS or TTS exists in diabetic patients, and both entrapment and other factors such as metabolic or vascular causes affect DPN.

Finally, our study was an sonographic examination only; therefore, it remains unknown exactly what causes increased the hypoechoic area or CSA. In addition, there were some limitations of our study that warrant discussion because advanced sonographic techniques such as color and power Doppler imaging were not used. However, sonography is a noninvasive method that can be used to evaluate detailed nerve structures. Further studies are needed to confirm these findings in larger groups of diabetic patients and in other types of neuropathy.

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グルコース感受性転写因子：ChREBP

Glucose Activated Transcription Factor : ChREBP

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グルコースはインスリンとともに細胞外の栄養状態を反映するシグナル伝達物質としての側面がある．ChREBP（carbohydrate response element binding protein）は再摂食時に見られる脂質合成系遺伝子の誘導を司る転写因子である．ChREBPはリン酸化により転写活性が抑制され，脱リン酸化により転写活性が亢進する．ChREBPは肝臓における脂肪合成の60%を説明し，肝臓での栄養貯蔵をグリコーゲンでなく中性脂肪として蓄えるよう調節する．ChREBPの抑制はメタボリックシンドローム病態の改善につながるため，今後グルコースによるChREBP活性化機構に加えて，脂肪細胞や膵β細胞などの臓器での役割を含めた研究の展開が重要と思われる．

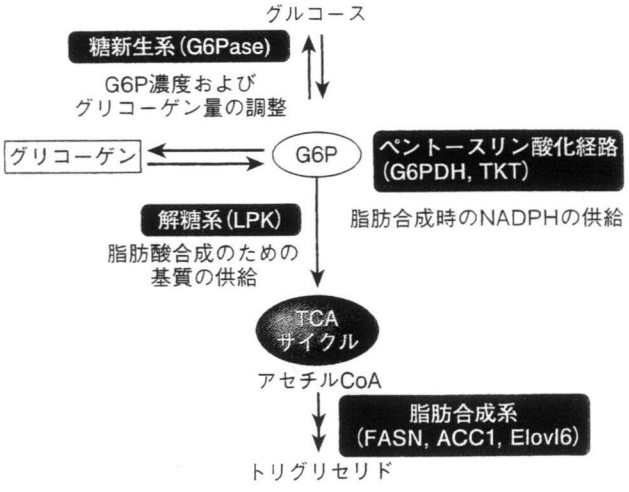
key words　ChREBP，脂肪酸合成

はじめに

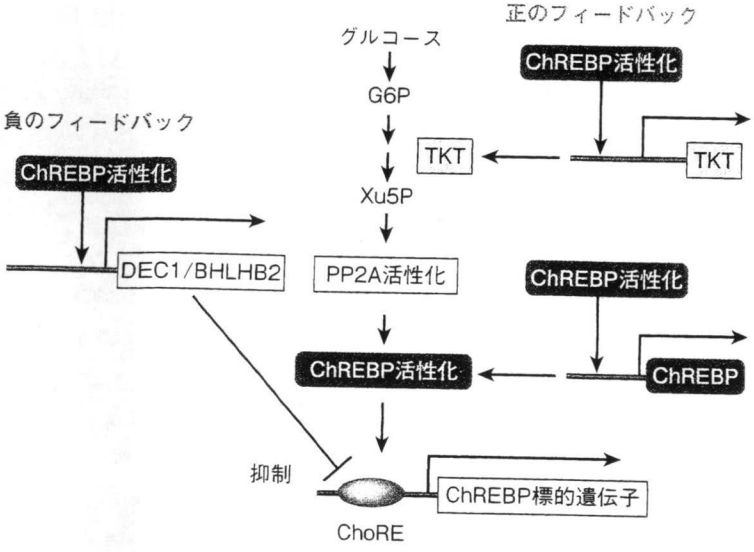
絶食後の再摂食時（refeeding）に解糖系酵素（liver type pyruvate kinase；LPK）や脂肪合成系酵素〔FASN（fatty acid synthase）やACC1（acetyl CoA carboxylase 1）〕の遺伝子発現が急速に誘導される[1]．この際にインスリンシグナルに加え，グルコースシグナルが重要な役割を果たす．ChREBP（carbohydrate response element binding protein）は，*Lpk*遺伝子のプロモーターに存在するグルコース応答領域（ChoRE）に結合する転写因子として，Uyedaらにより同定された[2]．ChREBPは肝臓のほか，膵島，腎臓，脂肪組織，筋肉など主として脂肪酸合成を行う臓器で強く発現する[3]．ChREBPの標的遺伝子は，上述の解糖系酵素遺伝子や脂肪合成系酵素遺伝子に加えて，ペントースリン酸経路の遺伝子，糖新生系酵素遺伝子，時計遺伝子など多岐にわたる[4)~7)]（図1）．

I　ChREBPのグルコースによる活性化および非活性化機構

ChREBPは，グルコースシグナルにより活性化され，転写因子Mlx（Max like protein X）とヘテロダイマーを形成し，標的遺伝子のプロモーター領域に存在するChoRE（コンセンサス配列；CACGTGN5CACGTG）に結合する[4), 6), 7)]．グルコースによるChREBPの活性化機構においては，キシルロース5リン酸（Xu5P）がグルコースシグナル伝達分子とし



■図1　ChREBPの標的遺伝子
ChREBPは解糖系酵素（liver type pyruvate kinase；LPK），ペントースリン酸化経路（glucose-6-phosphate dehydrogenase；G6PDH，transketolase；TKT），糖新生系酵素（glucose 6 phophatas；G6Pase），脂肪合成系酵素（acetyl CoA carboxylase Ⅰ；ACC1，fatty acid synthase；FASN，Elovl6など）などの代謝経路を調節する．ChREBPの代謝酵素の発現調節により，過剰に摂取したグルコース（Glc）をグリコーゲンでなく効率の良い中性脂肪として効率良く貯蔵することができる．

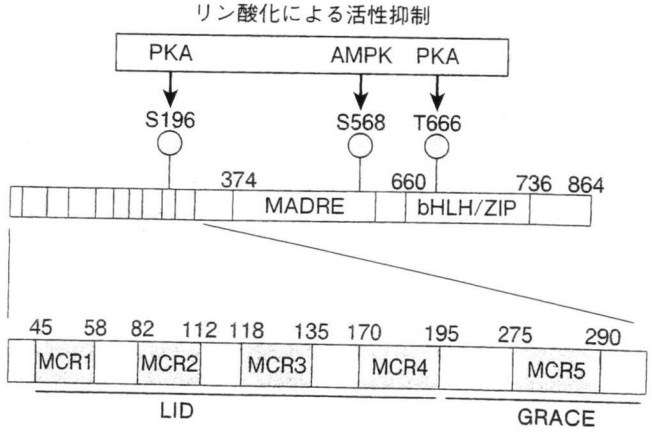


■図2 ChREBPとその標的遺伝子による正および負のフィードバック機構

ChREBPはChREBP自身およびTKT (transketolase) の発現を介して正のフィードバックを行い，転写抑制因子であるDEC1/BHLHB2の発現を介して負のフィードバックを行う．

て知られている．Xu5Pはプロテインホスファターゼ2A (PP2A) を活性化し，ChREBPを脱リン酸化することで細胞核に局在し，標的遺伝子のChoREに結合する[8]．また，Xu5PによるPP2Aの活性化により，6ホスホフルクト2キナーゼ (6PF2K) の活性化，ひいては解糖系酵素であるホスホフルクトキナーゼ (PFK) の活性化が生じる[9]．解糖系酵素 (PFK) の活性化と転写因子ChREBPの活性化が同じシグナル分子であるXu5Pにより生じることは，時相の違いはあるものの，解糖系から脂質合成系への基質を供給するうえで非常に合理的である．さらに，Xu5Pの産生酵素であるトランスケトラーゼ (TKT) はChREBPにより転写調節を受け，*ChREBP*遺伝子自身もまたChREBPにより発現が誘導される[6]．すなわち，ChREBPとTKTとの間に正の発現フィードが存在する (図2)．また，時計関連遺伝子であるDEC1/BHLHB2とChREBPの間に負のフィードバックが存在するため，ChREBP自身の標的遺伝子によりChREBPの転写活性が正および負の調節を受ける可能性が考えられる[5] (図2)．一方，転写因子LXRがグルコース (Glc) やグルコース6リン酸 (G6P) により活性化され，脂肪合成系酵素を誘導するとの報告がなされた[10]．しかしながら，①2-デオキシグルコース刺激では脂肪合成系酵素遺伝子の発現が誘導されないこと，②*LXR*のノックアウトマウスでも，グルコースによる脂肪酸合成系酵素 (FASN，ACC1) の発現誘導に影響がないこと，③HepG2細胞のようなグルコース反応性の遺伝子調節を見るのに不適切な細胞が使われていることから，Glc/Glc-6-P-LXR経路は生体において，必ずしも主要な経路ではないと思われる[1), 11)]．

他方，ChREBPの不活性化機構にはPKA (protein kinase A) やAMPK (AMP-activated protein kinase) によるChREBPのリン酸化が関与することが報告されている[12), 13)]．空腹時や低血糖の状態は脂肪酸合成が抑制された状態であり，グルカゴンやアドレナリンの血中濃度が増加し (PKAの活性化)，細胞内AMP濃度も増加しているため (AMPKの活性化)，ChREBPの不活性化と合致している[13)]．また，多価不飽和脂肪酸によるグルコキナーゼの不活性化を介したChREBPの抑制機序も報告されている[14)]．上記で示されたように，ChREBPの転写活性はリン酸化および脱リン酸化により調節を受けることに加えて，ChREBPの欠質変異体の解析によりChREBPのN末端にはLID (low glucose inhibitory domain) やGRACE (glucose response activation conserved element) といったグルコース感受性モジュールが同定されている[5), 15)]



■図3 ChREBPタンパク質の機能局在

ChREBPには，196，568，666などのリン酸化部位に加えて，ChREBPのN末端に存在するMCR (Mondo conserve region) によりChREBP転写活性の機能調節が行われる．bHLH/ZIP；basic helix loop helix /leucin zipper，GRACE；glucose response activation conserved element，LID；low glucose inhibitory domain，MADRE；middle activation domain as in RelB．

(図3). これはMondoAやMlxといった他のChREBPファミリーでも保存されているMCR (Mondo conserve region) と呼ばれる領域である[6), 15)~17)] (図3). さらに, MCR領域に対する詳細な解析から, MCR2およびMCR3領域がグルコース反応性に関わる領域であること, MCR3領域が14-3-3タンパク質に結合し, 細胞内局在を決定していること, MCR2とMCR5が低グルコース濃度におけるChREBP転写活性の抑制に関与していることなどが明らかになった. すなわち, ChREBPの核内への局在化と標的遺伝子プロモーター内のChoREへの結合からなるChREBP活性化機構は上記のようなメカニズムにより制御されていることが明らかにされた[15)~17)]. 今後, グルコースによるChREBPの活性化機構 (とりわけDNA結合能の調節) がより詳細に明らかになれば, 後述するメタボリックシンドロームの病態の治療薬の開発に有益と思われる.

Ⅱ ChREBPの病態生理学的意義

1. 肝臓における役割

ChREBPは前述のとおり, 解糖系酵素である肝型ピルビン酸キナーゼ (LPK) や脂肪合成系酵素であるアセチルCoAカルボキシラーゼ (ACC1) や脂肪酸合成酵素 (FASN) の発現, さらには脂肪酸合成の際にNADPHを供給するうえで重要なペントースリン酸経路の酵素であるグルコース6リン酸脱水素酵素 (G6PDH) やTKTの発現を正に調節する[6)]. *ChREBP*のノックアウトマウスでは, 解糖系からの基質の供給の低下と脂肪酸合成系酵素の発現量の低下により肝臓における脂肪合成が正常マウスの40%程度に低下する[3)]. さらに, 脂肪酸合成能の低下に一致して, 細胞質内のNADP/NADPH比はノックアウトマウスで高い[18)]. 他方, インスリンシグナルにより活性化され, 脂肪酸合成系酵素の発現を調節する*SREBP1c*のノックアウトマウスでは肝臓における脂肪酸合成能は正常の50%程度である. 上記の結果は, グルコースにより活性化されるChREBPと, インスリンにより活性化されるSREBP1cにより脂肪酸合成が協調的に調節されることを意味する[1)].

ChREBPが肝臓における脂肪酸合成の60%を調節することから, ChREBPの転写活性の抑制は肥満糖尿病病態の改善につながると考え, 筆者らは肥満糖尿病モデルマウスである*ob/ob*マウスと*ChREBP*ノックアウトマウスを交配させ, *ob/ob ChREBP*$^{-/-}$マウスを作製した[19)]. 同マウスでは, *ob/ob*マウスと比べて, ChREBPの標的遺伝子 (*Lpk*, *FASN*, *ACC1*など) のmRNA発現量の低下とともに, 耐糖能障害の改善, 高中性脂肪血症の改善, 脂肪肝の改善, 白色脂肪重量の低下および体重の改善が見られた. 同様の効果は, *ChREBP*のshRNA (small hairpin RNA) を*ob/ob*マウスで発現させた場合や糖尿病になりやすい正常加齢マウスにMlxの抑制体を発現させた場合にも見られた[6), 20)]. したがって, ChREBPはメタボリックシンドロームの治療標的として重要であることが示唆された.

さらに, *ob/ob ChREBP*$^{-/-}$マウスで見られた面白い表現型の1つにグリコーゲン蓄積によると思われる肝腫大がある[19)]. 同マウスと*ob/ob*マウスのグリコーゲンの量を比較したとき, グラム当たりのグリコーゲン含量は2.4倍である. しかしながら, 肝臓の重量は約1.7倍に増加している. そのため, 肝臓全体では約4倍のグリコーゲンを蓄積していることになる. 重量当たりのグリコーゲンは高浸透圧のため, グリコーゲン1g蓄えるためには, 3m*l*の水分が必要となる (実質上グリコーゲン1gは1kcalを供給できることとなる) ため, エネルギー貯蔵のうえで非常に効率が悪い. 肝臓に貯蔵されたカロリーを*ob/ob*マウスと*ob/ob ChREBP*$^{-/-}$マウスとで比較すると, グリコーゲン (300 vs.1200kcal) および中性脂肪 (1971 vs. 432kcal) となる. したがってChREBPは, 肝臓において効率の悪いグリコーゲンとして蓄えるのでなく, 効率の良い中性脂肪として蓄えるように調節する転写因子であると言える[1), 19)]. なお, 上記のようなグリコーゲンの蓄積ならびに肝腫大は, *ChREBP* shRNAやMlxの抑制体ではこのような効果が見られなかった[6), 20)]. おそらくはアデノウイルスは短期間 (数日) の効果であること, 作用としてノックアウトマウスに比べるとChREBPの転写活性抑制効果が弱いことが考えられる. したがって, ChREBPの転写活性をほどほどに抑制する薬剤は, メタボリックシンドロームの改善薬として有望であると考えられる.

ChREBPもSREBP1cも共通して, 脂肪合成系酵素やペントースリン酸経路など脂肪合成系に重要な遺伝子の発現を調節する. しかしながら, 解糖系酵素や糖新生系酵素の発現調節は両者で異なる. 解糖系に関しては, SREBP1cはグルコキナーゼの調節を司るのに対し, ChREBPはLPKの発現を調節する[1)]. 糖新生系についても, ChREBPは肝臓からのブドウ糖放出や肝臓のグリコーゲン量を制御する*G6Pase*の発現を調節している[6), 19), 21)]. 肝臓特異的*SREBP1*ノックアウトマウスと*ob/ob*マウスの二重交配動物では, 脂肪肝の改善のみで肝臓グリコーゲンの過剰な蓄積や耐糖能障害の改

善は見られない．SREBP1cとChREBPの*G6Pase*遺伝子に対する発現調節の違いは，両マウスで見られる耐糖能改善効果の違いを反映していると思われる[1]．

2．他臓器における役割

1）脂肪組織

ChREBPは肝臓以外でも，腎臓，脂肪組織，筋肉などの臓器および膵島での発現が知られている[2]．ChREBP mRNAの発現量は3T3L1細胞の脂肪細胞への分化の際に発現が誘導される[22]．また，再摂食時のラット脂肪組織において，ChREBP mRNAの発現量が増加する．しかしながら，脂肪細胞の主要な機能は脂肪合成でなく脂肪の貯蔵であることや，ChREBPには脂肪合成調節以外の重要な役割があると思われる．今後，脂肪細胞の分化やアディポサイトカインの発現調節におけるChREBPの役割の解明が必要と思われる．

2）膵β細胞

ChREBPは膵β細胞でも強く発現する．マウスインスリノーマ細胞であるINS-1細胞では，ChREBPの過剰発現は*Lpk*や*FASN*などの遺伝子発現を誘導する[23]．インスリン分泌には影響を与えないが，筆者らは糖尿病内科医としての立場から膵β細胞の糖毒性および脂肪毒性における役割について現在研究を進めている．

おわりに

ChREBPは肝臓における脂肪酸合成の60%を説明する重要な転写因子である．ChREBPの活性調節には，現在提示されているリン酸化・脱リン酸化モデルに加えて，*ChREBP*遺伝子の発現調節やChREBPタンパク質の分解調節などの新たなモデルが提示されつつある．ChREBPの機能抑制は脂肪肝や耐糖能障害などのメタボリックシンドローム様病態の改善につながるため，ChREBPの活性調節機構の解明は新規の創薬ターゲットの手掛かりとなるであろう．

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Review Article II

綜 説

全ゲノム関連解析(GWAS)の成果をふまえた2型糖尿病遺伝子同定の現状と課題

堀川 幸男*

2006年に転写因子*TCF7L2*の多型が候補遺伝子座の同定から詳細なマイクロサテライト，SNPsタイピングにより2型糖尿病感受性多型として報告された．*TCF7L2*はそれまで糖尿病遺伝子として全く予想されていなかった遺伝子であり，これは最初に遺伝子ありきのリバースジェネティクスの常である．現在では設備的にも費用的にもゲノム全域でのSNPs解析が可能になり，全ゲノム関連解析(GWAS)が可能になり，*HHEX, CDKN2B, CDKAL1, IGF2BP2*などの領域の新規糖尿病感受性多型がまず欧米にて報告された．上記の遺伝子の感受性アリルはわが国でも糖尿病発症との関連が認められ，人種を超えた感受性アリルであることが証明された．またわが国においてもGWASが施行され，*KCNQ1*のイントロン15の多型が感受性多型として同定された．しかし従来の危険因子(肥満，家族歴など)に比べて，依然上記の遺伝子多型による糖尿病の発症予測の有用性は現時点で低いという報告もなされた．今後さらに糖尿病感受性遺伝子多型は増えるであろうが，これら関連解析で獲得できた発症オッズ比(易発症度)はあくまで集団でのデータであり，個々人においてはその遺伝素因の全貌が明らかにならない限り正確な意味を持たないことに注意すべきである．したがって現段階では，疾患感受性多型同定の意義は発症予測，予防というよりも，むしろ疾患発症メカニズム解明の手がかりを与えることであり，こちらは新規治療法の開発並びに創薬への展開にとって重要な役割を担っている．

Yukio HORIKAWA：Present status and future plan for identifying susceptibility gene to type 2 diabetes in the post genome-sequencing era. Diabetes Journal, 37：95～103, 2009

はじめに

今世紀に入り，まず欧米[1,2]にて疾患ごとに，ゲノム全域でのSNPsを用いた体系的関連解析が糖尿病を含め施行され，その成績が報告され始めた．ゲノム全域での10～30万個のSNPsが患者－対照で解析され，相対危険度の低い感受性遺伝子も同定できるようになった．同時期，わが国においてもゲノム研究の国家的コンソーシアムであるミレニアムプロジェクトが展開された．

全ゲノム関連解析(Genome-Wide Association Study：GWAS)が実現した背景には，タイピングコストの低下，タイピングアレイなど大規模タイピング技術の急速な発展があげられる．またタイピング技術の高速化とともに，国際ハップマップ計画(http：//hapmap.org /index.html.en)により，ヒト遺伝子多型マップの整備が進んだこともGWASに大きく寄与している．現在Phase Ⅲのハップマップデータ ではSNPs情報が公開されており，4民族(Caucasian，Japanese，Han Chinese，Yoruba)それぞれの400万個以上のSNPs情報や連鎖不平衡ブロックの構造をみることができ，研究者は効率的なタイピングを行うことができる．

2006年に転写因子*TCF7L2*の多型が，連鎖解析と同定された感受性遺伝子座の詳細なSNPsタイピングによって新規2型糖尿病感受性遺伝子多型として報告され，その後さまざまな人種において2型糖尿病との関連が報告されていた．その後の

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表1　日本人2型糖尿病における大規模関連解析

Gene	SNP ID	RAF /Case	RAF /Cont	*P*	OR	95% CI		RAF-C	OR-C
						upper	lower		
SLC30A8	rs13266634	0.60	0.56	1.7×10^{-5}	1.20	1.10	1.30	0.65	1.12
SLC30A8	rs3802177	0.61	0.57	2.7×10^{-5}	1.19	1.10	1.29		
HHEX	rs1111875	0.33	0.27	2.2×10^{-10}	1.33	1.22	1.45	0.53	1.13
HHEX	rs7923837	0.22	0.17	7.0×10^{-12}	1.43	1.29	1.58	0.62	1.22
CDKN2B	rs10811661	0.62	0.53	1.9×10^{-18}	1.44	1.32	1.56	0.83	1.20
IGF2BP2	rs4402960	0.35	0.31	1.1×10^{-4}	1.18	1.09	1.29	0.29	1.14
IGF2BP2	rs1470579	0.37	0.33	2.8×10^{-5}	1.20	1.10	1.30	0.30	1.17
CDKAL1	rs7754840	0.47	0.41	4.0×10^{-9}	1.27	1.18	1.38	0.31	1.12
CDKAL1	rs7756992	0.53	0.47	2.0×10^{-8}	1.26	1.16	1.36	0.26	1.2
TCF7L2	rs7903146	0.061	0.036	5.6×10^{-9}	1.76	1.45	2.13	0.18	1.37
KCNJ11	rs5219	0.37	0.36	0.066	1.08	0.99	1.17	0.46	1.14
PPARG	rs1801282	0.97	0.96	0.075	1.21	0.98	1.49	0.82	1.14
KCNQ1	rs2237892	0.68	0.59	1.2×10^{-20}	1.49	1.37	1.62	0.93	1.29
HNF1B	rs7501939	0.35	0.31	9.3×10^{-5}	1.18	1.09	1.29	0.45	1.10

RAF：risk allele frequency；RAF-C：risk allele frequency of Caucasians　　文献33)より引用改変

GWASにて，*HHEX, CDKN2B, CDKAL1, IGF2BP2*などの領域の糖尿病感受性多型が，*TCF7L2*リスク多型のGWASでの再現とともに欧米にて報告された[3,4]．上記の遺伝子の感受性アリルについてはわが国でも糖尿病発症との関連が認められ，人種を超えた糖尿病感受性アリルであることが証明された[5,6]．またわが国においても上述のミレニアムプロジェクトのGWASの結果，*KCNQ1*のイントロン15の多型が日本人2型糖尿病における重要な感受性多型として同定された[7](**表1**)．

I. GWASでの有意水準と検出力

まずGWASには適切なパワー(検出力)を有するサンプル数や*p*値(有意水準)の設定など研究デザインが重要である．Bonferroniのように0.05を検定数で割って*p*値を設定する多重検定補正や，事前，事後オッズから有意水準を想定する方法が使われている．2型糖尿病との真の関連を示す事後オッズは，「真の関連の事後オッズ＝事前オッズ×検出力/有意水準(尤度比)」で表される．つまり有意水準を決めると，本当に疾患と関連するかどうかの事後オッズは事前オッズとスタディの検出力によって決まる．たとえば，ある領域の事前オッズは1×10^{6}オーダー(百万個)の中から10個の遺伝子多型が真の関連があるくらいとすると，事前オッズは1×10^{-5}のオーダーと予測される．スタディのパワーを0.8とし，*p*値の閾値を8×10^{-7}と設定すれば(偽陽性0.8個)，真の関連遺伝子を検出する事後オッズは10となる．すなわち候補として同定された遺伝子多型は約91%真の糖尿病遺伝子多型ということになる．単一のGWASでこの基準をクリアできたのは，*TCF7L2*，*KCNQ1*と*FTO*のみである．たとえばORが1.37を呈する*TCF7L2*で考えてみても，*p*値を上記のように設定すると80%の検出力を得るためには欧米での危険アリル頻度(約30%)で最低で1,200名ずつくらいの患者，対照者のサンプルが必要になる計算になる．もし日本人の頻度(約5%)を当てはめたら5,700名ずつくらい必要になるのである．またprior probability(事前確率，*p*が小さければ事前オッズ)とオッズ比より，獲得された成績のFPRP(False Positive Report Probability)を提示する方法もある[8]．

以下に，GWASで同定された新規糖尿病感受性遺伝子のうちで，コーカシアンと日本人で関連再現が現在までに認められた遺伝子について主に概説する．