In contrast to the depression group, no significant differences in [oxyHb] increase during the task period, except a few seconds just after the start of the task, in the frontal channels were found between the schizophrenia and control groups during the word fluency task. The absence of significant differences was not in agreement with either the hypofrontality (Andreasen et al 1998) observed when the task performances of schizophrenic patients are poorer or hyperfrontality (Weinberger et al 2001) observed when the task performances are matched, but it was in agreement with the normal magnitude of frontal activation seen in verbal fluency tasks in some neuroimaging studies (for example, Frith et al 1995). Modified characteristics in the word fluency task used in the present study and nonsignificant difference in performances between the schizophrenic and control groups could partly explain the results. In the bilateral temporal channels, which are assumed to correspond to the perisylvian area, [oxyHb] increases were smaller than those in the control group. This result is consistent with results of previous PET and SPECT studies that showed rCBV reduction in the temporal cortex of schizophrenic patients (Ragland et al 2001; Riehemann et al 2001) and might reflect an impaired function of the temporal cortex.

Other findings in schizophrenia were that the [oxyHb] change difference between the schizophrenic and control subjects were found not during the task period but immediately after the start and end of the task period (i.e., the small trough of [oxyHb] at the start of the task period and the [oxyHb] re-increase in the posttask period, respectively). Both these results suggest the inefficient activation of the prefrontal cortex along the time course of the task demand in schizophrenic patients: for example, insufficient activation at the start of the task and unnecessary activation after the task was finished. Such a detailed examination of the rCBV change pattern along the time course was made possible by the high time resolution of the NIRS machine.

The cognitive task specificity associated with the above results should be noted. [OxyHb] increases in the frontal channels in the patient groups were smaller in the word fluency task but were larger in the finger-tapping task than those in the control group. These differences between the tasks suggest that the obtained findings cannot be explained by general and nonspecific factors, such as impaired vascular responsiveness irrespective of neural activation, but by the difference in neural activation in the frontal cortex. The altered activations of the frontal channels observed in the present study, therefore, are assumed to reflect cerebral blood flow aspects of the frontal lobe dysfunction in depression and schizophrenia.

The NIRS methodology used in the present study has limitations: NIRS enables measurement of Hb concentration changes 1) only as relative values but not as absolute values; 2) only in the cortex immediately beneath the probes but not in deeper brain structures; 3) with a high time resolution but with a poor spatial resolution in imaging methodologies for cerebral blood flow and metabolism; and 4) not only in the brain but also in more surface structures, such as the skin and skull. We examined the influence of the first limitation by superimposing the individual waveforms of [oxyHb] changes in each group, and found essentially similar time courses of [oxyHb] changes among the subjects in each group. These results confirm that summing the individual waveforms of [oxyHb] changes within each group generates a significant grand average waveform. The second and third limitations could be resolved in future studies on simultaneous measurements by NIRS and other neuroimaging methodologies, such as PET, SPECT, and fMRI. The fourth limitation can be resolved if blood volumes in the skin, muscle, and skull are constant. Hence, we monitored the movements of a subject's head and extremities during the experiment and excluded the data contaminated with such artifacts.

There are three points that should be improved in the present study. The first point is the limited cerebral regions that could be measured with NIRS probes. A considerable nonmeasured area existed between the areas covered by the frontal and temporal probes, owing to their arrangement on the skull, which prevented the examination of the lower posterior frontal cortex. The second point is the subjects' characteristics. The sample sizes were small, the gender ratios were somewhat skewed, and the patients' symptoms were rather mild. The third point is that all the patients were taking medications at the time of the examination, and it is possible that at least part of the observed findings could result from the effects of these psychotropic drugs. Further studies are needed to improve on these three points.

In conclusion, the characteristic patterns of [oxyHb] changes in the frontal lobe were assessed for depression and schizophrenia in this study. The findings coincided with the proposed frontal lobe dysfunction in depression and schizophrenia determined by PET, SPECT, and fMRI and clarified in more detail the dysfunction along the time course of [oxyHb], owing to the high time resolution of the NIRS machine. Near-infrared spectroscopy, with its noninvasiveness and high time resolution, could be a useful research tool for the examination of brain functions in psychiatric disorders when combined with other methodologies, as well as a clinically useful tool for the diagnosis and treatment of individual psychiatric patients in the near future.

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Sex and age dependencies of cerebral blood volume changes during cognitive activation: a multichannel near-infrared spectroscopy study

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In this study, we measured the change in cerebral hemoglobin concentrations during a cognitive task using multichannel near-infrared spectroscopy (NIRS), and investigated the relationship between regional cerebral blood volume and sex, age, and task performance. Thirty-nine healthy volunteers (24 males and 15 females; mean age, 33.0 years) participated after giving their informed consent and performed a word fluency task. The relative oxy-hemoglobin concentration ([oxy-Hb]) was measured using frontal and temporal probes with two sets of 24-channel NIRS machines. The effects of sex, age, and task performance on [oxy-Hb] changes were analyzed using analysis of covariance: with sex, age, and task performance as independent variables, and [oxy-Hb] changes as dependent variables, and years of education as covariates. The effects on [oxy-Hb] increase were significant in many channels in the frontal and temporal probes for sex, that is the most prominent effect, and in a few frontal channels for age: [oxy-Hb] increases were larger in males than in females, and in the young than in the middle-aged. The effects on [oxy-Hb] increase were not significant for task performance, but [oxy-Hb] increases in subjects with low performance tended to be larger than those in subjects with high performance. The results demonstrated that multichannel NIRS could detect cerebral activation during cognitive tasks and clarify sex- and age-dependent differences in such cerebral activation. Sex- and age-dependent differences in cerebral activation, as demonstrated in the present study, should be considered when interpreting cerebral blood volume, cerebral blood flow, and cerebral glucose metabolism data.

Keywords: Near-infrared spectroscopy; Cerebral blood volume; Sex differences; Aging; Performance; Word fluency test

Introduction

Recent advances in neuroimaging methodologies have enabled their application to the assessment of cognitive brain function in healthy subjects and diagnostic examination of neurological and psychiatric disorders. In interpreting obtained results, effects of demographic parameters such as sex and age should be incorporated in data analyses.

A number of neuroimaging studies investigated the effects of sex and age on cognitive brain function. The greater lateralization of brain activation in males than in females was reported in language tasks using functional magnetic resonance imaging (fMRI) (Shaywitz et al., 1995; Vikingstad et al., 2000) and positron emission tomography (PET) (Jaeger et al., 1998). An age-dependent decline of brain activation was also well reported in cognitive tasks such as a calculation task (NIRS) (Hock et al., 1995), WAIS-III (NIRS and fMRI) (Kwee and Nakada, 2003), and a color-word-stroop task (NIRS) (Schroeter et al., 2003).

The effects of sex and age on cognitive function could interact with the performance of the task employed. Sex and age dependencies of cognitive functions have been well established: females perform better in verbal tasks, whereas males excel in spatial tasks (Collins and Kimura, 1997; Halpern, 2000; Silverman et al., 1996); the elderly perform more poorly than younger subjects in various cognitive tasks (Grady and Craik, 2000). However, as far as the authors surveyed, sex and age effects on brain function in relation to their interaction with task performance have not been fully clarified. This can cause problems in the interpretation of sex and age effect results: sex- or age-related differences in brain function could be the direct effects of sex or age, or the indirect effects of different task performances due to sex and age.

Near-infrared spectroscopy (NIRS) is a recently developed neuroimaging methodology. Its noninvasiveness (Ito et al., 2000), portability, natural setting of examination, and low running cost have enabled the examination of male and female subjects over a wide age range, from infancy to old age while performing cognitive tasks. Therefore, the effects of sex, age, and task performance should be studied in more detail using NIRS data interpretation.

NIRS can detect changes in the concentration of cerebral blood hemoglobins, such as oxy-hemoglobin ([oxy-Hb]), deoxy-hemoglobin ([deoxy-Hb]) and total-hemoglobin ([total-Hb]), which is the sum of [oxy-Hb] and [deoxy-Hb]. The principle of NIRS is based on the modified Lambert-Beer Law stating that the absorption of near-infrared light by oxy- and deoxy-hemoglobin varies with wavelength. Both [oxy-Hb] increases and [deoxy-Hb] decreases in NIRS are interpreted to reflect cortical activation

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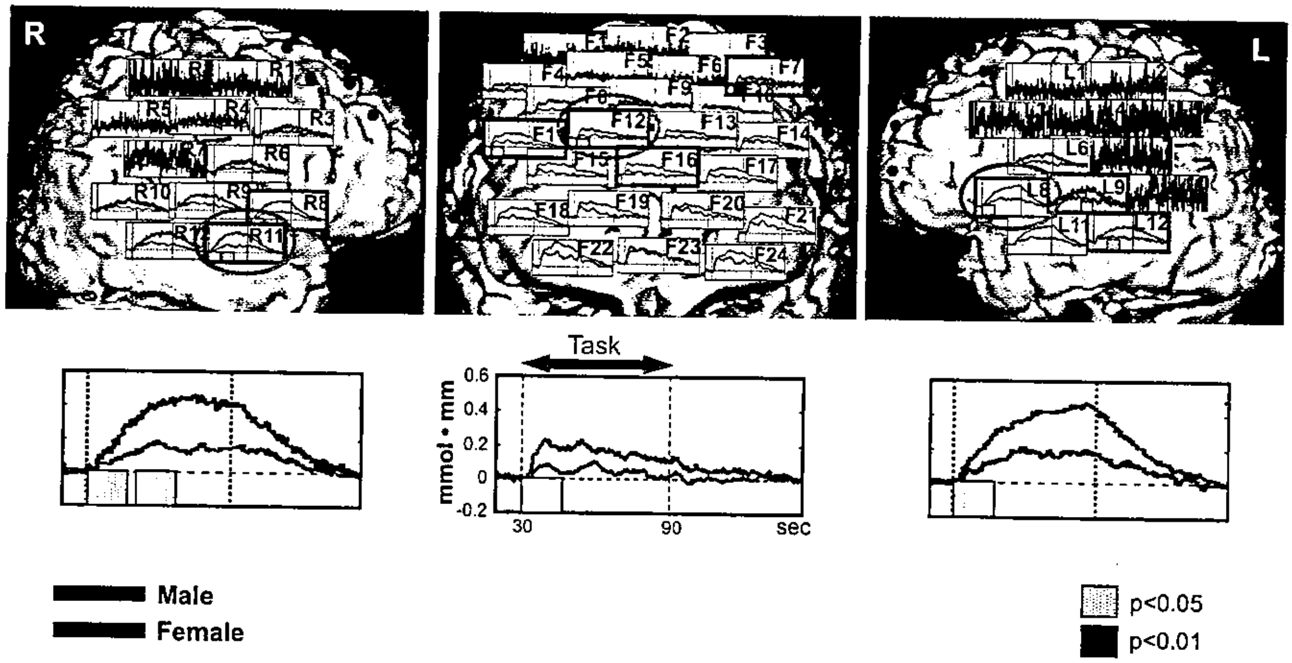

Fig. 1. Male group vs. female group. The upper figures show the grand average waveforms of oxy-hemoglobin changes obtained by NIRS in male (red line) and female subjects (blue line), superimposed on a reconstructed cerebral cortex image. The measurement channels with significant differences are displayed with thick red border lines and the time segments with significant differences in the channels are marked with red squares (filled squares, $P < 0.01$; unfilled squares, $P < 0.05$). Three representative channels (circled in yellow) are enlarged below.

because they have been shown to correspond to regional cerebral blood flow (CBF) in fMRI studies (Kleinschmidt et al., 1996; Toronov et al., 2001). Correlations of hemoglobin data in NIRS with CBF data simultaneously obtained using other methodologies have been shown to be stronger for [oxy-Hb] than for [deoxy-Hb] in a fMRI study (Strangman et al., 2002b) and a laser-Doppler study (Malonek et al., 1997). The differences are interpreted to be due to poorer contrast-to-noise ratios in the measurements of [deoxy-Hb] than in those of [oxy-Hb] (Strangman et al., 2002b). The technological principle of NIRS was reviewed in detail by Koizumi et al. (1999), Strangman et al. (2002a), and Obrig and Villringer (2003).

For human cognitive function, a number of NIRS studies have been published since 1993 (Chance et al., 1993; Hoshi and Tamura, 1993; Kato et al., 1993; Villringer et al., 1993) using various tasks: a confrontational writing task (Yamamoto et al., 1999), a speech listening task (Sato et al., 1999; Sakai et al., 2001), a calculation task (Hock et al., 1995), WAIS-III (Kwee and Nakada, 2003), and a word fluency (generation) task (Fallgatter et al., 1997; Herrmann et al., 2003; Hock et al., 1997; Matsuo et al., 2000, 2002; Suto et al., 2004; Watanabe et al., 1998). Some of these studies, using only one or two channels over a limited frontal area, investigated the effect of age, but not that of sex, on brain activation (Hock et al., 1995; Kwee and Nakada, 2003).

In the present study, we measured the changes in cerebral hemoglobin concentration using multichannel NIRS machines during a cognitive task, and investigated the relationship of regional cerebral blood volume (CBV) with sex, age, and task performance. The purpose of this study was to elucidate the effects of sex, age, and task performance on cerebral activation over larger brain areas along the task time course.

Materials and methods

Subjects

The subjects were 39 healthy volunteers (24 males and 15 females; mean age, 33.0 years; male mean age, 33.5 years ranging from 25 to 47; female mean age, 32.1 years ranging from 23 to 52; mean years of education, 16.8 years ranging from 12 to 22). Written informed consent was obtained from the subjects before the start of the investigation. All the subjects were right-handed as assessed by the Edinburgh Handedness Inventory (Oldfield, 1970). The handedness scores were mean 0.99, SD 0.05 for men and mean 0.96, SD 0.08 for women. None of the subjects had pre-existing neurological or psychiatric disorders. The present study was approved by the Institutional Review Board of Gunma University Graduate School of Medicine.

Task procedure

The subjects performed a word fluency task, which consisted of a 30-s pretask baseline period, a 60-s task period, and a 60-s post-task baseline period. The subjects were instructed in detail, in the instruction period before the NIRS measurements, to generate as many words as possible in the task period until they fully understood the task requirements. The initial syllables assigned were changed every 20 s (/a/, /ka/ and /sa/, respectively) during the 60-s task period. The words generated were monitored for correct and incorrect responses, and the number of correct words generated was determined as the subjects' task performance.

In the pretask and the post-task baseline periods, the subjects were instructed to repeat a train of syllables "/a/, /i/, /u/, /e/, /o/". The subjects were seated in a comfortable chair with their eyes open throughout the measurements. The timing of syllable changes was delivered to the subjects by the examiners' cue. The subjects were asked to avoid body movements such as neck movements, strong biting, and blinking during the NIRS measurements because they had been identified as most influential in the preliminary artifact-evoking study. The movements of the subjects were monitored throughout the examination.

NIRS measurements

NIRS machine

Relative [oxy-Hb], [deoxy-Hb], and [total-Hb] were measured with two sets of 24-channel NIRS machines (Hitachi ETG-100). The machine uses two wavelengths of the near-infrared light (780 and 830 nm), whose difference in the absorption spectrum enables the measurement of [oxy-Hb] and [deoxy-Hb] (Maki et al., 1995). The distance between pairs of emission and detector probes was set at 3.0 cm, which enabled cerebral blood volume measurements at a 2- to 3-cm depth from the skin of the head, that is, the surface of cerebral cortices (Hock et al., 1997; Toronov et al., 2001).

Probe positions and measurement points

The probes of the NIRS machines were fixed with thermoplastic shells and placed on the subject's frontal and bilateral temporal areas. The 16 probes on the subject's frontal area can measure the relative concentrations of hemoglobins at 24 measurement points in a 9 × 9 cm^2 area. The lowest probes in the frontal shell were positioned along the Fp_1–Fp_2 line according to the international 10/20 system used in electroencephalography. Each of the nine probes on the subject's bilateral temporal areas can measure the relative concentrations of hemoglobins at 12 measurement points in a 6 × 6 cm^2 area. The central probe in each temporal shell was positioned at the midpoint between the vertex and external ear hole. The measurement points were superimposed on a magnetic resonance image of a three-dimensionally reconstructed cerebral cortex of a subject. Measurement points were labeled as F1–F24 for the frontal channels, L1–L12 for the left temporal channels, and R1–R12 for the right temporal channels, from top to bottom.

Measurement parameters

The rate of data sampling was 0.1 s. Obtained data were analyzed using the "integral mode": the pretask baseline was determined as the mean over a 10-s period just before the task period, the post-task baseline was determined as the mean 5 s, 50 s after the task period, and linear fitting between the pre- and the post-task baselines was applied to data between the two baselines. The moving average method was adapted for analyzed data to remove short-term motion artifacts (moving average window: 1 s).

Data analyses

Data contaminated with artifacts due to body movements were excluded from further analyses. For data analyses, the task and the post-task periods were divided into six time segments along the time course: the task period into three segments ("task 1", "task 2", and "task 3" segments for the first, the second, and the third 20-s periods, respectively) and the post-task period into three segments ("post-task 1", "post-task 2", and "post-task 3" segments for the first 20-s, the second 20-s, and the third 15-s period, respectively). Obtained [oxy-Hb] data were averaged for each subject over these six time segments. Data from the channels positioned over a hair-covered area often showed a low signal-to-noise ratio because of the paucity of near-infrared light detected, hence were excluded from the analyses when the standard deviations of [oxy-Hb] during the pretask period exceeded 0.035: four channels in the frontal shell (F1, F2, F3, and F6) and seven left channels (L1, L2, L3, L4, L5, L7, and L10) and five right channels (R1, R2, R4, R5, and R7) in the temporal shells.

For the remaining 32 channels with a sufficient signal-to-noise ratio, the effects of sex, age and task performance on [oxy-Hb] changes were analyzed over the six time segments using the three-way analysis of covariance (ANCOVA): with sex (24 males and 15 females), age (19 young and 20 middle-aged subjects, divided by the median age of the subjects, 29.0 years; the young group: mean age 26.3, SD 1.6 years; the middle-aged group: mean age 39.4, SD 6.7 years), and task performance (19 subjects with low performance and 20 subjects with high performance, divided by the median performance of 17 words) as independent variables, and [oxy-Hb] changes during the six time segments as dependent variables, and years of education as covariates. Statistical analysis was performed using SPSS 11.0J software (Tokyo, Japan).

In addition, the effects of the language nature of the task, which was employed in the present study, were examined for the left–right hemisphere asymmetry of [oxy-Hb] changes by comparing the channel pairs of mirror-imaged positions using a t test.

Results

The three-way ANCOVA demonstrated significant main effects of two independent variables, sex, and age (Table 1). Significant main effects of sex were obtained in four frontal channels (F7, F11, F12, F16) out of 20 channels analyzed, in three left temporal channels (L8, L9, L12) out of five channels analyzed, and two right temporal channels (R8 and R11) out of seven channels analyzed (F = 4.4–8.9, df = 1, P = 0.046–0.006) (Fig. 1). In all the channels, [oxy-Hb] increases were larger in male than in female subjects. Time segments with significant differences were obtained only for task 1 in the frontal channels and from task 1 to task 3 in the temporal channels. Significant main effects of age were obtained only in four frontal channels (F11, F14, F15, F19) out of 20 channels analyzed (F = 4.7–6.2, df = 1, P = 0.037–0.018) (Fig. 2). In these four channels, [oxy-Hb] increases were larger in the young than in the middle-aged subjects. The time segment with a significant difference in [oxy-Hb] changes was confined only to the task 1 segment. No significant main effect of task performance was obtained, but the mean [oxy-Hb] increases tended to be larger in the subjects with low performance than in the subjects with high performance in the two frontal (F19; post-task 1, F21; post-task 2), one left temporal (L9; post-task 1), and two right temporal (R3; task 1, R11; task 3) channels (F = 2.9–3.8, df = 1, P = 0.98–0.062) (Fig. 3). Significant interactions among independent variables were obtained in two frontal and four temporal channels: the interaction of sex with age in the task 3 and post-task 1 segments in one frontal channel (F20; F = 5.1 and 4.9, df = 1, P = 0.032 and 0.035, respectively). The interaction of age with task performance in the task 1 and task 3 segments in one frontal channel (F5) and in the task 1 segment in one right temporal channel (R8; F = 4.4, df = 1, P = 0.045). The interaction of sex with task performance in one frontal (F5), one left (L9) and two right (R10 and R12) temporal channels (F = 4.2–6.5, df = 1, P = 0.048–0.016) from task 1 to task 3 segments and the interaction of sex with age and task

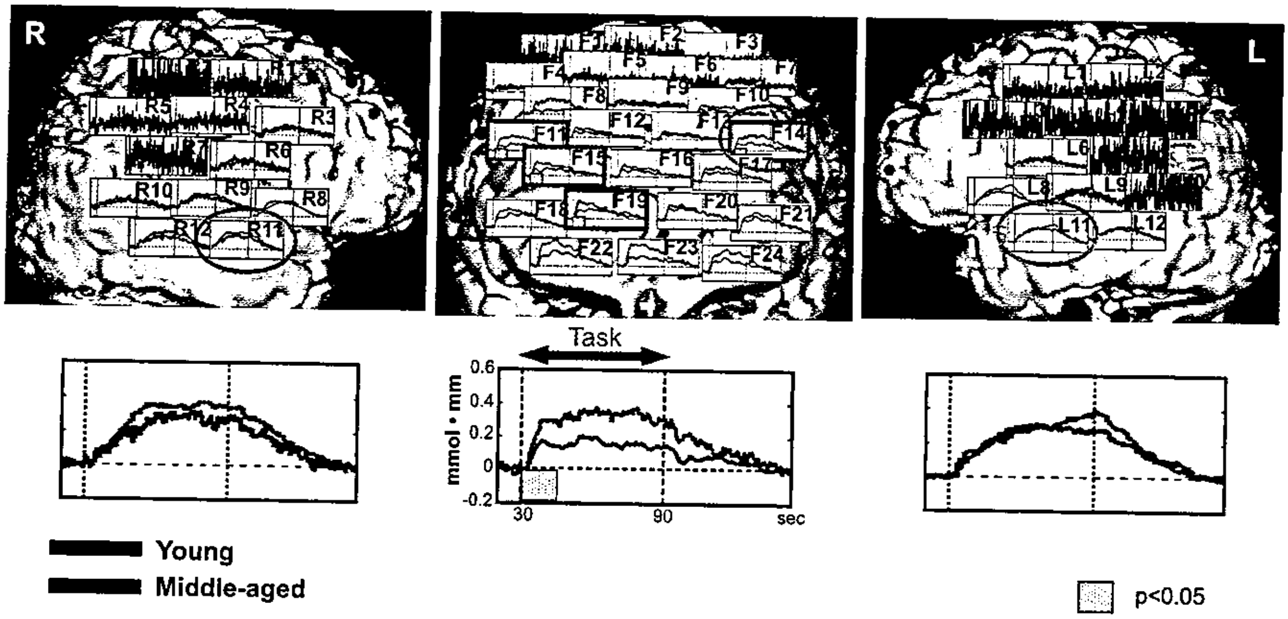

Fig. 2. Young group vs. middle-aged group. The upper figures show the grand average waveforms of oxy-hemoglobin changes obtained by NIRS in young (red line) and middle-aged subjects (blue line), superimposed on a reconstructed cerebral cortex image. The measurement channels with significant differences are displayed with thick red border lines and the time segments with significant differences in the channels are marked with red squares ($P < 0.05$). Three representative channels (circled in yellow) are enlarged below.

performance in the post-task 1 and post-task 2 segments in one right temporal channel (R8; $F = 5.0$ and 5.7, $df = 1$, $P = 0.034$ and 0.023, respectively). Covariate effects of the years of education were significant in eight frontal channels (F4, F5, F15, F18, F19, F20, F21, F23) and three left (L8, L11, L12) and six right (R6, R8, R9, R10, R11, R12) temporal channels mainly in post-task period (from task 3 to post-task 3) for positive correlations with [oxy-Hb] increases ($F = 4.2–26.0$, $df = 1$, $P = 0.049–0.000$).

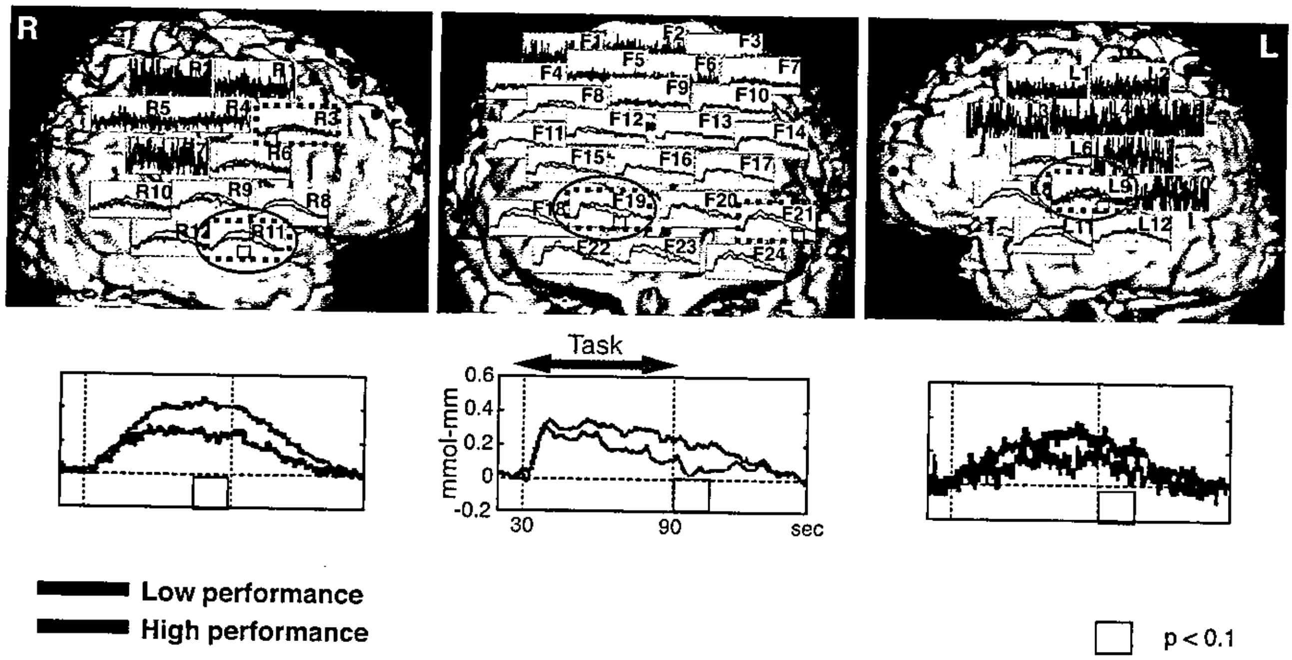

Fig. 3. Group with low performance vs. group with high performance. The upper figures show the grand average waveforms of oxy-hemoglobin changes obtained by NIRS in subjects with low performance (red line) and high performance (blue line), superimposed on a reconstructed cerebral cortex image. The measurement channels in which the mean [oxy-Hb] increases tended to be larger in the subjects with low performance than in the subjects with high performance are displayed with dotted red border lines and the time segments with differences in the channels are marked with red squares ($P < 0.1$). Three representative channels (circled in yellow) are enlarged below.

Table 1
Summary of the ANCOVA results for [oxy-Hb] changes

Channel	Time segment	Main effects			Interaction effects				Covariate
		sex (S)	age (A)	performance (P)	S × A	S × P	A × P	S × A × P	Education years
F5	task 1	1.9 (0.180)	2.5 (0.127)	0.2 (0.693)	0.2 (0.701)	4.4 (0.044)*	5.8 (0.023)*	1.5 (0.225)	1.7 (0.200)
	task 3	0.1 (0.784)	0.3 (0.603)	0.0 (0.996)	1.1 (0.292)	6.4 (0.017)*	5.1 (0.032)*	0.0 (0.937)	0.4 (0.526)
F7	task 1	8.9 (0.006)**	3.9 (0.059)	0.1 (0.733)	1.6 (0.215)	1.3 (0.263)	0.2 (0.655)	0.5 (0.480)	0.3 (0.592)
F11	task 1	5.0 (0.033)*	5.9 (0.022)*	0.1 (0.798)	1.5 (0.235)	0.6 (0.464)	0.4 (0.542)	0.0 (0.835)	0.1 (0.726)
F12	task 1	5.9 (0.021)*	3.3 (0.081)	1.5 (0.232)	1.9 (0.174)	1.2 (0.284)	0.1 (0.754)	0.5 (0.476)	0.0 (0.918)
F14	task 1	3.2 (0.083)	6.2 (0.018)*	0.2 (0.681)	0.2 (0.650)	0.1 (0.736)	0.1 (0.818)	0.0 (0.875)	0.6 (0.433)
F15	task 1	3.9 (0.058)	5.1 (0.031)*	0.1 (0.769)	0.4 (0.538)	0.9 (0.349)	0.0 (0.828)	0.0 (0.840)	0.0 (0.981)
F16	task 1	5.0 (0.033)*	3.0 (0.546)	0.1 (0.778)	2.3 (0.140)	0.1 (0.742)	0.0 (0.839)	0.1 (0.780)	0.0 (0.936)
F19	task 1	2.4 (0.133)	4.7 (0.037)*	0.2 (0.657)	0.2 (0.623)	0.0 (0.964)	0.3 (0.611)	0.1 (0.824)	0.1 (0.755)
F20	task 3	0.1 (0.724)	0.0 (0.898)	0.5 (0.498)	5.1 (0.032)*	1.3 (0.272)	0.9 (0.357)	0.2 (0.627)	1.5 (0.237)
	post-task 1	0.1 (0.802)	0.3 (0.586)	2.6 (0.116)	4.9 (0.035)*	0.1 (0.715)	0.4 (0.558)	0.1 (0.717)	1.7 (0.201)
L8	task 1	6.1 (0.020)*	0.0 (0.850)	0.6 (0.432)	0.0 (0.850)	0.9 (0.342)	0.0 (0.947)	0.0 (0.883)	0.1 (0.816)
L9	task 3	4.5 (0.043)*	0.1 (0.818)	2.6 (0.117)	0.7 (0.424)	4.3 (0.048)*	0.1 (0.741)	1.2 (0.288)	0.4 (0.550)
L12	task 2	4.4 (0.046)*	0.1 (0.706)	0.2 (0.655)	0.1 (0.742)	0.1 (0.750)	1.1 (0.292)	2.8 (0.104)	0.1 (0.966)
R8	task 1	4.4 (0.045)*	0.2 (0.630)	0.9 (0.348)	0.1 (0.777)	0.2 (0.660)	0.1 (0.809)	0.3 (0.566)	0.1 (0.817)
	task 2	4.8 (0.037)*	0.0 (0.986)	0.9 (0.345)	0.5 (0.466)	1.6 (0.212)	0.9 (0.339)	1.0 (0.337)	1.0 (0.328)
	post-task 1	1.4 (0.247)	0.2 (0.691)	0.9 (0.344)	0.5 (0.499)	2.5 (0.125)	4.4 (0.045)*	5.0 (0.034)*	13.3 (0.001)**
	post-task 2	0.0 (0.906)	1.3 (0.270)	2.0 (0.165)	0.1 (0.768)	0.6 (0.464)	3.3 (0.078)	5.7 (0.023)*	26.0 (0.000)**
R10	task 2	2.1 (0.156)	0.1 (0.760)	0.2 (0.649)	0.4 (0.527)	5.1 (0.031)*	0.0 (0.985)	1.3 (0.265)	0.1 (0.759)
	task 3	0.9 (0.343)	0.1 (0.815)	0.1 (0.709)	0.5 (0.480)	6.5 (0.016)*	1.1 (0.299)	0.8 (0.383)	2.9 (0.099)
R11	task 1	5.1 (0.031)*	0.0 (0.907)	0.6 (0.427)	0.1 (0.763)	0.3 (0.585)	1.3 (0.262)	0.0 (0.897)	0.2 (0.684)
	task 2	4.4 (0.045)*	0.2 (0.635)	1.5 (0.224)	0.1 (0.714)	2.7 (0.109)	2.9 (0.098)	0.3 (0.574)	3.6 (0.066)
R12	task 2	1.5 (0.228)	0.1 (0.753)	0.3 (0.575)	0.5 (0.490)	4.2 (0.048)*	0.5 (0.488)	0.6 (0.434)	2.9 (0.098)
	task 3	1.1 (0.302)	0.2 (0.645)	0.5 (0.480)	0.4 (0.510)	5.6 (0.024)*	1.3 (0.260)	0.6 (0.462)	7.2 (0.012)*

F and P values are shown only for the channels and time segments with statistical significance in main and interaction effects as 'F (P)'.
*$P < 0.05$.
**$P < 0.01$.

All the t tests examining the left–right hemisphere asymmetry of [oxy-Hb] changes showed no significant differences.

Discussion

The present study examined the effects of sex, age, and task performance on brain activation during a cognitive task using multichannel NIRS. The statistically significant main effects on [oxy-Hb] increase were obtained for sex in many channels in the frontal and temporal probes and for age in a few frontal channels: [oxy-Hb] increases were larger in males than in females, and in the young than in the middle-aged. On the other hand, the main effect of task performance on [oxy-Hb] increases was not significant, although [oxy-Hb] increases in subjects with low performance tended to be larger than those in subjects with high performance.

The obtained differences in cerebral activation for sex during the cognitive task are consistent with a previous PET study by Buckner et al. (1995) that demonstrated a larger prefrontal activation in male than in female subjects during a verb generation task. They interpreted the result as being derived from the difference in difficulty performing the same task between male and female subjects based on the better performance of the female subjects; in general, female subjects perform language tasks more efficiently than male subjects (Halpern, 2000). However, this explanation is not relevant for the present study because the task performance was equal between the male and the female subjects.

The age-dependent decrease in cerebral activation during the cognitive task is consistent with previous NIRS studies using a calculation task (Hock et al., 1995), WAIS III (Kwee and Nakada, 2003), and a color-word-stroop task (Schroeter et al., 2003), and with a PET study using a serial verbal learning test (Hazlett et al., 1998). The authors of these previous reports assumed the decrease to be the consequence of structural cortical changes (decrease of region-specific cortical thickness and shrinkage of neuronal dendrites), functional cortical changes (decline of frontal activation), or the decline of working memory utilization due to aging. The rather low age range of the subjects in the present study (23–52 years old) and the absence of a significant difference in the task performance between the young and the middle-aged subjects could exclude the first and the third interpretations mentioned above, respectively.

Larger [oxy-Hb] increases in the subjects with high performance, although the differences did not reach statistical significance, are consistent with the findings of task performance dependencies of cerebral metabolism activation in a PET study using a verbal fluency test (Parks et al., 1988), and CBV changes in an NIRS study using the calculation task (Hoshi and Tamura, 1993). The finding could be interpreted as indicating greater effort and hence less efficient processing in poor performers.

As for the laterality of brain activation in the language task, previous brain imaging studies reported a left lateralized pattern with some inconsistencies: both in male and female subjects (Buckner et al., 1995; Frost et al., 1999; Schlosser et al., 1998) or only in male subjects (Shaywitz et al., 1995; Vikingstad et al., 2000). The absence of such a left lateralized pattern in the present study could be explained by the task characteristics employed. The change in [oxy-Hb] was determined as the difference between the syllable-repeating condition in the pretask baseline period and the word-fluency condition in the task period. Such a measurement could result in subtracting the utterance process-related activation from the word generation-related activation, resulting in an altered lateralization of brain activation. In fact, orthographic, phonological, and semantic processing in language tasks were reported to be differentially lateralized in a fMRI study (Shaywitz et al., 1995).

The distinction between baseline CBF and activation-related changes in CBF and CBV should be stressed. The present study, as well as the studies mentioned above, examined activation-related changes in CBF and CBV using NIRS, PET, SPECT, and fMRI.

The effects of sex and age on baseline levels of CBF, CBV, and cerebral glucose metabolism measured at the resting state have been reported with rather different results from those of activation studies. Studies on sex differences in CBF, CBV, and cerebral glucose metabolism yielded variable results in the resting state as well as in the activation state. Compared with male subjects, cerebral perfusion and metabolism in female subjects were reported to be higher both in terms of global CBF (Gur et al., 1982; Rodriguez et al., 1988) and regional CBF in the temporal regions (Ragland et al., 2000), but to be lower in terms of cerebral glucose metabolism in the temporal-limbic region (Gur et al., 1995), and to be without significant difference in cerebral glucose metabolism (Azari et al., 1992). The results of these studies in the resting state should be taken into consideration when interpreting the results of activation studies: for example, increased baseline perfusion and metabolism could result in decreased activation due to the ceiling effect. However, as far as the authors surveyed, no studies that examined baseline and activation levels simultaneously in the same subjects have been carried out.

The age-dependent decrease in CBF, CBV, and cerebral glucose metabolism in the resting state as well as in the activation state, in contrast, has been frequently replicated (Marchal et al., 1992; Meltzer et al., 2000; Murphy et al., 1996; Pantano et al., 1984; Yamaguchi et al., 1986). The age-dependent decrease in cerebral perfusion and metabolism in the resting state is hypothesized to result from the combined effects of direct neuronal loss, cellular biological impairment, and functional deafferentation (Marchal et al., 1992) and such mechanisms could also underlie the age-dependent decrease in activation in terms of cerebral perfusion and metabolism.

Two limitations in the present study should be addressed. First, NIRS measures the hemoglobin concentration changes only as relative values. The estimation of hemoglobin concentration changes depends on the assumed near-infrared pathlength; hence, differences in this pathlength among the subjects could influence the obtained results. The result for age effect, at least, should be interpreted carefully because pathlength was reported to be weakly dependent on age, approximately 5% for 10 years at the most (Duncan et al., 1996), but not on sex (Duncan et al., 1995). Second, the NIRS probes employed in the present study covered only limited cerebral regions. We could not measure regional CBV over gap areas between the frontal and temporal probes as well as between the parietal and occipital areas, and the left gap area is assumed to correspond to Broca's area. Measurement of regional CBV in Broca's area could demonstrate different results regarding age, sex, and task performance dependencies. Further studies are required to address these limitations.

In conclusion, the present study demonstrated that multi-channel NIRS can detect cerebral activation during cognitive tasks and can also clarify sex- and age-dependent differences in such cerebral activation. Sex- and age-dependent differences in cerebral activation obtained in the present study should be considered when interpreting CBF, CBV, and cerebral glucose metabolism data.

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Characteristics of a Japanese Adult Twin Database of High School Graduates

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This paper profiles a unique cohort of adult Japanese twins. The database contains more than 700 twin pairs, aged 18 to 66 years, who are all graduates of the secondary school attached to the faculty of education of the University of Tokyo. This school was established in 1948, when the study of twins was burgeoning in Japan, and about 10 to 20 pairs of twins have been admitted there every year to participate in studies on twins in education and in related projects. The zygosity of all twins was determined carefully on the basis of various sources. Data from the perinatal period to adulthood were linkable using ID numbers. Follow-up surveys in the field of medical genetics were performed in 1985, 1989 and 1999. For the third survey, which was sent and received exclusively by mail, the distribution and collection process was also assessed in detail. The response rate was around 40%, which statistically was influenced mainly by previous participation and sex. The limitation of this cohort is its selection bias concerning socioeconomic status and its imbalance in favor of monozygotic pairs.

Although many twin registries have been established around the world, including those in Asian countries (Sumathipala et al., 2000; Sung et al., 2002; Yang et al., 2002), no nationwide or population-based twin registry exists in Japan.

Previously, we have reported elsewhere on the progress of a database of young Japanese twins born between 1968 and 1990 and their families (Ooki & Yokoyama, 2003) through using methods such as collecting data from mothers belonging to associations for the parents of twins.

It is considerably more difficult to collect systematic data on adult twins in Japan than young twins. Birth records are not allowed to be used in Japan as a tool to identify individuals. Additionally, Sumathipala et al. (2003) report that in Sri Lanka birth records are not effective for obtaining data on adults. Therefore, we used alternative data sources such as the historically unique data source specific to Japan which we describe in this paper.

Methods

Participants

The twins were all graduates of the secondary education school attached to the faculty of education of the University of Tokyo. This school is famous in Japan because many twins study there. The school was established in 1948, when the study of twins was burgeoning in Japan, and has always had a unique entrance system. The school collects applications from twins in addition to applications from the general student population. About 50 pairs of twins of all sex and zygosity combinations, aged 11 or 12 years and living in the Tokyo metropolitan area, take an examination every year, of which about 10 to 20 pairs are admitted. The entrance examination taken by twins is the same as that taken by the other applicants. Mothers of all of the twin applicants must complete and hand in a Twins Protocol Questionnaire, which gathers information on family structure, obstetrical findings on the mothers, and the twins' physical growth, zygosity, and motor and mental development from birth through 11 years of age. One parent of each applicant, usually the mother, participates in a medical interview conducted by two or three interviewers (including, from 1988 on, two of the present authors, Ooki and Asaka), in which their responses to the questionnaire are checked carefully. During their six years of enrolment, the twins participate in observational studies for educational and related projects. Zygosity is diagnosed strictly by the best possible way, depending on the year of entrance. For example, many anthropometric characteristics and blood groups were used in the early years. Recently, many genetic markers or DNA polymorphisms have been used (Ooki & Asaka, 2004). All data from the perinatal periods through childhood, school age, and adulthood were linkable theoretically using individual specific ID numbers, though in practice the linkages have been partial so far.

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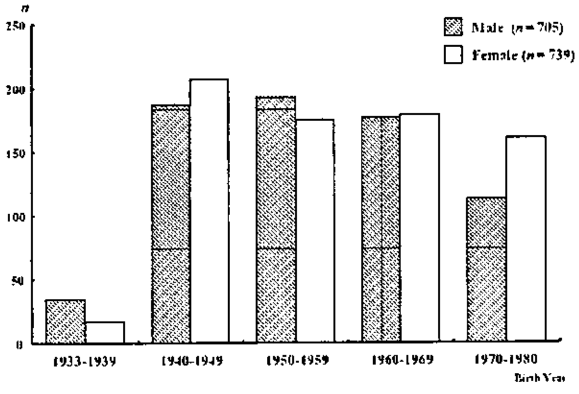

Figure 1
Birth-year distribution of the participants.

A total of 722 pairs of twins, consisting of 586 monozygotic (MZ) (283 male–male [MZM] and 303 female–female [MZF]) and 136 dizygotic (DZ) (44 male–male [DZSSM], 41 female–female [DZSSF], and 51 opposite-sex [DZOS]), have graduated from this school through 1999. Cumulative frequencies according to the combination of sex and zygosity are increasing almost proportionally year by year. The birth-year distribution of this cohort is shown in Figure 1.

Outline of Follow-Up Studies

The three follow-up studies are outlined in Table 1. The core items in all three surveys included questions about occupation, marital status, number of children, body weight and height, drinking habits, smoking habits, food preference, medical history. The third follow-up study was performed in 1999. Considering the 10-year gap between the second and third surveys, we substantially reconstructed the database for future studies (Ooki, 2002). The process of finding and contacting participants by mail was also examined.

Mailed Survey in the Third Follow-Up Study

Figure 2 provides a flowchart of the response and follow-up for the mailed survey. A total of 1444 twins (722 twin pairs) were identified as graduates through the end of the 1998–1999 school year. First, postcards containing a brief introduction to our research and an invitation to participate were sent to 1244 individuals, asking for their cooperation in the survey. As Table 2 shows, there were no marked differences in sex or zygosity as to the percentage of possible participants actually contacted. For 77 twins, we tried to get the address of one co-twin from the other, resulting in 14 more addresses. We then mailed the questionnaires, along with return envelopes, to 1215 individuals, and 1207 of those individuals received them. To raise the response rate, we asked again for the cooperation of 135 twins whose co-twin had already answered the questionnaire. This resulted in 57 more responses. Therefore, in total we received 435 responses.

Statistical Analysis

The response rate for the third follow-up study was summarized. Multiple logistic regression analysis was performed to clarify the factors affecting participation in the third follow-up survey. Each response in a completed questionnaire was determined as an independent variable. Zygosity, sex, and age at the time of the survey were determined as explanatory variables. Participation in the second survey was also considered. Data was not available on regular occupation and marital status of nonrespondents for all three surveys. Therefore, it was not possible to perform logistic regression analysis taking these factors into consideration because of insufficient informative sample size. We compared regular occupation and marital status of twins who completed all three surveys with those who did not complete all three surveys. All statistical analysis was performed using SAS for Windows (SAS Institute, 1997).

Results

The response rates to the questionnaire surveys according to zygosity and sex are shown in Table 2. Among same-sex pairs, the response rate was much lower among males than females. Among pairs, the response rate was highest in female MZ pairs and lowest in male DZ pairs. A total of 1215 questionnaires were mailed out with 1207 being received by participants. Of the 435 total respondents, 378 (Group 1) returned the answers directly while 57 other twins (Group 2) responded only after their co-

Table 1
Outline of Three Follow-Up Surveys

Survey	Year of survey	Number of all graduates	Age of graduates	Response rate (individuals)	Response rate (pairs)	Medical examination	Medical topics
First	1985	541 pairs	18–52	Unknown	28.6%(120/420)	44 pairs	Diabetes, personality
Second	1989	573 pairs	19–55	45.5% (435/957)	37.5%(167/445)	27 pairs	Bone mineral density, osteoporoses
Third	1999	722 pairs	19–66	36.0% (435/1207)	31.0%(174/562)	Not performed	Multiple risk factor syndrome

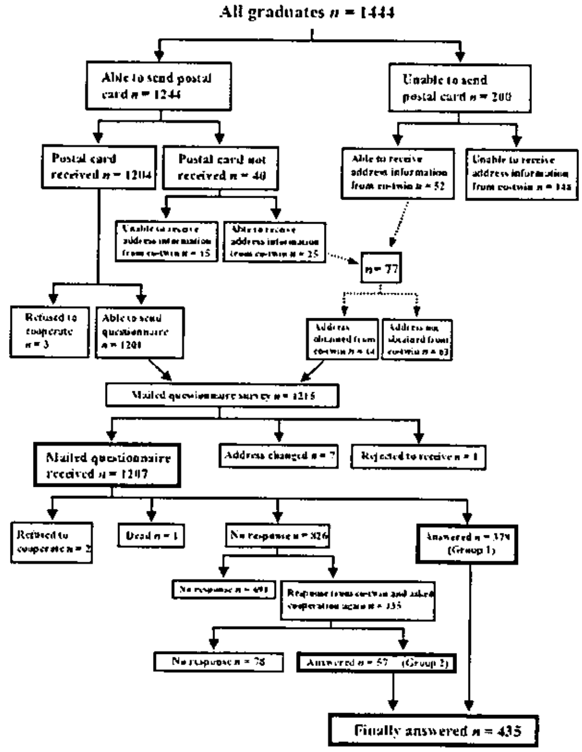

Figure 2

Summary of response and follow-up for the 1999 mailed survey.

twin asked them to. The response rate of females was higher than that of males in both groups.

The results of logistic regression analysis are as follows. Participation in the third survey depended mainly on sex ($p < .0001$, odds ratio = 2.174, 95% confidence intervals = 1.705 – 2.773). The response rate was much higher among females than males. If participation in the second survey was considered, this variable was the most influential ($p < .0001$, odds ratio = 4.295, 95% confidence intervals = 3.237 – 5.698), and in that case the effect of sex was diminished ($p < .0001$, odds ratio = 1.870, 95% confidence intervals = 1.410 – 2.480). No other variable, such as age at time of survey and zygosity met the .01 significance level for entry into the model.

Figure 3 summarizes participation in all three surveys. Data from single participants, a twin who responded without a response from the co-twin, in the first survey were excluded because of a lack of records. Thirty-six pairs participated in all three surveys. Of these, 33 pairs were MZ (5 male pairs and 28 female pairs) and 3 pairs were DZ (2 female pairs and 1 opposite-sex pair). The characteristics of the 272 (74 + 40 + 158) twins who did not respond to the third survey but had responded to at least one of the first two surveys were compared with 94 twins who remained in all three surveys. Significant sex difference was observed between the drop-out group and remainder group ($p < .0001$). More females (35%; 73/208) than males (13%; 21/158) remained in the survey. No significant difference was observed as to zygosity, marital status, regular occupation (yes or no), and age at time of survey between drop-out group and remainder group.

According to the third survey, at least 80.9% (352/435) of the twins expressed their intention to cooperate to some extent in the future. The future-cooperation rate among MZ (81.9%) was slightly

Table 2

Response Rate in the Third Follow-Up Survey According to Sex and Zygosity

	Monozygotic		Same-sex dizygotic		Opposite-sex dizygotic		Total
	Male	Female	Male	Female	Male	Female	
Participants (individual twins)	566	606	88	82	51	51	1444
Number of possible participants actually contacted (%)	80.9	86.1	85.2	84.1	80.4	82.4	83.6
Response rate (individual twins) (%)	26.6	45.6	21.3	30.4	43.9	47.6	36.0
	(122/458)	(238/522)	(16/75)	(21/69)	(18/41)	(20/42)	(435/1207)
Group 1*	23.1	40.8	17.3	24.6	31.7	38.1	31.3
	(106/458)	(213/522)	(13/75)	(17/69)	(13/41)	(16/42)	(378/1207)
Group 2**	28.6	54.3	27.3	80.0	50.0	57.1	42.2
	(16/56)	(25/46)	(3/11)	(4/5)	(5/10)	(4/7)	(57/135)
Response rate (complete twin pairs) (%)	18.7	42.9	11.4	30.3	39.5		31.0
	(39/209)	(106/247)	(4/35)	(10/33)	(15/38)		(174/562)

Note: *Returned questionnaire without prompting.
**Returned questionnaire after prompting by a co-twin.

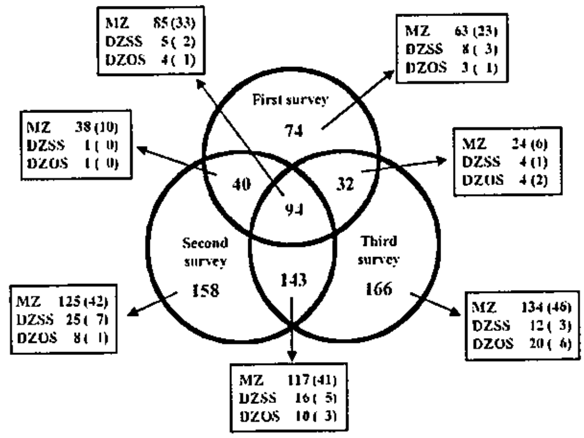

Figure 3

Circumstances of participation in the three follow-up surveys.

Note: Number of individuals are shown with the number of complete pairs in parentheses.

higher than that among DZ (76.0%). There was no sex difference (80.8% for males and 81.0% for females).

Discussion

Sex was the most important factor in deciding participation in the third follow-up survey, if participation in the second survey was not considered. According to population-based studies of twins performed by Cockburn et al. (2001), this tendency was especially marked in the younger adult age group. Among age groups by sex in the third survey, the highest response rate, 61.4% (97/158), was obtained from females in their thirties, and the lowest, 15.2% (14/92), was from males in their twenties. Participation in the second survey, when considered, became the most influential factor. Moreover, no sex difference was observed in the third survey as to participants' intentions for future surveys, supporting the tendency of fixation of the respondents. It was comparatively difficult to collect data from younger males, especially in DZ twins, from this cohort.

As shown in Figure 2, the effort to obtain address information for one co-twin from the other provided only minimal additional possible respondents.

In all surveys postage-paid envelopes were provided to respondents, so there was no need to stamp the envelopes individually. This may have contributed to the low response rate because, according to Duffy & Martin (2001), response rates increase by increasing the numbers of postage stamps.

Table 3 provides a description of the cohort. Though the sample is small, this cohort has several unique characteristics. Firstly, the zygosity of every participant was determined carefully on the basis of various sources. This is a very important feature, as zygosity classification using DNA markers is considerably troublesome in Japan, from an ethical viewpoint. Secondly, data from the perinatal period to adulthood can be linked by using individual ID numbers. This feature is advantageous for longitudinal studies or for examining the so-called fetal origin hypothesis.

The greatest limitation of this cohort is its selection biases based on the sampling process itself. All participants are graduates of the high school attached to the university. They lived in the Tokyo metropolitan area when they were enrolled at the school, and all passed the entrance examination. Participants are not representative of adults in general in Japan regarding socioeconomic status and some abilities. This selection bias would be fatal in some study designs. For example, the smoking rate was 41.7% for males and 8.4% for females in the third survey, which is much lower than in the Japanese general population, even considering age distribution.

Table 3

Outline of the Japanese Adult Twin Database

Name of cohort	Japanese adult twin database of high-school graduates
Country	Japan
Kind of ascertainment	Information from school and follow-up survey
Follow-up survey	Three times (see Table 1)
Opposite-sex twins	Yes
Number of pairs	722 (62 pairs are recruited newly in the next survey, planned for 2004. About 10 to 20 pairs will be added every year.)
Birth year	1933–1980
Primary interest	Genetic study of lifestyle (eating, smoking, drinking, and sleep habits, stress reaction, physical exercise, etc.) and lifestyle-related disease, cognitive ability, and longevity. Fetal origin hypothesis. Psychology as to twinship. Functional molecular genetics.
Comments	1. The zygosity of same-sex pairs was determined by detailed anthropometric similarity or DNA/genetic markers.
	2. Twins are followed longitudinally.
	3. All data from perinatal period to adulthood can be linked using individual ID numbers.
	4. Family data have been collected.
	5. Functional molecular genetic analysis of twin pairs who are discordant in relation to certain diseases are planned.

Moreover, there is a zygosity imbalance in favor of MZ twins, in contrast to the small sample size of DZ pairs, in relation to the MZ/DZ ratio in Japan (Imaizumi & Nonaka, 1997). This difference may also result from the entrance process. If only DZ twins who are similar in their abilities are admitted together, this could be a potential source of bias. It is difficult to estimate the long-term effects of these selection biases.

We need to maintain and make the most of this historically important cohort in Japan, but take into consideration these characteristics and limitations. The authors plan to study the functional molecular genetics of twin pairs who are discordant in relation to certain diseases; such twin studies have been rare in Japan.

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Comparison of Obstetric and Birthweight Characteristics Between the Two Largest Databases of Japanese Twins Measured in Childhood

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The purpose of this study is to compare obstetric and birthweight data of twin children from the two largest databases in Japan to estimate the difference between them in sample collection. The first group consisted of 1131 twin-pair school applicants, and the second group consisted of members' children from several maternal associations devoted to twins and included 951 pairs. All data were gathered by questionnaire. The mean birth years of the twins in these two databases were 1979 and 1995 respectively. The percentage of mothers treated with ovulation-stimulating drugs or in-vitro fertilization was markedly higher in the maternal associations group. Gestational age was around 1 week less in the maternal associations group, whereas birthweight according to gestational weeks and intrapair relative birth weight difference as a percentage according to zygosity showed little difference between both groups. We conclude that the obstetric and birthweight feature data from both groups should be considered to construct twin growth charts based on the methods of sample selection.

Many countries including Asian countries are constructing or have constructed large population-based twin registries. No systematic twin registry exists in Japan, however. Three main data sources are used to study the growth and development of multiples in Japan. First, vital statistics can be obtained; however access to individual information is prohibited. Second, data from large hospitals is used in the field of obstetrics, particularly for managing high-risk pregnancies. Twin data collection within this field is relatively easy; however, it has a selection bias towards high-risk infants, for example those with a very low birth weight.

To fill the gap between vital statistics and hospital data, a volunteer-based twin database was set up that is larger and less biased than hospital data and contains more detailed information than vital statistics. In addition, data regarding the condition of twins after birth has been gathered, a difficulty for both vital statistics and hospital data. The database consisted mainly of two independent groups. The purpose of this study is to compare the obstetric and birthweight characteristics of both samples to estimate the effect of different sample collection.

Materials and Methods

Subjects and Data Collection

The present sample consisted of two independent groups. The first group included 1131 mothers and their twin children living in the Tokyo metropolitan area. All of the twins in this group — the school applicant group — had applied between 1981 and 2004 to (though not necessarily enrolled in) the secondary school attached to the Faculty of Education at the University of Tokyo. The second group — the maternal associations group — consisted of 951 mothers from several associations for parents of multiples throughout Japan. The twins' age at data collection was between 11 and 12 years in the school applicant group, and between 0 and 15 years (mean 5.9) in the maternal associations group.

Mailed or hand-delivered questionnaires of nearly the same format were used to collect the data from the two groups. These included questions about family structure; obstetrical findings on the mothers; the twins' physical growth, zygosity, motor, language, and mental development; the twins' and parents' medical histories; and any behavioral problems the twins had had (Ooki & Yokoyama, 2003, 2004). Data on the socioeconomic and educational status of the parents were not obtained because of ethical limi-

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Table 1
Basic Characteristics of Present Database

			School applicants	Maternal associations
N			1131 pairs	951 pairs
Method of data collection			Handed questionnaire and interview	Mailed questionnaire
Year of data collection			1981–2004	2001–2004
District			Tokyo metropolitan area	All around Japan
Birth year of twin pairs		Mean ± *SD* (range)	1979 ± 7 (1968–1992)	1995 ± 4 (1986–2003)
Sex of twin individuals	Male/female		1057/1205	982/920
Zygosity	Monozygotic	MM/FF	336/418	235/210
	Dizygotic	MM/FF/MF/FM	77/69/70/57	95/109/95/113
	Suspended	MM/FF	33/36	46/31
	Insufficient information	MM/FF	19/16	11/6
Age of twin pairs at data collection (years)		Mean ± *SD* (range)	11.9 ± 0.4 (11–12)	5.9 ± 3.8 (0–15)
Maternal age at twins birth (year)[a]		Mean ± *SD* (range)	29.1±3.9 (19–43)	30.7 ± 3.8 (21–41)
Paternal age at twins birth (year)[b]		Mean ± *SD* (range)	31.9 ± 4.7 (19–53)	33.3 ± 4.5 (22–50)
Previous abortion		0	786 (69.5%)	744 (78.2%)
		1	205 (18.1%)	166 (17.5%)
		2–	50 (4.4%)	40 (4.2%)
		Unknown	90 (8.0%)	1 (0.1%)
Treatment of infertility (ovulation stimulating drugs, in-vitro fertilization, and other types of assisted conception)		Yes	31 (2.7%)	292 (30.7%)
		No	1015 (89.7%)	640 (67.3%)
		Unknown	85 (7.5%)	19 (2.0%)
Gestation (weeks)[c]		Mean ± SD	37.9 ± 2.2	36.9 ± 2.3
Parity		1	591 (52.3%)	655 (68.9%)
		2	424 (37.5%)	296 (31.1%)[d]
		3–5	115 (10.2%)	
		Unknown	1 (0.1%)	0 (0%)
Neonatal condition (twin individuals)		Healthy	1778 (78.6%)	1491 (78.4%)
		Hyposthenia (not so healthy)	304 (13.4%)	205 (10.8%)
		Neonatal asphyxia	125 (5.5%)	130 (6.8%)
		Unknown	55 (2.4%)	76 (4.0%)

Note: [a] 4 missing values as to school applicants. [b] 14 missing values as to school applicants, and 5 missing values as to maternal associations. [c] 15 missing values as to school applicants, and 14 missing values as to material associations. [d] All multiparity. *SD:* Standard Deviation.

tations and Japanese restrictions on epidemiological data collection.

Obstetric findings were obtained from the *Maternal and Child Health Handbook* (which is presented by the Ministry of Health, Labor and Welfare to all pregnant women and whose format varies depending on the policies of each city). No information on chorionicity was gathered.

The zygosity of the twins was determined primarily by a questionnaire (Ooki & Asaka, 2004) that was completed by the mothers in both groups. Zygosity — monozygotic (MZ), unclassified (UZ) and dizygotic (DZ) — was determined according to similarity scores calculated using five questions about physical similarity and the confusion of identity between the twins. In a previous study in which zygosity determination by DNA/genetic markers was regarded as the gold standard, the accuracy of the zygosity questionnaire was 97.5% (Ooki & Asaka, 2004), although around 10% of pairs were unclassified. A trade-off exists between high accuracy on the one hand and a high percentage of unclassified pairs on the other.

Informed consent regarding the statistical analysis of the school applicant group's data was obtained using written documents as part of the application process. The mothers of the maternal associations group all cooperated voluntarily with the study through the associations or through personal introductions.

Statistical Analysis

The basic characteristics of the samples were summarized, including zygosity, birth year of the twins, maternal and paternal age at the time of the birth of the twins, and gestational age.

Birthweights were then analyzed according to the subject's group and sex. Fiftieth percentiles by gesta-

Table 2

Birthweight Characteristics of Both Groups According to Sex

	School applicants		Maternal associations	
	Male	Female	Male	Female
N	1053[a]	1200[b]	977[c]	916[d]
Mean (g)	2503	2452	2374	2274
Standard deviation (g)	455	427	445	443
Skewness	−0.03	−0.11	−0.70	−0.40
Kurtosis	0.08	0.28	1.39	0.65
Coefficient of variation	18	17	19	19
Median (g)	2500	2470	2410	2308
Range (g)	2910	3011	2925	2915
Range between 25 and 75 percentiles (g)	585	554	520	556
Fitting of normal distribution[e]	$p < 0.05$	$p < 0.01$	$p < 0.01$	$p < 0.01$
LBW (%)	48.2	52.0	58.7	68.6
VLBW (%)	1.2	1.9	4.1	5.2

Note: [a]4 missing values. [b]5 missing values. [c]5 missing values. [d]4 missing values. [e]Kolmogorov-Smirnov test. LBW: low birth weight < 2500. VLBW: very low birth weight, birthweight < 1500.

tional age were calculated. Smoothing of the growth curves was performed for both groups using a cubic spline function, and the curves compared with the birthweight norms for twins in Japan (Kato, 2004) calculated by using the vital statistics of around 65,000 pairs of twins.

Birthweight discordancy was also analyzed in order to estimate the selection bias through which severely discordant twins are unknowingly excluded from the twin database. For each twin pair, the intrapair relative birthweight difference (RBWD) was calculated as a percentage of the absolute difference of birthweight divided by heavier birthweight (Sadrzadeh et al., 2001), and then compared between the two groups. The mean RBWDs of MZ and DZ same-sex twin pairs within each group were tested using Student's *t* test. The mean RBWDs of twin pairs between groups were also compared to test the selection bias of severely discordant twin pairs. This selection bias was also tested using Student's *t* test.

Statistical analyses were all performed using SAS for Windows (1997). Smoothing of growth curves was performed using the PROC TRANSREG program by specifying the 'pspline' model.

Results

The basic characteristics of the subjects including the obstetric findings of the mothers are summarized in Table 1. The percentage of mothers treated with ovulation-stimulating drugs or in-vitro fertilization was much higher in the maternal associations group. The number of gestational weeks for the maternal associations group was one week less than that of the school applicant group. The mean birth year of the school applicant group was 1979, and 1995 for the maternal association group.

Basic statistics of birthweight are summarized in Table 2. Irrespective of sex, the birthweight of the school applicant group was more than 100g heavier than that of the maternal associations group. For the maternal associations group, twins conceived spontaneously (n = 632 pairs) had shorter gestations, slightly lower birthweights, and higher maternal ages at birth compared with twins conceived with assistance (n = 287 pairs): gestation, 36.8 weeks versus 37.2 weeks ($p < .05$); birthweight, 2306 g versus 2355 g ($p < .05$); maternal age, 30.2 versus 31.7 years

Table 3

Mean and Median Relative Birth Weight Difference in Percentage, Frequencies of Discordant Twins, and Intraclass Correlations According to Zygosity and Sex Combination

	School applicants RBWD						Maternal associations RBWD						Difference of RBWD between Two Groups	
	N	Mean	*SD*	Median	DT (%)	Intraclass correlation	*N*	Mean	*SD*	Median	DT (%)	Intraclass correlation	Variance	*p*
MZ	749	10.53	8.55	8.31	6.9	0.650	442	10.80	8.71	8.87	7.0	0.692	Equal	0.6062
MZM	334	10.44	8.47	8.34	6.6	0.658	234	11.25	8.88	9.35	7.3	0.648	Equal	0.2722
MZF	415	10.61	8.62	8.27	7.2	0.641	208	10.29	8.52	8.71	6.7	0.729	Equal	0.6654
DZSS	145	12.61	10.04	11.19	12.4	0.574	203	13.34	10.85	10.92	11.8	0.572	Equal	0.5269
DZM	77	14.67	10.67	13.21	18.2	0.564	94	13.45	10.95	11.52	11.7	0.556	Equal	0.4625
DZF	68	10.28	8.79	8.81	5.9	0.594	109	13.24	10.82	10.24	11.9	0.571	Equal	0.0591
DZOS	127	12.86	8.30	12.77	10.2	0.485	207	12.15	8.66	10.91	9.7	0.607	Equal	0.4603
SS(all)	997	10.92	8.83	8.81	7.7	0.637	739	11.71	9.50	9.78	8.8	0.652	Unequal	0.0797
SS(MZ + DZ)	894	10.87	8.84	8.68	7.8	0.636	645	11.60	9.50	9.64	8.5	0.652	Unequal	0.1272
SS(unclassified zygosity)	103	11.35	8.84	10.34	6.8	0.631	94	12.44	9.49	11.21	10.6	0.649	Equal	0.4067

Note: RBWD: Relative birth weight difference. DT: Discordant twins (RBWD ≥ 25%). MZM: Monozygotic males. MZF: Monozygotic females. DZM: Dizygotic males. DZF: Dizygotic females. DZSS: Dizygotic same sex. DZOS: Dizygotic opposite sex. SS: Same sex. The difference of mean RBWD between groups were tested using Student's *t* test.

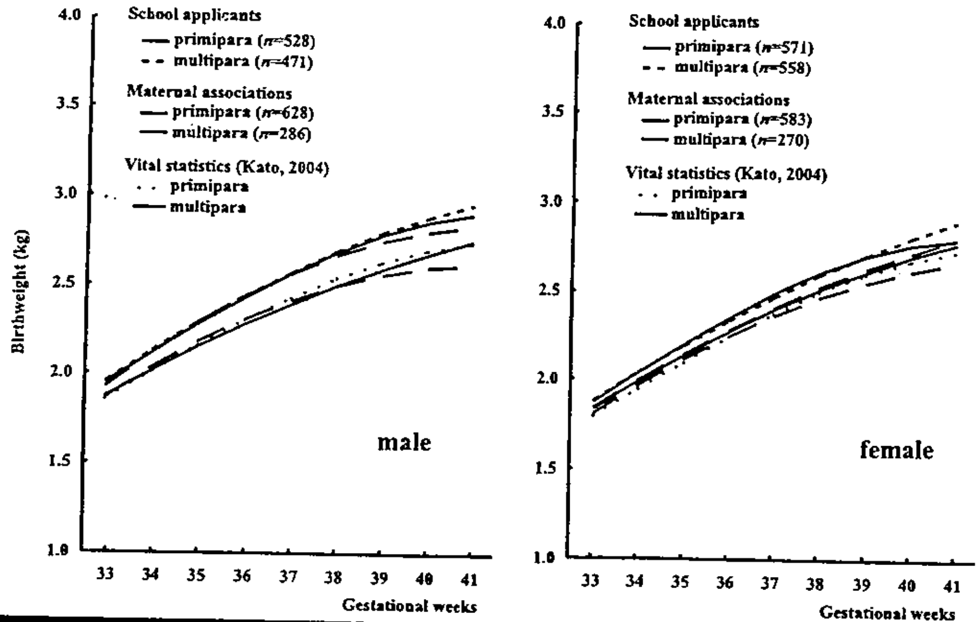

Figure 1

50th percentiles of birthweight according to gestational weeks.

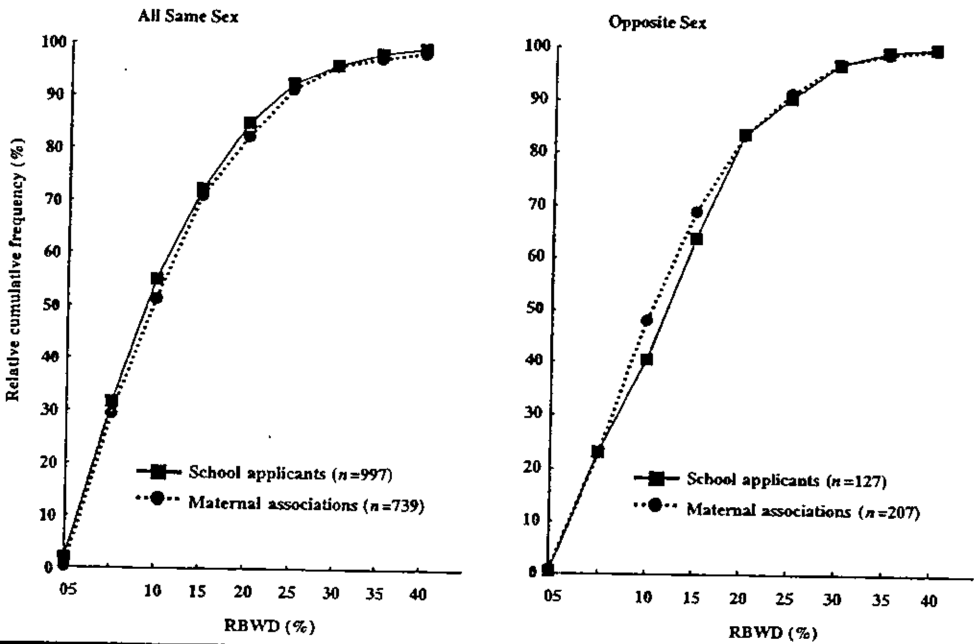

Figure 2

Curve of relative cumulative frequency of RBWD according to sex combination.

Note: RBWD = relative birthweight difference in percentage. All same sex pairs include pairs with unclassified zygosity.

($p < .0001$). MZ twins conceived spontaneously were lighter than DZ twins conceived spontaneously in the school applicant group (2469 g vs. 2553 g, $p = .0003$). This tendency was not observed in the maternal associations group (2305 g vs. 2328 g, *ns*).

The smoothed curves of the birthweight 50th percentile according to gestational weeks separately for sex and parity are shown in Figure 1. Little difference was observed between the school applicant group, the maternal associations group, and the vital statistics.

The curves of the relative cumulative frequency of RBWD according to same-sex pairs and opposite-sex pairs are shown in Figure 2. At the 30% level of RBWD, the relative cumulative frequency of both groups reached between 96% and 97%, irrespective of the sex combination of the pairs. Mean and median

RBWD in percentage, frequencies of discordant twin pairs and intraclass correlations according to zygosity and sex combination are shown in Table 3. The RBWD of MZ pairs was significantly lower than that of DZ same-sex pairs in both the school applicant group (p = .0206) and the maternal associations group (p = .0037), whereas no significant difference in RBWD was found between the two groups regarding zygosity and sex combinations.

Discussion

Data from the maternal associations and school applicant groups were gathered partly to evaluate the representativeness of the school applicants' data that had already been collected. Features of the school applicant group are as follows: first, both twins were alive and showed no marked growth disturbance at ages 11 and 12 years. Second, the twins lived only in the Tokyo Metropolitan area. Third, all in the group applied to take an entrance examination for a university-affiliated school and appear to have performed well in their schooling up to that point, which could exclude children with disabilities or low birthweight twins. These features may have an advantage regarding intrauterine growth, although the direct effect of these positive selection biases is difficult to specify.

The total MZ/DZ ratio for the school applicant group was 2.76 (754/273), and that of the maternal associations group was 1.08 (445/412). According to Imaizumi and Nonaka (1997), the Japanese MZ/DZ ratio has decreased from 1.90 in 1979 to 1.09 in 1994. The MZ proportion was much higher in the school applicant group, partly reflecting the higher proportion of spontaneous MZ twinning in this period in Japan, but also partly reflecting selection biases based on the sampling process itself whereby MZ pairs are more likely to be applicants.

There was a clear birth year difference between the groups. The mean birth year of the school applicant group was around 16 years earlier than that of the maternal associations group. There was a tendency towards higher maternal and paternal age, higher percentage of primiparity, and considerably higher frequency of treatment using ovulation-stimulating drugs or in-vitro fertilization in the maternal associations group in comparison with the school applicant group. These reflect recent birth trends in Japan including singleton births, reflected in the difference in twins' birth years between the two groups.

The maternal associations group birthweight was lower than that of the school applicant group, as shown in Table 2, partly reflecting the earlier gestational age of the twins in the maternal associations group. This finding may be partly attributed to the recent tendency in Japanese obstetrics to keep maternal body weight gains from becoming too great. Nevertheless, the birthweight of both groups according to gestational weeks was not extremely different to that of the general twin population in Japan (Kato, 2004), as shown in Figure 1.

Many reports have indicated differences between twins conceived spontaneously and those conceived with assistance in maternal and birth characteristics such as socioeconomic and educational status, maternal age, gestation, and birthweight (Helmerhorst, et al., 2004; Zaib-un-Nisa et al., 2003). The maternal associations group clearly reflected some of these differences. Detailed analyses of these characteristics are the next step in the study.

Data on biometrical birth parameters might be influenced by birth year and recruitment methods. According to the results of Sadrzadeh et al. (2001), who analyzed potential biases regarding birthweight in the historical and contemporary twin databases of the Australian Twin Registry (Treloar et al., 2000), the Netherlands Twin Registry (Boomsma, 1998), and the East Flanders Prospective Twin Survey (Loos et al., 1998), each twin registry has its own features represented by birthweight data. MZ twins are subject to negative selection in historical databases through which severely discordant twins are unintentionally excluded, whereas the hypothesis that MZ twins are less discordant for birthweight in a volunteer-based twin registry than in a population-based twin registry had to be rejected.

At the 25%, 30%, 35% and 40% levels of RBWD, the relative cumulative frequency was nearly the same for both groups irrespective of sex combination, as shown in Figure 2. This suggests that there is no fatal selection bias of severely discordant twin pairs in the school applicant group. Some unexpected selection biases may exist, however. We conclude that the obstetric and birthweight features of both groups based on the methods of sample selection should be considered to construct growth charts of twins using this data.

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