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Original Article

Health-related and diabetes-related quality of life in Japanese children and adolescents with type 1 and type 2 diabetes

Nobue Nakamura,[1] Nozomu Sasaki,[2] Kaichi Kida,[3†] Nobuo Matsuura,[4] for the Study Group of Health Sciences Research, Ministry of Health, Labor and Welfare, Japan*

[1]*Department of Child Nursing, School of Nursing, Chiba University, Chiba,* [2]*Department of Pediatrics, Saitama Medical College, Gero,* [3]*Ehime University School of Medicine, Matsuyama, and* [4]*Department of Early Childhood Education, Seitoku University, Matsudo, Japan*

Abstract ***Background***: The aim of this study was to assess (i) the health-related quality of life (HR-QOL) of primary, junior and high school children with type 1 and type 2 diabetes and to compare it with that of healthy school children; and (ii) to compare the diabetes-related QOL (DR-QOL) and the QOL of parents of children with diabetes, between type 1 and type 2 diabetes in Japan.

Methods: Overall, 471 patients aged 9–18 years (368 with type 1 and 103 with type 2 diabetes) and their parents were involved. QOL was assessed using a self-administered questionnaire.

Results: The total score for HR-QOL of primary and junior school children with type 1 diabetes was significantly higher than that of those with type 2 diabetes and healthy controls. However, there were no significant differences in high school children. Some subscales regarding HR-QOL were significantly lower for children with type 2 diabetes than for children with type 1 diabetes or healthy controls. The DR-QOL of children with type 1 and type 2 diabetes did not significantly differ. The Family Burden and Family Involvement were significantly greater in parents of children with type 1 diabetes. There were significantly positive correlations between HR-QOL and DR-QOL in both groups. In type 1 diabetes only, there were significant negative correlations between glycated hemoglobin and some subscales of the HR-QOL and QOL of parents of children with diabetes, and weak positive correlation between glycated hemoglobin and Family Burden.

Conclusions: The HR-QOL of school children with type 1 diabetes was higher than that of those with type 2 diabetes and healthy school children. The QOL of school children with type 1 diabetes was not impaired.

Key words childhood diabetes, diabetes-related QOL, health-related QOL, parents-diabetes QOL, quality of life.

Correspondence: Nobuo Matsuura, MD, PhD , Department of Early Childhood Education, Seitoku University, Iwase 550, Matsudo 271-8555, Japan. Email: seimatsu@seitoku.ac.jp

*List of the study group of Health Sciences Research, Ministry of Health, Labor and Welfare, Japan: Matsuura N, Kitasato University; Igarashi H, Igarashi Clinic; Uchigata Y, Tokyo Women's Medical College; Amemiya S, Yamanashi University; Miyamoto S, Chiba Children's Hospital; Onigata K, Gunma University; Yokota I, Tokushima University; Miki Y, Tokyo University; Jinno K, Hiroshima JR Hospital; Sasaki N, Saitama Medical College; Ohki Y, Nippon Medical College; Kikuchi N, Yokohama City University; Owada M, Nihhon University Surugadai Hospital; Kohno A, Fukuoka Children's Hospital; Masuda H, National Mie Hospital; Okada T, Kochi University; Nishiyama S, Kumamoto University; Nakamura N, Chiba University; Kida K, Ehime University; Asayama K, Sangyo Medical College; Arisaka O, Dokkyo Medical College; Uchiyama H, Niigata University; Ohzeki T, Tottori University; Kinugawa A, Kyoto Medical College; Okada T, Nihon University; Sugihara S, Tokyo Women's Medical University Medical Center East; and Tamai T, Osaka Medical College.

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The goals of treatment for children with type 1 diabetes are to obtain normal growth and development, to avoid diabetic complications through good metabolic control and to help them live life the same as healthy children at the present and in the future. At the same time, it is necessary to evaluate how the patients and their parents feel about diabetes-related daily procedures such as insulin injection and self-monitoring of blood glucose. Negative feelings for diabetes-related daily procedures are associated with poor quality of life (QOL). Moreover it is important to compare how children with diabetes feel about everyday events with how healthy school children feel.

The annual incidence of type 1 diabetes in the age group from 1 to 14 years was reported to be 2.0–2.5/100 000/year in Japanese children, which is much lower than that in Caucasians.[1] However, the incidence of type 2 diabetes has been steeply increasing in adolescents in Japan, and now exceeds that of type 1 in the age group more than 12 years old.[2] In Japan, more than 90% of patients with diabetes have type 2 diabetes. Therefore many Japanese people confuse type 1 and type 2 diabetes, which is unfortunate for children with type 1 diabetes and their parents. The understanding of type 1 diabetes has been spreading recently

through public education. Moreover, newly developed insulin analogs have been introduced and a multiple insulin injection regimen was also developed.[3,4] Currently, almost all children with type 1 diabetes over 12 years are on a multiple daily injection regimen.

Although an international corroboration study has shown that the long-term prognosis of mortality is poor in Japan,[5] it was remarkably improved in a follow-up corroboration study of children with type 1 diabetes.[6] In contrast, the long-term prognosis of youth-onset type 2 diabetes was found to be poor.[7]

The QOL of those with type 1 diabetes was evaluated in the Hvidøre study, in which 64 cases from Japan were evaluated.[8] However, the QOL for type 2 diabetes, particularly compared to that for type 1 diabetes and healthy control children, has not been evaluated previously. Therefore it is important to compare how children with diabetes versus healthy school children feel about everyday events. Moreover, diabetes-specific family conflict diminishes QOL for a child with diabetes as reported by Laffel *et al.*[9] There is a possibility that the QOL of diabetic children is affected by their parents' QOL. A nationwide survey of QOL of type 1 and type 2 diabetes in Japanese children was therefore conducted with the aid of the Ministry of Health, Labor and Welfare, Japan.

The aims of our study were (i) to assess the health-related quality of life (HR-QOL) of primary, junior and high school children with type 1 and type 2 diabetes and to compare them with healthy school children; and (ii) to compare the diabetes-related QOL (DR-QOL) of children, and the QOL of parents of children, with type 1 and type 2 diabetes.

Methods

Participants

The participants were children with type 1 and type 2 diabetes who were diagnosed before the age of 18 years and were between 9 and 22 years old at the time of this study. Questionnaires were given to 1189 children by their doctors during their visits to 48 hospitals. The questionnaires from 645 children (306 from primary and junior school children and 337 from high school children or older) were collected. The recovery rate was 54.2%. In this study, 471 of 645 (73.0%) patients under 18 years of age (primary, junior and high school children) and their parents were subjects for analysis to compare with healthy controls.

The participants' characteristics are shown in Table 1. There were 368 and 103 children with type 1 and type 2 diabetes, whose mean ages were 14.0 ± 2.6 and 14.8 ± 2.3 years and mean durations of diabetes were 6.5 ± 3.9 and 3.0 ± 2.3 years, respectively. Type 1 diabetic children had a significantly longer duration of diabetes than type 2 diabetic children ($t = 9.01$, $P < 0.0001$). The mean glycated hemoglobin (HbA1c) level in type 1 diabetes was 8.0 ± 1.5%, which was significantly higher than that of type 2 diabetes (7.1 ± 2.1%). The mean body mass index (BMI) was 20.9 ± 3.2 kg/m^2 for type 1 diabetes versus 26.7 ± 5.3 kg/m^2 for type 2 diabetes. Type 1 diabetic children had a significantly lower BMI than type 2 diabetic children ($t = -13.73$, $P < 0.0001$). The guardians included 419 mothers (89.0%), 42 fathers (8.9%) and 10 grandparents (2.1%). There were two parents in their twenties (0.4%), 92 in their thirties (19.6%), 311 in their forties (66.0%), 59 in their fifties (12.5%) and seven unknown (1.5%).

Healthy samples

Age- and gender-matched healthy school children aged 9–18 years whose HR-QOL was reported previously were used as healthy controls.[10,11] The demographics of healthy controls are shown in Table 1.

Measures of QOL

Health-related QOL (HR-QOL)

The questionnaire to assess HR-QOL for primary and junior school children (younger group)[10,12] and for high school children (older group)[11] were developed by three specialists in child care and six pediatricians (younger group) and three specialists in child care and three pediatricians (older group). Reliability and validity of both questionnaires were determined using factor analysis, test–retest and Cronbach's alpha coefficients. Questions were scored from 1–5; a higher score indicating better QOL.

The HR-QOL questionnaire for the younger group contained 37 items, with six subscales: anxiety (nine items), family (seven items), friends (five items), school (four items), general health (five items), and strength, diligence, and self-esteem (seven items). Cronbach's alpha coefficient for the questionnaire was 0.90, and for the subscales from 0.59 to 0.81 in this study. The HR-QOL questionnaire for the older group contained 40 items, with eight subscales: friends (six items), school (seven items),

Table 1 Participants' characteristics

	Type 1 diabetes $n = 368$	Type 2 diabetes $n = 103$	Healthy sample $n = 368$
Male	$n = 138$ 37.5%	$n = 43$ 41.7%	$n = 138$ 37.5%
Female	$n = 230$ 62.5%	$n = 60$ 58.3%	$n = 230$ 62.5%
Age (range 9–18)*	14.0 ± 2.6 years	14.5 ± 2.3 years	14.1 ± 2.6 years
Duration of diabetes***	6.5 ± 3.9 (3 months–18 years)	3.0 ± 2.3 (3 months–14 years)	
HbA1c***	8.0 ± 1.5 (4.9–14.9) %	7.1 ± 2.1 (4.3–12.9) %	
BMI***	20.9 ± 3.2 (12.6–29.7)kg/m^2	26.7 ± 5.3 (15.8–42.7) kg/m^2	

Non-paired t-test: *$P < 0.05$; ***$P < 0.001$. BMI, body mass index; HbA1c, glycated hemoglobin.

mental health (seven items), parental and economic states (five items), relationship with boy(girl)friends and self-esteem (six items), vitality (four items), anxiety regarding admission to high school or employment (four items), and sibling (two items). Cronbach's alpha coefficient for the questionnaire was 0.91, with subscale values from 0.53 to 0.83 in this study.

Diabetes-related QOL (DR-QOL)

In this study, "satisfaction with life" developed in the Hvidøre Study[13] was utilized to assess DR-QOL. The "satisfaction with life" contained seven items. Questions were scored from 1 to 5, a higher score indicating a better QOL in this study. Cronbach's alpha coefficient was 0.77.

Family burden related to diabetes (F-Burden)

The original scale, which contained five items, was used in the Hvidøre study.[13] Questions were scored from 1 to 5, a higher score indicating a higher burden (poorer QOL). Cronbach's alpha coefficient was 0.83 in this study.

QOL of parents of children with diabetes (PDQOL)

The questionnaire originally used for DR-QOL in the Hvidøre study on childhood diabetes,[13] modified by Umeda and Nakamura for the QOL of parents of children with diabetes, was used.[14] The PDQOL for diabetic control contained seven items, and for the QOL of parents themselves, contained 10 items. Questions were scored from 1 to 5, a higher score indicating better QOL in this study. Cronbach's alpha coefficient for the questionnaire was 0.86 in this study.

Family involvement in diabetes management (F-Involvement)

The questionnaire was developed by the authors. This questionnaire contained 37 items about insulin injection (five items), self-monitoring blood glucose (SMBG) (four items), management of hypoglycemia (five items), diet (eight items), exercise (five items), contact with school (four items) and outpatient visits (six items), which were answered yes or no. A yes answer was scored as 1 point and all items were added. A higher score indicated a higher involvement in diabetes management. Cronbach's alpha coefficient for the total score was 0.82.

Demographic data

Demographic data concerning age, school grade, gender, height, weight and HbA1c were obtained from children. The parents' age was also obtained.

Statistical analysis

Statistical analysis was performed with SPSS version 11.5 for Windows. Non-paired *t*-tests were used for comparison of the HR-QOL, DR-QOL, PDQOL, F-Burden and F-Involvement scores between type 1 and type 2 diabetes. HR-QOL in different groups (healthy children and type 1 or type 2 diabetes) was compared using one-way ANOVA. The relationships between HR-QOL, DR-QOL, PDQOL, F-Burden, F-Involvement and HbA1c levels were analyzed using Pearson's product moment correlation coefficient.

Ethical considerations

The study was approved by the Medical Ethical Committee of the Kitasato University/Kitasato Hospital, to which chief investigator N. Matsuura belonged.

Results

1. HR-QOL of type 1 and type 2 diabetic children and healthy children

The HR-QOL of primary and junior school children with type 1 and type 2 diabetes and healthy children is shown in Table 2. The total score of HR-QOL for children with type 1 diabetes was significantly higher than that of children with type 2 diabetes and healthy controls ($F = 6.06$, $P < 0.01$). Among the six subscales, "strength/diligence/self-esteem" ($F = 8.05$, $P < 0.001$) and "anxiety" ($F = 4.34$, $P < 0.05$) for children with type 1 diabetes were significantly higher than for children with type 2 diabetes and healthy controls. Age-related total HR-QOL is shown in Fig. 1. Total HR-QOL declined with age for all groups.

HR-QOL of high school children is shown in Table 3. There were no significant differences between type 1, type 2 and healthy controls in the total score. Among the eight subscales, "school" was significantly higher for children with type 1 diabetes than for children with type 2 diabetes and healthy controls ($F = 3.94$, $P < 0.05$), and "parent/economy states" ($F = 2.90$,

Table 2 Comparison of health-related quality of life of primary and junior school children with type 1 and type 2 diabetes and healthy school children

	Primary/junior school children with type 1 diabetes $n = 233$	Primary/junior school children with type 2 diabetes $n = 60$	Primary/junior school healthy children $n = 233$	F
Factor 1:anxiety	29.7 ± 7.1	27.9 ± 7.8	27.8 ± 7.1	4.34*
Factor 2:family	25.6 ± 5.0	24.0 ± 5.3	25.0 ± 5.5	2.05
Factor 3:friends	20.3 ± 3.3	19.5 ± 3.3	19.9 ± 3.5	1.57
Factor 4:school	15.2 ± 3.2	14.0 ± 3.1	14.3 ± 3.3	1.74
Factor 5:general health	19.1 ± 3.3	18.2 ± 3.1	19.2 ± 3.2	1.32
Factor 6:strength/ diligence/self-esteem	24.6 ± 4.8	22.2 ± 5.2	23.0 ± 4.8	8.05***
Total score	134.5 ± 19.6	126.7 ± 18.5	129.1 ± 18.9	6.06**

ANOVA: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

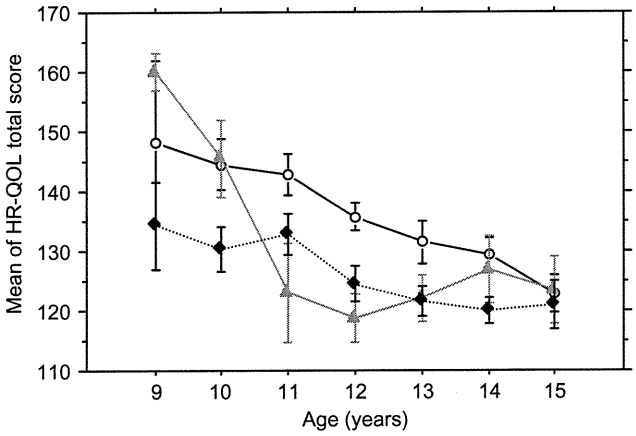

Fig. 1 Total health-related quality of life (HR-QOL) scores of primary and junior school children with (○) type 1 and (▲) type 2 diabetes and of (◆) healthy school children.

$P < 0.05$) and "siblings" (F = 3.67, $P < 0.05$) were significantly lower for children with type 2 diabetes than for healthy controls. In high school children, age-related total HR-QOL was invariable for all groups.

2. Comparison of DR-QOL and PDQOL, F-Burden, F-Involvement for type 1 and type 2 diabetes

The DR-QOL in primary and junior school children and high school children with type 1 and type 2 diabetes were not significantly different (Table 4). The PDQOL for type 1 and type 2 diabetes were not significantly different as compared using the non-paired *t*-test. However, the F-Burden (t = 3.97, $P < 0.001$) and F-Involvement (t = 8.40, $P < 0.001$) were significantly higher for subjects with type 1 diabetes than for those with type 2 diabetes.

The F-Burden (r = −0.14, $P < 0.05$) and the F-Involvement (r = −0.55, $P < 0.001$) of parents of children with type 1 diabetes declined with age. Although the F-Involvement of parents of children with type 2 diabetes declined with age (r = −0.35, $P < 0.001$), the F-Burden did not.

3. Correlation of HR-QOL, DR-QOL, PDQOL, F-Burden, F-Involvement and HbA1c for children with type 1 and type 2 diabetes

(1) Primary and junior school children with type 1 and type 2 diabetes

There were significant positive correlations between HR-QOL and DR-QOL for type 1 (r = 0.50, $P < 0.001$) and type 2 (r = 0.43, $P < 0.01$) diabetes. There was a significant negative correlation between PDQOL and F-Burden for type 1 (r = −0.58, $P < 0.001$) and type 2 diabetes (r = −0.53, $P < 0.001$). Moreover, in children with type 1 diabetes only, there were significant positive correlations between HR-QOL and PDQOL (r = 0.30, $P < 0.001$), and F-Involvement (r = 0.21, $P < 0.01$), and a significant negative correlation with F-Burden (r = −0.23, $P < 0.01$). Additionally, in children with type 1 diabetes only, there were significant correlations between DR-QOL and PDQOL (r = 0.31, $P < 0.001$),

Table 3 Comparison of health-related quality of life of high school children with type 1 and type 2 diabetes and healthy high school children

	High school children with type 1 diabetes $n = 135$	High school children with type 2 diabetes $n = 43$	Healthy high school children $n = 135$	F
Factor 1:friends	22.5 ± 4.5	21.2 ± 3.7	22.3 ± 3.7	1.91
Factor 2:school	24.5 ± 5.2	23.7 ± 4.7	22.9 ± 4.9	3.94*
Factor 3:mental health	22.7 ± 5.1	20.6 ± 4.4	22.6 ± 4.9	2.74
Factor 4:parent/economy states	16.8 ± 4.0	16.0 ± 3.6	17.4 ± 3.9	2.90*
Factor 5:relationship with boy(girl)friend/self-esteem	17.1 ± 4.7	16.6 ± 4.5	16.9 ± 4.0	0.32
Factor 6:vitality	14.4 ± 3.0	13.8 ± 3.0	14.3 ± 2.7	0.59
Factor 7:anxiety regarding admission to higher school/employment	7.8 ± 3.1	7.2 ± 2.7	7.1 ± 2.7	1.07
Factor 8:sibling(s)	5.9 ± 2.4	5.1 ± 2.2	6.2 ± 2.4	3.67*
Total score	131.9 ± 22.1	124.6 ± 18.5	129.7 ± 17.4	2.01

ANOVA: *$P < 0.05$.

Table 4 Comparison of diabetes-related quality of life (QOL), QOL of parents of children with diabetes, family burden related to diabetes and family involvement in diabetes management for type 1 and type 2 diabetes

	Primary/junior/high school children with type 1 diabetes $n = 368$	Primary/junior/high school children with type 2 diabetes $n = 103$	T	*P*
Diabetes-related QOL:	20.7 ± 4.9	21.7 ± 3.5	−1.71	0.098
QOL of parents of children with diabetes	55.2 ± 9.2	56.6 ± 8.7	−1.20	0.242
Family burden related to diabetes	12.4 ± 4.1	10.5 ± 3.4	3.97	<0.001
Family involvement	14.1 ± 6.5	8.0 ± 3.8	8.40	<0.001

Non-paired *t*-test.

and F-Burden (r = –0.29, $P < 0.001$), and between F-Involvement and F-Burden (r = 0.32, $P < 0.001$).

There was a significant negative correlation between HbA1c and the "general health" subscale of the HR-QOL (r = –0.18, $P < 0.01$) and PDQOL (r = –0.15, $P < 0.05$), and positive correlation with F-Burden (r = 0.15, $P < 0.05$) for type 1 diabetes. However, there were no significant correlations between these parameters for type 2 diabetes.

(2) High school children with type 1 and type 2 diabetes

There were significant positive correlations between HR-QOL and DR-QOL for type 1 (r = 0.52, $P < 0.001$) and type 2 (r = 0.36, $P < 0.05$) diabetes. There was a significant negative correlation between PDQOL and F-Burden for type 1 (r = –0.46, $P < 0.001$) and type 2 diabetes (r = –0.42, $P < 0.05$). Moreover, in type 1 diabetes only, there were significant correlations between HR-QOL and PDQOL (r = 0.38, $P < 0.001$) and F-Burden (r = –0.31, $P < 0.001$). Additionally, in type 1 diabetes only, there were significant correlations between DR-QOL and PDQOL (r = 0.29, $P < 0.001$), and F-Burden (r = –0.23, $P < 0.01$), that is, a higher QOL of children reflected a higher QOL of parents. There was a significant positive correlation between F-Involvement and F-Burden for type 1 diabetes in the younger group (r = 0.26, $P < 0.01$). There were negative correlations between HbA1c and "parent/economy states", "vitality", subscales of HR-QOL (r = –0.20, $P < 0.05$), and DR-QOL (r = –0.18, $P < 0.05$) and positive correlation with F-Burden in type 1 diabetes (r = 0.22, $P < 0.05$).

Discussion

In this paper, we found significantly higher total scores and higher scores for two subscales of HR-QOL in primary and junior school children with type 1 diabetes than for those with type 2 diabetes and healthy controls. In high school children, the total score of HR-QOL was not significantly different between the type 1 group, the type 2 group and the healthy controls. Among eight subscales, the score for "school" was significantly higher in high school children with type 1 diabetes than for healthy controls, and those for "parent/economy states" and "siblings" of high school children with type 2 diabetes were significantly lower than for those with healthy high school children.

The HR-QOL of children with type 1 diabetes is higher than that of children with type 2 diabetes, as reported by Varni *et al.*[15] In a previous study, we showed that children and adolescents with type 1 diabetes were more supported by friends with diabetes and medical personnel. As a consequence, children with type 1 diabetes have higher motivation to enhance adherence to self-care behaviors and self-esteem than children with type 2 diabetes[16] In the daily life of children with type 1 diabetes, there are more difficulties to maintain medical treatments such as insulin injection than for children with type 2 diabetes. However, they maintain higher levels of QOL through support by parents and school personnel. In contrast, many children with type 2 diabetes without insulin injection do not receive special care or support from school personnel. In addition, most children with type 2 diabetes are obese, which lowers self-esteem and QOL.

Until now, there has not been a report that indicates a higher HR-QOL for children with type 1 diabetes than for healthy children. Although the HR-QOL of children and adolescents with diabetes is poorer than that of children in the general population,[17–19] Varni *et al.*[15] reported no significant difference in QOL for "physical health" and "social functioning"; however, significantly lower QOL values were found for the "total score", "psychosocial health", "emotional functioning", and "school functioning" in children with type 1 diabetes than in healthy school children. Laffel *et al.*[9] also reported that there was no significant difference in QOL for adolescents with type 1 diabetes than in healthy adolescents. They concluded that painful medical procedures such as insulin injection and SMBG did not affect QOL in children with diabetes. The reasons for the difference from our present report need to be considered.

There could be some reasons why HR-QOL in children with type 1 diabetes was better than that of healthy controls and children with type 2 diabetes. The medical procedures such as insulin injection and reactions to hypoglycemia in primary school children with type 1 diabetes are not well established. As a consequence, children with type 1 diabetes receive a lot of help from school personnel and friends. When children with type 1 diabetes have enough support, insulin injection, SMBG and promotion of exercise enhance their strength and self-esteem, which elevates the "strength/diligence/self-esteem" of their HR-QOL. In high school children with type 1 diabetes, only the "school" subscale of HR-QOL was significantly elevated compared to healthy high school children. The total score and other subscale scores were not significantly different. While most high school children with type 1 diabetes experience school life the same as healthy peers with the understanding and support of school nurses and teachers, it is possible for them to manage diabetes-related daily procedures. They become independent from their parents and teachers and they don't want to be worried about their disease. This could be the reason why the difference from healthy controls disappeared at high school age.

In this study, we found a weak correlation between the level of QOL and HbA1c only in children with type 1 diabetes. In the Hvidøre study, a high correlation between the level of QOL and HbA1c was observed.[8] In our study, no correlation was observed between HbA1c and QOL in children with type 2 diabetes whose HbA1c was low. But many of these children were obese and their QOL was rather low. In children and adolescents with type 1 diabetes, lower HbA1c was correlated with the better HR-QOL at some subscale. These results are the same as those of the Hvidøre study and reports from other studies.[8,20,21] There are various factors that affect the DR-QOL of children and adolescents with type 1 diabetes. Regional and urban residences are reported to be such factors.[22] Socioeconomic status and psychological problems such as depression also affect QOL together with metabolic control.[23] Warm and caring behavior on the part of the family related to self-care also influence QOL and metabolic control.[24]

The parents of children with type 1 diabetes had high F-Involvement and F-Burden compared to those of children with type 2 diabetes. The age-dependence of these scores decreased with age, which was also observed in the Hvidøre study.[8,25]

Moreover, there was a positive correlation between F-Involvement and F-Burden, which could not be observed in the parents of children with type 2 diabetes. In addition to this, there was a positive correlation between the HbA1c level and F-Burden of type 1 diabetes. Type 1 diabetes occurred at a younger age, required more medical procedures such as injection of insulin and management of hypoglycemia, and more outpatient clinic visits than type 2 diabetes. The parents of children with type 1 diabetes required more involvement in diabetic procedures and were more involved in metabolic control, which strongly affected the QOL of the parents.

The PDQOL was not different between parents of children with type 1 and type 2 diabetes. The parents of children with type 1 diabetes were more involved in diabetes management and felt a burden related to diabetes which lowered their QOL. At the same time, the parents felt satisfaction when achieving diabetes management, which heightened their QOL.

From our study, it can be emphasized that we have to provide more support for parents of children with type 1 diabetes. At the same time, we have to provide more support for children with type 2 diabetes, which will improve their QOL as well.

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Nocturnal Blood Glucose and IGFBP-1 Changes in Type 1 Diabetes: Differences in the Dawn Phenomenon between Insulin Regimens

Authors

H. Yagasaki[1], K. Kobayashi[1], T. Saitou[1], K. Nagamine[1], Y. Mitsui[1], M. Mochizuki[1], K. Kobayashi[1], H. Cho[2], K. Ohyama[1], S. Amemiya[3], S. Nakazawa[1]

Affiliations

[1] Department of Pediatrics, Faculty of Medicine, University of Yamanashi, Yamanashi, Japan
[2] Department of Pediatrics, Kawasaki Municipal Hospital, Kanagawa, Japan
[3] Department of Pediatrics, Faculty of Medicine, Saitama Medical School, Saitama, Japan

Key words
- insulin-like growth factor binding protein-1
- dawn phenomenon
- type 1 diabetes mellitus

Abstract

Objective: Insulin-like growth factor binding protein-1 (IGFBP-1) is known to regulate the bioavailability of insulin-like growth factor (IGF) and the levels of IGFBP-1 are increased in the morning in patients with type 1 diabetes mellitus. We investigated the nocturnal fluctuations of glucose, IGFBP-1, and free IGF-1 levels with three insulin regimens.

Research Design and Methods: Forty-eight type 1 diabetes patients were divided into three groups according to their basal insulin therapy (continuous subcutaneous insulin infusion [CSII], insulin glargine, NPH insulin). Blood samples were obtained every 2 h between 2300 h and 0700 h to measure plasma glucose, IGFBP-1 and free IGF-1 levels.

Results: The dawn phenomenon was more frequent with NPH (62.1%) than with glargine (16.6%, $p<0.05$) and CSII (14.3%, $p<0.05$). In the NPH group, the serum IGFBP-1 levels were markedly increased from 21.0±3.6 ng/ml at 2300 h to 200.3±21.8 ng/ml at 0700 h and free IGF-1 levels were inversely decreased; these changes were partially suppressed in the CSII and glargine groups.

Conclusions: The use of insulin regimens that provide sufficient insulin levels in the early morning can suppress the dawn phenomenon, leading to improved glycemic control. The increase in circulating IGFBP-1 in the morning, as a result of waning of insulin action, lowers free IGF-1 levels and may cause insulin resistance.

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Correspondence
H. Yagasaki, MD
Department of Pediatrics
Faculty of Medicine University of Yamanashi
1110 Shimokato, Chuo
409-3898 Yamanashi
Japan
Tel.: +81 55-273-9606
Fax: +81 55-273-6745
yagasaki@mwd.biglobe.ne.jp

Introduction

Patients with type 1 diabetes mellitus are in an insulin-deficient state due to pancreatic β-cell dysfunction. Therefore, they need insulin replacement therapy, which is predominantly achieved via subcutaneous injection of recombinant insulin. However, nocturnal hypoglycemia is one of the limiting factors for strict insulin treatment, because severe hypoglycemia often causes neurological outcomes such as convulsion or neuropsychological dysfunction. Because of this fear of nocturnal hypoglycemia, patients are often unwilling to inject the appropriate dose of insulin, and thus experience insulin insufficiency in the early morning because of the early waning of insulin activity. Because of this fear of nocturnal hypoglycemia, patients often decrease their insulin doses at bedtime and thus experience insulin insufficiency in the early morning.

The dawn phenomenon was first reported in 1981 (Schmidt et al., 1981) and is characterized by a marked increase in glucose levels in the morning (dawn), which is caused by a combination of the waning of the insulin (commonly NPH insulin) injected the previous night in addition to an increase in insulin resistance, which has been demonstrated by the glucose clamp method (van Cauter et al., 1989). The onset of insulin resistance at dawn is believed to be due to nocturnal surges of counterregulatory hormones such as growth hormone (Perriello et al., 1990). In addition, Kobayashi et al. reported that IGF binding protein (IGFBP)-1 also plays a role in nocturnal glycemic control (Kobayashi et al., 1997).

IGFBP-1 is produced mainly in the liver and its transcription is inhibited by insulin. Thus, IGFBP-1 and insulin both play important roles in the regulation of short-term IGF-1 bioavailability (Lee et al., 1997; Lang et al., 2003). The levels of IGFBP-1 fluctuate with a peak before breakfast, and patients with type 1 diabetes often show extremely high IGFBP-1 levels compared with

normal subjects, as a result of hepatic insulin insufficiency (Hilding et al., 1995).
Modern insulin management using an insulin pump (continuous subcutaneous insulin infusion, CSII) (Pickup and Keen, 2002) or long-acting insulin analogs such as insulin glargine (Rosenstock et al., 2000) or insulin detemir provides stable and peakless insulin levels, particularly compared with NPH insulin. These modern insulin regimens reduced the risk of hypoglycemic events and, in turn, allow for better glycemic control. In terms of nocturnal glycemic control, some reports have indicated that the dawn phenomenon is decreased with CSII or glargine (Pickup and Renard, 2008).
Here, we investigated the nocturnal fluctuations in glucose, IGFBP-1 and free IGF-1 levels with three basal insulin regimens (CSII, insulin glargine, NPH insulin) that are commonly used by patients with type 1 diabetes in Japan. The aim of this study was to compare the nocturnal IGFBP-1 overproduction and blood glucose fluctuation control in terms of onset of the dawn phenomenon with these insulin regimens.

Research Design and Methods

Subjects

We enrolled 62 Japanese type 1 diabetes mellitus patients from the University of Yamanashi, who were being treated with intensive insulin therapy to achieve near-normal glycemic control while avoiding severe hypoglycemia. Children were enrolled after parental or patient written informed consent was obtained, and adults provided written informed consent. This study was approved by the ethical committee of University of Yamanashi.
Exclusion criteria included diabetes diagnosed within the past 6 months (2 patients), poor glycemic control with HbA1c level >10.0% (5 patients), frequent hypoglycemia (1 patient), acute or chronic illness (1 patient) or ketoacidosis (0 patient). Patients who ate a midnight snack as a result of fear of hypoglycemia were also excluded (5 patients). As a result, 48 patients participated in and completed the study, of which 20 were male and 28 were female. The mean age of the patients was 14.4 years (range 9.9–24.5 years) and the mean time since diagnosis was 6.0 years (0.7–18.8 years). The mean HbA1c level was 7.9% (5.7–9.8%) and the mean body mass index was 21.2 kg/m^2 (15.5–24.9 kg/m^2), and the mean body mass index–standard deviation score (BMI-SDS) was 0.7 (−1.8 to 2.7 SD).

Clinical study design

Patients were classified according to their basal insulin therapy with either NPH (29 patients), glargine (12 patients) or CSII (7 patients) (**Table 1**). The groups were comparable in terms of age, diabetes duration, insulin doses and HbA1c levels (NS between groups), but different in terms of sex. In the NPH group, all patients received multiple daily injection (MDI) with once daily NPH injection at bedtime and preprandial regular insulin (11 patients) or insulin analog (aspart, 8 patients; lispro, 10 patients lispro). In the glargine group, all patients also received MDI with once daily injection of glargine (Lantus®, Sanofi-Aventis, Japan) at bedtime, and their preprandial insulin was insulin analog only (aspart, 5 patients; lispro, 7 patients). In the CSII group, all of the patients were female and were treated with insulin aspart administered by Medtronic Mini Med 505 (2 patients) or NIPRO (5 patients) insulin pumps. Their insulin pumps were set, by the physician, to deliver a constant basal insulin rate during the experimental period. The basal CSII dose was set at 45–60% of the total daily insulin dose and was comparable with the bedtime doses of insulin used in the MDI groups.
At the research center, the subjects administered their preprandial insulin injection, including a bolus infusion in the CSII group, and ate dinner at 1800 h, which was calculated by a dietitian to be appropriate for the patient's age in terms of calories. NPH or glargine was administered at 2100 h, and breakfast was at 0800 h. None of the patients in any group ate a bedtime snack. An intravenous catheter filled with physiological saline was inserted and blood samples were obtained every 2 h from 2300 h to 0700 h, without waking the patient up, for measurement of plasma glucose, IGFBP-1 and free IGF-1 levels. The HbA1c, IGF-1 and IGFBP-3 levels were determined at 0700 h. We assessed the nocturnal profiles of these factors and the appearance of the dawn phenomenon in each group.

Table 1 Baseline characteristics of the patients according to the basal insulin regimen.

	NPH (n=29)	Glargine (n=12)	CSII (n=7)
males/females (n)	13/16	7/5	0/7
age (years)	14.0±0.5	14.9±0.7	15.0±0.6
time since diagnosis (years)	5.6±0.8	7.4±1.4	5.3±1.1
height (cm)	152.1±2.0	154.0±3.4	153.4±0.9
weight (kg)	48.9 ±2.1	53.1±3.6	51.2 ±2.1
body mass index (kg/m^2)	20.8±0.5	22.2±1.0	21.7±0.7
body mass index-standard deviation scores	0.6±0.2	0.9±0.3	0.8±0.3

Data are n or mean ± SEM
There were no differences between groups in terms of any of the baseline characteristics

Definition of dawn phenomenon

Dawn phenomenon was defined as previously described (Kobayashi et al., 1997) as: 1) change in plasma glucose from 0500 h to 0700 h of >20 mg/dl; 2) plasma glucose level at 0700 h of >140 mg/dl; and 3) no antecedent hypoglycemia.

Assays

Blood samples were centrifuged (2000×g, 5 min, 4 °C) rapidly after sampling, and plasma or serum were aliquoted and frozen at −20 °C. The plasma glucose concentration was measured by the glucose electrode method (Glucose Auto and Stat, ARKRAY, Japan). HbA1c was measured by high performance liquid chromatography (Hi Auto H1c, ARKRAY). Serum IGFBP-1 and free IGF-1 concentrations were measured by immunoradiometric assays (Diagnostics Systems Laboratories, Webster, TX, USA). The sensitivity of the IGFBP-1 assay was 2.0 ng/ml. Intraassay imprecision was 6.5% at 7.0 ng/ml and 5.3% at 70.0 ng/ml. The sensitivity of the free IGF-1 assay was 0.4 ng/ml. Intraassay imprecision was 4.9% at 0.02 ng/ml and 7.8% at 2.0 ng/ml, respectively.

Statistical analysis

Clinical results are presented as means±standard error of the mean. BMI-SDS scores were calculated based on a previous report of BMI standardized centile curves in Japanese children and adolescents (Inokuchi et al., 2006). The distributions of the data were examined for normality using the Kolmogorov-Smir-

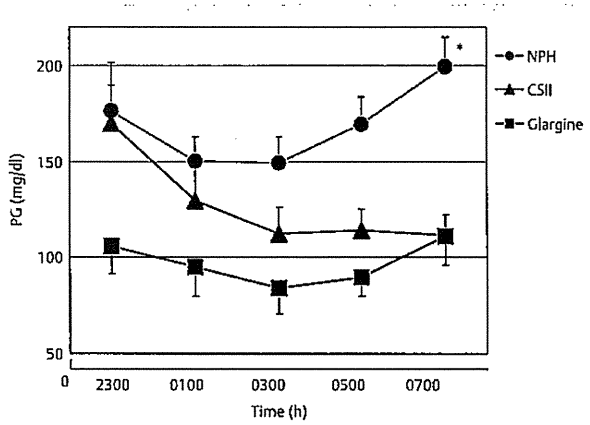

Fig. 1 Nocturnal blood glucose profiles in patients with type 1 diabetes treated with NPH insulin (n=29, black circles), CSII (n=7, black triangles) or insulin glargine (n=12, black squares) based regimens. Data are means±SEM. *p<0.001, blood glucose levels with NPH at 0300h vs. 0700h.

nov goodness of fit test. Log transformation of IGFBP-1 was necessary for statistical testing purpose, but we present non-transformed values for clear comprehension. The Mann-Whitney U test was used to compare the three groups. Cross-correlation was used to determine the association between IGFBP-1 and free IGF-1.

Results

Insulin doses

As shown in **Table 2**, the mean daily dose of insulin was comparable in both groups (although numerically higher in the glargine group), but the proportion of insulin administered as basal insulin was significantly higher in the CSII group than in the NPH or glargine groups.

Nocturnal glycemic profiles

The nocturnal changes in blood glucose levels are shown in ○ **Fig. 1**. At bedtime (2300h), the blood glucose level was 170.0±31.8mg/dl in the CSII group, which was comparable to that in the NPH group (176.5±13.2mg/dl). In the NPH group, the blood glucose level was 149.5±13.6mg/dl at 0300h, which increased significantly to 199.4±15.3mg/dl at 0700h (p<0.001). In the CSII group, the mean glucose level during the morning was stable from at 0300h (112.5±13.6mg/dl) to 0700h (111.2±11.3mg/dl). In the glargine group, there was a mild increase in nocturnal glucose levels between 0300h and 0700h (84.2±13.6mg/dl to 111.2±15.6mg/dl), but the blood glucose level at 2300h was the lowest of all three groups. The dawn phenomenon was significantly more frequent in the NPH group (62.1%) than in the glargine (16.6%, p<0.05) or CSII (14.3%, p<0.05) groups (**Table 2**).

Nocturnal IGFBP-1 and free IGF-1 profiles

Patients in the NPH group were younger than those in the other groups, and the mean total IGF-1 levels were distributed in levels corresponding to the age of the patients in each group (**Table 1**). Nevertheless, the circulating IGFBP-3 levels, the primary IGF-1 carrier protein, were comparable between the three groups.

Table 2 Effects of the three insulin regimens on total IGFBP-1 and IGF-1 levels and dawn phenomenon.

	NPH (n=29)	Glargine (n=12)	CSII (n=7)
basal insulin rate (%)	31.5±1.4	32.3±1.4	54.1±2.7*
daily insulin dose (U/kg)	1.1±0.1	1.3±0.1	1.1±0.1
HbA1c (%)	8.0±0.3	7.8±0.3	8.1±0.5
total IGF-1 (ng/ml)	244.0±19.6	306.2±26.6	351.3±30.2
IGFBP-3 (μg/ml)	4.2±0.1	4.0±0.2	4.2±0.1
Dawn phenomenon (%, n)	62.1 (18/29)	16.6 (2/12)**	14.3 (1/7)**

Data are n or mean±SEM
* p<0.05 vs. NPH and glargine groups
** p<0.05 vs. NPH group

The nocturnal IGFBP-1 and free IGF-1 profiles are shown in ○ **Fig. 2**. The IGFBP-1 levels were inversely correlated with free IGF-1 levels at 0700h in all patients (r=−0.302, p<0.05). In all groups, the IGFBP-1 levels were markedly higher than the reference range (15–50ng/ml) and were particularly higher in the morning. Of note, the IGFBP-1 levels in the NPH group increased by about 10-fold between 2300h and 0700h (from 21.0±3.6ng/ml to 200.3±21.8ng/ml), and the free IGF-1 levels inversely decreased by about half over the same time (from 7.5±1.1ng/ml to 3.3±0.6ng/ml). In the CSII group, the IGFBP-1 levels increased from 19.6±5.0ng/ml to 95.2±17.9ng/ml (p<0.001 vs. NPH) and the free IGF-1 levels were stable (5.7±1.3ng/ml at 2300h and 5.3±1.4ng/ml at 0300h). Glargine partly suppressed the morning rise in IGFBP-1 (25.0±7.2ng/ml at 2300h; 53.1±6.3ng/ml at 0700h; p<0.001 vs. NPH) and the free IGF-1 levels remained stable between 2300h (4.5±0.5ng/ml) and 0700h (4.3±0.7mg/ml).

Discussion

In this study, we investigated the nocturnal fluctuations in glucose, IGFBP-1 and free IGF-1 levels in patients with type 1 diabetes, and compared these profiles between three basal insulin regimens that are commonly used in Japan (CSII, insulin glargine, NPH insulin). We found that the dawn-time glucose rise was significant in patients with NPH, with 60% of patients exhibiting the dawn phenomenon, and the circulating IGFBP-1 levels were markedly increased before breakfast and the free IGF-1 levels were substantially decreased. In the CSII group (the insulin pumps were set to deliver a constant basal insulin rate), the dawn-time glucose levels were stable and the dawn phenomenon was less frequent than in the NPH group. The nocturnal IGFBP-1 levels were also stable with CSII and the morning IGFBP-1 level was lower than those in patients with NPH. In the glargine group, the dawn-time glucose levels were stable and the dawn phenomenon was less frequent than in the NPH group. The nocturnal IGFBP-1 levels exhibited a normal morning rise in the glargine group.

The results of this study should be considered after taking into account limitations of the study. First, this was an open-label, non-randomized, cross sectional study, in which patients continued their prior insulin regimen without optimization of their insulin doses prior to the study. Second, the glucose levels at 2300h were markedly different between the glargine group and the CSII and NPH group. As a result, direct comparison of the

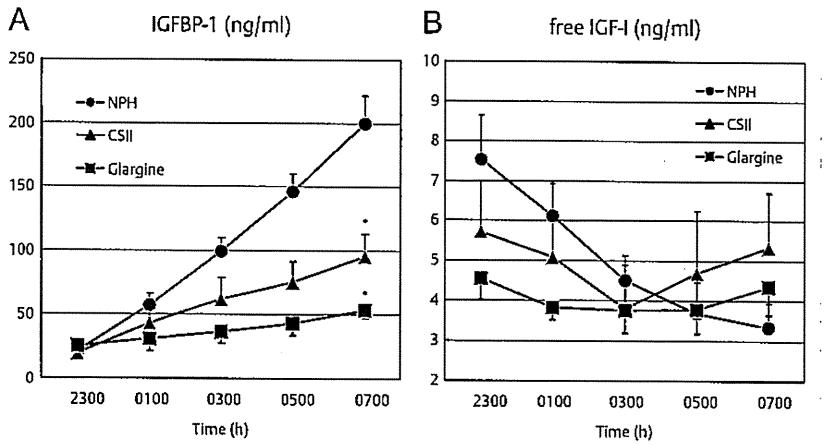

Fig. 2 Nocturnal IGFBP-1 (A) and free IGF-1 (B) profiles in patients treated with NPH insulin (n=29, black circles), CSII (n=7, black triangles) or insulin glargine ((n=12, black squares) based regimens. Data are means ± SEM. *p<0.05 vs. NPH group at 0700 h.

findings between the glargine group and the CSII and NPH groups should be made with care. This finding was surprising and it is unclear why this occurred, because the total insulin doses, evening meal size and preprandial insulin doses were comparable in all three groups. However, the high glucose level in CSII group may be attributable to significantly higher proportion of basal insulin in the CSII group (54.1%) compared with the NPH (31.5%) and glargine groups (32.4%). Therefore, it is possible that the prandial dose was inadequate in the CSII group, meaning postprandial hyperglycemia was not corrected as quickly as in the glargine group. Alternatively, the basal doses of insulin were not optimally titrated, particularly in the CSII and NPH groups because of the fear of hypoglycemia, for example. In this study, we could not differentiate between the waning of the insulin effect and the effect of insulin resistance on dawn phenomenon, because we could not assess the exact timing of the GH surge because blood samples were taken every 2 h. A future study in which blood samples are taken more frequently, perhaps every 5–10 min, would allow accurate identification of the timing of the GH surge and its associated effect on IGF-1, IGFBP-1 and glucose levels. In addition, we could not measure the nocturnal free insulin profile, because assays for insulin analogs (insulin aspart, lispro or glargine) are often inaccurate because of limited cross-reactivity for each analog. Despite these limitations, we believe that this study provides valuable information regarding the effect of insulin regimens on the dawn phenomenon. These findings need to be further investigated in a randomized, controlled cross-over study design, with titration of insulin doses in advance of the study day to limit the influence of sub-optimal glycemic control and blood samples should be obtained more frequently to identify the GH surge.

The main cause of dawn phenomenon is considered to be the waning of insulin action, particularly in NPH insulin-based MDI regimens, in which the insulin doses are often lower than recommended to prevent unwanted hypoglycemia. The second cause of the dawn phenomenon is insulin resistance, which is mediated by increased GH surges. The morning surge in IGFBP-1 levels, which binds to IGF-1, may also play a role in the dawn phenomenon. A study by Frystyk et al. showed that the highest IGFBP-1 levels (15–50 ng/ml) in healthy children are observed before breakfast (Frystyk et al., 2003), but the IGFBP-1 levels in our patients with type 1 diabetes were in the range 50–300 ng/ml at the same time, particularly in the NPH group. In children and adolescents with poorly controlled type 1 diabetes, circulating total IGF-1 levels are often inappropriately low compared with the high GH levels, possibly derived from an acquired state of hepatic GH resistance (Dunger et al., 2005). Therefore, normalization of the GH-IGF-1 axis is essential for prevention of poor glycemic control.

Insulin pump therapy and insulin glargine could maintain stable insulin levels compared with NPH. Of note, the dose of NPH insulin used in this study was not sufficient to maintain the required level of insulin until breakfast, whereas both insulin glargine and CSII maintains insulin at the appropriate level and were able to suppress IGFBP-1 release. It is of interest to determine the effect of insulin detemir on nocturnal IGFBP-1 and IGF-1 levels and the dawn phenomenon, particularly because insulin detemir can be used either once daily or twice daily as part of an MDI regimen. Understanding the effect of detemir dosing on the dawn phenomenon will be of value to help clinicians and patients to use insulin detemir in the best possible way for the patient (Le Floch et al., 2009).

We conclude that the dawn phenomenon is induced by the waning of insulin effect during the early morning before breakfast and was prevented in this study by CSII and glargine therapy. A decrease in free IGF-1, due to binding with IGFBP-1, leads to a loss of its metabolic effect and may affect insulin resistance in the morning. Insulinopenia affects the IGFBP-1 level; thus, a stable basal insulin level should be maintained, irrespective of the regimen used. Modern insulin regimens based on CSII or basal insulin analogs, which offer profiles without marked peaks in activity and with protracted duration of action provide all-night insulin availability with a lower risk of hypoglycemia compared with NPH insulin. Appropriate insulin therapy to maintain stable insulin levels overnight and in the morning will allow better glycemic control and prevent the dawn phenomenon. Patients who regularly experience the dawn phenomenon should consider either changing their insulin dose, or switching to an alternative insulin regimen that provides more stable insulin levels to minimize the impact of the dawn phenomenon on their overall glycemic control.

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Conflict of interest: None.

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Pro198Leu missense polymorphism of the glutathione peroxidase 1 gene might be a common genetic predisposition of distal symmetric polyneuropathy and macrovascular disease in Japanese type 2 diabetic patients

Shohei Matsuno, Hideyuki Sasaki*, Hiroshi Yamasaki, Hiroyuki Yamaoka, Kenichi Ogawa, Muneki Nakatani, Tohru Hamanishi, Asako Doi, Yoshio Nakano, Hisao Wakasaki, Hiroto Furuta, Masahiro Nishi, Takashi Akamizu, Kishio Nanjo

ABSTRACT

Aims/Introduction: We have previously reported that the Pro198Leu missense polymorphism in the glutathione peroxidase 1 (GPx-1) gene was associated with frequent macrovascular disease (MVD). Our goal was to examine whether the GPx-1 genotype is associated with diabetic neuropathy.

Materials and Methods: We determined the GPx-1 genotype in 173 Japanese type 2 diabetic patients who received medical interviews, physical examinations, nerve conduction studies, quantitative vibratory perception (QVP), head-up tilt and heart rate variability tests by polymerase chain reaction-restriction fragment-length polymorphism. Diabetic sensorimotor distal symmetric polyneuropathy (DSPN) and diabetic autonomic neuropathy (DAN) were evaluated separately. DSPN and DAN were defined by two or more abnormalities of neuropathic leg symptoms, diminished Achilles tendon reflexes or impaired QVP in toes, and two autonomic dysfunctions, respectively. The association of the GPx-1 genotype with DSPN, DAN, MVD and other clinical manifestations was analyzed.

Results: The prevalence of DSPN, impaired QVP and painful leg cramps in patients having a genotype with Pro/Leu at the codon 198 (Pro/Leu type) was significantly higher than those with Pro/Pro type. As a result of multivariate analyses that contained the GPx-1 genotype as an independent variable, the Pro/Leu type was extracted as a significant risk factor of DSPN, QVP impairment and MVD. The statistical significance did not disappear, even after proteinuria, retinopathy and a history of MVD were introduced as independent variables. In contrast, the GPx-1 genotype was not associated with DAN.

Conclusions: The Pro198Leu missense polymorphism of the GPx-1 gene might have a common genetic predisposition to DSPN and MVD. (J Diabetes Invest, doi: 10.1111/j.2040-1124.2011.00127.x, 2011)

KEY WORDS: Glutathione peroxidase 1 gene, Diabetic distal symmetric polyneuropathy, Macrovascular disease

INTRODUCTION

It is well known that macrovascular diseases (MVD), such as myocardial and cerebral infarction, are more common in patients with impaired glucose tolerance[1,2]. Recently, a higher prevalence of polyneuropathy in patients with impaired glucose tolerance, as compared with healthy subjects, has been reported[3]. The similarities in risk factors of diabetic polyneuropathy and MVD have also been reported[4,5]. These findings suggest that there might be a common underlying etiological mechanism in both complications.

One of the most plausible common etiological factors of these complications is the excess of oxidative stress. Elevated reactive oxygen species produced by hyperglycemia induces an oxidation of low-density lipoprotein, and an induction of monocyte chemoattractant protein 1 and adhesion molecules. These mechanisms seem to be mainly implicated in the development of MVD[6]. Excessive oxidative stress is also implicated in the development of diabetic neuropathy[7]. Thus, oxidative stress and the related molecular derangements are widely thought to be a common underlying cause of diabetic complications[8]. We have previously reported that the Pro198Leu missense polymorphism of the glutathione peroxidase 1 (GPx-1) gene is associated with a reduction in transcription and enzyme activity of GPx-1, which is an important anti-oxidative enzyme[9]. Furthermore, we and other investigators have reported significant associations

The First Department of Medicine, Wakayama Medical University, Wakayama, Japan
*Corresponding author. Hideyuki Sasaki Tel.: +81-73-441-0625 Fax: +81-73-445-9436
E-mail address: sasaki-h@wakayama-med.ac.jp
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between the polymorphism of the GPx-1 gene and MVD[9,10]. A recent study reported a significant association between a GPx-1 gene variant, which was deferent from our study, and peripheral neuropathy in Caucasian subjects[11]. However, to date, there has been no study to show the association between the GPx-1 gene polymorphism and diabetic neuropathy in Japanese patients. In the present study, we aimed to examine whether Pro198Leu polymorphism in the GPx-1 gene is associated with not only MVD, but also diabetic neuropathy.

Diabetic neuropathy is roughly classified into three types: (i) chronic sensorimotor distal symmetric polyneuropathy (DSPN); (ii) diabetic autonomic neuropathy (DAN); and (iii) focal and multifocal neuropathies, according to a statement by the American Diabetes Association[12]. Although DSPN and DAN are specific complications of diabetes, focal and multifocal neuropathies are not. As we have observed that exacerbating factors of sensory and autonomic functions were different[13], subtypes of diabetic neuropathy, DSPN and DAN were separately evaluated. Additionally, various quantitative neurological functions, such as vibratory perception thresholds, nerve conduction parameters and autonomic functions, were also individually evaluated. Then associations between the GPx-1 gene polymorphism and these subtypes of diabetic neuropathy or quantitative neurological functions were investigated.

MATERIALS AND METHODS

Study Design and Participants

A total of 173 unrelated Japanese type 2 diabetic patients (54 outpatients and 119 hospitalized patients of Wakayama Medical University Hospital) who received serial neurological examinations and agreed to be involved in the genetic study were enrolled after giving written informed consent. The study was approved by the ethics committee of Wakayama Medical University and carried out in accordance with the Helsinki Declaration (revised in 2000). In order to evaluate neurological functions accurately, aged patients (more than 70 years) and patients with severe liver or renal dysfunction, cerebrovascular disease with residual neurological deficits, peripheral arterial disease (second degree of Fontaine classification or more) or other neurological diseases were excluded. Diabetes was diagnosed according to the criteria set by the World Health Organization. Hypertension was defined by a blood pressure > 130/80 mmHg or receiving antihypertensive treatment. Patients with total cholesterol > 5.17 mmol/L (200 mg/dL) and/or triglycerides > 1.70 mmol/L (150 mg/dL) and/or high-density lipoprotein cholesterol < 1.03 mmol/L (40 mg/dL) or those on antihyperlipidemic medication were defined as dyslipidemic. MVD was defined by a previous history of cardiovascular disease and/or stroke without residual neurological deficits. Fair and poor glycemic controls were defined as HbA_{1c} (%) < 8.4% and HbA_{1c} (%) ≥ 8.4%, respectively. The value for HbA_{1c} (%) was estimated as a National Glycohemoglobin Standardization Program (NGSP) equivalent value (%) calculated by the formula HbA_{1c} (%) = HbA_{1c} (Japan Diabetes Society [JDS]) (%) + 0.4%, considering the relational expression of HbA_{1c} (JDS) (%) measured by the previous Japanese standard substance and measurement methods and HbA_{1c} (NGSP)[14].

Genotyping of GPx-1

Genomic DNA was isolated from peripheral blood according to standard procedures. The GPx-1 genotype for the Pro198Leu missense polymorphism in exon 2 was analyzed by polymerase chain reaction-restriction fragment-length polymorphism using *HaeIII* as a restriction enzyme, as previously described[9]. The genotypes with Pro/Leu and Pro/Pro at the codon 198 are described as Pro/Leu type and Pro/Pro type, respectively.

Assessment of Neurological Functions

To evaluate the clinical diabetic neuropathy, the sensorimotor and autonomic symptoms were evaluated. Subjective symptoms were ascertained using five criteria: 'numbness in toe and sole'; 'pain in feet, particularly below the knee: pain in feet'; 'painful leg cramp occurring two or more times in a month: painful leg cramp'; 'dizziness on standing: orthostatic dizziness'; and 'frequent constipation/diarrhea or their alternation: frequent constipation/diarrhea'. In the present study, numbness means an uncomfortable sensation with or without ordinary stimulation and dullness in perception inclusively. Painful muscle cramp was defined as a spasm of the calf muscle with severe pain. Achilles tendon reflex (ATR) in the knee-standing position was also examined bilaterally. Furthermore, four objective and quantitative tests were carried out to assess the sensory, motor and autonomic nerve functions as previously described[15]. All examinations were carried out in a temperature-controlled room at 25°C.

Quantitative Vibratory Perception Threshold

Quantitative vibratory perception threshold (QVP) at 125 Hz was assessed using a vibratory sensation meter (AU-02A; RION Company, Tokyo, Japan), whose output level could be changed from −10 to 40 dB (0 dB ref 0–3 m/s^2)[16]. First, the patient put the plantar aspect of their big toe on a vibrating plate and was shown the vibration output level from minimum to maximum. Then the patient was asked to respond by saying 'buzzing' when they felt vibration during a gradual increase of vibratory stimulation. When the patient responded at the same output level twice or more, we regarded that as the perceptible threshold. Measurements were carried out bilaterally and an average of the two sides was used for analysis.

Autonomic Nerve Function Tests (Head-up Tilt Test and Heart Rate Variability Test)

Sympathetic vasomotor function was evaluated by a head-up tilt test using a tilt table (Sakai, Tokyo, Japan) and an automatic sphygmomanometer (BP-88; Colin Company, Tokyo, Japan). Orthostasis-induced decreases in systolic blood pressure after passive standing for 5 min in a 70° head-up position (ΔBP) were examined.

Parasympathetic cardiovagal function was also evaluated by a heart rate variability test. Coefficients of variation of R-R intervals on electrocardiogram after 15 min resting in the supine position (CVR-R) were determined with an electrocardiograph (Autocardiner FCP-2201; Fukuda Denshi, Tokyo, Japan).

Nerve Conduction Study

Motor nerve conduction velocity (MCV) between the wrist and elbow, compound muscle action potential (CMAP) of the ulnar nerve, sensory nerve velocity (SCV) between the wrist and elbow, and sensory nerve action potential (SNAP) of the median nerve were measured bilaterally using standard methods with an electromyograph (Synax 1200; NEC, Tokyo, Japan). Electric stimuli were produced at supramaximal intensity. The CMAP and SNAP produced by the wrist stimulation were evaluated. Skin temperature was measured at the forearms and was maintained at 32°C.

Decision of Abnormality and Subtypes of Diabetic Neuropathy

QVP, MCV, CAMP, SCV, SNAP and logarithmic CVR-R were distributed normally, values exceeding the range of means ± 2 SD of the age-matched healthy subjects in our institution were judged as impaired. Abnormal ΔBP was defined by the American Autonomic Society criteria[17]. Namely, a fall in systolic blood pressure of more than 20 mmHg and/or a fall in diastolic blood pressure of more than 10 mmHg was judged to be an abnormal value. We then classified various neurological manifestations into two subtypes of diabetic neuropathy, distal symmetric polyneuropathy (DSPN) and diabetic autonomic neuropathy (DAN). DSPN was defined by two or more abnormalities in specific neuropathic leg symptoms (numbness in toes and soles, and/or pain in feet), bilaterally diminished ATR and impaired QVP. Painful leg cramp was not included as a neuropathic symptom. For example, this symptom is recognized as a sign of a circulatory disturbance in the questionnaire of Michigan Neuropathy Screening Instruments (MNSI) announced from the website of the Michigan Diabetes Research and Training Center. Neurological Symptom Score (NSC) in the Mayo Clinic also does not contain painful muscle cramp as a sensory symptom[18]. DAN was diagnosed by the two autonomic dysfunctions, impaired CVR-R and abnormal ΔBP.

Statistical Analysis

All statistical analyses were carried out with the StatView program for Windows (version 5.01; SAS Institute, Cary, NC, USA). Differences of clinical data, neuropathic symptoms, ATR, various nerve function data and subtypes of diabetic neuropathy between the two diabetic groups divided based on the GPx-1 genotype were analyzed by ANOVA and χ^2-test.

Multiple logistic regression analyses were carried out to verify the associations between clinical manifestations of diabetic neuropathy and clinical background factors, including the GPx-1 genotype. DSPN, DAN and painful leg cramps were set as dependent variables for the analyses. Eight clinical background factors (age, sex, duration of diabetes, hypertension, dyslipidemia, glycemic control, body mass index [BMI] and GPx-1 genotype: Pro/Pro = 0, Pro/Leu = 1) were selected as independent variables (model 1). Additional analyses, which added proteinuria and retinopathy as independent variables, were also carried out (model 2). In order to negate the influence of MVD on diabetic neuropathy, another analysis was carried out (model 3) in which the history of MVD was added as an independent variable of model 2. An association between MVD and clinical background factors was also evaluated by the two analyses (model 1 and 2).

Multiple regression analyses were also used to determine independent associations between the GPx-1 genotype and six actual results of nerve function tests using the same three sets of independent variables (model 1, 2, 3). A P-value of <0.05 was considered statistically significant.

RESULTS

GPX-1 Genotype

Genotype frequencies (%) of Pro/Pro type, Pro/Leu type and Leu/Leu type were 86.1, 13.9 and 0 in all diabetic patients, respectively. Genotype distributions did not significantly differ from Hardy–Weinberg equilibrium expectations. The frequency of Pro/Leu type in diabetic patients with DSPN was significantly higher than that in the patients without DSPN (17/79 = 21.5% vs 7/94 = 7.5%, P = 0.0076). The frequencies of Pro/Leu type in diabetic patients with DAN was not significantly different from those in patients without DAN (3/25 = 12.0% vs 21/148 = 14.2%, P = 0.7696).

Relationships Between GPx-1 Genotype and Clinical, Neurological Data

Patients were divided into two groups (Pro/Pro type and Pro/Leu type) based on the codon 198 polymorphism, and clinical and neurological features were then compared. Clinical characteristics of the two diabetic groups, such as age, sex, duration of diabetes, therapy, BMI, hypertension, dyslipidemia, recent HbA_{1c}, proteinuria, retinopathy and history of MVD are shown in Table 1. Though the prevalence of MVD tended to be higher in Pro/Leu type than Pro/Pro type (P = 00510), there was no significant difference in clinical characteristics.

The data of subjective symptoms, ATR, subtypes of diabetic neuropathy and quantitative nerve functions are also shown in Table 1. As a subjective symptom, the prevalence of painful leg cramps in Pro/Leu type was significantly higher than that in Pro/Pro type. Among the two subtypes of diabetic neuropathy, only DSPN showed a significantly higher prevalence in Pro/Leu type compared with Pro/Pro type (70.8 vs 41.6, P = 0.0076). In the quantitative neurological data, statistically significant differences between Pro/Leu type and Pro/Pro type were observed in QVP with a prevalence of impaired QVP. In contrast, there was no significant difference in the autonomic or nerve conduction functions between Pro/Leu and Pro/Pro type.

Table 1 | Comparison of clinical characteristics and neurological functions between two diabetic groups divided based on GPx-1 genotype (n = 173)

	Pro/Leu type	Pro/Pro type	P-value
n	24	149	
Clinical characteristics			
Age (year)	55.6 ± 13.0	54.9 ± 10.3	0.8503
Gender (Male/Female)	13/11	85/64	0.7916
Duration of diabetes (years)	12.9 ± 7.8	11.3 ± 7.7	0.3433
Therapy (insulin/OHA/diet and exercise)	1/5/18 (4.2/20.8/75.0)	6/42/101 (4.0/28.2/67.8)	0.7524
BMI (kg/m^2)	23.1 ± 4.2	23.9 ± 3.9	0.3163
Hypertension	10/24 (41.7)	67/149 (45.0)	0.7627
Dyslipidemia	10/24 (41.7)	72/149 (48.3)	0.5445
HbA_{1c} (%)	9.83 ± 2.22	9.11 ± 2.06	0.1134
Proteinuria (no/intermittent/persistent)	16/4/4 (66.6/16.7/16.7)	102/19/28 (68.5/12.7/18.8)	0.8614
Retinopathy (no/simple/pre-, proliferative)	10/4/10 (41.7/16.6/41.7)	78/22/49 (52.4/14.7/32.9)	0.6124
History of macrovascular disease (MVD)	5/24 (20.8)	12/149 (8.1)	0.0510
Subjective symptoms and Achilles tendon reflex (ATR)			
Numbness in toes and soles	9/24 (37.5)	52/149 (34.9)	0.8045
Pain in feet	3/24 (12.5)	16/149 (10.7)	0.7978
Painful leg cramp	14/24 (58.3)	36/149 (24.2)	**0.0006**
Orthostatic dizziness	4/24 (16.7)	27/149 (18.4)	0.8411
Frequent constipation/diarrhea	1/24 (4.2)	13/149 (8.7)	0.4429
Diminished ATRs	19/24 (79.2)	94/149 (64.8)	0.1669
Subtypes of diabetic neuropathy and quantitative nerve functions			
DSPN (Distal symmetric polyneuropathy)	17/24 (70.8)	62/149 (41.6)	**0.0076**
DAN (diabetic autonomic neuropathy)	3/24 (12.5)	22/149 (14.8)	0.7696
QVP (dB)	26.0 ± 7.2	20.4 ± 10.3	**0.0114**
Prevalence of impaired QVP	17/24 (70.8)	56/149 (37.6)	**0.0022**
CVR-R (%)	1.98 ± 0.92	1.96 ± 1.06	0.9591
Prevalence of impaired CVR-R	13/24 (56.5)	68/149 (46.9)	0.3908
ΔBP (mmHg)	7.79 ± 12.49	11.02 ± 14.51	0.3057
Orthostatic hypotension	4/24 (16.7)	35/149 (23.5)	0.4579
MCV (m/s)	50.9 ± 3.9	50.4 ± 6.8	0.7506
Prevalence of impaired MCV	5/24 (20.8)	50/149 (33.6)	0.2141
CMAP (mV)	7.12 ± 3.36	7.10 ± 2.74	0.9734
Prevalence of impaired CMAP	5/24 (20.8)	16/149 (10.7)	0.1599
SCV (m/s)	56.4 ± 5.2	57.3 ± 5.9	0.5187
Prevalence of impaired SCV	10/24 (41.7)	58/149 (38.9)	0.7987
SNAP (μV)	18.4 ± 14.9	21.1 ± 14.3	0.3993
Prevalence of impaired SNAP	8/24 (33.3)	39/149 (26.2)	0.4644

Numbers in parenthesis indicate the percentage. OHA, oral hypoglycemic agents; BMI, body mass index; ATR, Achilles tendon reflex; QVP, quantitative vibratory perception thresholds; CVR-R, correlation coefficient of R-R intervals in electrocardiogram; BP, blood pressure; CMAP, compound muscle action potential; SCV, sensory nerve conduction velocity; SNAP, sensory nerve action potential; Pro/Leu type, genotype with Pro/Leu at the codon 198 of glutathione peroxidase 1 gene; Pro/Pro type, genotype with Pro/Pro at the codon 198 of glutathione peroxidase 1 gene. The value for HbA_{1c} (%) was estimated as an NGSP equivalent value (%) calculated by the formula HbA_{1c} (%) = HbA_{1c} (JDS) (%) + 0.4%. Statistically significant P-value was shown by boldfaced type.

Multivariate Analyses

Relationships between the GPx-1 genotype and a subtype of diabetic neuropathy, painful leg cramp and MVD were analyzed by multiple logistic regression analyses and are shown in Table 2. Multiple logistic regression analysis showed that the GPx-1 genotype (Pro/Leu type) was a significant risk factor for DSPN, painful leg cramps and MVD independent from age, sex, duration, hypertension, dyslipidemia, recent glycemic control and BMI (model 1). The GPx-1 genotype (Pro/Leu type) was a significant risk factor for DSPN, painful leg cramps and MVD, even if proteinuria and retinopathy were added as independent variables of the analyses (model 2). In contrast, Pro/Leu type was not identified as a significant risk factor for DAN in the two regression models.

Associations between the GPx-1 genotype and quantitative neurological functions analyzed by multiple regression analyses are shown in Table 3. Though the GPx-1 genotype (Pro/Leu type) was identified as a significant exacerbation factor of QVP independent from age, sex, duration, hypertension, dyslipidemia, recent glycemic control and BMI, no significant relationship between the GPx-1 genotype and other autonomic or nerve

Table 2 | Relationships between the glutathione peroxidase 1 gene polymorphism and subtype of diabetic neuropathy, painful leg cramp, macrovascular disease evaluated by multiple logistic regression analysis

Independent variables	Model 1 dependent variables				Model 2 dependent variables			
	Subtypes of diabetic neuropathy		Painful leg cramp	History of MVD	Subtypes of diabetic neuropathy		Painful leg cramp	History of MVD
	DSPN	DAN			DSPN	DAN		
R^2 (*P*-value)	R^2 = 0.122 (*P* = 0.0003)	R^2 = 0.137 (*P* < 0.0001)	R^2 = 0.086 (*P* = 0.0217)	R^2 = 0.209 (*P* = 0.0031	R^2 = 0.210 (*P* < 0.0001)	R^2 = 0.530 (*P* < 0.0001)	R^2 = 0.102 (*P* = 0.0202)	R^2 = 0.215 (*P* = 0.0078)
	Adjusted OR (95% CI) *P*-value	Adjusted OR (95% CI) *P*-value	Adjusted OR (95% CI) *P*-value	Adjusted OR (95% CI) *P*-value	Adjusted OR (95% CI) *P*-value	Adjusted OR (95% CI) *P*-value	Adjusted OR (95% CI) *P*-value	Adjusted OR (95% CI) *P*-value
Age (years)	1.010 (0.976–1.044) 0.5783	**1.039 (1.003–1.076) 0.0345**	0.998 (0.963–1.034) 0.9125	**1.118 (1.022–1.224) 0.0152**	1.010 (0.974–1.047) 0.5809	1.001 (0.934–1.072) 0.9806	0.977 (0.961–1.033) 0.8620	**1.122 (1.023–1.231) 0.0151**
Sex (female: 0, male: 1)	1.054 (0.534–2.077) 0.8800	1.098 (0.552–2.186) 0.7892	0.561 (0.273–1.152) 0.1154	**7.212 (1.609–32.334) 0.0098**	1.099 (0.518–2.329) 0.8059	1.883 (0.458–7.743) 0.3803	0.488 (0.229–1.037) 0.0621	**6.342 (1.375–29.265) 0.0179**
Duration (years) (≥5: 0. 6–15: 1, 16≤: 2)	**1.997 (1.252–3.188) 0.0037**	**2.003 (1.254–3.200) 0.0037**	0.981 (0.598–1.609) 0.9395	1.123 (0.518–2.434) 0.7684	1.343 (0.795–2.270) 0.2706	0.978 (0.409–2.336) 0.9596	1.133 (0.650–1.976) 0.6587	1.031 (0.255–2.335) 0.9413
Hypertension (no: 0, yes: 1)	**2.477 (1.199–5.119) 0.0143**	1.823 (0.881–3.776) 0.1057	0.774 (0.361–1.660) 0.5104	1.590 (0.510–4.961) 0.4244	2.117 (0.958–4.679) 0.0637	2.595 (0.706–9.535) 0.1508	0.753 (0.339–1.674) 0.4864	1.395 (0.429–4.543) 0.5801
Dyslipidemia (no: 0, yes: 1)	**0.412 (0.205–0.830) 0.0130**	**0.463 (0.231–0.931) 0.0308**	0.573 (0.274–1.197) 0.1384	0.801 (0.254–2.524) 0.7050	**0.465 (0.218–0.989) 0.0467**	0.842 (0.212–3.344) 0.8070	0.510 (0.239–1.088) 0.0816	0.741 (0.229–2.393) 0.6160
Glycemic control (~fair: 0, poor: 1)	1.383 (0.699–2.736) 0.3513	1.664 (0.828–3.344) 0.1526	1.572 (0.756–3.269) 0.2261	0.791 (0.261–3.270) 0.1745	1.728 (0.821–3.638) 0.1496	**9.232 (1.991–43.945) 0.0046**	1.410 (0.669–2.971) 0.3663	0.728 (0.234–2.265) 0.5832
BMI (kg/m^2) (>22: 0. 22–25: 1, 25<: 2)	0.845 (0.552–1.294) 0.4387	**0.572 (0.369–0.885) 0.0122**	0.980 (0.626–1.535) 0.9301	1.624 (0.807–3.270) 0.1745	0.863 (0.547–1.363) 0.5285	**0.158 (0.055–0.456) 0.0006**	0.985 (0.627–1.549) 0.9486	1.606 (0.797–3.236) 0.3330
GPx-1 genotype (Pro/Pro : 0, Pro/Leu :1)	**3.390 (1.252–9.181) 0.0163**	0.891 (0.338–2.351) 0.8157	**4.333 (1.718–10.929) 0.0019**	**3.886 (1.078–14.009) 0.0380**	**3.303 (1.175–9.285) 0.0234**	0.340 (0.045–2.571) 0.2957	**4.653 (1.813–11.943) 0.0014**	**3.782 (1.044–13.703) 0.0428**
Proteinuria (no: 0, i ntermittent: 1, persistent: 2)					0.694 (0.399–1.205) 0.1941	0.664 (0.302–1.459) 0.3078	1.545 (0.879–2.714) 0.1303	1.350 (0.621–2.936) 0.4494
Retinopathy (no: 0, simple: 1, PPDR~: 2)					**2.919 (1.764–4.831) <0.0001**	**39.232 (5.792–265.745) 0.0002**	0.659 (0.388–1.119) 0.1228	1.009 (0.491–2.076) 0.9802

BMI, body mass index; CI, confidence interval; DAN, diabetic autonomic neuropathy; DSPN, distal symmetric polyneuropathy; GPx-1, glutathione peroxidase 1 gene; MVD, macrovascular disease, OR, odds ratio; PPDR, preproliferative diabetic retinopathy; R^2, decision coefficient. Statistically significant *P*-value was shown by boldfaced type.

Table 3 | Relationships between Pro198Leu polymorphism of glutathione peroxidase 1 gene and quantitative neurological functions evaluated by multiple regression analysis

Independent variables	Model 1 dependent variables							Model 2 dependent variables						
	Vibration	Autonomic functions		Nerve conduction parameters				Vibration	Autonomic functions		Nerve conduction parameters			
	QVP	CVR–R	ΔBP	MCV	CMAP	SCV	SNAP	QVP	CVR–R	ΔBP	MCV	CMAP	SCV	SNAP
R^2 (*P*-value)	R^2 = 0.182 (*P* < 0.0001)	R^2 = 0.202 (*P* < 0.0001)	R^2 = 0.143 (*P* = 0.0012)	R^2 = 0.101 (*P* = 0.0254)	R^2 = 0.049 (*P* = 0.3987)	R^2 = 0.093 (*P* = 0.0522)	R^2 = 0.286 (*P* < 0.0001)	R^2 = 0.320 (*P* < 0.0001)	R^2 = 0.288 (*P* < 0.0001)	R^2 = 0.266 (*P* < 0.0001)	R^2 = 0.254 (*P* < 0.0001)	R^2 = 0.083 (*P* = 0.1588)	R^2 = 0.182 (*P* = 0.0005)	R^2 = 0.419 (*P* < 0.0001)
	β (*P*-value)	β (*P*-value)	β (*P*-value)	β (*P*-value)	β (*P*-value)	β (*P*-value)	β (*P*-value)	β (*P*-value)	β (*P*-value)	β (*P*-value)	β (*P*-value)	β (*P*-value)	β (*P*-value)	β (*P*-value)
Age (years)	**0.281** (**0.0004**)	**−0.296** (**0.0003**)	0.079 (0.3281)	−0.121 (0.1445)			**−0.326** (**<0.0001**)	**0.288** (**<0.0001**)	**−0.303** (**<0.0001**)	0.088 (0.2425)	−0.133 (0.0823)		−0.080 (0.3241)	**−0.338** (**<0.0001**)
Sex (female: 0, male: 1)	0.062 (0.3950)	0.049 (0.5027)	0.063 (0.4036)	−0.142 (0.0681)			**−0.169** (**0.0157**)	0.072 (0.2984)	0.064 (0.3781)	0.048 (0.5058)	−0.093 (0.2049)		−0.025 (0.7484)	**−0.164** (**0.0122**)
Duration (years) (≥5: 0. 6–15: 1, 16≤: 2)	0.114 (0.1398)	**−0.215** (**0.0015**)	0.178 (0.0247)	−0.061 (0.4510)			**−0.265** (**0.0004**)	−0.062 (0.4192)	−0.118 (0.1406)	0.014 (0.8588)	0.100 (0.2184)		0.084 (0.3350)	−0.089 (0.2228)
Hypertension (no: 0, yes: 1)	0.120 (0.1149)	0.040 (0.5958)	**0.200** (**0.0109**)	−0.064 (0.4235)			−0.069 (0.3355)	0.058 (0.4249)	0.105 (0.1619)	0.122 (0.1052)	0.045 (0.5568)		−0.099 (0.2235)	0.004 (0.9516)
Dyslipidemia (no: 0, yes: 1)	−0.071 (0.3417)	0.037 (0.6153)	−0.129 (0.0899)	0.032 (0.6847)			0.115 (0.1032)	−0.016 (0.8112)	0.006 (0.9343)	−0.090 (0.2120)	0.001 (0.9954)		−0.011 (0.8882)	0.067 (0.3026)
Glycemic control (∼fair: 0, poor: 1)	−0.007 (0.9271)	−0.103 (0.1572)	0.104 (0.1601)	**−0.191** (**0.0135**)			−0.089 (0.1923)	0.025 (0.7038)	−0.117 (0.0938)	0.123 (0.0791)	**−0.196** (**0.0062**)		**−0.246** (**0.0014**)	−0.101 (0.1093)
BMI (kg/m^2) (>22: 0. 22–25: 1, 25<: 2)	−0.072 (0.3443)	0.071 (0.3489)	**−0.205** (**0.0091**)	**0.164** (**0.0429**)			−0.091 (0.2088)	−0.063 (0.3725)	0.062 (0.3891)	**−0.197** (**0.0074**)	**0.163** (0.0286)		0.145 (0.0671)	−0.092 (0.1605)
GPx-1 genotype (Pro/Pro: 0, Pro/Leu: 1)	**0.176** (**0.0145**)	0.036 (0.6111)	−0.111 (0.1292)	0.047 (0.5339)			−0.044 (0.5124)	**0.154** (**0.0196**)	0.050 (0.4617)	−0.129 (0.0596)	0.060 (0.3849)		−0.009 (0.8987)	−0.021 (0.7360)
Retinopathy (no: 0, simple: 1, PPDR∼: 2)								**0.455** (**<0.0001**)	**−0.306** (**0.0006**)	**0.380** (**<0.0001**)	**−0.320** (**0.0003**)		**−0.298** (**0.0017**)	**−0.409** (**<0.0001**)
Proteinuria (no:0, intermittent: 1, persistent: 2)								−0.091 (0.2517)	−0.041 (0.6236)	0.028 (0.7315)	**−0.181** (**0.0322**)		−0.068 (0.4482)	−0.001 (0.9862)

The significant regression formula on compound muscle action potential (CMAP) and sensory nerve velocity (SCV) were not obtained in model 1. The significant regression formula on CMAP was not obtained in model 2. β, Standard regression coefficient; BMI, body mass index; ΔBP, orthostasis-induced decreases in systolic blood pressure at standing; QVP, quantitative vibratory perception thresholds; CVR–R, coefficient of variation of RR intervals on electrocardiogram after 15 min resting; GPx-1, glutathione peroxidase 1 gene; MCV, motor nerve conduction velocity; PPDR, preproliferative diabetic retinopathy; SCV, sensory nerve conduction velocity; SNAP, sensory nerve action potential, R^2, decision coefficient. Statistically significant *P*-value was shown by boldfaced type.

conduction parameters was proven (model 1). Virtually the same result was obtained from the reanalysis to which proteinuria and retinopathy were added as independent variables (model 2).

Table 4 shows the result of the multiple logistic regression and multiple regression analyses that contains a history of MVD as an independent variable (model 3). As in the results of model 1 and 2, significant associations of the GPx-1 genotype with DSPN, painful leg cramp and QVP were also observed in this model.

DISCUSSION

In the present study, we showed the following four major findings. First, the frequency of Pro/Leu type of the GPx-1 genotype in diabetic patients with DSPN was significantly higher than that in the patients without DSPN. Second, the frequencies of painful leg cramp, DSPN and impaired QVP in the patients with Pro/Leu type were significantly higher than those in the patients with Pro/Pro type, respectively. Third, Pro/Leu type was a significant risk factor associated with painful leg cramp, DSPN and history of MVD, but it was not associated with DAN. Fourth, though Pro/Leu type was a significant exacerbation factor of QVP, it had no association with other neurological functions.

Our first finding is that there is a significantly higher frequency of the Pro/Leu type in diabetic patients with DSPN compared with those without DSPN. The genotype frequency (%) of the Pro/Leu type in the Japanese population is quite similar to the present study (15.1 vs 13.9)[19]. So, Pro198Leu polymorphism of the GPx-1 gene seems to be relevant to the development of diabetic complications, but not to the onset of diabetes itself.

Our second finding indicates the possible relationship between the GPx-1 genotype and several manifestations of diabetic neuropathy by univariation analysis. The diabetic patients with Pro/Leu type were susceptible to impaired QVP in the toe, DSPN and painful leg cramps.

Our third and fourth findings confirmed this by multivariate analysis. The statistically significant associations between the GPx-1 genotype and DSPN, painful leg cramp, history of MVD and QVP impairment did not disappear, even if it was adjusted for microangiopathies (proteinuria and retinopathy), which are closely related to diabetic neuropathy[20]. The associations between the GPx-1 genotype and DSPN, painful leg cramp and QVP impairment also kept statistical significance, even after the adjustment for the history of MVD.

On the other hand, we could not observe any significant relationship between the GPx-1 genotype and DAN, autonomic and nerve conduction functions. In general, DSPN and DAN are considered to reflect mainly the large and small diameter nerve fiber dysfunctions, respectively[21]. Thus, the lack of association of the GPx-1 genotype with DAN and autonomic functions might show that the etiological factors of DSPN differ from those of DAN.

In contrast, impairment of nerve conduction is thought to be a reliable marker of DSPN. The amplitude and conduction abnormalities are most prominent in the distal segments of nerves in the legs; the potential for sensory nerve action in the sural nerve is especially sensitive and useful in identifying early abnormalities[22]. As we did not carry out nerve conduction studies in the lower limbs, accurate nerve conduction functions seemed not to be evaluated sufficiently in our study. Associations between the GPx-1 genotype and nerve conduction data in the lower limbs might provide different results. A more plausible explanation of this issue is that the GPx-1 genotype is mainly associated with QVT impairment, which is a part of the manifestation of DSPN. Actually, our data showed a strong association between the GPx-1 genotype and QVP, whereas a significant association with the GPx-1 genotype, diminished ATR and sensory symptoms was not proven. QVP reflects the functions of the peripheral and central nervous system, and it can be impaired by causes other than neuropathy, such as peripheral arterial disease. However, we suppose that the GPx-1 genotype affects the peripheral nerve function of vibratory sensation to some degree, because the patients with clinical peripheral arterial disease were excluded from the present study and the association of the GPx-1 genotype with QVP was independent from the history of MVD. Furthermore, a recent study using DCCT/EDIC participants proved that QVP is a sensitive measure of peripheral neuropathy[23]. Considering all of the aforementioned findings, we might be able to conclude that Pro198Leu polymorphism of the GPx-1 gene might be a candidate for the common genetic predisposition to MVD and DSPN, especially with an impairment of vibratory perception.

Two possible pathophysiological mechanisms of the association between the GPx-1 genotype and QVP impairment can be considered. One possible mechanism is impaired microcirculation in the peripheral nerve caused by vascular endothelial dysfunction elicited through accelerated oxidative stress in patients with a Pro/Leu genotype. We have previously reported that anti-oxidative activity of GPx-1 decreased in the Pro/Leu genotype[9]. Significant relationships between the GPx-1 genotype and painful leg cramps might support this possibility, because painful leg cramps are considered to reflect a circulatory disturbance in the leg and are frequently experienced in cold ischemic conditions. Another possible mechanism is direct nerve damage as a result of elevated oxidative stress. Neurotoxicity of excessive oxidative stress is widely recognized in experimental diabetic neuropathy. At present, the precise mechanism of the harmful effects of Pro/Leu genotype of the GPx-1 gene on QVT impairment is uncertain.

We have also shown a significant relationship between the GPx-1 genotype and the prevalence of painful leg cramps. Because painful leg cramps can be associated with various disorders, such as neurological, muscular, metabolic, endocrine and vascular diseases, a significant association between the GPx-1 genotype and painful leg cramps might not reflect DSPN. Furthermore, the prevalence of painful leg cramps occurring in self-administered questionnaires in 1524 diabetic patients under a primary care physician (25.5%) was not different from that in 501 non-diabetic subjects (29.4%) who underwent a corporate health screening examination (Nakatani M, Sasaki H, Kurisu S, Yamaoka H, Matsuno S, Ogawa K, Yamasaki H, Wakasaki H, Furuta H, Nishi M, Akamizu T, Nanjo K, 2011, unpublished

Table 4 | Relationships between glutathione peroxidase 1 gene polymorphism and subtype of diabetic neuropathy, painful leg cramp and quantitative neurological functions evaluated by multiple logistic regression and multiple regression analysis

Independent variables	Model 3 dependent variables			Model 3 dependent variables						
	Subtypes of diabetic neuropathy		Painful leg cramp	Vibration	Autonomic functions		Nerve conduction parameters			
	DSPN	DAN		QVP	CVR–R	ΔBP	MCV	CMAP	SCV	SNAP
R^2 (*P*-value)	R^2 = 0.210 (*P* < 0.0001)	R^2 = 0.530 (*P* < 0.0001)	R^2 = 0.103 (*P* = 0.0289)	R^2 = 0.320 (*P* < 0.0001)	R^2 = 0.303 (*P* < 0.0001)	R^2 = 0.266 (*P* < 0.0001)	R^2 = 0.254 (*P* < 0.0001)	R^2 = 0.093 (*P* = 0.1411)	R^2 = 0.189 (*P* = 0.0006)	R^2 = 0.419 (*P* < 0.0001)
	Adjusted OR (95% CI) *P*-value	Adjusted OR (95% CI) *P*-value	Adjusted OR (95% CI) *P*-value	β (*P*-value)	β (*P*-value)	β (*P*-value)	β (*P*-value)	β (*P*-value)	β (*P*-value)	β (*P*-value)
Age (years)	1.010 (0.974–1.048) 0.5938	1.002 (0.933–1.076) 0.9619	0.995 (0.959–1.032) 0.7785	**0.292 (0.0001)**	**−0.278 (0.0003)**	0.091 (0.2337)	−0.135 (0.0833)	–	−0.096 (0.2446)	**−0.342 (<0.0001)**
Sex (female: 0, male: 1)	1.095 (0.514–2.335) 0.8136	1.904 (0.455–7.969) 0.3781	0.468 (0.217–1.010) 0.0529	0.076 (0.2664)	0.090 (0.2177)	0.051 (0.4859)	−0.095 (0.2033)	–	−0.039 (0.6258)	**−0.168 (0.0122)**
Duration (years) (≥5: 0. 6–15: 1, 16≤: 2)	1.343 (0.794–2.269) 0.2713	0.976 (0.408–2.336) 0.9567	1.139 (0.652–1.989) 0.6473	−0.062 (0.4217)	−0.118 (0.1386)	0.014 (0.8577)	0.100 (0.2203)	–	0.083 (0.3434)	−0.089 (0.2237)
Hypertension (no: 0, yes: 1)	2.115 (0.956–4.677) 0.0644	2.598 (0.708–9.531) 0.1499	0.742 (0.333–1.654) 0.4650	0.058 (0.4217)	0.108 (0.1472)	0.123 (0.1046)	0.044 (0.5625)	–	−0.105 (0.1978)	0.003 (0.9595)
Dyslipidemia (no: 0, yes: 1)	**0.466 (0.218–0.996) 0.0486**	0.833 (0.206–3.370) 0.7979	0.516 (0.241–1.102) 0.0875	−0.017 (0.8013)	−0.003 (0.9711)	−0.091 (0.2092)	0.001 (0.9866)	–	−0.007 (0.9303)	0.068 (0.2982)
Glycemic control (~fair: 0, poor: 1)	1.731 (0.821–3.650) 0.1494	**9.470 (1.968–45.577) 0.0050**	1.412 (0.672–2.985) 0.3600	0.025 (0.7139)	−0.125 (0.0725)	0.122 (0.0823)	**−0.195 (0.0066)**	–	**−0.239 (0.0019)**	−0.100 (0.1148)
BMI (kg/m^2) (>22: 0. 22–25: 1, 25<: 2)	0.862 (0.545–1.364) 0.5263	**0.159 (0.055–0.457) 0.0007**	0.978 (0.621–1.540) 0.9235	−0.061 (0.3870)	0.072 (0.3212)	**−0.196 (0.0082)**	**0.162 (0.0308)**	–	0.135 (0.0894)	−0.093 (0.1569)
GPx-1 genotype (Pro/Pro: 0, Pro/Leu: 1)	**3.286 (1.156–9.346) 0.0257**	0.352 (0.042–2.965) 0.3366	**4.469 (1.725–11.578) 0.0021**	**0.157 (0.0194)**	0.064 (0.3454)	−0.126 (0.0696)	0.058 (0.4070)	–	−0.025 (0.7420)	−0.023 (0.7082)
Proteinuria (no: 0, intermittent: 1, persistent: 2)	0.692 (0.379–1.207) 0.1948	0.670 (0.299–1.503) 0.3314	1.529 (0.869–2.691) 0.1408	−0.090 (0.2624)	−0.030 (0.7158)	0.030 (0.7192)	**−0.182 (0.0322)**	–	−0.081 (0.3672)	−0.003 (0.9682)
Retinopathy (no: 0, simple: 1, PPDR~: 2)	**2.921 (1.764–4.835) <0.0001**	**38.537 (5.553–267.423) 0.0002**	0.655 (0.384–1.115) 0.1187	**0.455 (<0.0001)**	**−0.307 (0.0006)**	**0.380 (<0.0001)**	**−0.320 (0.0004)**	–	**−0.293 (0.0020)**	**−0.409 (<0.0001)**
History of MVD (no: 0, yes: 1)	1.042 (0.279–3.653) 0.9491	0.890 (0.82–9.632) 0.9235	1.438 (0.421–4.909) 0.5621	−0.018 (0.7950)	−0.128 (0.0736)	−0.018 (0.3834)	0.012 (0.8722)	–	0.086 (0.2765)	0.0181 (0.7868)

The significant regression formula on compound muscle action potential (CMAP) were not obtained in model 3. β, Standard regression coefficient; BMI, body mass index; ΔBP, orthostasis-induced decreases in systolic blood pressure at standing; CI, confidence interval; CVR-R, coefficient of variation of RR intervals on electrocardiogram after 15 min resting; DAN, diabetic autonomic neuropathy; DSPN, distal symmetric polyneuropathy; MCV, motor nerve conduction velocity; MVD, macrovascular disease, OR, odds ratio; PPDR, preproliferative diabetic retinopathy; QVP, quantitative vibratory perception thresholds; R^2, decision coefficient; SCV, sensory nerve conduction velocity; SNAP, sensory nerve action potential. Statistically significant *P*-value was shown by boldfaced type.

data). Therefore, the observed relationship between painful leg cramps and the Pro/Leu genotype might not be exclusively confined to a diabetic population.

As for common risk factors of MVD and diabetic neuropathy, several investigators reported common risk factors, such as obesity, dyslipidemia and hypertension. Most of the reports were epidemiological studies, and diabetic neuropathy was correspondent to the DSPN of the present study, though autonomic or nerve conduction functions were not carefully evaluated[3–5]. It might be speculated that the GPx-1 gene polymorphism could affect the increasing prevalence of DSPN though the deteriorating effect of vibratory perception.

Further studies, such as a prospective observational study, are necessary to confirm the association of the Pro198Leu polymorphism of the GPx-1 gene with diabetic neuropathy.

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