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Abnormal folic acid-homocysteine metabolism as maternal risk factors for Down syndrome in Japan

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■ **Summary** *Background* Japan has been considered as “a folate sufficient area”, since traditional Japanese food contains an adequate amount of folic acid. However, the recent westernized food style of young Japanese mothers may affect the intake of folic acid among them. This food style may contribute to the occurrence of Down syndrome, which has proved to be linked to abnormal folate and homocysteine metabolisms. *Aim of the study* To preliminarily evaluate the levels of folic acid, homocysteine and other relevant factors which are associated with folate metabolism, among Japanese women who had pregnancies affected by Down syndrome. *Methods* Blood samples from 31 women who had pregnancies affected by Down syndrome (DS) were obtained. 60 age-matched control blood samples were also obtained from mothers who had not experienced miscarriages or abnormal pregnancies (CONT). Plasma homocysteine and serum folic acid, vitamin B12, and B6 were measured and compared between DS and CONT. Furthermore, the frequency of MTHFR polymor-

phism (C677T) was also investigated. *Results* Plasma levels of homocysteine were significantly increased in DS mothers ($p = 0.004$). In contrast, serum levels of folic acid were significantly decreased in DS mothers ($p = 0.0001$). There were no significant differences in the vitamin B12 and B6 levels between DS and CONT. Also, the frequency of 5,10-methylenetetrahydrofolate reductase gene (MTHFR) homozygous polymorphism showed no differences between DS and CONT. *Conclusion* Different levels of serum folic acid and plasma homocysteine between both groups may contribute to the occurrence of Down syndrome even in Japan. Although there was no significant difference in the frequency of MTHFR polymorphism between the groups, probably because of an inadequate number of samples, further studies may contribute to the understanding of the occurrence of Down syndrome in Japan.

■ **Key words** folic acid – down syndrome – homocysteine – 5,10-methylenetetrahydrofolate reductase gene (MTHFR)

Introduction

Down syndrome (DS [MIM 190685]) is the most common genetic cause of human mental retardation, with an

incidence of approximately 1 in 600 to 1000 live births [1]. It is estimated that 1 in 150 conceptions have trisomy 21 and that 80 % of these are lost during early pregnancy. It has long been recognized that the risk of having a child with trisomy 21 increases with maternal age [2].

For example, the risk of having a live-born with Down syndrome at a maternal age of 30 is 1 in 1000 and at a maternal age of 40 is 9 in 1000 [3, 4].

Down syndrome has also been linked to the abnormal metabolism of homocysteine, which is led by variants in a critical folate metabolizing enzyme [5]. A thermolabile variant of 5,10-methylenetetrahydrofolate reductase (*MTHFR*) has been described in which a cytosine-to-thymine nucleotide (C677T) occurs, causing relatively reduced enzyme activity [6].

Although several studies have been performed on the causal relationship between homocysteine metabolism and Down syndrome in Western countries, there are scant data on folate metabolism in Japan, since Japan has been considered to be a "folate sufficient area". However, recent westernized food choices among young mothers may affect the incidence of Down syndrome in Japan.

In this study, we focused on the levels of homocysteine, folic acid and other relevant factors which contribute to homocysteine metabolism among mothers of Down syndrome children in Japan.

Materials and methods

Study participants

Before the study, ethical approval was obtained from the special committee of the Nagasaki University School of Medicine (project registration no. 0207160007). Blood samples from 31 women who had a pregnancy affected by Down syndrome (DS) were obtained at Nagasaki University Hospital. Mean age was 42.9 years. Blood samples of control mothers, who had experienced no miscarriages or abnormal pregnancies, were obtained at several clinics in Nagasaki city (see Acknowledgement). In all cases, informed consent was obtained. There was no significant difference between each group's mean age (42.9 ± 8.3 and 42.9 ± 9.5 years old, respectively, $p = 0.74$).

Hematochemical analyses

Plasma homocysteine, serum folate and vitamins B12 and B6 were measured in mothers of children with Down syndrome and in control mothers. Plasma homocysteine and serum vitamin B6 were measured using high performance liquid chromatography (HPLC). Serum folic acid and vitamin B12 were measured using the chemiluminescent immunoassay radioimmunoassay (CLIA) method.

Genotype analyses

Genomic DNA was extracted from blood cells using a QIAamp DNA blood mini kit (Qiagen, Tokyo, Japan). The *MTHFR* was amplified by polymerase chain reaction using the following set of primers:

sense: 5' – CTG GGA AGA ACT CAG CGA AC – 3'

antisense: 5' – GGA AGG TGC AAG ATC AGA GC – 3'

The presence of the C677T mutation within the *MTHFR* gene creates a *HinfI* restriction site that is detected by the appearance of 226 and 165 base pair fragments on 2 % agarose gel.

Statistical analysis

Data were analyzed using the Statistical Analysis System, version 6.12 (SAS Institute, Inc. Cary, NC). Results of continuous data (e.g., folic acid, homocysteine, and vitamins B12 and B6) are expressed as median \pm SD. Comparisons between groups were evaluated with the Mann Whitney test. For count data (e.g., the number of individuals with each genotype), comparisons of percentage between groups were evaluated with the χ^2 test.

Results and discussion

As shown in Fig. 1a, plasma levels of homocysteine were significantly higher in mothers of DS children compared with the control group (8.65 ± 2.09 nmol/ml vs. 7.40 ± 1.77 nmol/ml, $p = 0.004$). Serum levels of folic acid were significantly lower in mothers of DS children (6.20 ± 1.38 ng/ml vs. 9.10 ± 3.60 ng/ml, $p = 0.0001$) (Fig. 1b). In contrast, there were no significant differences in vitamin B12 and B6 levels between the groups (data not shown). Furthermore, analysis of the frequency of *MTHFR* homozygous polymorphism showed no significant differences between the groups (10.6 % vs 7.1 %, $p = 0.85$), probably due to an inadequate number of samples.

In this study, we demonstrated the possibility that mother's folate status may be associated with the occurrence of Down syndrome in Japan. Less attention has been paid to the importance of folate intake during pregnancy in this country since Japan has been considered to be a "folate rich country" based upon its traditional food style that includes abundant vegetables and rice. On the other hand, it has been noted that food choice among young Japanese has been rapidly westernized [7]. Already in the United States, the Food and Drug Administration (FDA) recommended the addition of folic acid to enrich grain products in 1986 and made compliance mandatory by 1998 [8]. In the future, such an interventional policy to reduce the occurrence of Down syndrome may also be needed in Japan.

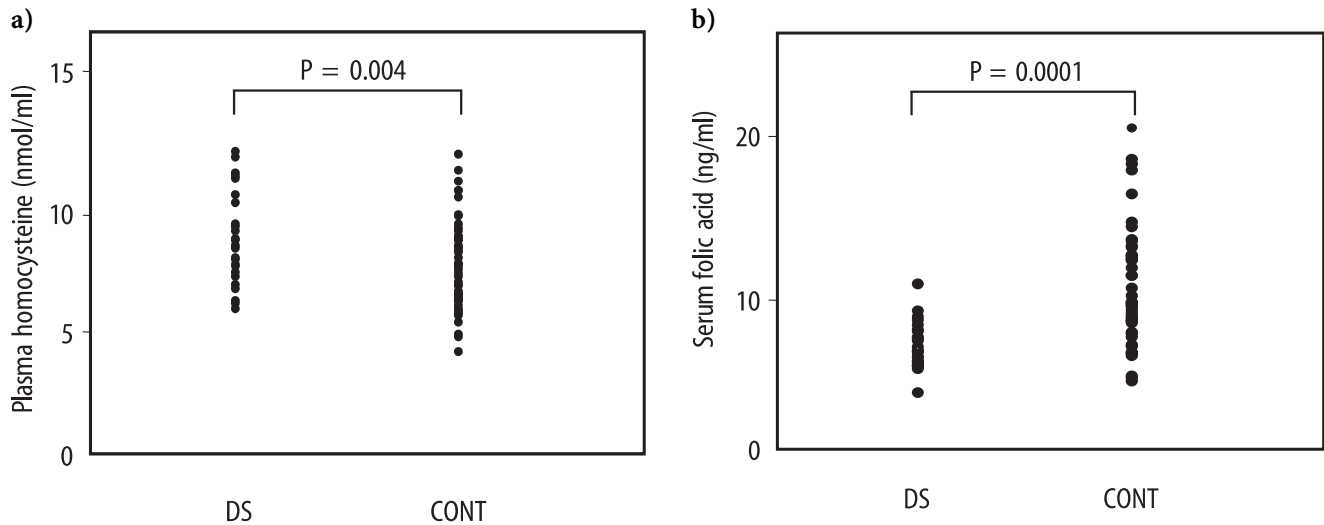


Fig. 1 The comparison of plasma homocysteine (a) and serum folic acid (b) levels between case and control mothers

Although there are no data about dietary and/or supplement intake of folic acid in either group, our preliminary study suggests that low levels of serum folic acid and plasma homocysteine may contribute to the occurrence of Down syndrome in Japan. Besides the evaluation of folate intake, contribution of genetic background should also be considered. Furthermore, nutritional ed-

ucation to emphasize the importance of folate intake may be beneficial for the effective prevention of Down syndrome in Japan.

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