frequency of the E66Q allele as a functional variant in Korean newborns. *J Hum Genet* 2010; **55:** 512–517.
31. Togawa T, Tsukimura T, Kodama T, *et al.* Fabry disease: biochemical, pathological and structural studies of the alpha-galactosidase A with E66Q amino acid substitution. *Mol Genet Metab* 2012; **105:** 615–620.
32. Yasuda M, Shabbeer J, Benson SD, Maire I, Burnett RM, Desnick RJ. Fabry disease: characterization of alpha-galactosidase A double mutations and the D313Y plasma enzyme pseudodeficiency allele. *Hum Mutat* 2003; **22:** 486–492.
33. Crutchfield KE, Patronas NJ, Dambrosia JM, *et al.* Quantitative analysis of cerebral vasculopathy in patients with Fabry disease. *Neurology* 1998; **50:** 1746–1749.
34. Moore DF, Kaneski CR, Askari H, Schiffmann R. The cerebral vasculopathy of Fabry disease. *J Neurol Sci* 2007; **257:** 258–263.

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ORIGINAL ARTICLE

Newborn screening for Fabry disease in Japan: prevalence and genotypes of Fabry disease in a pilot study

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Fabry disease (FD) is an X-linked lysosomal storage disorder caused by a deficiency of α-galactosidase A (GLA) activity. Enzyme replacement therapy (ERT) for FD is available, and newborn mass screening for FD is being implemented. Here, we undertook a pilot study of newborn mass screening for FD in Japan. GLA activity in dried blood spots was measured using a fluorescence assay and confirmed by measurement of GLA activity in white blood cells (WBCs) in infants with abnormally low GLA activity. This was followed up by genetic testing. A total of 21 170 neonates were enrolled in the study. Of these, seven (five boys, two girls) had low GLA activities, which were verified by the WBC GLA activity assay. Thus, the initial fluorescence assay was suitable for newborn mass screening for FD. Pathogenic mutations of the *GLA* gene, that is, V199M and IVS4+919G>A, were found in two boys and one boy, respectively. Functional mutations, E66Q and c. −10C>T: g.1170C>T, were found in two boys and one girl, respectively. The prevalence of test-positive newborns was 1/3024, while that of those with a pathogenic mutation was 1/7057. The numbers are higher than those previously anticipated. Standardized management for FD found during newborn mass screening, including an ERT regimen, remains to be established.
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Keywords: enzyme replacement therapy; Fabry disease; genetic counseling; mass screening; metabolic disorder; newborn screening; X-linked recessive lysosomal storage disorder

INTRODUCTION

Fabry disease (FD: MIM 301500) is an X-linked inherited lysosomal storage disorder caused by a deficiency in α-galactosidase A (GLA) activity.[1] This deficiency leads to the accumulation of globotriaosylceramide in different tissues and can cause progressive malfunctions in systemic organs, such as the skin, eyes, kidneys, ears, lungs, heart and brain.[1–3]

Male patients with classic early-onset FD usually have very low GLA activity and are generally asymptomatic in early childhood (onset symptoms are reported at a mean age of 9 years).[4,5] The major clinical symptoms of classical early-onset FD include pain in the peripheral extremities, angiokeratoma, hypohidrosis, corneal opacity, and renal, cardiac and cerebrovascular diseases.[1] In contrast, patients with late-onset FD exhibit residual GLA activity and milder clinical manifestations than those with classical early-onset FD.[1] Heterozygous females with FD have wide clinical manifestation spectra, ranging from asymptomatic to severely affected.[6]

In 2001, in the USA and EU, enzyme replacement therapy (ERT) was approved for the treatment of FD; Japan began using ERT in 2004. In all three of these regions, ERT has been shown to be effective in alleviating many of the signs and symptoms of the disease and in slowing or even reversing disease progression.[7–9] Several studies have demonstrated that ERT must be administered before the occurrence of renal or cardiac failure in order to achieve optimal results.[10–12] As the importance of early treatment is now generally recognized, newborn mass screening for FD is being implemented in several countries. Such newborn mass screening, however, had not yet been undertaken in Japan; hence, the prevalence and genotypes of FD in association with GLA activity have not been studied in the Japanese population.

In this work, we present the results of a pilot study for newborn mass screening for FD in Fukuoka City and its vicinity in Japan.

SUBJECTS AND METHODS

Subjects

This study was conducted from April 2007 to April 2010 in Fukuoka City and its vicinity in Japan. Among newborns who took the conventional newborn mass screening during the study period, only newborns whose parent gave their written consent to participate were enrolled in the study. Conventional newborn mass screening has taken place as a local administrative service nationwide in Japan to screen six disorders: cretinism, congenital adrenal hypertrophy, galactosemia, phenylketonuria, homocystinemia and maple syrup

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urine disease. Virtually all newborns born in Japan take this conventional newborn screening. The dried blood spots that remained after completion of the conventional newborn mass screening were used for this study. The sex of newborns enrolled in the study was judged according to information written on the filter papers; this information was not available for some of the enrolled newborns because of incomplete descriptions.

Methods

Venous blood was collected from neonates on days 4–6 after birth, transferred to filter paper, and dried at room temperature for the conventional newborn screening and this study. A small circle of 3 mm in diameter was punched out of the dried blood spot and used for mass screening for FD.[3,13,14]

In the mass-screening study, GLA activity was determined using a fluorescent substrate, as described previously.[3,13,14] In brief, 40 µl McIlvan buffer (0.1 M citrate: 0.2 M NaH_2PO_4, 36.8:63.2 v/v, pH 6.0) was added to each well of 96-microwell plates. Punched dried blood spots were added to the buffer and processed for extraction at room temperature for 2 h. Aliquots of 30 µl of blood extract were transferred to new 96-microwell plates. An aliquot of 100 µl of the reaction mixture (3.5 mM 4-methylumbelliferone (4-MU) galactosylpyranoside, 100 mM citrate, 200 mM phosphate and 100 mM *N*-acetylgalactosamine, pH 4.4) was added to each well of the 96-microwell plates and incubated at 37 °C for 24 h. The reaction was terminated with 150 µl termination solution (300 mM glycine, NaOH, pH 10.6) immediately after the reaction. The fluorescence intensity from 4-MUs in the wells was measured with a fluorescence plate reader (Bio-Tek, Winooski, VT, USA) at 450 nm. One unit (AgalU) of enzymatic activity was equal to 0.34 pmol of 4-methylumbelliferyl-D-galactopyranoside cleaved per hour per disc. When GLA activity was identified as being abnormally low (i.e., less than the cutoff value of 20 AgalU), a second measurement was taken 2 or 3 weeks later to verify the initial measurement.[14] Newborns whose GLA activity had been verified as <20 AgalU were brought to the Department of Pediatrics of Fukuoka University or Kyushu University.

Cases in which GLA activity was found to be <20 AgalU in the mass screening, white blood cell (WBC) GLA activity was measured by a fluorometric enzyme assay. Briefly, whole blood was collected from the neonates and immediately treated with EDTA-2Na. The assay mix included 50 µl leukocyte lysate (WBC pellet prepared from 5 ml blood treated with EDTA-2Na was lysed by sonication in 1.0 ml water) and 50 µl of the reaction mixture (8.0 mM 4-methylumbelliferyl-α-D-galactopyranoside, 100 mM citrate phosphate buffer and 200 mM *N*-acetylgalactosamine, pH 4.5). This was incubated for 1 h at 37 °C. The reaction was terminated with 1.5 ml of 200 mM glycine buffer (pH 10.7) immediately after the reaction was completed. The fluorescence intensity from 4-MUs was measured with a fluorescent plate reader (Jasco Co, Tokyo, Japan) at 365 and 450 nm.

For genetic analysis, total genomic DNA was extracted from leukocytes of patients. All seven exons of the *GLA* gene were amplified by PCR, and the amplification products were analyzed by direct sequencing.[14] Detailed information on the PCR protocol is available upon request.

Before testing, counseling was provided as to the nature of the disease, its future medical management and risk of recurrence. Informed consent from each child's parent(s) for newborn mass screening for FD was at birth and that for WBC GLA activity assay and genetic analysis were obtained at their first visit to our hospitals.

All studies were approved by the Ethical Committees of Kumamoto University and Fukuoka University.

RESULTS

During the 37-month-period from April 2007 to April 2010, a total of 39 224 newborns took the conventional newborn mass screening. Among these, 21 170 (54.0%) newborns were enrolled in this pilot study after written informed consent was provided by the parent(s). The enrollees included 10 827 boys, 10 343 girls. Among the 21 170 newborns, seven (five boys and two girls) showed GLA activity <20 AgalU in the mass-screening test. Six newborns (four boys and two girls) were referred to Fukuoka University Hospital, while one boy (Case 5) was referred to Kyushu University for further examinations. The parents of one girl (Case 7) refused additional medical examinations; therefore, this child was not included in further analyses (Figure 1). All newborns had been delivered without incident at term. Cases 1 and 2 were brothers.

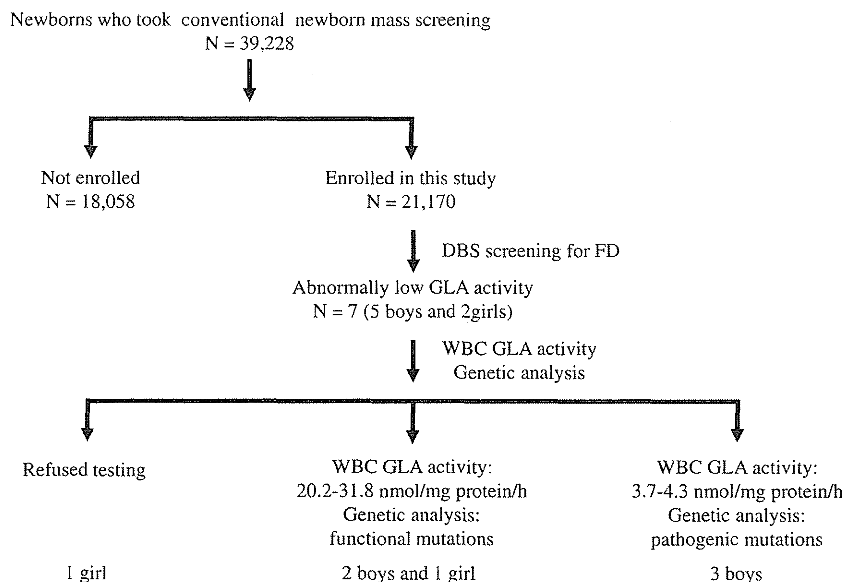

Figure 1 Flow chart of newborn screening for Fabry disease. During a 37-month period from April 2007 to April 2010, 39 224 newborns took the conventional newborn mass screening test in Fukuoka City and its vicinity; this test is given to all newborns in Japan. Among these, 21 170 (54.0%) newborns were enrolled in this pilot study with written informed consent. Seven newborns (five boys and two girls) showed an α-galactosidase A activity <20 AgalU (cutoff value) in the mass screening. The parents of one girl (Case 7) refused additional medical examinations. Four newborns had their white blood cell GLA activities measured, while all six underwent genetic testing. Pathogenic mutations, that is, V199M and IVS4+919G>A, were found in two boys and one boy, respectively. Functional mutations or polymorphisms (see text), that is, E66Q and heterozygous c.−10C>T: g.1170C>T, were found in two boys and one girl, respectively.

One boy (Case 5) presented with neonatal hyperbilirubinemia, high-pitched crying, a dysmorphic face (low-set ears and small mouth), brachydactyly (left shorter fifth finger) and hypotonia. Karyotyping revealed that this boy had a chromosomal abnormality, 46,XY,der(3)t(3;4)(p26;p14). However, we believe that this condition was unrelated to the presence of FD because the *GLA* gene locus was not involved in his chromosomal abnormality (Table 1).

The mother of one boy (Case 3) had a history of familial renal disease, but the other six subjects had no histories that would suggest a risk of FD. Additional details of the characteristics of familial renal disease in the mother of Case 3 were not available. Except for the dysmorphic features observed in Case 5, none of other six children presented with abnormalities at 1 month of age. The newborns' parents and other family members with GLA deficiency were counseled and offered medical evaluations and medical follow-up.

WBC GLA activity was very low (3.7 and 4.3 nmol mg^{-1} protein per hour) in two boys (Cases 1 and 3). In contrast, in one boy and one girl (Cases 4 and 6, respectively), WBC GLA activity was markedly higher, at 31.8 and 20.2 nmol mg^{-1} protein per hour, respectively, but still well below the normal range of 49.8–116.4 nmol mg^{-1} protein per hour as determined from 48 healthy volunteers. WBC GLA activity was not measured for two boys (Cases 2 and 5) (Table 1).

Genetic analyses found V199M and IVS4 + 919G > A mutations in two brothers (Cases 1 and 2) and one boy (Case 3), respectively. V199M is considered pathogenic and is thought to cause the classical phenotype of FD,[15] while IVS4 + 919G > A is also considered pathogenic, but is thought to cause the late-onset phenotype.[16,17] E66Q and heterozygous c. −10C > T: g.1170C > T mutations were found in two boys (Cases 4 and 5) and one girl (Case 6), respectively. These are both functional mutations and are not thought to cause FD, that is, they are considered polymorphisms, but were subjected to further investigations[18–22] (Table 1).

Cases 1–4 were last followed up when the patients were 2–3 years old, by then none of them showed any symptoms of FD.

DISCUSSION

This pilot study of newborn mass screening for FD comprised a large cohort of 21 170 newborns, most of them were of Japanese ethnicity. The screening used a fluorescent GLA activity measurement from a dried blood spot. A total of seven newborns tested positive with respect to low GLA activity (cutoff value: < 20 AgalU) and four of these newborns were verified to have low GLA activity by a WBC GLA activity measurement. Thus, the prevalence of positive testing in this screening was 1/3024. Subsequent genetic analysis revealed that six newborns harbored different mutations in the *GLA* gene. Given that only V199M and IVS4 + 919G > A are thought to be pathogenic, the prevalence of individuals with pathogenic mutations in our cohort was 1/7057, as three newborns had such mutations. These findings support the contention that the mass-screening system used in this study was legitimate and hence useful for the early diagnosis of FD. At the same time, however, these findings raised concerns about the timing of ERT.

The prevalence of FD in this study (1/7057) suggests that the disease may be far more prevalent than previously thought and much more common than the 1/40 000 males estimated by Desnick *et al.*[1] Nevertheless, this number is lower than those reported in newborn screenings in other countries: 1/1100 boys in Italy (with a 11:1 ratio of late-onset to classic phenotype);[23] 1/3859 boys and girls in Austria (no instances of classic phenotype, but one case in which the phenotype could not be determined)[24] and 1/1250 boys in Taiwan (86% with the late-onset mutation IVS4 + 919G > A).[17] These differences may have to do with the genetic backgrounds of the newborns examined in each study.

GLA activities measured by the fluorescence assay were in accordance with GLA activities measured from WBC samples, an accepted method for GLA measurement in the diagnosis of FD. This again supports the notion that our screening system, which utilizes a fluorescence assay to measure GLA activity, is reliable. WBC GLA activities were very low, that is, 3.7 and 4.3 nmol mg^{-1} protein per hour, in Cases 1 and 3, respectively. The mutations identified in these newborns were V199M and IVS4 + 919G > A in the *GLA* gene, which are considered pathogenic mutations and are thought to cause classical and late-onset phenotypes of FD, respectively. In contrast, WBC GLA activity was markedly higher, at 31.8 and 20.2 nmol mg^{-1} protein per hour, in Cases 4 and 6, respectively, but still well below the normal range of 49.8–116.4 nmol mg^{-1} protein per hour. The mutations identified in these two cases are functional mutations and are considered polymorphisms that lead to low GLA activity but may not evolve into FD; however, whether they are totally benign

Table 1 Case summary

		GLA activity			*GLA mutation*		
Case	*Sex*	*First measurement (AgalU)*	*Second measurement (AgalU)*	*WBC measure GLA activity*[a] *(nmol mg^{-1} protein per hour)*	*Location*	*Mutation*	*Deduced phenotype*
1	M	4.4	5.2	3.7	Exon4	V199M	Classic
2	M	5.0	ND	ND	Exon4	V199M	Classic
3	M	8.2	9.2	4.3	Intron4	IVS4 + 919 G > A	Late-onset
4	M	13.6	16.3	31.8	Exon2	E66Q	Normal
5[b]	M	12.0	14.8[c]	ND	Exon2	E66Q	Normal
6	F	13.1	16.4	20.2	5′UTR	g.1170C > T (c. −10C > T)	Normal
7	F	15.1	17.9	ND	ND		

Abbreviations: GLA, α-galactosidase A; ND, not determined; UTR, untranslated region; WBC, white blood cell.
Seven newborns tested positive in a pilot newborn mass screening for FD. Of these, The parents of one girl (Case 7) refused additional medical examinations; four underwent WBC GLA activity measurements (Cases 1, 3, 4 and 6); and all six underwent genetic testing for mutations in the *GLA* gene. Cases 1 and 2 were brothers. Case 5 had a chromosomal abnormality and underwent GLA measurements four times.
[a]Normal range of GLA activity in WBCs was 49.8–116.4 nmol mg^{-1} protein per hour ($n = 48$ healthy volunteers).
[b]46,XY,der(3)t(3;4)(p26;p14) chromosomal abnormality was identified, but was considered unrelated to FD because the *GLA* gene locus was not affected by the chromosomal abnormality.
[c]Third measurement: 22.9, fourth measurement (1 year later): 10.7.

polymorphisms is still controversial.[18–22] Overall, the GLA activity measurements in this study seemed to reflect the nature of the identified *GLA* gene mutations.

Given that newborn mass screening for FD is conducted to allow for early diagnosis and, in turn, early treatment with ERT, this early diagnosis raises a number of concerns. First, the timing of ERT is still controversial, and there is uncertainty as to when ERT should be initiated in neonates (many of whom will be asymptomatic). Several studies have demonstrated that ERT must be administered before instances of renal or cardiac failure in order to achieve optimal results.[10–12] However, Ross[25] insists that 'premature treatment may cause more harm than good' as a result of 'side-effects' and 'medicalizing of a normal childhood.' Others, instead, indicate that there is no evidence that early ERT is ineffective or that it harms the patient.[26–30] Indeed, the findings that are currently available on the efficacy and side effects of ERT are based on a small sampling and are the result of very few ERT follow-up reports for young children. Therefore, the implementation of mass screening for FD, which has only recently been initiated in very limited regions of the world, should provide significant insights into these controversies. In addition, it will be necessary to accumulate enough long-term follow-up data on the efficacy and safety of ERT to conclusively determine the effectiveness of ERT in newborns diagnosed with FD during newborn mass screening; as the prevalence of FD is very low, this process may take many years.

Second, in relation to the first concern raised above, neither GLA activity nor genotypes necessarily correlate with phenotypes in terms of severity and onset of the disease. This is particularly the case in heterozygous females who may have significantly reduced enzyme activity but no symptoms. Of course, positive results in newborn screening followed by further examinations, including WBC GLA measurement and genetic analyses, enable ERT to be initiated at first onset of the condition and alert the child's female relatives to the necessity of future medical examinations. Serum or urinary globotriaosylceramide or serum globotriaosyl spingosine levels may give us better clues for anticipating the severity and onset of the disease identified in newborn mass screening, although they were not investigated in the present study.

Third, there are also ethical issues associated with early screening for FD. Newborns who tested positive in newborn mass screening may be given a presymptomatic diagnosis by genetic testing. According to the Japanese Association of Medical Science, presymptomatic diagnosis by genetic testing should only be performed after the examinee has sufficiently understood the available preventive measures and therapeutic strategies.[31] Obviously, imparting such information to a neonate is impossible, but providing a parent with sufficient counseling to making an informed decision is essential. Thus, careful genetic counseling is required, preferentially from a knowledgeable geneticist.

Likewise, adequate education on the symptoms and disease progression for FD should also be provided to parents and their relatives. On their first visit to our hospitals, parents were unfamiliar with FD, and, to ease their concerns, it was necessary to provide them detailed information about its nature, future medical management and risk of recurrence. Furthermore, as FD is an X-linked inherited disease, inadequate counseling may create a burden to mothers and even affect family relationships, which may lead to serious consequences, such as divorce. From our experience with the seven cases reported in this study, some parents, for whatever reason, were surprised that FD might be serious, while others were overly alarmed by the disease.

To overcome all concerns raised, certain guidelines for management of FD, including when ERT is appropriate, should be established based on evidence collected as a result of accumulating experiences with FD identified in newborn mass screening. Although FD is quite rare, it is feasible to establish such guidelines, as guidelines are available for similar rare diseases, that is, Gaucher disease[32] and Pompe disease.[33] These worldwide guidelines have proven to be extremely useful.[32,33] In addition, these guidelines could aid in the collection of global data that would assist in determining not only the appropriate age for ERT, but also the nature and duration of follow-up care and genetic counseling, providing, in short, a means of coordinating both research and treatment.

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1 Desnick, R. J., Ioannou, Y. A. & Eng, C. M. *Alpha-galactosidase A Deficiency: Fabry Disease, in The Metabolic and Molecular Bases of Inherited Disease.* 8th edn (eds Scriver, C. R., Beaudet, A. L., Sly, W. S. & Valle, D.) 3733–3774 (McGraw-Hill, New York, NY, 2001).
2 Mehta, A., Beck, M., Eyskens, F., Feliciani, C., Kantola, I., Ramaswami, U. *et al.* Fabry disease: a review of current management strategies. *QJM.* **103**, 641–659 (2010).
3 Nakamura, K., Hattori, K. & Endo, F. Newborn screening for lysosomal storage disorders. *Am. J. Med. Genet. C. Semin. Med. Genet.* **157**, 63–71 (2011).
4 Ramaswami, U., Whybra, C., Parini, R., Pintos-Morell, G., Mehta, A., Sunder-Plassmann, G. *et al.* Clinical manifestations of Fabry disease in children: data from the Fabry Outcome Survey. *Acta. Paediatr.* **95**, 86–92 (2006).
5 Eng, C. M., Fletcher, J., Wilcox, W. R., Waldek, S., Scott, C. R., Sillence, D. O. *et al.* Fabry disease: baseline medical characteristics of a cohort of 1765 males and females in the Fabry Registry. *J. Inherit. Metab. Dis.* **30**, 184–192 (2007).
6 Matsuzawa, F., Aikawa, S., Doi, H., Okumiya, T. & Sakuraba, H. Fabry disease: correlation between structural changes in alpha-galactosidase, and clinical and biochemical phenotypes. *Hum. Genet.* **117**, 317–328 (2005).
7 Eng, C. M., Guffon, N., Wilcox, W. R., Germain, D. P., Lee, P., Waldek, S. *et al.* Safety and efficacy of recombinant human alpha-galactosidase A—replacement therapy in Fabry's disease. *N. Engl. J. Med.* **345**, 9–16 (2001).
8 Schiffmann, R., Kopp, J. B., Austin, H. A. 3rd, Sabnis, S., Moore, D. F., Weibel, T. *et al.* Enzyme replacement therapy in Fabry disease: a randomized controlled trial. *JAMA* **285**, 2743–2749 (2001).
9 Beck, M., Ricci, R., Widmer, U., Dehout, F., de Lorenzo, A. G., Kampmann, C. *et al.* Fabry disease: overall effects of agalsidase alfa treatment. *Eur. J. Clin. Invest.* **34**, 838–844 (2004).
10 Wilcox, W. R., Banikazemi, M., Guffon, N., Waldek, S., Lee, P., Linthorst, G. E. *et al.* Long-term safety and efficacy of enzyme replacement therapy for Fabry disease. *Am. J. Hum. Genet.* **75**, 65–74 (2004).
11 Banikazemi, M., Bultas, J., Waldek, S., Wilcox, W. R., Whitley, C. B., McDonald, M. *et al.* Agalsidase-beta therapy for advanced Fabry disease: a randomized trial. *Ann. Intern. Med.* **146**, 77–86 (2007).
12 Weidemann, F., Breunig, F., Beer, M., Sandstede, J., Turschner, O., Voelker, W. *et al.* Improvement of cardiac function during enzyme replacement therapy in patients with Fabry disease: a prospective strain rate imaging study. *Circulation* **108**, 1299–1301 (2003).

13 Chamoles, N. A., Blanco, M. & Gaggioli, D. Fabry disease: enzymatic diagnosis in dried blood spots on filter paper. *Clin. Chim. Acta.* **308,** 195–196 (2001).
14 Nakamura, K., Sekijima, Y., Nakamura, K., Hattori, K., Nagamatsu, K., Shimizu, Y. *et al.* Cerebral hemorrhage in Fabry's disease. *J. Hum. Genet.* **55,** 259–261 (2010).
15 Shabbeer, J., Yasuda, M., Luca, E. & Desnick, R. J. Fabry disease: 45 novel mutations in the alpha-galactosidase A gene causing the classical phenotype. *Mol. Genet. Metab.* **76,** 23–30 (2002).
16 Ishii, S., Nakao, S., Minamikawa-Tachino, R., Desnick, R. J. & Fan, J. Q. Alternative splicing in the alpha-galactosidase A gene: increased exon inclusion results in the Fabry cardiac phenotype. *Am. J. Hum. Genet.* **70,** 994–1002 (2002).
17 Hwu, W. L., Chien, Y. H., Lee, N. C., Chiang, S. C., Dobrovolny, R., Huang, A. C. *et al.* Newborn screening for Fabry disease in Taiwan reveals a high incidence of the lateronset *GLA* mutation c.936+919G4A (IVS4+919G4A). *Hum. Mutat.* **30,** 1397–1405 (2009).
18 Lee, B. H., Heo, S. H., Kim, G.-W., Park, J.-Y., Kim, W.-S., Kang, D. H. *et al.* Mutations of the *GLA* gene in Korean patients with Fabry disease and frequency of the E66Q allele as a functional variant in Korean newborns. *J. Hum. Genet.* **55,** 512–517 (2010).
19 Togawa, T., Tsukimura, T., Kodama, T., Tanaka, T., Kawashima, I., Saito, S. *et al.* Fabry disease: Biochemical, pathological and structural studies of the α-galactosidase A with E66Q amino acid substitution. *Mol. Genet. Metab.* **105,** 615–620 (2012).
20 Davies, J. P., Winchester, B. G. & Malcolm, S. Sequence variations in the first exon of alpha-galactosidase A. *J. Med. Genet.* **30,** 658–663 (1993).
21 Oliveira, J. P., Ferreira, S., Reguenga, C., Carvalho, F. & Månsson, J. E. The g.1170C>T polymorphism of the 5′ untranslated region of the human alpha-galactosidase gene is associated with decreased enzyme expression—evidence from a family study. *J. Inherit. Metab. Dis.* **31,** S405–S413 (2008).
22 Oliveira, J. P., Ferreira, S., Barceló, J., Gaspar, P., Carvalho, F., Sá Miranda, M. C. *et al.* Effect of single-nucleotide polymorphisms of the 5′ untranslated region of the human α-galactosidase gene on enzyme activity, and their frequencies in Portuguese caucasians. *J. Inherit. Metab. Dis.* **31,** S247–S253 (2008).
23 Spada, M., Pagliardini, S., Yasuda, M., Tukel, T., Thiagarajan, G., Sakuraba, H. *et al.* High incidence of later-onset Fabry disease revealed by newborn screening. *Am. J. Hum. Genet.* **79,** 31–40 (2006).
24 Mechtler, T. P., Stary, S., Metz, T. F., De Jesús, V. R., Greber-Platzer, S., Pollak, A. *et al.* Neonatal screening for lysosomal storage disorders: feasibility and incidence from a nationwide study in Austria. *Lancet* **379,** 335–341 (2012).
25 Ross, L. F. Newborn screening for lysosomal storage diseases: an ethical and policy analysis. *J. Inherit. Metab. Dis.* **35,** 627–634 (2012).
26 Ries, M., Clarke, J. T., Whybra, C., Timmons, M., Robinson, C., Schlaggar, B. L. *et al.* Enzyme-replacement therapy with agalsidase alfa in children with Fabry disease. *Pediatrics* **118,** 924–932 (2006).
27 Ries, M., Clarke, J. T., Whybra, C., Mehta, A., Loveday, K. S., Brady, R. O. *et al.* Enzyme replacement in Fabry disease: pharmacokinetics and pharmacodynamics of agalsidase alpha in children and adolescents. *J. Clin. Pharmacol.* **47,** 1222–1230 (2007).
28 Schiffmann, R., Martin, R. A., Reimschisel, T., Johnson, K., Castaneda, V., Lien, Y. H. *et al.* Four-year prospective clinical trial of agalsidase alfa in children with Fabry disease. *J. Pediatr.* **156,** 832–837 (2010).
29 Ramaswami, U., Wendt, S., Pintos-Morell, G., Parini, R., Whybra, C., Leon Leal, J. A. *et al.* Enzyme replacement therapy with agalsidase alfa in children with Fabry disease. *Acta. Paediatr.* **96,** 122–127 (2007).
30 Ramaswami, U. Update on role of agalsidase alfa in management of Fabry disease. *Drug Des. Devel. Ther.* **5,** 155–173 (2011).
31 The Japanese Association of Medical Sciences "Guidelines for Genetic Tests and Diagnoses in Medical Practice" http://jams.med.or.jp/guideline/genetics-diagnosis_e.pdf.
32 Martins, A. M., Valadares, E. R., Porta, G., Coelho, J., Semionato Filho, J., Pianovski, M. A. *et al.* Recommendations on diagnosis, treatment, and monitoring for Gaucher disease. *J. Pediatr.* **155,** S10–S18 (2009).
33 Cupler, E. J., Berger, K. I., Leshner, R. T., Wolfe, G. I., Han, J. J., Barohn, R. J. *et al.* Consensus treatment recommendations for late-onset Pompe disease. *Muscle Nerve* **45,** 319–333 (2012).

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CLINICAL STUDY

Identification of a Novel Mutation and Prevalence Study for Fabry Disease in Japanese Dialysis Patients

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Abstract

Fabry disease—a genetic disorder characterized by the accumulation of globotriaosylceramide in cell lysosomes resulting from an X-linked deficiency of α-galactosidase A activity—presents with multiorgan manifestations, including progressive renal disease. Recently, its prevalence has been reported to be higher in hemodialysis (HD) patients than in the general population. We, therefore, examined patients on maintenance dialysis living in the Nagasaki Prefecture, Japan, to clarify the prevalence of Fabry disease. We screened 933 patients on maintenance dialysis, who were residents of Nagasaki Prefecture in Japan, for α-galactosidase A activity using a dried blood spot on filter paper. Patients with low α-galactosidase A activity were clinically assessed; subsequently, genetic analysis of the *α-Galactosidase A* gene (MIM:30064) was performed in these patients. Of the 933 patients, 55 had low α-galactosidase A activity; of these, one male and two females had *α-Galactosidase A* mutations. The prevalence of Fabry disease was thus 0.32%, which was similar to that reported previously. However, one mutation was newly identified, while the E66Q mutation observed in two patients was as previously identified. These two patients with the E66Q mutation were excluded because of the possibility of polymorphism; the prevalence of Fabry disease in the HD population was finally calculated to be 0.11%. The prevalence of Fabry disease in patients on maintenance dialysis living in Nagasaki Prefecture was 0.32%. Dried blood spot screening was considered as a simple and effective method for screening patients on maintenance dialysis for Fabry disease.

Keywords: α-Galactosidase A, dialysis patients, dried blood spot screening, Fabry disease, mutation

INTRODUCTION

Fabry disease is an X-linked recessive lysosomal storage disorder caused by mutations in *α-Galactosidase A* (*α-Gal A*) gene (MIM:30064) that lead to deficient activity of this enzyme.[1] Absence or reduced activity of α-galactosidase A (α-Gal A) leads to the intracellular accumulation of glycosphingolipids, mainly globotriaosylceramide, in various tissues.[2] Distinctive cytoplasmic inclusions can be observed in renal epithelial cells, endothelial cells, pericytes, vascular smooth muscle cells, cardiomyocytes, and neurons of the autonomic nervous system.[3] The clinical manifestations of Fabry disease differ between the classic and variant forms in hemizygotes. Male patients with the classic form present with acroparesthesia, hypohidrosis, corneal opacities, stroke, cardiac abnormalities, and renal disorders. The mortality rate in patients with the classic form is extremely high. The variant form of Fabry disease presents with manifestations limited to the heart[4–6] or kidneys[7–9] with few or none of the clinical symptoms observed in the classic form. These patients have relatively higher plasma α-Gal A activity and a milder phenotype than those with the classic form. However, most of such patients are at risk for end-stage renal disease (ESRD), eventually requiring renal replacement therapy. It has been reported that the prevalence of Fabry disease is 1 in 40,000–117,000 males.[3,10] Because not all patients undergo renal biopsy before renal replacement therapy, the above-mentioned prevalence may be lower than the

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actual prevalence. To date, several reports have indicated that in dialysis patients, the prevalence of Fabry disease may be as high as 0.5–1.2%.[11–17] Furthermore, it appears that the prevalence of Fabry disease in females is also much higher among hemodialysis (HD) patients. Because specific enzyme replacement therapy (ERT) is now available for patients with Fabry disease,[18] the identification of such patients is critical.

This study aimed to define the prevalence of Fabry disease in patients from the Nagasaki Prefecture, Japan, undergoing chronic HD or continuous ambulatory peritoneal dialysis (CAPD) by a screening test using a dried blood spot on filter paper. In addition, we also examined the clinical characteristics of these patients and investigated the mutations in the *α-Gal A* gene in patients with confirmed low enzyme activity.

MATERIALS AND METHODS

Study Population and Protocol

Our selection criterion was patients on maintenance dialysis (HD and CAPD) at Nagasaki University Hospital and its affiliated hospitals in Nagasaki Prefecture, Japan. After obtaining informed consent, we screened 933 patients (557 men and 376 women) on maintenance dialysis from December 2006 to March 2009. The patients we screened in this study account for approximately a quarter of all maintenance dialysis patients in Nagasaki. This study was designed with the following objectives: (1) to screen patients on maintenance dialysis for Fabry disease by an enzyme activity assay using a dried blood spot, (2) to confirm positive blood spot test results by repeated assays, and (3) to confirm the diagnosis of Fabry disease by molecular analysis of the *α-Gal A* gene. The study protocol was approved by the Human Ethics Review Committee of Nagasaki University School of Medicine, and written informed consent was obtained from each subject. The results of the enzyme activity assay were directly communicated to the patient by the doctor. We provided genetic information regarding Fabry disease to the patients in whom the deficiency of α-Gal A activity was confirmed; genotyping was performed for those patients who provided written informed consent. Once diagnosed with Fabry disease, the patient was referred to the genetic counselor of the Nagasaki University Hospital for genetic counseling if the patient desired the same.

Measurement of α-Galactosidase A Activity

Venous blood was collected from patients before the initiation of renal replacement therapy. Four drops of blood were spotted on filter paper, allowed to dry at room temperature, and stored at 2–4°C until they were sent to Kumamoto University (Kumamoto, Japan) for analysis. Blood spot α-Gal A activity was determined using a fluorescent substrate, as previously described by Chamoles et al.[19] Briefly, 40 μL of McIlvaine buffer (0.1 M citrate:0.2 M NaH_2PO_4, 36.8:63.2; pH 6.0) was added to 96-microwell plates. Then, 3 mm punched dried blood spots were added to the buffer and processed for extraction at room temperature for 2 h. Subsequently, 30 μL of this blood extract was transferred into another 96-microwell plate, and 100 μL of the reaction mixture (3.5 mM 4-MU galactosylpyranoside, 100 mM citrate, 200 mM phosphate, 100 mM *N*-acetylgalactosamine) was added to each well and incubated at 37°C for 24 h. The reaction was terminated with 150 μL of termination solution (300 mM glycine, NaOH, pH 10.6) immediately after the reaction. The fluorescence intensity from the 4-methylumbelliferones in the wells was measured at 450 nm using a fluorescent plate reader (BIO-TEK, Winooski, VT, USA). One unit (Agal U) of enzymatic activity was equal to 0.34 pmol of 4-methylumbelliferyl-D-galactopyranoside cleaved per hour per disc. Samples with activities <20 Agal U were retested by the same method. Samples with activities less than the same cut-off were considered as "screening positive." Patients showing low α-Gal A activity in both assays were clinically assessed, and a genetic study of the *α-Gal A* gene was performed, provided that the patient provided consent.

Genetic Study of *α-Galactosidase A* Gene

Genomic DNA was extracted from peripheral blood leucocytes for *α-Gal A* mutation analyses.[20] DNA regions of the *α-Gal A* gene were analyzed by polymerase chain reaction after amplifying each of the even *α-Gal A* exons and sequencing the opposite strand.

Statistical Analysis

Values are presented as the mean ± SD.

RESULTS

α-Galactosidase A Activity and Genetic Analysis

The average α-Gal A activity of the patients was 23.4 ± 15.4 Agal U. The first assay revealed 178 of the 933 dialysis patients to have low plasma α-Gal A activity (14.6 ± 3.3 Agal U), while the mean enzyme activity of 755 patients was within the normal range at 26.0 ± 16.3 Agal U. Among the 178 patients with low activity, 55 (24 men and 31 women) were screened for low enzyme activity by a repeated assay using the dried blood spot (14.0 ± 3.0 Agal U). We performed genetic analysis in 36 patients who consented to the same. The results revealed missense mutations in one male and two females (Figures 1 and 2). The average α-Gal A activity of these three patients was 11.9 ± 3.3 Agal U, while that of the remaining 33 patients was 14.9 ± 2.6 Agal U. Patients 2 and 3 had the same mutation (c.196G>C, p.E66Q) that has been described previously (11, 21). Patient 1 had a missense mutation (c.218C>A, p.A73E) that, to the best of our knowledge, has not been previously reported.

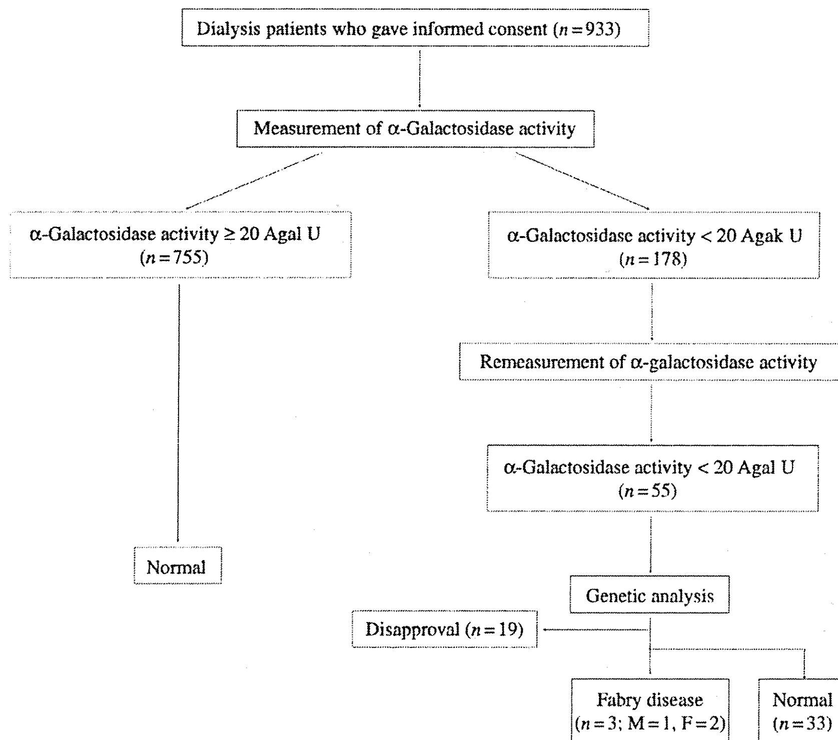

Figure 1. Flow chart for screening dialysis patients for Fabry disease.

Clinical Evaluation of the Patients

Table 1 shows the clinical characteristics of the three patients newly diagnosed with Fabry disease. Patient 1 was diagnosed with ESRD in 2006 and with initiation of CAPD and diagnosis of left ventricular hypertrophy (LVH) in 2007. The patient's mother had a history of renal disease and the elder brother suffered from hearing impairment. In 2008, the patient suffered a stroke, and the dialysis modality was changed from CAPD to HD. Unfortunately, the patient refused to undergo ERT; the consent for having her family screened for Fabry disease was also refused.

Patient 2 was diagnosed with focal segmental glomerulosclerosis by renal biopsy in 1976; CAPD was started in 1995. At 2 months after CAPD initiation, the dialysis modality was changed to HD because of fungal peritonitis. Sequence analysis of the *α-Gal A* gene in this patient revealed that his two daughters also had the same missense mutation (E66Q). The patient accepted ERT and has suffered no side effects thus far.

Patient 3 had a history of acute myocardial infarction in 1998 with renal insufficiency diagnosed. She was started on maintenance HD in 2001. Subsequent to genetic analysis, the patient is undergoing ERT with no side effects reported thus far. Family screening and genetic analysis also showed the same heterozygous mutation in her two daughters.

DISCUSSION

Fabry disease is considered as a rare disorder, with the estimated prevalence of classic hemizygous disease being 1 in 40,000–117,000 males (0.0025–0.00085%).[3,10] However, Fabry disease is reported as the cause of ESRD in 0.0167% and 0.0188% of dialysis patients in the United States.[16] To date, a few variant types of Fabry disease have been identified; their manifestations are primarily limited to the heart, kidneys, or brain.[8,20–24] It is, however, very difficult to identify Fabry disease in dialysis patients because patients with variants of Fabry disease often lack symptoms that are observed in the classic form. Recently, several studies have investigated the prevalence of Fabry disease in dialysis patients. Kotanko et al.[14] found Fabry disease in 4 out of 2480 (0.16%) Austrian patients on maintenance dialysis. More recently, Merta et al.[15] found Fabry disease in 5 out of 3370 (0.15%) HD patients in the Czech Republic. Based on the findings of these studies, the prevalence of Fabry disease in dialysis patients appears to be 10–50 times higher than that in the general population. Further, the prevalence of Fabry disease among Japanese patients on maintenance dialysis has been reported to be 0.16–1.2%.[8,11,12,17]

In our study, we examined the prevalence of Fabry disease in patients from the Nagasaki Prefecture

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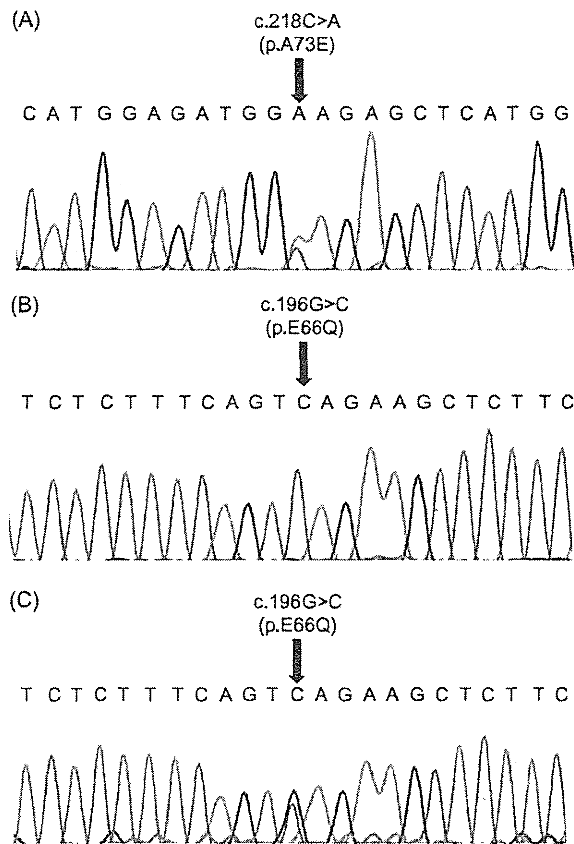

Figure 2. Direct nucleotide sequencing of PCR-amplified DNA from the *α-Gal A* gene. (A) Sense strand of the DNA from patient 1. Arrow indicates nucleotide 218, where a C>A transversion resulted in the amino acid substitution p.Ala73Glu. (B, C) Sense strands of the DNA from patient 2 (B) and patient 3 (C). Arrow indicates nucleotide 196, where a G>C transversion resulted in the amino acid substitution p.Glu66Gln.

Table 1.

Characteristics	Patient 1	Patient 2	Patient 3
Age (years)	58	53	57
Gender	Female	Male	Female
α-Galactosidase A activity	7.9	12.6	14.9
Dialysis duration (years)	1	12	7
Arterial blood pressure (mmHg)	163/78	160/90	150/85
Cerebrovascular damage	+	−	−
Acroparesthesia	−	−	−
Hypohidrosis	−	−	−
Angiokeratoma	−	−	−
Corneal opacities	−	−	−
α-Galactosidase A mutation	−	−	−
Nucleotide	c.218C>A	c.196G>C	c.196G>C
Amino acid	p.A73E	p.E66Q	p.E66Q

undergoing HD or CAPD, assessing a dried blood spot on filter paper in the screening test. Among 933 patients, 3 patients were diagnosed with Fabry disease (0.32%); this prevalence was similar to that reported previously for dialysis patients.[8,11,12,17] We believe that there are no regional idiosyncrasies of Fabry disease in terms of prevalence rate or inherited mutations, which can be explained by the fact that Fabry disease is panethnic. In our study, one patient had the novel A73E mutation, while two patients had the previously reported E66Q mutation. All three patients had low α-Gal A activity, and Fabry disease had not been previously diagnosed in any of them. In addition, the E66Q patients had no symptoms of Fabry disease apart from renal failure and LVH. The family members (children) of these two patients, who were asymptomatic, were also identified as heterozygotes for Fabry disease. Previous reports included patients with E66Q mutations, indicating the substitution of glutamine for glutamic acid at residue 66;[11,17,21] this mutation is currently under debate as to whether it is a disease-caused mutation or not. Lee et al.[25] reported that the allele frequency of E66Q was approximately 1% among 833 newborns in Korea, suggesting that E66Q is a polymorphism because no globotriaosylceramide is found in patients with E66Q. The current definition of Fabry disease is a measured decrease or deficit of α-Gal A enzyme activity or the detection of any mutation of the *α-Gal A* gene. In view of this definition, patients with E66Q should be included among those with Fabry disease. Thus, the frequency of Fabry disease in this study would be 0.32% if all the three patients were included. However, if E66Q were indeed a polymorphism, the previously reported prevalence of Fabry disease would need to be reviewed. After excluding the E66Q mutation, the prevalence of Fabry disease in our HD patients would be 0.11% (1/933 patients).

α-Gal A can be measured in a variety of materials such as dried whole blood spots, blood leukocytes, serum, or plasma. Since approximately 300,000 patients are under maintenance dialysis in Japan, large-scale screening of all of these patients would be difficult. However, diagnosis of this condition is important because an efficacious treatment—ERT—is now available. Therefore, it is important to have an easy, safe, economical, and simple method for the screening of Fabry disease. Most of the previous screening studies used a plasma α-Gal A assay, and others measured α-Gal A activity in leukocytes. The dried blood spot test, which we used in the present study, is easy to carry out because it can be performed directly using whole blood, which only needs to be dried, and it is inexpensive, whereas serum or plasma must be centrifuged and frozen to be sent for testing, the resulting cost of which would be higher than that of the dried blood spot test. Therefore, we believe that the dried blood spot test is a useful and simple screening tool for the detection of Fabry disease; hence, we used dried blood spots for the α-Gal A test in our study. Recently, however, it has been reported that the average α-Gal A activity in dried blood spot samples prepared using EDTA tubes was higher when compared with those spotted directly irrespective of disease status.[26] Therefore, further studies would be

necessary for validating this assay to determine the best method for screening Fabry disease.

ERT is now available for patients with Fabry disease in many countries. ERT can delay disease progression in the heart and kidneys; however, early diagnosis prior to the onset of irreversible pathologic changes is essential for successful treatment. In our study, two out of the three patients diagnosed with Fabry disease were females. Fabry disease management guidelines in 2006 recommended that female patients should be offered ERT if they manifest significant symptoms or show evidence of progressive organ involvement.[27] Even for patients on maintenance dialysis, it is crucial to anticipate and treat the manifestations of cardiac and cerebrovascular involvements.

In conclusion, we consider the dried blood spot test to be a useful and simple screening tool for the detection of Fabry disease. It is important to identify patients with Fabry disease and the complications of the disease as early as possible for an early intervention, such as ERT, which may delay the disease progression.

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

[1] Brady RO, Gal AE, Bradley RM, Martensson E, Warshaw AL, Laster L. Enzymatic defect in Fabry's disease. Ceramide trihexosidase deficiency. *N Engl J Med.* 1967;276:1163–1167.

[2] Sweeley CC, Klionsky B. Fabry's disease: Classification as a sphingolipidosis and partial characterization of a novel glycolipid. *J Biol Chem.* 1963;238:3148–3150.

[3] Desnick RJ, Ioannou YA, Eng CM. α-Galactosidase A deficiency: Fabry disease. In: Scriber CR, Beauder AL, Sly WS, et al., eds. *The Metabolic Bases of Inherited Disease.* 8th ed. New York: Mc Graw-Hill; 2001:3733–3774.

[4] Nakao S, Takenaka T, Maeda M, et al. An atypical variant of Fabry's disease in men with left ventricular hypertrophy. *N Engl J Med.* 1995;333:288–293.

[5] Sakuraba H, Oshima A, Fukuhara Y, et al. Identification of point mutations in the alpha-galactosidase A gene in classical and atypical hemizygotes with Fabry disease. *Am J Hum Genet.* 1990;47:784–789.

[6] von Scheidt W, Eng CM, Fitzmaurice TF, et al. An atypical variant of Fabry's disease with manifestations confined to the myocardium. *N Engl J Med.* 1991;324:395–399.

[7] Meroni M, Spisni C, Tazzari S, et al. Isolated glomerular proteinuria as the only clinical manifestation of Fabry's disease in an adult male. *Nephrol Dial Transplant.* 1997;12:221–223.

[8] Nakao S, Kodama C, Takenaka T, et al. Fabry disease: Detection of undiagnosed hemodialysis patients and identification of a "renal variant" phenotype. *Kidney Int.* 2003; 64:801–807.

[9] Sawada K, Mizoguchi K, Hishida A, et al. Point mutation in the alpha-galactosidase A gene of atypical Fabry disease with only nephropathy. *Clin Nephrol.* 1996;45:289–294.

[10] Meikle PJ, Hopwood JJ, Clague AE, Carey WF. Prevalence of lysosomal storage disorders. *JAMA.* 1999;281:249–254.

[11] Tanaka M, Ohashi T, Kobayashi M, et al. Identification of Fabry's disease by the screening of alpha-galactosidase A activity in male and female hemodialysis patients. *Clin Nephrol.* 2005;64:281–287.

[12] Ichinose M, Nakayama M, Ohashi T, Utsunomiya Y, Kobayashi M, Eto Y. Significance of screening for Fabry disease among male dialysis patients. *Clin Exp Nephrol.* 2005;9:228–232.

[13] Linthorst GE, Hollak CE, Korevaar JC, Van Manen JG, Aerts JM, Boeschoten EW. Alpha-galactosidase A deficiency in Dutch patients on dialysis: A critical appraisal of screening for Fabry disease. *Nephrol Dial Transplant.* 2003; 18:1581–1584.

[14] Kotanko P, Kramar R, Devrnja D, et al. Results of a nationwide screening for Anderson-Fabry disease among dialysis patients. *J Am Soc Nephrol.* 2004;15:1323–1329.

[15] Merta M, Reiterova J, Ledvinova J, et al. A nationwide blood spot screening study for Fabry disease in the Czech Republic hemodialysis patient population. *Nephrol Dial Transplant.* 2007;22:179–186.

[16] Thadhani R, Wolf M, West ML, et al. Patients with Fabry disease on dialysis in the United States. *Kidney Int.* 2002;61:249–255.

[17] Fujii H, Kono K, Goto S, et al. Prevalence and cardiovascular features of Japanese hemodialysis patients with Fabry disease. *Am J Nephrol.* 2009;30:527–535.

[18] Eng CM, Guffon N, Wilcox WR, et al. Safety and efficacy of recombinant human alpha-galactosidase A—replacement therapy in Fabry's disease. *N Engl J Med.* 2001;345:9–16.

[19] Chamoles NA, Blanco M, Gaggioli D. Fabry disease: Enzymatic diagnosis in dried blood spots on filter paper. *Clin Chim Acta.* 2001;308:195–196.

[20] Nakamura K, Robertson M, Liu G, et al. Complete heart block and sudden death in mice overexpressing calreticulin. *J Clin Invest.* 2001;107:1245–1253.

[21] Shimotori M, Maruyama H, Nakamura G, et al. Novel mutations of the GLA gene in Japanese patients with Fabry disease and their functional characterization by active site specific chaperone. *Hum Mutat.* 2008;29:331.

[22] Rolfs A, Böttcher T, Zschiesche M, et al. Prevalence of Fabry disease in patients with cryptogenic stroke: A prospective study. *Lancet.* 2005;366:1794–1796.

[23] Chimenti C, Pieroni M, Morgante E, et al. Prevalence of Fabry disease in female patients with late-onset hypertrophic cardiomyopathy. *Circulation.* 2004;110:1047–1053.

[24] Monserrat L, Gimeno-Blanes JR, Marín F, et al. Prevalence of Fabry disease in a cohort of 508 unrelated patients with hypertrophic cardiomyopathy. *J Am Coll Cardiol.* 2007; 50:2399–2403.

[25] Lee BH, Heo SH, Kim GH, et al. Mutations of the GLA gene in Korean patients with Fabry disease and frequency of the E66Q allele as a functional variant in Korean newborns. *J Hum Genet.* 2010;55:512–517.

[26] Olivova P, van der Veen K, Cullen E, et al. Effect of sample collection on α-galactosidase A enzyme activity measurements in dried blood spots on filter paper. *Clin Chim Acta.* 2009; 403:159–162.

[27] Eng CM, Germain DP, Banikazemi M, et al. Fabry disease: Guidelines for the evaluation and management of multi-organ system involvement. *Genet Med.* 2006;8:539–548.

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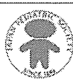
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Original Article

Effect of body mass index-z score on adverse levels of cardiovascular disease risk factors

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Abstract ***Background***: Cardiovascular disease (CVD) risk factors are associated with body mass index z-score (BMISD) and/or insulin resistance (IR). However, the correlation between adverse levels of these risk factors and BMISD, and the effect of IR on these associations are not fully understood in children. The aim of this study was to evaluate the association between adverse levels of CVD risk factors and BMISD, and the effect of IR on these associations in schoolchildren.
Methods: Conventional CVD risk factors, C-reactive protein (CRP), uric acid (UA) and adiponectin were determined in 757 boys and 494 girls aged between 7 and 12 years. IR was assessed by the homeostasis model approximation index.
Results: BMISD were linearly associated with relative risks having adverse levels of all factors, except for glucose and low-density lipoprotein cholesterol (LDL-C) in boys, and except for glucose, LDL-C and adiponectin in girls ($P <$ 0.01–0.001). These associations were weakened after adjustment for IR, but still significant in cases of UA and CRP in boys and UA, high-density lipoprotein cholesterol and CRP in girls ($P <$ 0.01–0.001).
Conclusion: The relative risk of having adverse levels of most CVD risk factors in school children increased across the entire range of BMISD. IR contributed to most of these relative risks, but BMISD itself also contributed to these relative risks. To prevent future development of CVD, it might be important for schoolchildren to maintain BMISD within normal range. However, in cases of hyper LDL-cholesterolemia, we should consider causes other than BMISD.

Key words adipocytokine, cardiovascular disease risk factors, hypercholesterolemia, insulin resistance, obesity.

Many epidemiological studies have shown that overweight and obesity are increasing globally in both children and adults.[1] In Japan, the prevalence of obesity in schoolchildren has steadily increased in recent decades, possibly due to changes in dietary patterns and lifestyles among these children.[2] Must *et al.* reported that the risk of morbidity from coronary heart disease and atherosclerosis was increased among men and women who had been overweight as adolescents of 13–18 years old.[3] Given this finding, it seems rational to consider that the incidence of atherosclerotic cardiovascular disease (CVD) in Japan could increase dramatically in the near future. In contrast, a recent study reported that the overweight and obese show no increased risk for total mortality and cardiovascular mortality compared with those with a normal body mass index (BMI):[4] severely obese patients did not have increased total mortality, but they had the highest risk for cardiovascular mortality. These results suggest that the metabolic aberrations that coexisted with overweight and obesity may be more important than overweight and obesity themselves. In this regard, Barter *et al.* reported that overweight people with normal plasma lipids might be at relatively low risk for developing diabetes and cardiovascular disease.[5]

In our previous studies, we showed that abnormal CVD risk factors, such as small dense low-density lipoproteins, dyslipidemia, hyperinsulinemia, high levels of inflammatory markers and low levels of adiponectin, were found in schoolchildren.[6–9] In addition, low-density lipoprotein particle size and serum concentrations of these CVD risk factors were closely associated with BMI.[6–9] However, these abnormal CVD risk factors may occur regardless of BMI.[7] As reported previously, genetic predispositions appear to contribute more to dyslipidemia in children than they do in adults.[7,10] Thus, it is important to clarify whether abnormal CVD risk factors are merely complications of overweight or obesity. It is generally accepted that many comorbidities with obesity, such as diabetes, dyslipidemia and hypertension, are attributed to insulin resistance.[11] Thus, in the present study, we investigated the correlations between adverse levels of CVD risk factors and BMI z-score (BMISD), and the effect of insulin resistance on these associations in Japanese schoolchildren.

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Methods

Subjects

We studied 1251 Japanese children (757 boys and 494 girls) aged 7–12 years, who underwent screening and were enrolled in a care

program for lifestyle-related diseases in Okinawa and Kumamoto, Japan. Sex-maturity stages in the children studied were equal or less than Tanner stage 3 (Tanner stage was evaluated by inspection of mammary development in girls, and by asking condition of pubic hair in boys and this evaluation was performed by a pediatrician). BMI was calculated as weight [kg]/height^2 [m^2]. BMISD adjusted for age and sex were obtained based on data on Japanese schoolchildren provided in 2000 by the Ministry of Education, Culture, Sports, Science and Technology (unpublished data). We employed BMISD to continuously evaluate BMI in the studied schoolchildren. None of the children was receiving therapy for weight reduction, or drugs that might affect lipid metabolism, and none had a smoking habit. Venous blood was drawn after an overnight fast. Informed consent was obtained from the parents of all of the children. This study was approved by the ethics committee of the Ryukyu University.

Laboratory measurements

The serum concentration of C-reactive protein (hCRP) was measured by a highly sensitive immunoturbidimetric assay using reagents and calibrators from Dade Behring Marbura GmbH (Marburg, Germany). The lower limit of detection for serum CRP concentration was 0.05 mg/L. Adiponectin was measured by enzyme-linked immunosorbent assays (R&D Systems, Minneapolis, MN, USA). Serum insulin was measured by two-step sandwich enzyme-linked immunosorbent assay (ELISA) (SRL Inc., Hachioji, Japan). Routine chemical methods were used to determine the serum concentrations of total cholesterol (TC), high-density-lipoprotein cholesterol (HDL-C), triglycerides (TG), uric acid and glucose. Low-density-lipoprotein cholesterol (LDL-C) was calculated as [TC – HDL-C – TG/5]. Apolipoprotein B (apoB) was measured by the turbidity immunoassay method.[12] Insulin resistance was calculated using the homeostasis model approximation index (HOMA-IR).[13]

Statistical evaluation

The significance of differences between boys and girls was determined by the Mann–Whitney *U*-test. Serum concentrations of insulin, TG and hCRP were markedly skewed. Thus, these parameters were normalized by log-transformation. Pearson and partial correlation coefficients were then computed to assess the associations between BMISD and various parameters. The logistic model was used to evaluate linear associations between adverse levels of variables and BMISD (continuous). The relative risks to have adverse variables (odds ratio) were adjusted for age by a multiple logistic regression analysis.

Results

Several indexes of overweight and/or abdominal obesity have been proposed for children, as in the case for adults. Among these, waist–height ratio is more strongly associated with CVD risk factors than is BMI;[14] however, a recent report found that BMISD and waist–height ratio did not differ in their ability to identify adverse risk factors.[15] Because waist circumference was not measured in our schoolchildren, we employed BMISD to evaluate the correlation between adverse levels of CVD risk factors and BMI.

As shown in Table 1, significant sex differences were found for several parameters; thus, we separated the data for boys and girls in the following analysis. Because age was significantly correlated with BMISD (boys: r = 0138, $P < 0.001$; and girls: r = 0.139, $P < 0.01$), age was adjusted by partial correlation. Table 2 shows age-adjusted correlations between BMISD and 10 parameters. All parameters except glucose were significantly associated with BMISD in both boys and girls ($P < 0.01$-0.001). BMISD showed a positive correlation with LDL-C and a negative correlation with HDL-C; therefore, we did not examine its correlation with TC. HOMA-IR and serum concentrations of insulin showed stronger correlations with BMISD than those of other factors in both boys and girls. HOMA-IR has recently been validated as a surrogate maker of insulin resistance, even in children.[16,17]

We then evaluated the correlation between adverse levels of CVD risk factors (except for glucose) and BMISD with a multiple logistic regression analysis. To date, there are no criteria to define adverse levels of these CVD risk factors in Japanese

Table 1 Clinical and chemical data

	Boys (*n* = 757)	*P*-value	Girls (*n* = 494)
Age (years)	10.0 ± 1.1	(NS)	10.0 ± 1.1
BMI SD	1.64 ± 1.12	($P < 0.01$)	1.46 ± 1.12
TC (mg/dL)‡	182 ± 29	($P < 0.01$)	176 ± 28
TG (mg/dL)§	79 ± 59	(NS)	80 ± 46
LDL-C (mg/dL)‡	107 ± 25	(NS)	104 ± 25
HDL-C (mg/dL)‡	59 ± 12	($P < 0.01$)	56 ± 11
ApoB (mg/dL)	79 ± 18	(NS)	77 ± 18
Glucose (mg/dL)†	90 ± 6	($P < 0.01$)	89 ± 7
Insulin (μU/mL)	12.21 ± 8.96	($P < 0.01$)	14.10 ± 9.79
HOMA-IR	2.75 ± 2.24	($P < 0.01$)	3.12 ± 2.36
Uric acid (mg/dL)	4.9 ± 1.0	(NS)	4.8 ± 1.0
Adiponectin (μg/mL)	8.7 ± 3.6	(NS)	8.6 ± 3.7
hCRP (mg/L)	1.65 ± 4.56	(NS)	1.24 ± 3.12

Values are expressed as mean ± standard deviation. †To convert to mmol/L, divided by 18. ‡To convert to mmol/L, multiply by 0.0259. §To convert to mmol/L, multiply by 0.0113. ApoB, apolipoprotein B; BMI, body mass index; hCRP, serum concentration of C-reactive protein; HDL-C, high-density-lipoprotein cholesterol; HOMA-IR, homeostasis model approximation index; LDL-C, low-density-lipoprotein cholesterol; NS, not significant; TG, triglycerides; TC, total cholesterol.

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Table 2 Age-adjusted correlations between body mass index z-score and cardiovascular disease risk factors

	Boys		Girls	
	r	*P*	r	*P*
Log TG	**0.177**	**<0.001**	**0.218**	**<0.001**
LDL-C	**0.107**	**<0.01**	**0.150**	**<0.01**
HDL-C	**−0.277**	**<0.001**	**−0.399**	**<0.001**
ApoB	**0.178**	**<0.001**	**0.239**	**<0.001**
Glucose	0.045	0.213	0.068	0.135
Log insulin	**0.568**	**<0.001**	**0.647**	**<0.001**
Log HOMA-IR	**0.561**	**<0.001**	**0.634**	**<0.001**
Uric acid	**0.370**	**<0.001**	**0.437**	**<0.001**
Adiponectin	**−0.264**	**<0.001**	**−0.303**	**<0.001**
Log hCRP	**0.464**	**<0.001**	**0.333**	**<0.001**

Bold indicates significant associations ($P < 0.05$). ApoB, apolipoprotein B; hCRP, serum concentration of C-reactive protein; HDL-C, high-density-lipoprotein cholesterol; HOMA-IR, homeostasis model approximation index; LDL-C, low-density-lipoprotein cholesterol; TG, triglycerides.

school children. Thus, when levels of CVD risk factors were greater than those of the 90th percentiles of our subjects, we tentatively considered the children to have adverse levels, except for HDL-C and adiponectin (boys: insulin > 20.8 μU/mL, HOMA-IR > 4.39, TG > 145 mg/dL, LDL-C > 138 mg/dL, apoB > 101 mg/dL, uric acid > 6.3 mg/dL and hCRP > 3.41 mg/L; girls: insulin > 26.64 μU/mL, HOMA-IR > 5.62, TG > 148 mg/dL, LDL-C 133 mg/dL, apoB > 98 mg/dL, uric acid > 5.9 mg/dL and hCRP > 2.39 mg/L). HDL-C and adiponectin were considered to be adverse levels when their levels were less than those of the 10th percentiles (boys: HDL < 44 mg/dL and adiponectin < 4.2 μg/mL; girls: HDL-C < 43 mg/dL and adiponectin < 4.1 μg/mL). As shown in Table 3, we observed no linear association of BMISD with adverse levels of LDL-C in boys. The relative risk of having adverse levels of other CVD risk factors increased with increasing BMISD. Table 4 shows the case of girls. In contrast to the case of boys, BMISD did not show a linear correlation with adverse levels of adiponectin. As shown in Table 2, HOMA-IR showed stronger correlations with BMISD than those of other CVD risk factors in both boys and girls. Thus, to examine whether the correlations of adverse levels of CVD risk factors with BMISD were independent of insulin resistance, the findings were adjusted for HOMA-IR, in addition to age. After adjustment for HOMA-IR and age (Table 5), the relative risk of having adverse levels of uric acid and hCRP increased with increasing BMISD in boys. Significant associations of adverse levels of HDL-C, TG, apoB and adiponectin with BMISD were eliminated in boys after adjustment. In girls, the relative risk of having adverse levels of uric acid, HDL-C and

Table 3 Age-adjusted associations between body mass index z-score and adverse levels (above the 90th percentile or below the 10th percentile; HDL-C and adiponectin) in boys as assessed by a multiple logistic regression analysis

Dependent variable	β	Wald χ^2	*P*-value	Exp (β)	95%CI
LDL-C	0.133	1.64	0.201	1.14	0.93–1.40
HDL-C	**0.351**	**11.56**	**<0.001**	**1.42**	**1.16–1.74**
TG	**0.230**	**4.87**	**0.027**	**1.26**	**1.03–1.54**
ApoB	**0.228**	**4.85**	**0.028**	**1.26**	**1.03–1.54**
Insulin	**1.009**	**68.55**	**<0.001**	**2.74**	**2.16–3.48**
HOMA-IR	**0.894**	**33.84**	**<0.001**	**2.44**	**1.95–3.07**
Uric acid	**0.680**	**40.99**	**<0.001**	**1.97**	**1.60–2.43**
Adiponectin	**0.387**	**14.49**	**<0.001**	**1.47**	**1.21–1.80**
hCRP	**0.745**	**45.05**	**<0.001**	**2.11**	**1.69–2.62**

Bold type indicates a significant correlation ($P < 0.05$). ApoB, apolipoprotein B; CI, confidence interval; hCRP, serum concentration of C-reactive protein; HDL-C, high-density-lipoprotein cholesterol; HOMA-IR, homeostasis model approximation index; LDL-C, low-density-lipoprotein cholesterol; TG, triglycerides.

Table 4 Age-adjusted associations between body mass index z-score and adverse levels (above the 90th percentile or below the 10th percentile; HDL-C and adiponectin) in girls as assessed by a multiple logistic regression analysis

Dependent variable	β	Wald χ^2	*P*-value	Exp (β)	95%CI
LDL-C	0.216	2.65	0.103	1.24	0.96–1.61
HDL-C	**0.831**	**32.98**	**<0.001**	**2.30**	**1.73–3.05**
TG	**0.451**	**11.21**	**<0.001**	**1.57**	**1.21–2.04**
ApoB	**0.392**	**8.62**	**<0.01**	**1.48**	**1.14–1.92**
Insulin	**0.846**	**33.28**	**<0.001**	**2.33**	**1.75–3.11**
HOMA-IR	**0.947**	**39.92**	**<0.001**	**2.58**	**1.92–3.46**
Uric acid	**0.931**	**42.75**	**<0.001**	**2.54**	**1.92–3.36**
Adiponectin	0.203	2.53	0.112	1.23	0.95–1.57
hCRP	**0.643**	**15.73**	**<0.001**	**1.90**	**1.39–2.62**

Bold type indicates a significant correlation ($P < 0.05$). ApoB, apolipoprotein B; CI, confidence interval; hCRP, serum concentration of C-reactive protein; HDL-C, high-density-lipoprotein cholesterol; HOMA-IR, homeostasis model approximation index; LDL-C, low-density-lipoprotein cholesterol; TG, triglycerides.

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Table 5 Age- and homeostasis model approximation index-adjusted significant associations between body mass index z-score and adverse levels (above the 90th percentile or below the 10th percentile; HDL-C and adiponectin) in schoolchildren as assessed by a multiple logistic regression analysis

Dependent variable	β	Wald χ^2	*P*-value	Exp (β)	95%CI
Boys					
hCRP	0.666	27.46	<0.001	1.95	1.52–2.50
Uric Acid	0.559	20.85	<0.001	1.75	1.38–2.22
Girls					
Uric acid	0.827	25.28	<0.001	2.29	1.66–3.16
HDL-C	0.591	12.49	<0.001	1.81	1.30–2.51
hCRP	0.530	9.48	<0.01	1.70	1.21–2.38

CI, confidence interval; hCRP, serum concentration of C-reactive protein; HDL-C, high-density-lipoprotein cholesterol.

hCRP showed an increase with increasing BMISD. Significant associations of adverse levels of TG and apoB with BMISD were eliminated in girls after adjustment.

Discussion

Based on the findings of the present study, adverse levels of CVD risk factors can be divided into three groups (Table 6): (i) independent of BMISD (boys: glucose and LDL-C; girls: glucose, LDL-C and adiponectin); (ii) dependent on BMISD and independent of insulin resistance (boys: uric acid and hCRP; girls: uric acid, HDL-C and hCRP); and (iii) dependent on BMISD and insulin resistance (boys: insulin, HOMA-IR, HDL-C, TG, apoB and adiponectin; girls: insulin, HOMA-IR, TG and apoB).

It is generally accepted that many comorbidities with obesity, such as diabetes, dyslipidemia and hypertension, are attributed to insulin resistance.[11] In the present study, BMISD was strongly correlated with insulin resistance. The relative risk of having an adverse level of insulin resistance was linearly increased across the normal range. The risk of an adverse level of insulin resistance was significantly higher for children at BMISD 1.0 compared with those at BMISD 0.0, with an odds ratio of adverse level of insulin resistance ranging from 2.44 to 2.58 (Tables 3 and 4). The present findings suggest that in schoolchildren, a slight shift of BMISD from normal ranges affects insulin resistance.

Correlations of BMISD and adverse levels of CVD risk factors are generally reported only in studies regarding hypertension,[18,19] in which the risk of hypertension has been found to be significantly higher in obese children than in non-obese children, with an odds ratio of hypertension ranging from 2.4 to 2.5; however, it has been noted that the prevalence of hypertension in children increases across the entire range of BMI values and cannot be defined by a simple threshold effect. The effects of BMISD on adverse levels of LDL-C, HDL-C, TG and apoB are not yet clear. Unexpectedly, LDL-C was not associated with BMISD in both boys and girls. However, adverse levels of TG and apoB were associated with BMISD in both boys and girls. These significant associations were lost after adjustment for insulin resistance. Different findings of LDL-C and apoB were consistent with our previous report that LDL size was inversely associated with BMI in school children.[6] In the case of HDL-C, a strong association between BMISD and adverse level of HDL-C was found in both boys and girls; however, after adjustment for insulin resistance, a significant association was only retained in girls. As reported previously, hypercholesterolemia (hyper LDL-C) in school children commonly occurs regardless of BMISD.[6,7] Familial hypercholesterolemia and familial combined hyperlipidemia should not be overlooked in school children with overweight and obesity. Effect of genetic factors on hyper LDL-C may be greater than that of environmental factors. In addition to hyper LDL-C in school children, low HDL-C in schoolgirls should not be diagnosed as complications of overweight and obesity before clarifying the genetic background.

Serum concentrations of adiponectin were inversely correlated with BMISD in both boys and girls. However, the association between the relative risk of having an adverse level of adiponectin and BMISD was only significant in boys. This association was completely dependent on insulin resistance. In other words, relative risk of an adverse level of adiponectin is not increased in obese boys without insulin resistance, thereby indicating a close correlation between adverse adiponectin level and insulin resistance in schoolboys. In girls, factors other than BMISD and insulin resistance seemed to regulate adverse levels

Table 6 Correlation between BMISD and adverse levels of cardiovascular disease risk factors

	Boys	Girls
Independent of BMISD	Glucose, LDL-C	Glucose, LDL-C, Adiponectin
Dependent on BMISD		
Independent on IR	Uric acid, hCRP	Uric acid, HDL-C, hCRP
Dependent of IR	Insulin, HOMA-IR, HDL-C, TG, apoB, adiponectin	Insulin, HOMA-IR, TG, apoB

apoB, apolipoprotein B; BMISD, body mass index z-score; hCRP, serum concentration of C-reactive protein; HDL-C, high-density-lipoprotein cholesterol; HOMA-IR, homeostasis model approximation index; IR, insulin resistance; LDL-C, low-density-lipoprotein cholesterol; TG, triglycerides.

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of adiponectin. Recently, Magge *et al.* also reported similar findings that adiponectin levels are independent of insulin resistance in adolescents.[20]

With respect to hCRP, the association between the relative risk of adverse levels of hCRP and BMISD was unaffected by the adjustment for insulin resistance in both boys and girls. The underlying mechanism behind the correlation between hCRP and BMISD has not been clarified in the present study; however, our data suggest that subclinical inflammation as expressed by hCRP did occur even in school children, and that the degree of inflammation was associated with BMISD. According to a recent report, serum concentrations of uric acid are associated with all-cause and cardiovascular disease mortality.[21] Association between an adverse level of uric acid and BMISD was unexpectedly high and was unaffected by insulin resistance in both boys and girls. In addition, the association was unaffected by hCRP (data not shown). Although further studies are needed to clarify the physiological role of uric acid in children, the strong association between the relative risk of having adverse levels of uric acid and BMISD should be highlighted as a complication of overweight and obesity.

Conclusion

In the present study, hyper LDL-cholesterolemia in school children cannot be explained by BMISD. However, the relative risk of having adverse levels of other CVD risk factors in school children increases across the entire range of BMISD. Relative risks of adverse levels of UA and CRP in boys, and those of UA, HDL-C and CRP in girls are independent of insulin resistance. Not only obese children but also overweight children seem to be high-risk for the future development of CVD. To prevent future development of CVD, it is quite important for school children to maintain BMISD within normal range. However, we should also consider causes other than BMISD, especially in cases of hyper LDL-cholesterolemia in school children.

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References

1 Bessesen DH. Update on obesity. *J. Clin. Endocrinol. Metab.* 2008; **93**: 2027–34.
2 Kitagawa T, Owada M, Urakami T, Yamauchi K. Increased incidence of non-insulin dependent diabetes mellitus among Japanese schoolchildren correlates with an increased intake of animal protein and fat. *Clin. Pediatr.* 1998; **37**: 111–15.
3 Must A, Jacques PF, Dallal GE, Bajema CJ, Dietz WH. Long-term morbidity and mortality of overweight adolescent. A follow-up of the Harvard Growth Study of 1922 to 1935. *N. Engl. J. Med.* 1992; **327**: 1350–5.
4 Romero-Corral A, Montori VM, Somers VK *et al.* Association of bodyweight with total mortality and with cardiovascular events in coronary artery disease: a systemic review of cohort studies. *Lancet* 2006; **368**: 666–78.
5 Barter P, McPherson YR, Song K *et al.* Serum insulin and inflammatory markers in overweight individuals with and without dyslipidemia. *J. Clin. Endocrinol. Metab.* 2007; **92**: 2041–5.
6 Shimabukuro T, Subagawa M, Ohta T. Low-density lipoprotein particle size and its regulatory factors in school children. *J. Clin. Endocrinol. Metab.* 2004; **489**: 2923–7.
7 Asato Y, Katsuren K, Ohshiro T, Kikawa K, Shimabukuro T, Ohta T. Relationship between lipid abnormalities and insulin resistance in Japanese schoolchildren. *Arterioscler. Thromb. Vasc. Biol.* 2006; **26**: 2781–6.
8 Yoshida T, Kaneshi T, Shimabukuro T, Sunagawa M, Ohta T. Serum C-reactive protein and its relation to cardiovascular risk factors and adipocytokines in Japanese children. *J. Clin. Endocrinol. Metab.* 2006; **91**: 2133–7.
9 Kaneshi T, Yoshida T, Ohshiro T, Nagasaki H, Asato Y, Ohta T. Birth weight and risk factors for cardiovascular diseases in Japanese school children. *Pediatr. Int.* 2007; **49**: 138–43.
10 Ohta T, Kiwaki K, Endo F, Umehashi H, Matsuda I. Dyslipidemia in young Japanese children: its relation to familial hypercholesterolemia and familial combined hyperlipidemia. *Pediatr. Int.* 2002; **44**: 602–7.
11 Kahn BB, Flier JS. Obesity and insulin resistance. *J. Clin. Invest.* 2000; **106**: 473–81.
12 Ikeda T, Shibuya U, Sugiuchi H, Araki S, Uji Y, Okabe H. Automated immunoturbidimetric analysis of six serum apolipoproteins: correlation with radial immunodiffusion assays. *J. Clin. Lab. Anal.* 1991; **5**: 90–5.
13 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; **28**: 412–19.
14 Hara M, Saitou E, Iwata F, Okada T, Harada K. Waist-to-height ratio is the best predictor of cardiovascular disease risk factors in Japanese schoolchildren. *J. Atheroscler. Thromb.* 2002; **9**: 127–32.
15 Freedman DS, Kahn HA, Mei Z *et al.* Relation of body mass index and waist-to-height ratio to cardiovascular disease risk factors in children and adolescents: the Bogalusa Heart Study. *Am. J. Clin. Nutr.* 2007; **86**: 33–40.
16 Conwell LS, Trost SG, Brown WJ, Batch JA. Indexes of insulin resistance and secretion in obese children and adolescents. *Diabetes Care* 2004; **27**: 314–19.
17 Keskin M, Kurtoglu S, Kendirci M, Atabek ME, Yazici C. Homeostasis model assessment is more reliable than the fasting glucose/insulin ratio and quantitative insulin sensitivity check index for assessing insulin resistance among obese children and adolescents. *Pediatrics* 2005; **115**: e500–e503.
18 Freedman DS, Mei Z, Srinivasan SR, Berenson GS, Dietz WH. Cardiovascular risk factors and excess adiposity among overweight children and adolescents: the Bogalusa Heart Study. *J. Pediatr.* 2007; **150**: 12–17.
19 Sorof J, Daniels S. Obesity hypertension in children. A problem of epidemic proportions. *Hypertension* 2002; **40**: 441–7.
20 Magge SN, Stettler N, Koren D *et al.* Adiponectin is associated with favorable lipoprotein profile, independent of BMI and insulin resistance, in adolescents. *J. Clin. Endocrinol. Metab.* 2011; **96**: 1549–54.
21 Meisinger C, Koenig W, Baumert J, Doring A. Uric acid levels are associated with all-cause and cardiovascular disease mortality independent of systemic inflammation in men from the general population. The MONICA/KORA cohort study. *Arterioscler. Thromb. Vasc. Biol.* 2008; **28**: 1186–92.

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新しい新生児代謝スクリーニング時代に適応した先天代謝異常症の診断基準作成と治療ガイドラインの作成および新たな薬剤開発に向けた調査研究班

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