Table 2
Oral anomalies in seven patients

	Deletion				Mutation			Total	
Patient	1	2	3	4	5	6	7	Deletion	Mutation
High-arched palate	+	+	–	+	+	–	+	3/4	2/3
Fused teeth	–	+	+	–	–	–	–	2/4	0/3
Hypodontia	–	+	+	–	–	–	+	2/4	1/3
Macrodontia	+	+	+	+	–	+	–	4/4	1/3
Dental caries	+ (10/23)	+ (10/19)	+ (15/19)	+ (16/22)	+ (7/22)	+ (4/24)	– (0/22)	4/4	2/3
Malocclusion	+ Crowding, narrow dental arch	–	–	–	–	+ Open bite	–	1/4	1/4

Parenthesis represents the number of dental caries/the total of present teeth.

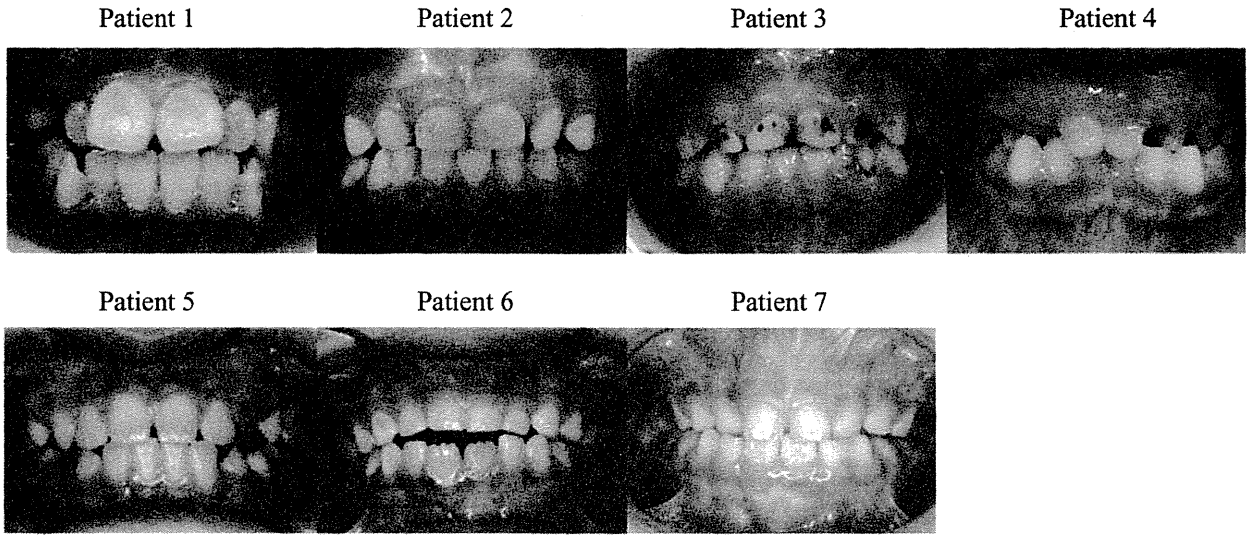

Fig. 2. Oral photographs of seven patients. Patients 1–4: deletion, patients 5–7: mutation.

as a component of syndromes such as KBG syndrome, "polydactyly, postaxial, with dental and vertebral anomalies", XXY and XYY male, and hemihyperplasia [17–21]. However, to our knowledge, macrodontia has not been described previously in NF1. Although macrodontia was also found in one patient with a mutation, it was relatively mild compared to the deletion patients, with only three large teeth having a width that exceeded 2 SD. Thus, macrodontia can be considered a distinctive feature of patients with *NF1* deletion. Dental caries was observed in both *NF1* deletion (4/4) and mutation (2/3) patients. However, patients with *NF1* deletion showed apparently severe caries (average number of dental caries 12.8) than those with *NF1* mutation (average number 5.5). Tucker et al. [5], on the basis of a questionnaire study of 37 families of children with NF1, reported that individuals with NF1 had a significantly higher average number of dental caries (8.1 ± 6.6) than their siblings without NF1 (5.5 ± 5.8). Unfortunately, no genetic investigation of the *NF1* gene was performed in their study. The authors mentioned some possibilities to account for increased dental caries in NF1 patients, including vitamin D deficiency, reduced bone mineralization (osteopenia or osteoporosis), and misregulation of various growth factor receptors. In addition, the author proposed that impaired mental capacity in NF1 patients might be a risk factor for excessive caries due to poor oral care. Our deletion patients tended to show poor oral hygiene indicated by the high plaque levels on oral examination. This could be explained by the reduced ability to perform dental care due to mental retardation. Nonetheless, the cause is still unknown and further studies are necessary. Malocclusion was not frequently seen in the patients examined. Patient 1 with

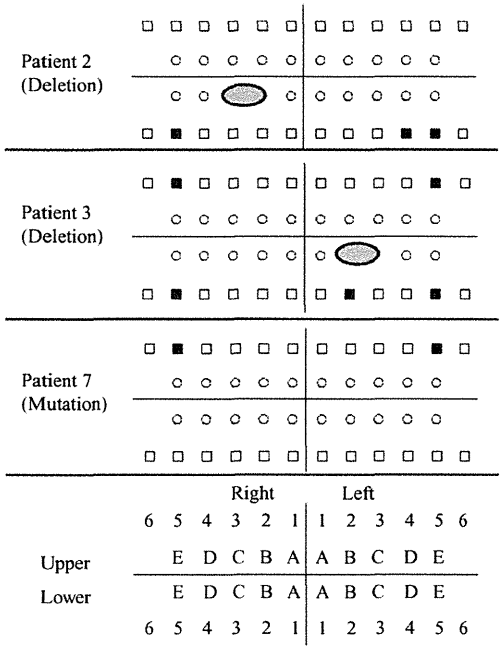

Fig. 3. Fused primary teeth and missing permanent teeth noted in patients 2, 3 with NF1 deletion, and missing permanent teeth observed in patient 7 with NF1 mutation. ⬭, Fused primary teeth. ■, Congenitally missing permanent tooth.

NF1 deletion had crowding, and patient 6 exhibited open bite. The former might be associated with macrodontia and a narrow dental arch, while the latter was likely due to tongue thrusting. Lateral cephalometric analysis showed a tendency toward a dolichofacial pattern in the patients with *NF1* deletion with maxillary protrusion, and a tendency toward labioclination of the maxillary central incisors in those with *NF1* mutation.

Grisart et al. [22] reported a family with microduplication of the identical *NF1* microdeletion region, in which patients showed moderate to borderline normal mental impairment, early onset of baldness and dental enamel hypoplasia. The author hypothesized that gene(s) responsible for dental enamel hypoplasia might reside in the deleted interval, although no candidate gene has been identified.

In conclusion, we evaluated seven NF1 patients, four with *NF1* deletion and three with *NF1* mutation, and found that fused teeth, macrodontia and excessive dental caries are distinctive manifestations of *NF1* deletion. Providing comprehensive dental care from early infancy would be very important to prevent dental caries especially in patients with *NF1* deletion.

Table 3
Size of the teeth in seven patients

Patient	Deletion								Mutation					
	1		2		3		4		5		6		7	
	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
Primary teeth														
Maxillary														
Central incisor			2.05	2.00	2.97	1.43	−0.41	−0.57			0.30	−0.51	0.45	−0.08
Lateral incisor			1.37	1.40	1.22	1.08	0.46	−1.51			1.22	1.27	−0.37	−0.29
Cuspid			2.28	2.19	2.30	2.82	−0.21	−0.67			0.12	0.15	−0.93	−0.93
First molar			1.71	1.73	3.03	1.78	1.43	1.65			2.38	2.13	0.83	0.15
Second molar	2.16		1.29	1.12	2.30	2.78	0.48	−0.62	−0.78		1.24	1.00	−0.59	0.32
Mandibular														
Central incisor			0.67	0.93	2.14	2.14	0.55	−0.55						
Lateral incisor				1.26	1.76		2.00	2.31			0.21	0.86	−0.82	−0.97
Cuspid				2.44	0.71		−0.43	0.36			0.43	2.75	0.15	0.44
First molar			1.77	1.81	1.80	2.24	2.27	2.24		0.19	0.36	1.27	−1.56	−1.23
Second molar	1.33	1.13	0.71	0.71	1.24	1.78	−1.40	−1.67	−0.69	−0.61	−0.38	−0.55	0.94	0.78
Permanent teeth														
Maxillary														
Central incisor	4.78	4.39							−0.59	−0.74				
Lateral incisor	3.27	2.78							0.65	0.39				
Cuspid		3.77												
First premolar		3.69												
First molar	1.20	1.53							−2.59	−1.61	−0.57			
Mandibular														
Central incisor	4.03	4.19							−0.30	−0.40	1.14	1.33	−0.12	1.00
Lateral incisor	3.10	4.00							−0.33	0.09				
Cuspid	3.03	3.05												
First molar	1.82	1.52							−1.78	−0.98				

Tooth size represents the distance from the medial to distal. Unit, SD.

Table 4
Dental arch measurements in seven patients

	Deletion				Mutation		
Patient	1	2	3	4	5	6	7
Maxillary							
W_C		2.04	2.11	−1.09		0.15	−0.42
W_E		0.32	2.27	−2.10		−1.22	−1.73
L_{AE}		1.27	0.76	−0.37		0.77	0.25
W_3	No data				No data		
W_6	−2.19				1.77		
L_{16}	2.37				0.66		
Mandibular							
W_C		No data	No data	0.48		2.69	0.88
W_E		0.40	−0.12	0.34		0.53	−0.96
L_{AE}		−0.36	−0.68	−2.18		No data	No data
W_3	No data				No data		
W_6	−2.78				1.95		
L_{16}	2.07				1.51		

The W_C, W_3, W_E, W_6 represents the distance between the primary cuspids, the cuspids, the primary second molars, the first molars. The L_{AE} represents the length from the distal surface of the primary second molars to the primary incisors central point. The L_{16} represents the length from the mesial surface of the first molars to the incisors central point. Unit, SD.

Table 5
Lateral cephalometric analysis in seven patients

	Deletion				Mutation		
Patient	1	2	3	4	5	6	7
Skeletal							
Convexity	−0.83	2.35	5.63	3.14	−0.61	1.16	−1.49
A-B plane	1.53	−0.31	−0.52	−0.46	1.53	−1.98	1.68
SNA	−0.71	−0.32	2.74	1.49	1.24	−1.56	−0.49
SNB	−0.38	−1.02	1.29	1.02	1.74	−3.55	−0.18
Facial angle	0.14	−1.54	−1.08	−0.77	3.52	1.23	0.23
SNP	−0.74	−1.27	0.06	−0.16	1.41	−2.36	2.04
Y-axis	0.80	1.77	2.06	2.19	−2.38	−2.66	1.58
SN-S-Gn	1.89	1.82	1.09	1.40	−0.94	1.40	0.78
Mandibular plane	2.51	2.65	1.62	2.87	−2.91	−1.76	0.55
Gonial angle	0.39	0.96	−1.32	2.32	−1.12	−0.34	−0.11
GZN	1.85	0.61	1.51	0.39	0.63	1.44	0.13
FH to SN	0.96	−0.21	−1.56	−0.90	1.31	3.94	−1.22
Denture							
U-1 to FH plane	1.18	−0.31	0.07	−3.41	3.84	4.66	1.22
U-1 to SN plane	0.77	−0.34	0.66	1.30	3.14	2.10	1.87
L-1 to mandibular	−0.47	0.80	1.65	−1.85	−0.64	2.04	0.09
Interincisal	−1.36	−1.09	−1.41	−0.86	−1.21	−3.50	−1.33
Occlusal plane	−0.06	1.00	2.02	0.88	−2.64	−1.69	2.04

Unit, SD.

Acknowledgement

The authors are grateful to Prof. Takahide Maeda for his helpful advice. We also thank to Dr. Kenji Shimizu and Dr. Yasuo Takahashi for their invaluable assistance.

This study was funded in part by a Grant for the Support of Projects for Strategic Research at Private Universities by the Ministry of Education, Culture, Sports, Science and Technology (MEXT; 2008–2012), and by a grant from the Ministry of Health, Labor and Welfare, Japan.

References

[1] Friedman JM. Epidemiology of neurofibromatosis type 1. Am J Med Genet 1999; 89: 1–6.

[2] Crawford AH, Schorry EK. Neurofibromatosis in children: the role of the orthopaedist. J Am Acad Orthop Surg 1999; 7: 217–30.

[3] Shapiro SD, Abramovitch K, Van Dis ML, Skoczylas LJ, Langlais RP, Jorgenson RJ, et al. Neurofibromatosis: oral and radiographic manifestations. Oral Surg Oral Med Oral Pathol 1984; 58: 493–8.

[4] Friedrich RE, Giese M, Schmelzle R, Mautner VF, Scheuer HA. Jaw malformations plus displacement and numerical aberrations of teeth in neurofibromatosis type 1: a descriptive analysis of 48 patients based on panoramic radiographs and oral findings. J Craniomaxillofac Surg 2003; 31: 1–9.

[5] Tucker T, Birch P, Savoy DM, Friedman JM. Increased dental caries in people with neurofibromatosis 1. Clin Genet 2007; 72: 524–27.

[6] Lammert M, Friedrich RE, Friedman JM, Mautner VF, Tucker T. Early primary tooth eruption in neurofibromatosis 1 individuals. Eur J Oral Sci 2007; 115: 425–26.

[7] Visnapuu V, Peltonen S, Ellilä T, Kerosuo E, Väänänen K, Happonen RP, et al. Periapical cemental dysplasia is common in women with NF1. Eur J Med Genet 2007; 50: 274–80.

[8] Venturin M, Guarnieri P, Natacci F, Stabile M, Tenconi R, Clementi M, et al. Mental retardation and cardiovascular malformations in NF1 microdeleted patients point to candidate genes in 17q11.2. J Med Genet 2004; 41: 35–41.

[9] Kluwe L, Siebert R, Gesk S, Friedrich RE, Tinschert S, Kehrer-Sawatzki H, et al. Screening 500 unselected neurofibromatosis 1 patients for deletions of the NF1 gene. Hum Mutat 2004; 23: 111–6.

[10] Kayes LM, Burke W, Riccardi VM, Bennett R, Ehrlish P, Rubenstein A, et al. Deletions spanning the neurofibromatosis 1 gene: identification and phenotype of five patients. Am J Hum Genet 1994; 54: 424–36.

[11] Cnossen MH, van der Est MN, Breuning MH, van Asperen CJ, Breslau-Siderius EJ, van der Ploeg AT, et al. Deletions spanning the neurofibromatosis type 1 gene: implications for genotype-phenotype correlations in neurofibromatosis type 1? Hum Mutat 1997; 9: 458–64.

[12] Lepping KA, Kaplan P, Viskochil D, Weaver M, Ortenberg J, Stepheens K. Familial neurofibromatosis 1 microdeletions: cosegregation with distinct facial phenotype and early onset of cutaneous neurofibromata. Am J Med Genet 1997; 73: 197–204.

[13] Tonsgard JH, Yelavarthi KK, Cushner S, Short MP, Lindgren V. Do NF1 gene deletions result in a characteristic phenotype? Am J Med Genet 1997; 73: 80–6.

[14] Mautner VF, Kluwe L, Friedrich RE, Roehl AC, Bammert S, Högel J, et al. Clinical characterisation of 29 neurofibromatosis type-1 patients with molecularly ascertained 1.4 Mb type-1 NF1 deletions. J Med Genet 2010; 47: 623–30.

[15] Iizuka T, Ishikawa F. Normal standards for various cephalometric analysis in Japanese adults. Nippon Kyousei Shika Gakkai Zasshi 1957; 16: 4–12 (in Japanese).

[16] Shi S, Machida Y, Yonezu T. The prevalence of fused teeth in Japanese and Chinese children. Shika Gakuho 1993; 93: 631–8 (in Japanese).

[17] Herrmann J, Pallister PD, Tiddy W, Opitz JM. The KBG syndrome-a syndrome of short stature, characteristic facies, mental retardation, macrodontia and skeletal anomalies. Birth Defects Orig Artic Ser 1975; 11: 7–18.

[18] Rogers JG, Levin LS, Dorst JP, Temtamy SA. A postaxial polydactyly-dental-vertebral syndrome. J Pediatr 1977; 90: 230–35.

[19] Alvesalo L, Portin P. 47, XXY males: sex chromosomes and tooth size. Am J Hum Genet 1980; 32: 955–9.

[20] Alvesalo L, Osborne RH, Kari M. The 47,XYY male, Y chromosome, and tooth size. Am J Hum Genet 1975; 27: 53–61.

[21] Alvesalo L, Kari M. Sizes of deciduous teeth in 47, XYY males. Am J Hum Genet 1977; 29: 486–9.

[22] Grisart B, Rack K, Vidrequin S, Hilbert P, Deltenre P, Verellen-Dumoulin C, et al. NF1 microduplication first clinical report: association with mild mental retardation, early onset of baldness and dental enamel hypoplasia? Eur J Hum Genet 2008; 16: 305–11.

ORIGINAL ARTICLE

Attitudes toward non-invasive prenatal diagnosis among pregnant women and health professionals in Japan

Junko Yotsumoto[1,2], Akihiko Sekizawa[1]*, Keiko Koide[1], Yuditiya Purwosunu[1], Kiyotake Ichizuka[1], Ryu Matsuoka[1], Hiroshi Kawame[2] and Takashi Okai[1]

[1]Department of Obstetrics and Gynecology, Showa University School of Medicine, Tokyo, Japan
[2]Department of Genetic Counseling, Ochanomizu University Graduate School of Humanities and Sciences, Tokyo, Japan
*Correspondence to: Akihiko Sekizawa. E-mail: sekizawa@med.showa-u.ac.jp

ABSTRACT

Objective This study aims to assess the attitudes toward non-invasive prenatal diagnosis (NIPD) and NIPD problems in clinical practice in Japan.

Methods A mail-in survey using a self-reported questionnaire was conducted among pregnant women and health professionals. The questionnaire enquired about attitudes, concerns, and expectations regarding NIPD.

Results The responses from 252 respondents revealed that pregnant women have more positive attitudes toward NIPD than health professionals. In addition, there were wide discrepancies in concerns and expectations about NIPD, between medical professionals and pregnant women. The respondents with less NIPD knowledge had a more positive attitude toward the clinical application of NIPD. There was concern expressed by clinical geneticists whether an NIPD test should be performed or not when there is a lack of knowledge about the NIPD. All of the health professionals emphasized the importance of providing genetic counseling prior to and after the testing.

Conclusion Pregnant women place importance on the safety and non-invasiveness of the NIPD tests, whereas medical professionals consider the diagnostic accuracy and reliability of the test to be the most important. Health professionals pointed out that the tests might be frequently performed without the pregnant women having adequate knowledge or counseling. © 2012 John Wiley & Sons, Ltd.

Funding sources: This study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sport, and Culture of Japan (nos. 23592417 and 23591602).
Conflicts of interest: None declared

INTRODUCTION

Despite recent human genetic breakthroughs and their applications in clinical practice, the prevention of genetic conditions in newborn still relies on screening, prenatal diagnosis, and termination of affected pregnancies. The decision to consent to prenatal diagnostic tests may be affected by a variety of factors, such as the country's culture, its health system, its abortion laws, and the information provided by the test, as well as parental level of awareness and education.[1–4] It has been recognized that a prenatal health professional's attitudes may influence a pregnant woman's decision to consent to a prenatal diagnosis.[5–7]

Invasive procedures such as amniocentesis and chorionic villus sampling (CVS) have been clinically available but involve small and definite risks to the fetus and/or the mother. To reduce the risk, a combination of maternal serum parameters and ultrasonographic screening is currently used to assess the risk of fetal aneuploidy. The development of new, potentially non-invasive approaches involves the examination of fetal cells or cell-free DNA within the maternal circulation as a source of fetal DNA. The enrichment of nucleated red blood cells using lectin and a subsequent auto-detection system has improved the accuracy of the diagnosis of fetal aneuploidy[8,9] and is now under evaluation in clinical trials. Another approach utilizes cell-free (extracellular) fetal DNA or RNA. The use of such procedures for determining the Rhesus factor status in RhD-negative pregnant women has been translated into clinical practice.[10] In 2008, a next-generation sequencing technology was applied to cell-free DNA analysis in maternal plasma.[11,12] The next-generation sequencing test was proven to be highly accurate for detecting fetal trisomy-21 in plasma,[13] and it has been commercially approved for the non-invasive prenatal diagnosis (NIPD) of trisomy-21 in both the USA and China.

In Japan, prenatal genetic screening is performed in fewer than 5% of all pregnancies and mostly performed as second-trimester maternal serum screening. First-trimester screening is still uncommon in Japan. After maternal serum screening was introduced in the 1990s, the Ministry of Health, Labour, and Welfare of Japan published clinical practice guidelines

entitled "Opinions Concerning Tests using Maternal Serum Markers" in 1999. The guidelines concluded that the implementation of second-trimester maternal serum screening is not a necessity. Additionally, obstetricians were not obliged to actively inform pregnant women about second-trimester maternal serum screening. The guidelines also concluded that not all pregnant women should be informed about the availability of such testing. Nevertheless, when a pregnant woman requests an explanation about the test, the medical professional should carefully explain about the prenatal diagnosis. The consensus about the ultrasonography detection of soft markers, such as nuchal translucency, is followed by the Japan Society of Obstetrics and Gynecology because there were concerns about the ethical, social, and psychological aspects of the tests. For example, the test results might lead pregnant women to misunderstand the information or may make them anxious about the results expressed as part of an individual risk assessment.[14] Therefore, there is concern about whether the test should be performed as mass screening to detect fetal abnormalities. For these reasons, Japanese health professionals tend not to actively inform pregnant women about prenatal screening tests (e.g., serum and ultrasonography) and diagnostic tests (e.g., amniocentesis and chorionic villus sampling). However, as pregnant women have learned more about genetic screening and prenatal diagnosis, there has recently been a greater demand for such testing.

New NIPD testing will be better in safety, robustness, and early time performed in pregnancy than invasive test. In the clinical setting, there is concern that informing parents about the prenatal screening can cause acceptance of this test and its results without sufficient consideration, causing an increase in elective abortion.[7,15–17] Little is known about the attitudes of pregnant women regarding NIPD.[18–21] Although Japan still has various limitations regarding the performance of both prenatal diagnosis and screening, it is important to know the opinions and social consciousness of pregnant women and health professionals to further develop NIPD testing systems in Japan.

METHODS

Self-report questionnaires were sent to clinical geneticists (pediatricians and obstetricians), midwives or nurses, and genetic counselors belonging to the same hospitals. Questionnaires were given to pregnant women when they visited the outpatient clinic at Showa University Hospital, Tokyo, and Shonan-Tobu Hospital, Kanagawa, Japan, between July and August 2011. A cover letter accompanied each questionnaire, explaining the purpose of the study, its confidentiality, and the method to follow when replying. Furthermore, in the supplement, we explain NIPD testing, which will become available as a first-trimester diagnostic test, as well as its risks, advantages, and disadvantages. The medical ethical committee of Showa University approved this study. Prior to the study, ten health professionals tested the questionnaire (the medical ethical committee of Showa University also approved this preliminary study). On the basis of the pilot test, the questionnaire required only minor grammatical changes.

The questionnaire was composed of seven sections. The first section collected sociodemographic data about the respondents and contained the information about the NIPD. The other six sections consisted questions relating to the following: (i) agreement or disagreement with the clinical application of NIPD; (ii) expectations about NIPD testing; (iii) concerns about the clinical application of NIPD; (iv) NIPD testing methods; (v) attitudes toward the clinical application of NIPD if the pregnant women have adequate or inadequate knowledge; and (vi) suitable targets of the NIPD testing. The survey used a 5-point Likert scale as follows: 1, fully disagree; 2, disagree; 3, neither agree nor disagree; 4, agree; 5, fully agree. Then, the 5-point Likert scale was limited to three categories (fully disagree, neither agree nor disagree, and fully agree) to avoid small data pools for some responses. A pregnant woman was assumed to have adequate knowledge when she knew the risks, advantages, and disadvantages of NIPD, as described in the questionnaire supplement. The data were analyzed using the Statistical Package for Social Sciences software program version 17. Cronbach's coefficient alpha test was used to clarify the validity of the test. The Chi-square test, Mann–Whitney test, Kruskal–Wallis test, and Friedman test were used in the statistical testing of differences between the groups.

RESULTS

Background characteristics

There were a total of 252 respondents consisting of 185 health professionals and 67 pregnant women. The response rate was 58% among obstetricians ($n=58$), 39% among pediatricians ($n=39$), and 45% among genetic counselors ($n=41$). The response rate of the pregnant women is unknown because the self-reported questionnaire was used at outpatient clinics. There were also 36 midwives or nurses in health professionals group. The demographic characteristics of the respondents are shown in Table 1.

Agreement or disagreement with the clinical application of NIPD

Table 2 shows that most of the health professionals and pregnant women agreed with the clinical application of NIPD. More pregnant women agree with the clinical application of NIPD than health professionals ($X^2=7.355$, $p<0.01$).

Expectations for NIPD

Table 3 shows the answers to the 11 questions related to the expectation for NIPD as follows: the better safety of the NIPD, the lower maternal physical and psychological stress related to the test's procedure, the earlier gestational age of the testing performed, the higher diagnostic accuracy, the ability to provide a definitive diagnosis, and the shorter processing time for the results (on the 5-point Likert scale). Pregnant women strongly expected the testing to be safe for the fetus and to result in less maternal physical and psychological stress. The medical professionals strongly expected the tests to be safe for the fetus, to have high diagnostic accuracy, and to provide a definitive diagnosis. It was revealed that the

Table 1 **The demographic characteristics of the respondents**

		Health professionals (N=185)	Pregnant women (N=67)
Gender	Female	98 (53%)	67 (100%)
	Male	81 (44%)	
	Not stated	6 (3%)	
Age	20–29	16 (8.6%)	10 (14.9%)
	30–39	34 (18.4%)	44 (65.7%)
	40–49	65 (35.1%)	10 (14.9%)
	50–59	57 (30.8%)	
	>60	10 (5.4%)	
	Not stated	3 (1.7%)	3 (4.5%)
Occupation	Obstetrician/clinical geneticist	58 (31.3%)	
	Pediatrician/clinical geneticist	39 (21.1%)	
	Genetic counselor	41 (22.2%)	
	Midwife or nurse	36 (19.5%)	
	Other	11 (5.9%)	

Table 2 **Agreement or disagreement with the clinical application of non-invasive prenatal diagnosis testing**

	Medical professionals	Pregnant women	Total
Agree	97 (78.9%)	38 (97.3%)	135 (83.3%)
Disagree	26 (21.1%)	1 (2.7%)	27 (16.7%)
Total	123	39	162

pregnant women expected more from the NIPD than the medical professionals in seven of the 11 items (Mann–Whitney test), including less maternal physical and psychological stress, items associated with an earlier diagnosis, and those associated with the safety of the NIPD tests.

Concerns about the clinical application of NIPD

Table 4 shows the responses to the 16 questions related to the concerns about the clinical applications of NIPD (on a 5-points Likert scale). Medical professionals had numerous concerns, especially with regard to the test's implementation if the pregnant women have inadequate knowledge of the NIPD, insufficient counseling before the testing, the occurrence of unexpected results and confusion, and increased test participation without full consideration. On the other hand, the pregnant women had concerns about an unequal access to the test (because of the cost), test's implementation without NIPD knowledge, an increase in abortions, and an increase in test participation without full consideration. Health professionals are significantly more concerned about NIPD than the pregnant women in ten of the 15 items ($p < 0.001$) as follows: increased test participation

Table 3 **Expectations about non-invasive prenatal diagnosis testing**

	Averaged rank[a]		
	Medical professionals	Pregnant women	*p*-value[b]
Safe for the fetus	8.47	7.83	0.045
Less maternal physical and physiological stress (because of peripheral blood sampling)	6.86	7.15	0.000
Can be diagnosed early in pregnancy (~10 weeks)	6.38	5.10	0.233
High diagnostic accuracy (compared with amniocentesis)	7.22	6.56	0.144
Definitive diagnosis (instead of screening)	7.20	6.69	0.065
Shorter processing time for results (about 1 week)	6.75	5.52	0.277
Earlier results are better because they reduce concerns about the baby	4.33	6.71	0.000
Earlier results lead to a better relationship between the mother and the newborn	3.27	4.40	0.000
Earlier treatment/consideration can be performed if an abnormality is found	5.03	5.95	0.000
In case of abortion, there is less maternal stress because of earlier gestation	4.66	4.36	0.003
Results are easy to understand	5.83	5.73	0.001

[a]Friedman test: In each group of medical professionals and pregnant women, a rank sum analysis was performed. The Friedman test (non-parametric statistical test) is used to detect differences in paired multiple items and is indicated by the rank sum result.
[b]Mann–Whitney test: The difference between medical professionals and pregnant women was analyzed.

Table 4 **Concerns about the clinical application of NIPD testing**

	Averaged rank[a]		
	Medical professionals	Pregnant women	p-value[b]
Increase in test participation without full consideration	10.41	9.56	0.000
Autonomous option of the test is restricted	6.88	6.17	0.000
Unequal access to the test due to cost	6.96	10.82	0.052
More pregnant women would receive an unexpected result (because of the frequency of testing), causing confusion	11.25	10.07	0.000
Counseling is insufficient because of the non-invasiveness of the testing	11.30	7.59	0.000
Abortions would become more prevalent	7.61	10.38	0.947
Autonomous decisions to have a baby with a chromosomal abnormality will be limited	6.47	9.36	0.992
Testing would be performed more often with inadequate physician's knowledge	10.67	7.24	0.000
Testing would be performed with inadequate pregnant women's knowledge	11.40	10.59	0.000
NIPD cannot diagnose chromosomal structural abnormalities (e.g., translocation)	9.01	7.63	0.000
NIPD can lead to discrimination against newborns with chromosomal abnormalities because of decreased prevalence	5.51	7.51	0.427
Unnecessary testing would be performed	8.82	7.78	0.000
Testing will develop into broad and global genetic screening	8.22	7.41	0.000
Testing will develop into mass screening	6.95	8.02	0.082
Testing will develop for non-medical purposes (e.g., gender determination)	7.08	6.93	0.000
The technology may be diverted for the sake of developing a "designer baby"	7.48	8.97	0.07

NIPD, non-invasive prenatal diagnosis.
[a]Friedman test: In each group of medical professionals and pregnant women, the rank sum analysis was performed.
[b]Mann–Whitney test: The difference between medical professionals and pregnant women was analyzed.

without full consideration, inadequate counseling prior to the test, implementation of such tests without NIPD knowledge among medical professionals and pregnant women, and using such tests when not necessary.

Clinical application of NIPD testing

In this section, we asked medical professionals and pregnant women whether each survey participant accepts the clinical setting that all pregnant women be allowed to undergo NIPD testing if they want to have the test. The attitudes of the pregnant women and the healthcare professionals differed in regard to this question (Table 5). Among the pregnant women, 97.8% agreed that women should be allowed to undergo the test if they want it, whereas only 35.9% of the healthcare professionals agreed ($X^2 = 49.049$, $p < 0.001$).

We also asked whether NIPD testing should be limited to women with a significant risk for having a fetus with a genetic condition (Table 6). Both groups showed a similar tendency: namely, a total of 84.8% of the health professionals and 77.8% of the pregnant women agreed that this test should be performed in high-risk cases ($p = 0.217$).

Table 5 **Pregnant women are able to undergo non-invasive prenatal diagnosis testing if they so desire: medical professionals versus pregnant women**

	Medical professionals	Pregnant women	Total
Agree	42 (35.9%)	43 (97.8%)	85 (52.8%)
Disagree	75 (64.1%)	1 (2.2%)	76 (47.2%)
Total	117	44	161

Table 6 **Non-invasive prenatal diagnosis testing should be performed for pregnant women who have a significant risk for having a fetus with a genetic condition**

	Medical professionals (N = 185)	Pregnant women (N = 67)	Total
Agree	123 (84.8%)	28 (77.8%)	30 (16.6%)
Disagree	22 (15.2%)	8 (22.2%)	151 (83.4%)
Total	145	36	181

Attitudes about the clinical application of NIPD with or without sufficient knowledge about the test

Table 7 shows that respondents with inadequate NIPD knowledge tended to have a positive attitude toward the clinical application of NIPD (76.8%). Only 34.3% of respondents with adequate NIPD knowledge agreed that pregnant women be allowed to undergo NIPD test if they want to ($X^2 = 22.569$, $p < 0.0001$). Table 8 shows that 36.6% and 60.5% respondents, with inadequate or adequate NIPD knowledge, respectively, wanted their partner to undergo the test ($X^2 = 5.717$, $p < 0.05$).

Suitable targets for NIPD testing

In order of importance, the suitable targets for NIPD testing according to health professionals are as follows: childhood-onset

Table 7 **Acceptance of the clinical application of NIPD testing with and without knowledge about the NIPD**

	With knowledge about NIPD	Without knowledge about NIPD	Total
Agree	24 (34.3%)	43 (76.8%)	67 (53.2%)
Disagree	46 (65.7%)	13 (23.2%)	59 (46.8%)
Total	70	56	126

NIPD, non-invasive prenatal diagnosis.

Table 8 **Respondents hope to undergo the NIPD testing if they or their partner becomes pregnant**

	With knowledge about NIPD	Without knowledge about NIPD	Total
Agree	26 (36.6%)	23 (60.5%)	49 (45%)
Disagree	45 (63.4%)	15 (39.5%)	60 (55%)
Total	71	38	109

NIPD, non-invasive prenatal diagnosis.

Table 9 **Suitable targets for non-invasive prenatal diagnosis testing based on the opinion of medical professionals**

	Averaged rank[a]
Childhood-onset severe genetic diseases	11.13
At risk of severe X-linked diseases	10.91
RhD blood typing	10.64
Chromosomal aneuploidy (e.g., Down syndrome)	10.63
Chromosomal abnormality (e.g., microdeletion, duplication)	8.82
Chromosomal test equal to karyotyping quality	8.32
Adult-onset severe neurological disorder (e.g., Huntington's disease)	8.12
Adult-onset hereditary cancer	7.28
Carrier testing for genetic disorders	5.91
Mild phenotype genetic disorders (e.g., color blindness)	5.64
Broad and global identification of genetic conditions	5.24
Lifestyle-related diseases (e.g., diabetes)	4.77
Mass-screening applications	4.54
Gender identification without medical reason	3.03

[a]Friedman test.

severe genetic disorders, severe X-linked disorders, rhesus D blood typing, and chromosomal aneuploidy such as Down syndrome and trisomy-18 (Table 9). The healthcare professionals disagreed about the application of NIPD testing for gender determination without a medical reason and for adult-onset diseases as mass screening for all pregnant women.

DISCUSSION

The present study showed that most of the pregnant women and health professionals had positive attitudes toward the clinical application of NIPD, although around 20%, especially clinical geneticists, were reluctant to recommend NIPD usage in clinical practice. These positive attitudes could hasten the acceptance of the application of NIPD testing in clinical practice, especially in Japan. There were discrepancies in the expectations and indications for NIPD between healthcare professionals and pregnant women. In particular, the pregnant women expected most in fetal safety and less in maternal stress of NIPD. Meanwhile, medical professionals expected most in high diagnostic accuracy, the ability to provide a definitive diagnosis, and the safety of the testing. Furthermore, pregnant women considered the reduction of maternal stress during the test procedure and the ability to have an earlier diagnosis (to relieve anxiety) to be more important than did the health professionals. Medical professionals put more importance on the diagnostic accuracy and reliability of the test. These differences in expectations and concerns could help in the development of improved education strategies for both pregnant women and health professionals.

Regarding concerns about the clinical application of NIPD, healthcare professionals pointed out that the test may be performed without providing information about the advantages and limitations of NIPD because of the test's non-invasiveness. In this study, the pregnant women are concerned about unequal access to the test due to the cost and increase in abortions performed.

It is interesting to note that those respondents with inadequate knowledge of NIPD tended to appreciate NIPD testing. The cause of this tendency is unknown. There was a possibility that pregnant women might accept NIPD testing more easily because of an unquestioned acceptance of anything new.[18] Moreover, in this study, more pregnant women than health professionals agreed that pregnant women be allowed to undergo the test if they wish. In this section, when we presented the first statement, "All women are able to undergo NIPD testing if they want it," there was a discrepancy in opinions (97.8% pregnant women agreed, 35.9% health professionals agreed, $p < 0.001$). Then, in the second statement, "NIPD should be limited to women with a high risk of a genetic condition," there was no substantial discrepancy in opinions (77.8% pregnant women agreed, 84.8% health professionals agreed, $p = 0.217$). When presented with the second statement, the pregnant women might have reconsidered, and their attitudes were changed slightly. This also indicates that pregnant women might accept NIPD testing more easily because of an unquestioned acceptance.

This difference in tendencies could lead to several problems. If the test is conducted on pregnant women with inadequate knowledge about the purpose of the test and what kinds of information can be obtained, the test results might thus create either misunderstanding or anxiety.

Although the survey did not address these problems, providing sufficient information about NIPD to both pregnant women and health professionals might have a positive impact. Prior to NIPD testing, it is necessary that genetic counseling, including discussions about the benefits and limitations of testing, is provided to every pregnant woman. In this way, pregnant women could make an autonomous informed decision about using NIPD and the test's results.[22]

The NIPD technology became commercially available in the USA in 2011, but only the test for trisomy-21 is available as an advanced screening test with an extremely high detection rate and a low false-positive rate. As a result, the International Society

for Prenatal Diagnosis has announced a statement about "Prenatal Detection of Down Syndrome Using Massively Parallel Sequencing (MPS)" (http://cmg.informz.net/CMG-ISPD/data/images/ispd.rr.mps.24oct11.pdf). This statement indicates that MPS can be helpful for pregnant women who may have been determined to be at high risk by previously recommended screening methods under suitable genetic counseling and that the International Society for Prenatal Diagnosis does not endorse the *ad hoc* use of MPS testing in women at lower risk. However, the future directions of NIPD technology will likely hasten progress toward definitive testing because pregnant women are hoping for the establishment of safe and reliable tests. Therefore, at the time of this MPS clinical application, a counseling system should be implemented for pregnant women prior to and after testing. Pregnant women who are considering the MPS test need to receive detailed genetic counseling and to give their informed consent prior to the test. The present study suggests that genetic counseling prior to and after the testing is vital to addressing the challenges associated with building a new framework for genetic counseling for NIPD testing.

ACKNOWLEDGEMENTS

We thank Professor Takeo Kubota for his valuable advice about designing the study. We also thank Dr. Mieko Saeki for her help in conducting the questionnaire to the pregnant women in Shonan-Tobu Hospital. We also thank Noriko Ando, Natsumi Osafune, Ruriko Horio, Takako Hirose, and Mayuko Inuzuka for their help in the analysis of the results of the questionnaire.

WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

- New non-invasive prenatal diagnosis testing will be better than invasive test in safety, definitive result, robustness, and early time performed in pregnancy. There were concerns about the clinical application of the non-invasive prenatal diagnosis; however, the details of the concerns among both pregnant women and health professionals have not yet been assessed in Japan.

WHAT DOES THIS STUDY ADD?

- We revealed the attitudes toward non-invasive prenatal diagnosis (NIPD) among pregnant women and health professionals. There were discrepancies in concerns and expectations about NIPD, between the health professionals and the pregnant women. It is suggested that genetic counseling prior to and after the testing is vital to addressing the challenges in building a new framework for genetic counseling for NIPD testing.

REFERENCES

1. van Den Berg M, Timmermans DR, Ten Kate LP, van Vugt JM, van Der Wal G. Are pregnant women making informed choices about prenatal screening? Genet Med 2005;7:332–8.
2. Dahl K, Kesmodel U, Hvidman L, Olesen F. Informed consent: attitudes, knowledge and information concerning prenatal examinations. Acta Obstet Gynecol Scand 2006;85:1414–9.
3. van Den Berg M, Timmermans DR, Ten Kate LP, van Vugt JM, van Der Wal G. Informed decision making in the context of prenatal screening. Patient Educ Couns 2006;63:110–7.
4. van Den Heuvel A, Marteau TM. Cultural variation in values attached to informed choice in the context of prenatal diagnosis. Semin Fetal Neonatal Med 2008;13:99–102.
5. Dormandy E, Marteau TM. Uptake of a prenatal screening test: the role of healthcare professionals' attitudes towards the test. Prenat Diagn 2004;24:864–8.
6. Lewis SM, Cullinane FM, Carlin JB, Halliday JL. Women's and health professionals' preferences for prenatal testing for Down syndrome in Australia. Aust N Z J Obstet Gynaecol 2006;46:205–11.
7. Hall S, Chitty L, Dormandy E, Hollywood A, Wildschut HI, Fortuny A, Masturzo B, Santavy J, Kabra M, Ma R, Marteau TM. Undergoing prenatal screening for Down's syndrome: presentation of choice and information in Europe and Asia. Eur J Hum Genet 2007;15:563–9.
8. Sekizawa A, Purwosunu Y, Matsuoka R, Koide K, Okazaki S, Farina A, Saito H, Okai T. Recent advances in non-invasive prenatal DNA diagnosis through analysis of maternal blood. J Obstet Gynaecol Res 2007;33:747–64.
9. Sekizawa A, Purwosunu Y, Farina A, Okai T, Takabayashi H, Kita M, Yura H, Kitagawa M. Development of noninvasive fetal DNA diagnosis from nucleated erythrocytes circulating in maternal blood. Prenat Diagn 2007;27:846–8.
10. Wright CF, Burton H. The use of cell-free fetal nucleic acids in maternal blood for non-invasive prenatal diagnosis. Hum Reprod Update 2009;15:139–51.
11. Chiu RW, Chan KC, Gao Y, Zheng W, Leung TY, Foo CH, Xie B, Tsui NB, Lun FM, Zee BC, Lau TK, Cantor CR, Lo YM. Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma. Proc Natl Acad Sci U S A 2008;105:20458–63.
12. Fan HC, Blumenfeld YJ, Chitkara U, Hudgins L, Quake SR. Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood. Proc Natl Acad Sci U S A 2008;105:16266–71.
13. Palomaki GE, Kloza EM, Lambert-Messerlian GM, Haddow JE, Neveux LM, Ehrich M, Van Den Boom D, Bombard AT, Deciu C, Nelson SF, Canick JA. DNA sequencing of maternal plasma to detect Down syndrome: an international clinical validation study. Genet Med 2011;13:913–20.
14. Weinans MJ, Huijssoon AM, Tymstra T, Gerrits MC, Beekhuis JR, Mantingh A. How women deal with the results of serum screening for Down syndrome in the second trimester of pregnancy. Prenat Diagn 2000;20:705–8.
15. Green JM, Hewison J, Bekker HL, Bryant LD, Cuckle HS. Psychosocial aspects of genetic screening of pregnant women and newborns: a systematic review. Health Technol Assess 2004;8: iii, ix-x, 1–109.
16. De Jong A, Dondorp WJ, De Die-Smulders CE, Frints SG, De Wert GM. Non-invasive prenatal testing: ethical issues explored. Eur J Hum Genet 2009;18:272–7.
17. van Den Heuvel A, Chitty L, Dormandy E, Newson A, Attwood S, Ma R, Masturzo B, Pajkrt E, Marteau M. Is informed choice in prenatal testing universally valued? A population-based survey in Europe and Asia. BJOG 2009;116:880–5.
18. Kooij L, Tymstra T, Berg P. The attitude of women toward current and future possibilities of diagnostic testing in maternal blood using fetal DNA. Prenat Diagn 2009;29:164–8.
19. Kelly SE, Farrimond HR. Non-invasive prenatal genetic testing: a study of public attitudes. Public Health Genomics 2011;15:73–81.
20. Sayres LC, Allyse M, Norton ME, Cho MK. Cell-free fetal DNA testing: a pilot study of obstetric healthcare provider attitudes toward clinical implementation. Prenat Diagn 2011;31:1070–6.
21. Tischler R, Hudgins L, Blumenfeld YJ, Greely HT, Ormond KE. Noninvasive prenatal diagnosis: pregnant women's interest and expected uptake. Prenat Diagn 2011;31:1292–9.
22. Marteau TM, Dormandy E. Facilitating informed choice in prenatal testing: how well are we doing? Am J Med Genet 2001;106:185–90.

Brain magnetic resonance imaging and motor and intellectual functioning in 86 patients born at term with spastic diplegia

YURIKA NUMATA[1,2] | AKIRA ONUMA[1] | YASUKO KOBAYASHI[3] | IKUKO SATO-SHIRAI[1,2] | SOICHIRO TANAKA[1] | SATORU KOBAYASHI[1] | KEISUKE WAKUSAWA[1] | TAKEHIKO INUI[1] | SHIGEO KURE[2] | KAZUHIRO HAGINOYA[1]

1 Department of Pediatric Neurology, Takuto Rehabilitation Center for Children, Sendai; **2** Department of Pediatrics, Tohoku University School of Medicine, Sendai; **3** Department of Pediatrics, Nishitaga National Hospital, Sendai, Japan.

Correspondence to Dr Kazuhiro Haginoya at Department of Pediatric Neurology, Takuto Rehabilitation Center for Children, Yumoto Akiumachi, Taihaku-ku, Sendai 982-0241, Japan.
E-mail: khaginoya@kha.biglobe.ne.jp

This article is commented on by Vollmer on pages 103–104 of this issue.

PUBLICATION DATA
Accepted for publication 5th August 2012.
Published online 2nd November 2012.

ABBREVIATIONS
PVI Periventricular venous infarction
PVL Periventricular leukomalacia

AIM To investigate the association between magnetic resonance imaging (MRI) patterns and motor function, epileptic episodes, and IQ or developmental quotient in patients born at term with spastic diplegia.
METHOD Eighty-six patients born at term with cerebral palsy (CP) and spastic diplegia (54 males, 32 females; median age 20y, range 7–42y) among 829 patients with CP underwent brain MRI between 1990 and 2008. The MRI and clinical findings were analysed retrospectively. Intellectual disability was classified according to the Enjoji developmental test or the Wechsler Intelligence Scale for Children (3rd edition).
RESULTS The median ages at diagnosis of CP, assignment of Gross Motor Function Classification System (GMFCS) level, cognitive assessment, and MRI were 2 years (range 5mo–8y), 6 years (2y 8mo–19y), 6 years (1y 4mo–19y), and 7 years (10mo–30y) respectively. MRI included normal findings (41.9%), periventricular leukomalacia, hypomyelination, and porencephaly/periventricular venous infarction. The frequency of patients in GMFCS levels III to V and intellectual disability did not differ between those with normal and abnormal MRI findings. Patients with normal MRI findings had significantly fewer epileptic episodes than those with abnormal ones (p=0.001).
INTERPRETATION Varied MRI findings, as well as the presence of severe motor dysfunction and intellectual disability (despite normal MRI), suggest that patients born at term with spastic diplegia had heterogeneous and unidentified pathophysiology.

Spastic diplegia is a subgroup of cerebral palsy (CP) found both in preterm and term infants; however, the underlying causes differ. Preterm spastic diplegia is the result of periventricular leukomalacia (PVL),[1] whereas only 12% of children born at term with spastic diplegia have PVL.[2] In a study based on the data collected from the Surveillance of Cerebral Palsy in Europe collaboration, 45.7% of patients born at term with CP with a birthweight of 2500g or more had bilateral spastic palsy.[3] The ratio of patients with CP with severe impairments peaked in the 1980s, fell during 1990 to 1999, and was little changed in the 1990s compared with the late 1970s.[4] However, another recent population-based comparative analysis of children with CP born between 1990 and 1996, and between 1997 and 2003 showed an increase in those born at term with spastic diplegia from 9 (4/46) to 20% (5/25)[5] respectively. The pathogenesis of spastic diplegia in those born at term is poorly understood. Although some clinical and neuroradiological studies have reported magnetic resonance imaging (MRI) findings other than PVL,[1,6–9] most used small sample sizes and, thus, did not provide a broad perspective on the pathology of spastic diplegia in those born at term.

The present study investigated the relation between MRI and clinical findings in a large sample of children born at term with spastic diplegia.

METHOD

Patients

Between 1 September 1990 and 31 August 2008, 829 patients with CP underwent MRI at the Takuto Rehabilitation Center for Children, Sendai, Japan. Of those, we enrolled 86 term patients (54 males, 32 females; median age 20y, range 7–42y) with clinical evidence of spastic diplegia. The inclusion criteria were a diagnosis of CP, a diagnosis of spastic diplegia, and gestational age of 37 weeks or more. Spastic diplegia was defined as a type of spastic quadriplegia affecting the lower more than the upper extremities.[1,6,7] Brain MRI and clinical findings of the participants were retrospectively analysed. The present study was approved by the ethics

committee of the Takuto Rehabilitation Center for Children (number 22-2).

MRI analysis

MRI was performed using a Toshiba MRIT 50A scanner (0.5T; Toshiba, Tokyo, Japan) with 10mm multislice axial and sagittal T1-weighted spin-echo images (repetition time/echo time [TR/TE] 375/14) and axial T2-weighted spin-echo images (TR/TE 2000/120), or a Shimadzu-Marconi Magnex Eclipse scanner (1.5T; Shimadzu-Marconi, Kyoto, Japan) with 5mm multislice axial and sagittal T1-weighted images (TR/TE 500/20) and axial T2-weighted images (TR/TE 5000/72). Two authors (YN, IS-S) with no knowledge of clinical information independently evaluated the MRI findings. The MRI findings were categorized as shown in Table I and are illustrated in Figure 1. Hypomyelination was defined as a delayed or arrested normal myelinating process resulting in a persistent immature pattern of myelination in the cerebral white matter. Porencephaly was defined as fluid-filled cavities in the cerebral hemisphere connecting to the lateral ventricle. In the present study, there were no patients with porencephaly connected to the hemisphere surface. Periventricular venous infarction (PVI) was defined to the loss of periventricular tissues after venous infarction caused by subependymal haemorrhage.[10] Because porencephaly and PVI are probably based on the same pathophysiology, we combined them into the one category. Cerebellar atrophy was defined as a small cerebellum with prominent fissures between shrunken folia,[11] whereas cerebellar hypoplasia was defined as a small cerebellum with normally sized fissures and folia.[11] When MRI revealed mixed abnormalities, the diagnosis was based on the most prevalent and dominant findings as the cause of the spastic diplegia. Furthermore, patients with abnormalities in more than one radiological category were analysed. Disagreements in diagnosis were resolved by the third author (YK) and second author (AO).

What this paper adds

- Almost 42% of patients born at term with spastic diplegia had normal MRI findings.
- The frequency of patients with severe motor dysfunction and intellectual disability did not differ between those with normal and abnormal MRI findings.

Clinical assessment

The patients' age at diagnosis of CP, assignment of Gross Motor Function Classification System (GMFCS)[12] level, and cognitive assessment as well as the history of asphyxia were retrospectively analysed from the medical records.

Motor-deficit severity was classified into levels I to V according to the GMFCS; the levels were interpreted from the latest clinical records. We found it difficult to distinguish between GMFCS levels I and II clinically; thus, those levels were combined in the statistical analysis.

The children's developmental quotients (DQ) or intelligence quotients (IQ) were assessed using either the Enjoji developmental test, which evaluates physical abilities of the whole body, skilled hand motor activities, behaviour, interpersonal skills, speech ability, and language comprehension,[13] or the Wechsler Intelligence Scale for Children (3rd edition).[14] In cases of serial rating studies, we used the latest result to ensure an exact classification. The patients' DQ/IQs were graded and classified into three groups: normal, DQ/IQ≥70; mild, 50?DQ/IQ<70; severe, DQ/IQ<50.

Epilepsy was defined as the occurrence of at least two unprovoked epileptic seizures; neonatal seizures and febrile convulsions were not included.[15] The epilepsy outcome at the time of the present study was classified as good when the patient was seizure-free for more than 2 years, poor when the patient had more than two seizures per month despite appropriate treatment, and moderate if the patient was between the two classifications.

Table I: Magnetic resonance imaging classification and its distribution among patients born at term with spastic diplegia

Classification	Description	*n*	%
Normal	No abnormality detected	36	41.9
Periventricular leukomalacia	Symmetric reduction of the peri-trigonal white matter volume with a consecutive enlargement of the posterior horns, scalloped ventricular contours, periventricular gliosis and secondary atrophy of the posterior part of the body and the splenium of the corpus callosum	12	14.0
Malformation	Includes cortical dysplasia, schizencephaly, polymicrogyria, pachygyria, and heterotopias	3	3.5
Hypomyelination	Delayed or arrested normal myelinating process resulting in persistent immature pattern of myelination in the cerebral white matter	5	5.8
Cerebellar atrophy	Shrinkage of cerebellar vermis and hemisphere	2	2.3
Diffuse cortical atrophy	Diffuse reduction of the volume of cerebral gyri	4	4.7
Enlargement of the bilateral lateral ventricles	Enlargement of bilateral lateral ventricles except the findings of periventricular leukomalacia	12	14.0
Border-zone infarction	Ischemic changes in the watershed area between anterior and medial cerebral artery or between medial and posterior cerebral artery	3	3.5
Porencephaly/periventricular venous infarction	Fluid-filled cavities in the cerebral hemisphere connecting to the lateral ventricle/loss of periventricular tissues after venous infarction caused by subependymal haemorrhage	6	7.0
Thin corpus callosum	Thinning of the corpus callosum except the findings of periventricular leukomalacia and porencephary	1	1.1
Unclassifiable	Unable to be classified into one of the above groups	2	2.3
Total		86	

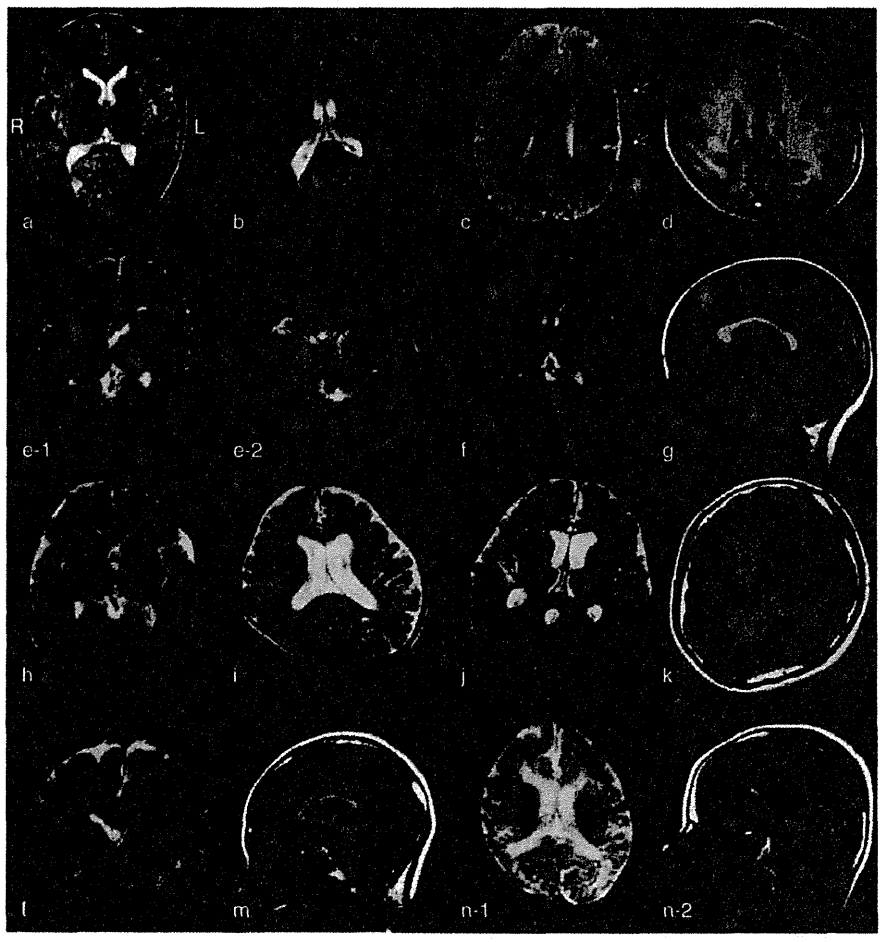

Figure 1: Magnetic resonance imaging findings of term patients with spastic diplegia. (a) Normal findings detected by T2-weighted spin-echo images (T2WI) at 7 years. (b) Periventricular leukomalacia by T2WI at 6 years. (c) Hemispheric polymicrogyria of the left hemisphere (arrows) by T2WI at 4 years. (d) Bilateral parieto-occipital schizencephaly by T1-weighted spin-echo images (T1WI) at 5 years. (e-1, e-2) Cerebral and cerebellar malformation by T1WI at 3 years. (f) Hypomyelination by T2WI performed at 10 years. (g) Cerebellar atrophy by T1WI at 10 years. (h) Diffuse cortical atrophy by T2WI at 10 years. (i) Enlargement of the bilateral lateral ventricles by T2WI at 2 years. (j) Bilateral frontal border-zone infarction by T1WI at 3 years. (k) Porencephaly by T1WI at 30 years. (l) Periventricular venous infarction by T2WI at 15 years. (m) Thin corpus callosum by T1WI at 12 years. (n-1, n-2) MRI images of the patients with hypomyelination, enlargement of the bilateral lateral ventricles, diffuse cortical atrophy, and thin corpus callosum at 7 years.

Table II: Correlation between normal or abnormal magnetic resonance imaging findings and Gross Motor Function Classification System (GMFCS) level, epilepsy, and intellectual disability

	All (*n*=86)		Normal (*n*=36)		Abnormal (*n*=50)		
	n	%	*n*	%	*n*	%	*p*
GMFCS level							
I or II	61	70.9	30	83	31	62	0.053
III–V	25	29.1	6	7	19	38	
Incidence of epilepsy	24	27.9	3	8	21	42	0.001
Outcome of epilepsy							
Good	14		3		11		
Moderate	4		0		4		
Poor	6		0		6		
Intellectual disability	52	60.5	19	51	33	66	0.266
Mild	15		7		8		
Severe	37		12		25		

Intellectual disability levels: normal, DQ/IQ≥70; mild, DQ≤50/IQ<70; severe, IQ/DQ<50.

Relationships between normal or abnormal MRI findings and GMFCS level, epilepsy, and intellectual disability are shown in Table II.

Statistical analysis

The association between factors in the children's clinical profile (motor function, epilepsy, and intellectual disability) and MRI findings was investigated. Statistical analyses were conducted using a χ^2 test. A p value <0.05 was deemed statistically significant.

RESULTS

The median ages at diagnosis of CP, assignment of GMFCS level, cognitive assessment, and MRI were 2 years (range 5mo–8y), 6 years (2y 8mo–19y), 6 years (1y 4mo–19y), and 7 years (10mo–30y) respectively. Seventeen patients experienced birth asphyxia, including those with normal MRI (n=4), PVL (n=5), diffuse cortical atrophy (n=2), hypomyelination (n=2), porencephaly/PVI (n=1), enlargement of the bilateral lateral ventricles (n=1), thin corpus callosum (n=1), and border-zone infarction (n=1).

MRI findings

The MRI results of the 86 patients born at term with spastic diplegia (Table I) revealed normal findings in 36 (41.9%) patients (Fig. 1a) and abnormal findings in 50 (58.1%). We examined 60 patients and 26 patients by 0.5T and 1.5T MRI respectively. The proportion of normal findings did not significantly differ between 0.5T and 1.5T MRI (p=1.0).

Of the abnormal MRI findings, PVL was observed in 12 patients (Fig. 1b), malformation was found in three, hemispheric polymicrogyria of the left hemisphere in one (Fig. 1c), bilateral parieto-occipital schizencephaly in one (Fig. 1d), and cerebral and cerebellar malformation in one (Fig. 1e-1, e-2). Hypomyelination was observed in five (Fig. 1f), cerebellar atrophy in two (Fig. 1g), diffuse cortical atrophy in four (Fig. 1h), enlargement of the bilateral lateral ventricles in 12 (Fig. 1i), border-zone infarction in three (Fig. 1j), porencephaly (Fig. 1k)/PVI (Fig. 1l) in six, and thin corpus callosum in one (Fig. 1m). Two patients were unclassified according to the above system.

Motor impairment

The associations between MRI findings and motor-deficit severity, epilepsy, and cognitive impairment are shown in Table II. Of the 86 patients, 61 (69%) were classified in GMFCS levels I or II. Of the 36 patients with normal MRI, 30 (83%) were graded in GMFCS levels I or II, whereas 30 of 50 patients (62%) with abnormal MRI findings were independently ambulatory (GMFCS levels I or II). These results indicate the frequency of patients with motor dysfunction did not differ between those with normal and abnormal MRI findings (p=0.053). The present study identified 25 patients in GMFCS levels III to V. Their MRI findings included normal findings (n=6), PVL (n=3), malformation (n=2), hypomyelination (n=3), cerebellar atrophy (n=2), diffuse cortical atrophy (n=2), enlargement of the bilateral lateral ventricles (n=2), border-zone infarction (n=1), porencephaly/PVI (n=2), thin corpus callosum (n=1), and unclassified (n=1). They contained five patients with abnormalities in more than one radiological category: one with cerebellar hypoplasia and pachygyria plus thin corpus callosum and enlargement of bilateral lateral ventricles; one with diffuse cortical atrophy plus enlargement of bilateral lateral ventricles; two with hypomyelination plus thin corpus callosum; and one with hypomyelination plus thin corpus callosum plus enlargement of bilateral lateral ventricles plus diffuse cortical atrophy (Fig. 1n-1, n-2). The first category was evaluated with the most dominant findings adopted as the MRI classification in Table II.

Epilepsy

Of the 86 patients, 24 (27.9%) had a history of epilepsy, including three of the 36 patients (8%) with normal MRI and 21 of the 50 patients (42%) with abnormal MRI findings. Thus, the frequency of epilepsy was significantly lower in patients with normal MRI findings than in those with abnormal ones (p=0.001). Among the 24 patients with epilepsy, 14 (58%) exhibited good control, four (17%) had moderate control, and six (25%) exhibited poor control. The MRI findings of the six patients with poorly controlled epilepsy were as follows: porencephaly/PVI (n=2), PVL (n=1), malformation (n=1), hypomyelination (n=1), and enlargement of the bilateral lateral ventricles (n=1). MRI of one patient who developed West syndrome revealed that hypomyelination overlapped diffuse cortical atrophy and enlargement of the bilateral lateral ventricles; the patient had severe intellectual disability, a poor epilepsy outcome, and was classified in GMFCS level V.

Intellectual disability

Of the 86 patients, 52 (60.5%) had an intellectual disability. In the group of 36 patients with normal MRI findings, 19 (51%) exhibited intellectual disability, including 12 (63%) with severe disability and seven (37%) with mild disability. Similarly, 33 of the 50 (66%) patients with abnormal MRI findings had intellectually disability; 25 (76%) severely and eight (24%) mildly. These results indicate that the MRI findings were not correlated with the frequency of intellectual disability in patients born at term with spastic diplegia (p=0.266).

Other characteristic findings

Two patients showed both cerebellar hemispheric and vermian atrophy. One had no history of asphyxia, the other had no perinatal record. They were referred to our hospital because of delayed motor development and crouched posture, and were diagnosed with spastic diplegia at the ages of 2 and 9 years 7 months respectively. Both had tendon-lengthening surgery around the age of 10 years. One had intellectual disability and epilepsy. One was non-ambulatory, whereas the other one was ambulatory with crutches.

Five patients (three males, two females) whose diagnosis of spastic diplegia was made between 2 and 3 years of age had hypomyelination. Their final MRI was studied at 3, 7, 8, 10, and 11 years respectively. MRI results showed severe hypomyelination with reduced white matter volume, whereas the

thalamus, brainstem, and cerebellum showed preserved myelination. Three patients had a history of birth asphyxia. Two of these showed ventricular dilatation and cortical atrophy. Four patients had severe intellectual disability. Three had epilepsy. Three patients were non-ambulatory, whereas two were able to walk independently. Auditory brainstem response, which correspond to the electrophysiological activity of the auditory system throughout the brainstem,[16] was abnormal in three patients in whom the upper brainstem components were not detected; two of these patients were siblings (one male, one female), suggesting an autosomal recessive trait. The *PLP1*, *GJC2*, and myelin basic protein gene analyses were normal in one patient. No patient showed clinical deterioration.

Eight patients had asymmetric MRI abnormalities. The MRI findings in these patients were porencephaly/PVI (n=5), malformation (hemispheric polymicrogyria; n=1), cerebellar atrophy (n=1), and enlargement of the bilateral lateral ventricles (n=1). Five patients with porencephaly/PVI and one patient with malformation had clinical asymmetry, which was dominant in the contralateral extremities.

Eight patients had abnormalities in more than one radiological category, as follows: enlargement of bilateral lateral ventricles plus thin corpus callosum (n=1) or diffuse cortical atrophy (n=1); malformation, which was cerebellar hypoplasia and pachygyria plus thin corpus callosum and enlargement of bilateral lateral ventricles (n=1); hypomyelination plus thin corpus callosum (n=2) plus enlargement of bilateral lateral ventricles (n=1) and diffuse cortical atrophy (n=1); and diffuse cortical atrophy plus enlargement of bilateral lateral ventricles (n=1). They included five patients in GMFCS levels III to V, four patients with epilepsy, and six patients with intellectual disability.

DISCUSSION

The results of the present study show that the MRI findings were more varied in those born at term with spastic diplegia than those born preterm with spastic diplegia, suggesting that the pathogenesis of spastic diplegia in those born at term is heterogeneous. A recent meta-analysis involving four studies of patients with spastic CP identified 51 term-born patients with spastic diplegia.[17] The MRI findings were classified as normal in 24 (47%), PVL in 13 (25.5%), brain maldevelopment in 6 (11.8%), grey matter lesions in four (7.8%), and miscellaneous in four (7.8%).[17] In our study, 36 patients (41.9%) were found to have normal MRI findings, which is consistent with previous reports (Table III). Our study demonstrated that patients born at term with spastic diplegia who had normal MRI findings showed significantly fewer epileptic episodes than those with abnormal MRI findings. Patients born at term with spastic diplegia and normal MRI findings tended to show less severe motor dysfunction than those with abnormal MRI findings, although it was not significant. However, the percentage of patients with intellectual disability did not differ between the normal (51%) and the abnormal (66%) MRI groups.

Previous studies of patients with preterm spastic diplegia have found that the severity of motor disability was correlated with the degree of white matter reduction.[6] However, an Australian population-based cohort study of CP by Robinson et al.[18] reported that spastic diplegia was the most common clinical motor impairment in patients with normal MRI findings (12/25). These findings suggest the existence of unknown pathophysiological processes. In our study, six patients were classified in GMFCS levels III to V despite their normal MRI findings. Additionally, the proportion of cerebellar atrophy and hypomyelination was high in the patients in GMFCS levels III to V.

Individuals with CP and epilepsy have been reported to have structural brain abnormalities.[19] Our results are consistent with these earlier reports. Previous studies have reported that 18 to 39% of patients with spastic diplegia develop epilepsy.[19–22] Among them, a comparative analysis of the frequency of epilepsy in those born preterm and at term with spastic diplegia revealed that epilepsy was more common in those born at term.[22] The predominance of deep white matter lesions in preterm infants is thought to be one of the factors explaining why preterm patients with spastic diplegia are less likely to develop epilepsy.[22,23] We found that the frequency of epilepsy was significantly higher in patients with abnormal MRI findings than in those with normal ones.

Table III: Patients born at term with spastic diplegia who had normal magnetic resonance imaging (MRI) and characteristics of spastic diplegia

References	Number born at term with spastic diplegia	Number born at term with spastic diplegia and normal MRI findings (%)	Characteristics of spastic diplegia (n born at term/preterm) (%)			
			Spastic diplegia (term/preterm)	Spastic diplegia (term/preterm) with severe motor impairment	Spastic diplegia (term/preterm) with epilepsy	Spastic diplegia (term/preterm) with intellectual disability
Koeda et al.[1]	7	4 (57)	18 (7/11)	–	–	–
Yokochi et al.[6]	3	2 (67)	34 (3/31)	20 (–/–) (58.8)	–	20 (–/–) (58.8)
Krägeloh-Mann et al.[9]	9	2 (22)	29 (9/20)	–	–	–
Hayakawa et al.[7]	20	11 (55)	63 (20/43)	6 (1/5) (9.5)	–	–
Okumura et al.[8]	13	4 (31)	81 (13/68)	–	–	–
Carlsson et al.[20]	23	–	59 (23/36)	–	21 (12/9) (35.6)	–
Kulak et al.[21]	17	–	40 (17/23)	5 (–/–) (12.5)	7 (–/–) (17.5)	30 (–/–) (75)
Present study	86	36 (41)	(86/–)	(25/–)	(24/–)	(52/–)

–, not examined.

Surprisingly, there was no difference in the frequency of intellectual disability between groups with normal and abnormal MRI findings. To the best of our knowledge, there are no reports on the relationship between severity of cognitive deficits and MRI findings in patients born at term with spastic diplegia. Reports comparing cognitive abnormalities in preterm and term-born children with spastic diplegia with PVL showed that visuoperceptual defects were common in preterm children.[24] Veelken et al.[22] reported that one-quarter of their children born at term with diplegia had severe intellectual disability (IQ<50), in contrast to 5% among preterm children with diplegia. In our study, the proportion of patients with intellectual disability, 60.5% (52/86) of all patients born at term with spastic diplegia, might be relatively high. Over 50% of patients born at term with spastic diplegia with normal MRI had intellectual disability. 'Normal MRI findings' may reflect as-yet undetected abnormalities by widely used MRI scanners. Furthermore, the use of advanced brain imaging techniques with an examination of genetic or early prenatal factors[25] may be helpful in elucidating the nature of spastic diplegia in those born at term.

PVL and porencephaly/PVI are categorized as preterm-type brain injuries because they are the result of vascular system immaturity, where vascular watershed areas are situated in the periventricular white matter.[26] This type of injury typically causes spastic diplegia based on the somatotopic organization of descending corticospinal tracts.[26] In the present study, PVL and porencephaly/PVI were observed in 18 patients with spastic diplegia; all but one, whose perinatal medical record was not available, showed no evidence of perinatal asphyxia, suggesting that PVL and porencephaly/PVI in the term infants occurred in utero early in the third trimester. Although five patients with porencephaly/PVI and one patient with malformation had asymmetric brain lesion and more severe contralateral palsy, they showed diplegia rather than hemiplegia, suggesting the presence of undetected lesions in the pyramidal tract.

In conclusion, the present study, which to our knowledge has used the largest reported sample size of patients born at term with spastic diplegia, found that (1) 41.9% of patients had normal MRI findings, (2) there was no difference in the incidence of severe motor dysfunction and intellectual disability between patients with abnormal and normal brain MRI findings, and (3) patients with no brain abnormalities exhibited significantly fewer epileptic episodes than those with abnormal MRI findings. Spastic diplegia in patients born at term with no brain abnormalities may be the result of unidentified pathophysiology that requires further clarification. The investigation of possible genetic or early prenatal factors may provide a better understanding of the pathogenesis of spastic diplegia in those born at term.

REFERENCES

1. Koeda T, Suganuma I, Kohno Y, Takamatsu T, Takeshita K. MR imaging of spastic diplegia. Comparative study between preterm and term infants. *Neuroradiology* 1990; **32:** 187–90.
2. Wu YW, Croen LA, Shah SJ, Newman TB, Najjar DV. Cerebral palsy in a term population: risk factors and neuroimaging findings. *Pediatrics* 2006; **118:** 690–7.
3. Sellier E, Surman G, Himmelmann K, et al. Trends in prevalence of cerebral palsy in children born with a birthweight of 2,500 g or over in Europe from 1980 to 1998. *Eur J Epidemiol* 2010; **25:** 635–42.
4. Surman G, Hemming K, Platt MJ, et al. Children with cerebral palsy: severity and trends over time. *Paediatr Perinat Epidemiol* 2009; **23:** 513–21.
5. Sigurdardóttir S, Thórkelsson T, Halldórsdóttir M, Thorarensen O, Vik T. Trends in prevalence and characteristics of cerebral palsy among Icelandic children born 1990 to 2003. *Dev Med Child Neurol* 2009; **51:** 356–63.
6. Yokochi K, Aiba K, Horie M, et al. Magnetic resonance imaging in children with spastic diplegia: correlation with the severity of their motor and mental abnormality. *Dev Med Child Neurol* 1991; **33:** 18–25.
7. Hayakawa K, Kanda T, Hashimoto K, et al. MR imaging of spastic diplegia. The importance of corpus callosum. *Acta Radiol* 1996; **37:** 830–6.
8. Okumura A, Kato T, Kuno K, Hayakawa F, Watanabe K. MRI findings in patients with spastic cerebral palsy. II: correlation with type of cerebral palsy. *Dev Med Child Neurol* 1997; **39:** 369–72.
9. Krägeloh-Mann I, Petersen D, Hagberg G, Vollmer B, Hagberg B, Michaelis R. Bilateral spastic cerebral palsy–MRI pathology and origin. Analysis from a representative series of 56 cases. *Dev Med Child Neurol* 1995; **37:** 379–97.
10. Perlman JM. White matter injury in the preterm infant: an important determination of abnormal neurodevelopment outcome. *Early Hum Dev* 1998; **53:** 99–120.
11. Barkovich AJ. Toxic and Metabolic Brain Disorders. In: Barkovich AJ, editor. Pediatric Neuroimaging. 3rd edn. Philadelphia, PA: Lippincott Williams & Wilkins, 2000: 143–5.
12. Palisano R, Rosenbaum P, Walter S, Russell D, Wood E, Galuppi B. Development and reliability of a system to classify gross motor function in children with cerebral palsy. *Dev Med Child Neurol* 1997; **39:** 214–23.
13. Orito Y, Oku H, Kubota S, et al. Thyroid function in early pregnancy in Japanese healthy women: relation to urinary iodine excretion, emesis, and fetal and child development. *J Clin Endocrinol Metab* 2009; **94:** 1683–8.
14. Wechsler D. Wechsler Intelligence Scale for Children. 3rd edn. London: Psychological Corporation, 1992.
15. Commission on Epidemiology and Prognosis of the International League Against Epilepsy. Guideline for epidemiologic studies on epilepsy. *Epilepsia* 1993; **34:** 592–6.
16. Guzzetta F, Conti G, Mercuri E. Auditory processing in infancy: do early abnormalities predict disorders of language and cognitive development? *Dev Med Child Neurol* 2011; **53:** 1085–90.
17. Krageloh-Mann I, Horber V. The role of magnetic resonance imaging in elucidating the pathogenesis of cerebral palsy: a systematic review. *Dev Med Child Neurol* 2007; **49:** 144–51.
18. Robinson MN, Peake LJ, Ditchfield MR, Reid SM, Lanigan A, Reddihough DS. Magnetic resonance imaging findings in a population-based cohort of children with cerebral palsy. *Dev Med Child Neurol* 2009; **51:** 39–45.
19. Kwong KL, Wong SN, So KT. Epilepsy in children with cerebral palsy. *Pediatr Neurol* 1998; **19:** 31–6.
20. Carlsson M, Hagberg G, Olsson I. Clinical and aetiological aspects of epilepsy in children with cerebral palsy. *Dev Med Child Neurol* 2003; **45:** 371–6.
21. Kulak W, Sobaniec W, Kubas B, et al. Spastic cerebral palsy: clinical magnetic resonance imaging correlation of 129 children. *J Child Neurol* 2007; **22:** 8–14.
22. Veelken N, Hagberg B, Hagberg G, Olow I. Diplegic cerebral palsy in Swedish term and preterm children. Differences in reduced optimality, relations to neurology and pathogenetic factors. *Neuropediatrics* 1983; **14:** 20–8.
23. Singhi P, Jagirdar S, Khandelwal N, Malhi P. Epilepsy in children with cerebral palsy. *J Child Neurol* 2003; **18:** 174–9.
24. Pagliano E, Fedrizzi E, Erbetta A, et al. Cognitive profiles and visuoperceptual abilities in preterm and term spastic diplegic children with periventricular leukomalacia. *J Child Neurol* 2007; **22:** 282–8.
25. O'Callaghan ME, MacLennan AH, Haan EA, Dekker G. The genomic basis of cerebral palsy: a HuGE systematic literature review. *Hum Genet* 2009; **126:** 149–72.
26. Khwaja O, Volpe JJ. Pathogenesis of cerebral white mater injury of prematurity. *Arch Dis Child Fetal Neonatal* 2008; **93:** 153–61.