Table 2. Change in incidence rates of suicide before and after the implementation in the study areas, displayed by age and gender

Subject		Age (years)	Baseline (January 1987–December 1994) *n*	Person-years	Incidence rate (/10^5)	Implementation (January 1995–December 2002) *n*	Person-years	Incidence rate (/10^5)	Corrected Mantel–Haenszel test: Breslow–Day test χ^2	d.f.	*P*-value	χ^2	d.f.	*P*-value	Age-adjusted incidence rate ratio	95% CI
Males	Intervention area															
	Yuri town	65–74	6	2760	217.4	6	3250	184.6	1.46	2	0.48	0.10	1	0.76	0.79	0.18–3.52
		75–84	3	1146	261.8	4	1591	251.4								
		≥85	1	236	423.7	0	413	0.0								
	Reference area															
	Chokai town	65–74	5	3293	151.8	5	4205	118.9	2.78	2	0.25	<0.001	1	0.99	0.91	–[†]
		75–84	4	1462	273.6	9	1829	492.1								
		≥85	1	252	396.8	0	416	0.0								
Females	Intervention area															
	Yuri town	65–74	6	3545	169.3	2	4075	49.1	0.19	2	0.91	10.0	1	0.002	0.24	0.10–0.58
		75–84	9	1908	471.7	3	2667	112.5								
		≥85	4	516	775.2	1	814	122.9								
	Reference area															
	Chokai town	65–74	8	4263	187.7	3	5380	55.8	1.29	2	0.53	2.39	1	0.12	0.54	0.25–1.18
		75–84	8	1949	410.5	9	2910	309.3								
		≥85	2	438	456.6	2	720	277.8								

[†]Uncalculated because χ^2 nearly equal to zero.

and over was observed (age-adjusted IRR, 0.24; 95% CI, 0.10–0.58) during the implementation, compared with the baseline. There was no evident change in the risk or in the number of male suicides before and after the implementation. In the reference area, there were no significant changes in the risks for either males or females.

Comparison of changes in incidence rates of the intervention and regional populations

The crude IRR for females aged 65 and over between the baseline and the implementation stage was 0.25 in Yuri town (95% CI, 0.10–0.63) and 0.76 in Akita prefecture (95% CI, 0.68–0.85). This shows a significant reduction in the relative risk of suicide in the prefectural region including the intervention area (Table 3).

General loglinear analysis for cell degrees added 0.5 because of the less than 10-degree estimated for the ratio of the crude IRR between the two stages in the town to that in its prefecture of 0.35 (95% CI, 0.14–0.84). This means that a reduction of the RR of suicide in the intervention area was greater than that in its prefecture area during our study period.

DISCUSSION

It is widely known that the lack of social support is the most common predictor of suicide.[6,7] A recent prospective, community-based study indicated that the absence of a relative or friend to confide in, increased the risk of depression and late-life suicide.[8] A recent Japanese review has also suggested that the importance of developing a community network is equal to that of detecting depression for improving suicide prevention in the elderly.[15] However, although the current successful approach of suicide prevention for the elderly has focused on the improved detection of depression on high-risk strategy, very few focused on population strategy such as the reinforcement of social support.

In the present study, a program focussing on these points of view was evaluated by the outcome from demographic data observed by public organizations. The suicide IR among elderly females, but not males, decreased in the intervention population with the implementation of the program based on population strategy through the group intervention. During the 8-year implementation, the suicide risk for females aged 65 and over was reduced by 76%. This result for females is similar to the 69% and 76% reduction in the Matsunoyama study[3] and the Joboji study using depression screening,[4] respectively. In contrast, the

Table 3. Comparison between incidence rate ratios of suicide for females (≥65 year-old) before and after the implementation in the intervention area (Yuri town, Japan) and in the region (Akita prefecture, Japan)

Area/region	Baseline (January 1987–December 1994)			Implementation (January 1995–December2002)					Ratio of incidence rate ratios[†]	95% CI
	n	Person-years	Incidence rate (/10^5)	*n*	Person-years	Incidence rate ratio	Incidence rate (/10^5)	95% CI		
Yuri town	19	5 969	318.3	6	7556	79.4	0.25	0.10-0.63	0.35[‡]	0.14–0.84[§]
Akita prefecture	596	937 076	63.6	616	1 270 634	48.5	0.76	0.68-0.85		

[†]Estimated as three-factor interaction parameter by general loglinear analysis on the assumption of the poisson distribution.
[‡]Calculated for cell degrees added 0.5 because of less than 10-degree.
[§]Estimated with χ^2 test (d.f. = 1).

present result of unchanged risk for males is different from the two previous results of reduced risk.

The present findings suggest that the interventions were effective in reducing suicide risk among the elderly females unless some biases resulting from this quasi-experimental design could significantly affect the risk. Since the present study adopted a non-randomized, concurrent comparative and time series design, the following biases could result. First, non-randomized assignment can reference no unmeasured confounding variables and could cause time-dependent confounding including a historical trend. However, the results of general loglinear analysis suggest that the reduction of suicide risk for the elderly females in the intervention area was greater than the regional historical trend. Second, the time series design could possibly lead to a regression effect towards the mean. A regression effect on the decrease of suicide rate in the intervention area could be suspected because the baseline rate in the study areas was higher than the regional average of its prefecture. Nevertheless, the suicide rate for the elderly was continuously high in the neighboring reference. This counters an explanation of regression towards the mean.

The present intervention program based on population strategy includes group activities, psychoeducation and self-assessment of depression. A recent neighboring investigation demonstrated that although there are many elderly people exhibiting a subclinical depressive state with recurring thoughts of suicide, the number of people who consult others is very low.[2] Psychoeducation and self-assessment appear to increase in knowledge. Group activities would also develop the face-to-face communication network. Thus, the combination of three programs should improve help-seeking behavior of participants, mostly females. Moreover, the combination may make the surroundings supportive against depression and suicide risk among all the elderly residents. In addition, the self-assessment was performed where suicide had previously occurred, so that this may suppress the contagion effect. Therefore, the decrease in suicide risk for females can be said as a result of integrated group intervention with three programs.

In the present study, a gender difference was observed where the suicide IR for elderly females decreased following the implementation, while the rate for elderly males was virtually unchanged. One possible explanation is that since the program was actively participational, it may be effective for females only, who are more likely to seek help for a mental health problem than males.[16,17] Another plausible explanation is that in the common pathway from depressive thought via depressive ideation, suicidal plan and impulsivity to suicide attempt,[18] the suicidal impulsivity would be more vulnerable in males than in females. This is partly because the male-related factors including serotonergic dysfunction, aggression, alcoholism and substance abuse affect impulsivity.[19] Other stages are unknown concerning the gender difference influencing suicidal behavior directly. For the above reasons, since the group activity and psychoeducation can ameliorate depressive thought or suicidal ideation, but not suicide impulsivity,[20] the present program may benefit females only.

The present study has several limitations. First, this study was conducted in a rural area with a relatively small population size, high suicide rate, and high percentage of elderly people. The results are, therefore, not immediately generalized to areas that differ in population size, suicide rate and percentage of elderly. However, elderly suicides were prevalent in sociodemographically similar areas to these study areas in Japan,[21] China, Taiwan and other countries.[1] The sample size was also sufficient to detect the reduced risk for elderly in the reference area. Second, we were unable to reference all potential confounders because of non-randomized assignment. However, for the variables probably related to suicide among the Japanese elderly, the main socioeconomic characteristics were similar between the intervention and reference. The social welfare service in the intervention area also appears to be similar to that in the reference because the study municipalities belong to Akita prefecture that uniformly planned the service. Finally, there was no available date that could specify the participants. We cannot, thus, clarify how many people of the intended population the intervention actually targeted. Nevertheless, there are indicators that the reduction by the definitely element strategies including the group intervention was successful for elderly females.

The present study has suggested that a suicide prevention program can be effective for elderly females, by using high quality local data from a public organization. Therefore, the present intervention would be a model of a community-based population strategy through group intervention designed to increase knowledge and to cultivate social relationships.

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Genomewide High-Density SNP Linkage Analysis of 236 Japanese Families Supports the Existence of Schizophrenia Susceptibility Loci on Chromosomes 1p, 14q, and 20p

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The Japanese Schizophrenia Sib-Pair Linkage Group (JSSLG) is a multisite collaborative study group that was organized to create a national resource for affected sib pair (ASP) studies of schizophrenia in Japan. We used a high-density single-nucleotide–polymorphism (SNP) genotyping assay, the Illumina BeadArray linkage mapping panel (version 4) comprising 5,861 SNPs, to perform a genomewide linkage analysis of JSSLG samples comprising 236 Japanese families with 268 nonindependent ASPs with schizophrenia. All subjects were Japanese. Among these families, 122 families comprised the same subjects analyzed with short tandem repeat markers. All the probands and their siblings, with the exception of seven siblings with schizoaffective disorder, had schizophrenia. After excluding SNPs with high linkage disequilibrium, we found significant evidence of linkage of schizophrenia to chromosome 1p21.2-1p13.2 (LOD = 3.39) and suggestive evidence of linkage to 14q11.2 (LOD = 2.87), 14q11.2-q13.2 (LOD = 2.33), and 20p12.1-p11.2 (LOD = 2.33). Although linkage to these regions has received little attention, these regions are included in or partially overlap the 10 regions reported by Lewis et al. that passed the two aggregate criteria of a meta-analysis. Results of the present study—which, to our knowledge, is the first genomewide analysis of schizophrenia in ASPs of a single Asian ethnicity that is comparable to the analyses done of ASPs of European descent—indicate the existence of schizophrenia susceptibility loci that are common to different ethnic groups but that likely have different ethnicity-specific effects.

Introduction

Schizophrenia (MIM 181500) is a common disorder, with a lifetime morbidity risk of 1%. A large number of family, twin, and adoption studies have revealed that individual differences in susceptibility are predominantly genetic, with a heritability of 0.70–0.85 and a 10-fold increased risk in siblings of probands (Levinson and Mowry 2000). More than 20 genome scans for susceptibility loci for schizophrenia have been completed, and evidence satisfying genomewide significance levels for linkage to schizophrenia was obtained for chromosome regions 6p24-p22 (MIM 600511) (Straub et al. 1995), 1q21-q22 (MIM 604906) (Brzustowicz et al. 2000), 13q32-q34 (MIM 603176) (Blouin et al. 1998), 10p14 (DeLisi et al. 2002*b*), and 10q25.3-q26.3 (Williams et al. 2003). Linkage for other regions—including 8p22-p21 (MIM 603013) (Kendler et al. 1996; Blouin et al. 1998), 6q21-q25 (MIM 603175) (Cao et al. 1997; Lindholm et al. 2001), 22q11-q12 (MIM 600850) (Pulver et al. 1994; Schizophrenia Linkage Collaborative Group

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for Chromosomes 3 and 8 1996), and 5q21-q33 (MIM 181510) (Bassett et al. 1988; Paunio et al. 2001)—has been reported multiple times. However, none of the above-named regions has been identified consistently in a majority of the genome scans. It is possible that loci with small populationwide effects hinder confirmation of linkage because replication of linkage data requires a larger sample population than the original data set (Suarez et al. 1994) and because the samples for most genome scans of schizophrenia have been small, typically 20–100 families.

Some problems of power and replication can be addressed by meta-analysis. Lewis and colleagues (2003) used the rank-based genome-scan meta-analysis (GSMA) method to analyze 20 complete genome scans for susceptibility loci for schizophrenia. In GSMA, the autosomes were divided into 30-cM bins, and the evidence of linkage in each study was rank ordered across bins with and without weights for sample size. The average ranks across studies were evaluated for statistically significant evidence of linkage in several ways. Lewis et al. (2003) concluded that schizophrenia loci are highly likely to be present in one or more of the following regions: 1p13.3-q23.3, 2p12-q23.3, 3p25.3-p22.1, 5q23.2-q34, 6pter-p21.1, 8p22-p21.1, 11q22.3-q24.1, 14pter-q13.1, 20p12.3-p11, and 22pter-q12.3, all of which met two aggregate criteria for linkage. Another meta-analysis found significant results only for chromosomes 8p, 13q, and 22q (Badner and Gershon 2002). However, meta-analysis has limitations (Levinson et al. 2003). One is that meta-analysis methods may not distinguish several weakly linked loci in the same region. This issue can be addressed by pooling the raw genotyping data for meta-analysis. Analysis of a multicenter sample of 779 pedigrees did not yield significant evidence of linkage of 22q to schizophrenia (Mowry et al. 2004); however, those authors suggested that collaborative pooling of data sets was limited by intersite differences in sampling frames, population ethnicity, and genotyping methods.

The largest genomewide linkage findings to date were reported by DeLisi and colleagues (2002*b*), who studied 294 families with 382 nonindependent affected sib pairs (ASPs) with schizophrenia or schizoaffective disorder from the United States, the United Kingdom, Italy, Chile, and Belgium. Williams and colleagues (2003) described linkage findings in 272 families with 353 nonindependent ASPs from the United Kingdom, Sweden, and the United States. Among these ASPs, 287 nonindependent ASPs in 231 families received a diagnosis of schizophrenia or schizoaffective disorder. Straub and colleagues (2002) described linkage findings in 270 families with 261 nonindependent ASPs with schizophrenia or poor-outcome schizoaffective disorder from Ireland and Northern Ireland. The Irish families were ethnically homogeneous, and most of the pedigrees in the other two studies were of European origin. The narrow ethnic distributions of these sample populations could have influenced the results, because an ethnically diverse study population has increased potential for variation, which could result in heterogeneity at certain susceptibility loci. A recent study found ethnic heterogeneity between European and East Asian populations in allelic association of the 102T/C polymorphism of the *HTR2A* gene (MIM 182135) with schizophrenia (Abdolmaleky et al. 2004). This type of heterogeneity compounds the recognized difficulty in characterizing genetically complex diseases for which the magnitude of the effect of any one locus is unknown.

The Japanese Schizophrenia Sib-Pair Linkage Group (JSSLG), a multisite collaborative study group, was established in 1997 as a national resource for genetic studies of schizophrenia. An initial genomewide linkage study was performed with 417 STR markers in 130 families; however, no loci with significant linkage to schizophrenia were detected (JSSLG 2003). We recruited additional families to participate in the JSSLG study and analyzed 236 families with 268 nonindependent ASPs with a high-density SNP linkage mapping set. High-density SNP linkage mapping systems provide significantly improved levels of information extraction with extremely high accuracy, particularly when founder genotypes are unavailable (Sawcer et al. 2004).

Material and Methods

Subjects

Linkage of genetic loci to schizophrenia was analyzed in Japanese families with at least two available siblings who had received the diagnosis of schizophrenia or schizoaffective disorder. A total of 236 families with 602 individuals were recruited at 24 centers across Japan (table 1). Of these, 122 families with 315 individuals comprised the same subjects analyzed by STR markers that we reported elsewhere (JSSLG 2003). Each family member received the diagnosis on the basis of the DSM-IV structured clinical interview. Each face-to-face interview was conducted by two experienced interviewers. In addition to direct interviews, all available medical records and information from relatives and hospital staff were considered. Inclusion criteria for this collaborative sample recruitment were DSM-IV-defined schizophrenia for probands and schizophrenia or schizoaffective disorder for affected siblings. Seven siblings with schizoaffective disorder were included. All participants and their parents were of Japanese descent. The study protocol was approved by the ethics committee of each institution, and written informed consent was obtained from all subjects.

Table 1

JSSLG Subjects

Center[a]	No. of Families	No. of JSSLG Families: Both Parents[b] 2 Affected Sibs	Both Parents[b] 3 Affected Sibs	One Parent[b] 2 Affected Sibs	One Parent[b] 3 Affected Sibs	No Parent[b] 2 Affected Sibs	No Parent[b] 3 Affected Sibs
Hakkaido University	2	0	0	0	0	1	1
Hirosaki University	1	0	0	0	0	0	1
Minami Hanamaki National Hospital	18	5	0	8	0	5	0
Tohoku University	1	0	0	0	0	1	0
Fukushima Medical University	4	0	0	2	0	2	0
Niigata University	19	3	0	2	0	14	0
University of Tsukuba	16	0	0	3	2	10	1
Teikyo University Ichihara Hospital	23	0	0	2	0	21	0
RIKEN Brain Science Institute	7	5	0	2	0	0	0
Juntendo University	3	2	0	1	0	0	0
Toho University	10	0	0	0	0	10	0
Tokyo Institute of Psychiatry	1	0	0	0	0	0	1
Nihon University	11	1	1	0	0	9	0
Teikyo University	4	0	0	2	0	2	0
National Center of Neurology and Psychiatry	5	0	0	0	0	5	0
Fujita Health University	7	0	0	0	0	7	0
Osaka Medical College	4	0	0	0	0	4	0
Okayama University	33	0	0	2	0	27	4
University of Occupational and Environmental Health	10	1	0	0	0	9	0
Kyushu University	2	0	0	0	0	2	0
Kurume University	7	0	0	0	0	7	0
Saga Medical School	22	5	1	5	1	10	0
Nagasaki University	19	7	0	7	1	3	1
Kagoshima University	7	0	1	1	0	5	0
Total	236	29	3	37	4	154	9

[a] In order of location from north to south.
[b] Available for genotyping.

Genotyping

The Illumina SNP-based Linkage Panel IV was used for genotyping. The panel includes 5,861 SNP markers distributed evenly across the genome. The average and median intervals between markers were 503 kb (0.64 cM) and 301 kb (0.35 cM), respectively. The largest interval between successfully genotyped markers was 4.9 Mb (8.8 cM) on chromosome Xp21. The Illumina markers were typed with the Illumina BeadStation 500G, in accordance with the manufacturer's standard recommendations.

Statistical Analysis

Multipoint linkage analysis was performed along the entire length of each chromosome with the MERLIN program (Center of Statistical Genetics) developed by Abecasis et al. (2002). Both the nonparametric linkage (NPL) *Z* score and nonparametric LOD score, calculated with the Kong and Cox (1997) linear model, were extracted from the MERLIN runs and were used to generate graphic plots of the whole-genome scan results. Because linkage disequilibrium (LD) between closely spaced SNP markers can falsely inflate linkage statistics, we used the SNPLINK program (Webb et al. 2005; Institute of Cancer Research), which removes LD from the marker sets in an automated fashion. Because the program considers LD between pairs of adjacent SNPs, the possibility of high LD between nonadjacent SNPs but low LD between adjacent SNPs, such as a situation in which there was high LD between SNPs 41 and 43 and low LD between SNPs 41 and 42 and between SNPs 42 and 43, was examined with the Haploview program. Because no empirical justification to remove LD by any criteria has been published, we tested the significant and suggestive regions, using a range of criteria from $r^2 = 0.4$, and gradually decreased the thresholds to $r^2 = 0.05$. The linkage panel includes 28 SNPs from the pseudoautosomal regions of the X chromosome (20 from the short arm; 8 from the long arm). Because no currently available multipoint linkage program can integrate data from X-linked and pseudoautosomal markers in a single analysis, each pseudoautosomal region was analyzed separately, as though it were an independent autosomal chromosome. The results of these analyses were then combined with those from the standard X-linked markers. Empirical *P* values were calculated for the NPL *Z* and LOD scores via simulation. MERLIN was used to

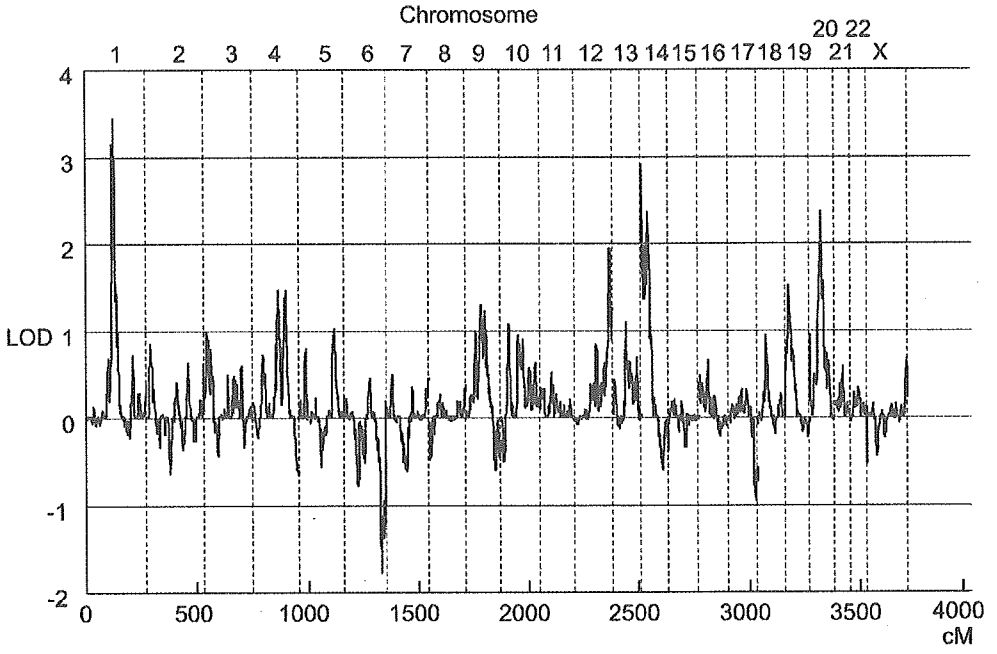

Figure 1 Multipoint nonparametric LOD score (Kong and Cox 1997) of genomewide scan for JSSLG ASPs with schizophrenia

generate 50,000 replicates of families identical to those in our sample. Markers with similar allele frequencies were also generated under the assumption of no linkage. Linkage analyses were then performed on these unlinked replicates, and peaks of NPL Z and LOD scores separated by at least 30 cM on each chromosome were recorded for each simulation. Simulation studies of our genome scan suggested that, on average, an NPL Z of 2.87 and a LOD of 2.06 per genome scan would have been expected, whereas an NPL Z of 3.48 and a LOD of 3.07 would have been expected to occur only once in every 20 genome scans in the absence of linkage. Therefore, these values correspond to "suggestive" and "significant" thresholds for genomewide significance, as defined by Lander and Kruglyak (1995). The GeneFinder program (Liang et al. 2001; Chiu et al. 2002; Glidden et al. 2003) was used to obtain 95% CIs for the locations of linked loci. The information content of the genotypes was estimated by MERLIN, with use of entropy information described by Kruglyak et al. (1996). Simulations suggested that our study had a power of >0.99, 0.79, 0.38, and 0.05 to detect a susceptibility locus of λs = 3, 2, 1.5, and 1.25 for schizophrenia, with a genomewide significance of $P = .05$.

Results

Among our Japanese family members, we observed an average minor-allele frequency of 0.29 and a mean heterozygosity of 0.37. These values were identical to those in Asian populations in the Illumina Linkage IV Panel. In our Japanese population, 125 SNPs were not polymorphic. The call rate (percentage of successful genotype calls among subjects) was used as a measure of quality. The average call rate was 98.5%, and we excluded 10 SNPs with call rates of <90%. The rate of Mendelian inconsistency or impossible recombination identified by the MERLIN program was 0.027% in the families with parents available for genotyping. Because the low heterozygosity of SNPs means that only 37% of genotyping errors will appear as Mendelian inconsistencies (Abecasis et al. 2002), the approximate genotyping error rate was estimated to be 0.073%.

Results of the linkage analysis are presented in figure 1. One region, 1p21.1, showed genomewide significance ($P < .05$) on the basis of simulation studies (LOD = 3.39; NPL Z = 3.96) with a 95% CI of 102.0–111.9 Mb (National Center for Biotechnology Information [NCBI] build 35). We also obtained suggestive evidence of linkage to chromosome 14q11.2 (LOD = 2.87; NPL Z = 3.14), with a 95% CI of 19.4–34.9 Mb; chromosome 14q12 (LOD = 2.33; NPL Z = 2.95), with a 95% CI of 19.4–34.9 Mb; and chromosome 20p11.2 (LOD = 2.33; NPL Z = 3.10), with a 95% CI of 16.0–33.2 Mb (table 2). Notable results were also obtained for chromosomes 4q24 (LOD = 1.44; NPL Z = 2.32), 4q31.3 (LOD = 1.44; NPL Z =

Table 2

Chromosome Regions with Genomewide Significant and Suggestive Linkage to Schizophrenia in 268 Nonindependent JSSLG ASPs

Peak SNP	Chromosome Region	Distance from pter Marker (cM)	Position[a] (Mb)	NPL Z (*P*)	LOD[b] (*P*)	95% CI SNP Region[a]	95% CI Position[a] (Mb)	95% CI Chromosome Region
rs2048839	1p21.1	126.18	105.7	3.96 (.00004)	3.39 (.00004)	*rs1445225–rs575208*	102.0–111.9	1p21.2-p13.2
rs1319956	14q11.2	.00	19.4	3.14 (.0009)	2.87 (.0001)	*rs1319956–rs8904*	19.4–34.9	14q11.2-q13.2
rs7149108	14q12	31.14	32.0	2.95 (.002)	2.33 (.0005)	*rs1319956–rs8904*	19.4–34.9	14q11.2-q13.2
rs7988	20p11.2	53.08	23.3	3.10 (.001)	2.33 (.0005)	*rs775133–rs663550*	16.0–33.2	20p12.1-q11.2

[a] NCBI build 35.
[b] Calculated with the Kong and Cox (1997) linear model.

2.42), 12q24.3 (LOD = 1.91; NPL Z = 2.67), and 19p13.3 (LOD = 1.49; NPL Z = 2.32).

Among 5,736 SNPs, 22 pairs of nonadjacent SNPs were in LD with $r^2 > 0.05$ but no adjacent SNPs were in LD with $r^2 < 0.05$. However, no such pairs were located in the significant and suggestive regions. The LOD scores were not changed by decreasing the thresholds to remove LD in the SNPLINK program, because pairs of adjacent SNPs showed high LD ($r^2 > 0.4$) or no or very low LD ($r^2 < 0.01$) in these regions.

Discussion

In our previous study of 130 families (JSSLG 2003), we did not observe any significant or suggestive evidence of linkage with schizophrenia. Of the 236 families examined in the present study, 122 had been analyzed previously. The present study revealed significant and suggestive evidence of linkage of specific chromosome regions to schizophrenia. The larger number of families and increased information extracted by the high-density SNP linkage system used in the present study may have contributed to the present results. The overall genetic linkage information content per 3-cM interval increased from 0.48 in our previous study (JSSLG 2003) to 0.72 in the present study. In addition to the increase in extractable information, high-throughput DNA typing technology is advantageous because it is accurate, fast, and requires little DNA. The genotyping error rate was ~0.073% in the present study. Although error rates are rarely published—and when they are expressed, the terminology varies greatly—it is noteworthy that microsatellite error rates of 0.1%–12.7% per reaction have been reported (Brzustowicz et al. 1993; Ginot et al. 1996; Ghosh et al. 1997; Ewen et al. 2000; Sobel et al. 2002; Weeks et al. 2002). Abecasis et al. (2001) reported that error rates of just 1% can reduce observed LOD scores by as much as 50%.

Our strongest finding was significant evidence of linkage of schizophrenia to the region 1p21-p13. To our knowledge, studies of linkage to schizophrenia have not focused on linkage to this region. However, this region overlaps a telomeric part of bin 1.6, which showed evidence of linkage to schizophrenia in the meta-analysis reported by Lewis et al. (2003) (table 2). A small peak LOD score for this region was observed in a cohort in the Central Valley of Costa Rica (DeLisi et al. 2002*a*). An NPL score of 2.72 for region 1p21 was observed in seven families with schizophrenia or schizophrenia spectrum personality disorders (Pulver et al. 2000). The *NTNG1* gene (MIM 608818) is located on 1p13.3 and may be a candidate gene for schizophrenia susceptibility. Association between specific haplotypes encompassing alternatively spliced exons of *NTNG1* and schizophrenia was observed in a Japanese population (Aoki-Suzuki et al. 2005).

Suggestive evidence of linkage to 14q11.2-q13.2 was also obtained in the present study. One region with NPL scores >2.0 in Arab-Israeli families was 14q11.1-q11.2 (Lerer et al. 2003). Potential linkage of schizophrenia to 14q13 was reported for the Maryland epidemiologic sample comprising 44 families of European descent (NPL = 2.57; P = .005) (Blouin et al. 1998). A mother and daughter who received the diagnoses of schizophrenia and schizophrenia comorbid with mild learning disability, respectively, possessed a balanced reciprocal translocation t(9,14)(q34.2;q13), and the *NPAS3* gene (MIM 609430) on 14q13.1 (32.5–33.3 Mb) was disrupted (Kamnasaran et al. 2003; Pickard et al. 2005). The region of 14q11.2-q13.2 is included in bin 14.1, which showed evidence of linkage in the meta-analysis reported by Lewis et al. (2003) (table 2).

Suggestive evidence of linkage to 20p11.2 was also obtained in the present study. Linkage of 20p11 with bipolar disorders has been reported (Radhakrishna et al. 2001; McInnis et al. 2003). This region is included in bin 20.2, which showed evidence of linkage with schizophrenia in the meta-analysis reported by Lewis et al. (2003) (table 2).

In the present study, all of the regions that showed significant and suggestive evidence of linkage to schizophrenia are included in or partially overlap the 10 regions that passed the two aggregate criteria of a meta-analysis (Lewis et al. 2003), although these regions have

not received much attention (Owen et al. 2004). Therefore, the presence of susceptibility loci for schizophrenia in both European and Asian populations in these regions is plausible, although these loci may have larger populationwide effects on schizophrenia in Asian populations than in European populations. Additional larger studies of Asian populations might validate the hypothesis (Hwu et al. 2005). In conclusion, the present JSSLG linkage study of Japanese families—which is one of the largest genomewide ASP analyses of a single ethnicity for schizophrenia to date and is comparable to genomewide ASP analyses of families of European descent with schizophrenia—supports the existence of schizophrenia susceptibility loci common to different ethnic groups but with possible ethnic-specific effects.

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Web Resources

The URLs for data presented herein are as follows:

Center of Statistical Genetics, http://csg.sph.umich.edu/ (for the MERLIN program)
GeneFinder, http://www.biostat.jhsph.edu/~wmchen/gf.html
Haploview, http://www.broad.mit.edu/mpg/haploview/
Institute of Cancer Research, http://www.icr.ac.uk/cancgen/molgen/MolPopGen_Bioinformatics.htm (for the SNPLINK program)
NCBI, http://www.ncbi.nlm.nih.gov/mapview/map_search.cgi?taxid=9606 (for map view build 35 and identification of candidate genes in locus of interest)
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for schizophrenia, 6p24-p22, 1q21-q22, 13q32-q34, 8p22-p21, 6q21-q25, 22q11-q12, 5q21-q33, *HTR2A*, *NTNG1*, and *NPAS3*)

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Association study of the *DISC1/TRAX* locus with schizophrenia in a Japanese population

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Abstract

Disrupted-in-Schizophrenia-1 (DISC1), identified by cytogenetic approaches in a pedigree with familial psychosis, is considered a candidate susceptibility gene for schizophrenia in some populations. In the pedigree, the *TRAX* gene, located adjacent to *DISC1* on the disrupted chromosome 1, may also contribute to the pathophysiology of the familial schizophrenia. We studied association of the *DISC1* and *TRAX* genes with schizophrenia in 338 Japanese by analyzing 15 single nucleotide polymorphisms (SNPs), including 12 SNPs in *DISC1* and three in *TRAX*, respectively. No significant difference was observed between the patients and controls in allelic frequencies or genotypic distributions of 15 SNPs. A weak trend for the association in genotypic distribution of one SNP in TRAX (major homo/hetero/minor homo: 0.324/0.431/0.245 vs. 0.293/0.526/0.181 for patients vs controls, $p=0.039$ in the 2×3 comparison) turned out to be insignificant after Bonferroni correction. Haplotype analysis did not support the association between the patients and controls. The present study suggests that the *DISC1/TRAX* locus may not have a major role in Japanese schizophrenia.

Keywords: Schizophrenia; DISC1; TRAX; Genetic association; Case-control

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1. Introduction

The significance of genetic factors in schizophrenia is evident from family, twin, and adoption studies (Gottesman, 1991). In a Scottish family, a hereditary chromosomal abnormality co-segregates with schizophrenia and other major mental illnesses. *Disrupted-in-Schizophrenia-1* (*DISC1*), which is located at 1q42, was identified as the sole gene with a disrupted open reading frame in the family (Miller et al., 2000; Blackwood et al., 2001). In addition, a frameshift mutation was identified in another family of schizophrenia, while the mutation was not detected in the control subjects (Sachs et al., 2005). The *Translin-Associated Factor X (TRAX; TSNAX)* gene is located adjacent to the 5′ side of the *DISC1* gene. Intergenic splicing and resultant fusion transcripts occur between the *TRAX* and *DISC1* genes (Miller et al., 2000). The genomic structure including these genes is well conserved in evolution (Taylor et al., 2003).

Functional studies of DISC1 protein suggest that DISC1 has cytoskeletal functions, especially during neurodevelopment (Ozeki et al., 2002; Morris et al., 2003; Miyoshi et al., 2003; Brandon et al., 2005). Of particular importance, mutant DISC1 that could occur in the Scottish patients impairs microtubular dynamics and neurite outgrowth in neuronal cultures (Ozeki et al., 2002). Together with Translin, TRAX also plays a role in brain development (Finkenstadt et al., 2000). These proteins may contribute to the developmental abnormalities implicated in the pathophysiology of schizophrenia (Sawa and Kamiya, 2003).

Several linkage and association studies have supported for the role of the *DISC1/TRAX* locus in schizophrenia in more than one ethnic group (Ekelund et al., 2001; Hennah et al., 2003; Hwu et al., 2003; Hodgkinson et al., 2004; Thomson et al., 2005; Callicott et al., 2005). Significant undertransmission of a common haplotype, spanning from intron 1 to exon 2 of the *DISC1* gene, was observed in affected individuals of a Finnish population (Hennah et al., 2003), which has been reproduced in Caucasians (Hodgkinson et al., 2004). A correlation between a coding single nucleotide polymorphism (SNP) in the *DISC1* gene and cognitive impairments in schizophrenia was also reported (Callicott et al., 2005). In contrast, there have been two studies that report a negative association between *DISC1* and schizophrenia (Devon et al., 2001; Kockelkorn et al., 2004).

Thus far, no Asian studies have extensively explored the association of the *DISC1/TRAX* locus with schizophrenia. A previous study by Kockelkorn et al. (2004) focused on a small number of SNPs in a very confined 5′ region of the *DISC1* gene, but not for the entire *DISC1/TRAX* locus. Here we investigated several single SNPs across the *DISC1* and *TRAX* genes and studied the association between the locus and schizophrenia in a Japanese population.

2. Subjects and methods

2.1. Subjects

In this study, Japanese patients and control subjects around Tokyo, Japan, were recruited: 338 unrelated patients with schizophrenia diagnosed by the DSM-IV criteria (183 males and 155 females; age, 46.3 ± 14.7 years, mean $\pm$ SD) and 338 sex-matched unrelated healthy volunteers (183 males and 155 females; age: 40.3 ± 11.3 years). The objective of the present study was clearly explained, and written informed consent was obtained from all subjects. The study was approved by the Ethical Committee of the Faculty of Medicine, the University of Tokyo.

2.2. SNP analysis

Genome-DNA was extracted from leukocytes by using the standard phenol–chloroform method. We sequenced all the coding exons and adjacent introns of the *DISC1* gene in 16 control subjects with Applied Biosystems 3730 DNA Analyzer (Applied Biosystems, CA), and identified 5 SNPs (SNPs 3–6 and 10) for the present study. Nine SNPs of *DISC1* (SNPs 1, 2, 7–9 and 11) and *TRAX* (SNPs A–C) were selected from the list of the Assays-on-Demand™ Products for ABI PRISM 7900HT. We primarily selected SNPs with minor alleles of higher frequencies (>20%) in the Japanese population, according to the database for the Assay-on-Demand™ Products. One SNP of the *DISC1* gene (SNP 12) was selected from the JSNP database of the Japanese population (Hirakawa et al., 2002). Locations of these 15 SNPs are summarized in Fig. 1.

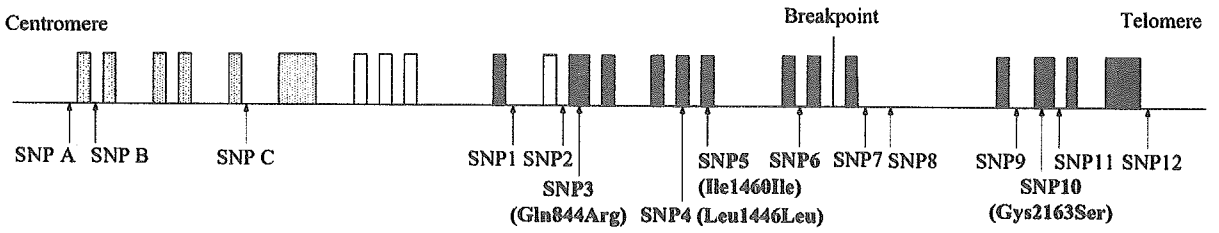

Fig. 1. Schematic diagram of the 1q42 region showing exonic structure of the *TRAX* (stippled boxes) and the *DISC1* (black boxes), as well as the location of the intergenic exons (white boxes). The analyzed SNPs are also shown with respect to the exonic structure of these genes. Five novel SNPs identified by our sequencing are described in bold.

The ten SNPs at introns (SNPs A–C of *TRAX* and SNPs 1, 2, 7–9, 11 and 12 of *DISC1*) were analyzed by using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, CA), while the five SNPs at exons (SNPs 3–5 and 10) or adjacent to an exon (SNP 6) were analyzed with restriction fragment length polymorphisms (RFLP).

2.3. Data analyses

Chi-square test was used to compare the SNP frequencies between the patients and the controls. Haplotypes of the SNPs and their frequencies were estimated by maximum-likelihood method with an expectation-maximization algorithm (Excoffier and Slatkin, 1995). Lewontin's D' was used to analyze pairwise linkage disequilibrium (LD) (Lewontin, 1964). Permutation p values were calculated in comparison of haplotype frequencies between the patients and controls (Fallin et al., 2001). The SNPAlyze 3.0Pro software (DYNACOM, Japan) was used to estimate haplotype frequencies, LD, and permutation p values.

3. Results

Allelic frequencies and genotypic distributions of the 15 SNPs compared between patients and controls are shown in Tables 1 and 2. The distributions of all 15 SNPs follow the Hardy–Weinberg equilibrium in the controls. In the patients, the distribution of SNP A was significantly deviated from Hardy–Weinberg equilibrium ($p=0.028$, uncorrected), while the distributions of the other 14 polymorphisms were within the values expected

Table 1
Allelic frequencies of 15 SNPs of the *DISC1* and *TRAX* genes

SNPs	dbSNP ID	Alleles (Major/minor)	Minor allele frequency		
			Schizophrenia[a]	Control[a]	p value[b]
SNP A	rs1630250	C/G	0.461 (306)	0.444 (321)	0.549
SNP B	rs1621135	A/T	0.525 (275)	0.482 (284)	0.150
SNP C	rs1655284	T/C	0.463 (284)	0.489 (274)	0.384
SNP 1	rs1865225	A/G	0.372 (286)	0.354 (277)	0.517
SNP 2	rs1572899	G/C	0.413 (271)	0.401 (268)	0.684
SNP 3	rs3738401	G/A	0.260 (308)	0.285 (335)	0.308
SNP 4	rs3738402	C/T	0.218 (331)	0.230 (335)	0.589
SNP 5	rs2492367	C/T	0.087 (320)	0.087 (334)	0.966
SNP 6	rs2273890	T/C	0.111 (320)	0.134 (332)	0.204
SNP 7	rs1000731	C/T	0.489 (313)	0.457 (328)	0.260
SNP 8	rs999710	G/A	0.391 (304)	0.407 (305)	0.590
SNP 9	rs843979	C/G	0.350 (308)	0.365 (312)	0.560
SNP 10	rs821616	A/T	0.103 (334)	0.138 (337)	0.051
SNP 11	rs3524	A/G	0.335 (312)	0.343 (315)	0.767
SNP 12	rs3737597	G/A	0.209 (311)	0.211 (327)	0.930

[a] Number of genotyped individuals for each SNP is given in parenthesis.
[b] Uncorrected values.

Table 2
Genotypic distributions of 15 SNPs of the *DISC1* and *TRAX* genes

SNPs	Genotypic distribution (major homo/hetero/minor homo)			
	Schizophrenia	Control	p value[a]	p value for recessive model (aa vs AA+Aa)[a,b]
SNP A	0.324/0.431/0.245	0.293/0.526/0.181	0.039	0.049
SNP B	0.240/0.469/0.291	0.261/0.514/0.225	0.208	0.076
SNP C	0.299/0.476/0.225	0.255/0.511/0.234	0.506	0.817
SNP 1	0.388/0.479/0.133	0.422/0.448/0.130	0.699	0.919
SNP 2	0.317/0.539/0.144	0.362/0.474/0.164	0.322	0.515
SNP 3	0.545/0.390/0.065	0.525/0.379/0.096	0.364	0.155
SNP 4	0.592/0.381/0.027	0.585/0.370/0.045	0.474	0.223
SNP 5	0.835/0.156/0.009	0.832/0.162/0.006	0.871	0.619
SNP 6	0.797/0.184/0.019	0.750/0.232/0.018	0.327	0.949
SNP 7	0.256/0.511/0.233	0.277/0.533/0.190	0.424	0.196
SNP 8	0.369/0.480/0.151	0.351/0.485/0.164	0.864	0.669
SNP 9	0.434/0.434/0.132	0.397/0.475/0.128	0.583	0.868
SNP 10	0.802/0.189/0.009	0.742/0.240/0.018	0.145	0.321
SNP 11	0.436/0.458/0.106	0.438/0.438/0.124	0.745	0.479
SNP 12	0.620/0.341/0.039	0.617/0.343/0.040	0.996	0.939

[a] Uncorrected values.
[b] "A" and "a" represent major and minor alleles at each SNP site, respectively.

from Hardy–Weinberg equilibrium. No significant difference was observed in the allelic frequency between the controls and patients. Genotypic distribution of SNP A was significantly different between the two groups ($p=0.039$ in the 2×3 comparison, and $p=0.049$ for the recessive model, uncorrected). The difference, however, did not reach statistical significance after correction for multiple comparisons of 15 SNPs. No significant difference was observed in the genotypic distributions of the other SNPs between the controls and patients. Analysis after dividing subjects by sex showed no significant difference (data not shown).

The pattern of LD is broadly similar in the patient and control groups. Three SNPs (A–C) of *TRAX* were in a tight LD both in the patients ($D'=0.98$–1.00) and controls ($D'=1.00$). Frequencies of the haplotypes consisting of the SNPs A–C were estimated. Four of these haplotypes occurred with a frequency of >2%, and two of them ('C-A-C' and 'G-T-T') accounted for >90% of variations. Permutation test suggested no significant difference in estimated frequencies of these four haplotypes between the controls and patients. No significant difference was observed in distributions of all estimated haplotypes between the controls and patients (global permutation $p=0.396$). Haplotypes of nine SNPs inside the *DISC1* gene, including SNPs 1–4, 7–9, 11, and 12, with the minor allele frequencies of >15%, were also evaluated, but no significant difference was observed in frequencies of any estimated haplotype or in distributions of all estimated haplotypes between the controls and patients (global permutation $p=0.377$).

4. Discussion

In the present study, we investigated the association of 15 SNPs in the *DISC1/TRAX* locus and schizophrenia. No significant difference was observed between the controls and patients in allelic frequencies or genotypic distributions of the 15 SNPs. A weak trend for association was observed in genotypic distribution of SNP A ($p=0.039$, uncorrected), however the difference was insignificant after Bonferroni correction. Permutation test showed no significant difference in estimated haplotype frequencies of the *DISC1* and *TRAX* genes between the controls and patients. Thus, the present study provides no positive evidence of association between the *DISC1/TRAX* locus and schizophrenia in a Japanese population.

Several studies have provided support for the association of *DISC1/TRAX* with schizophrenia. Hennah et al. (2003) observed a significant association of a common haplotype, containing two SNPs spanning from intron 1 to exon 2 of the gene, that was significantly undertransmitted to affected subjects ($p=0.0031$, uncorrected), especially in females ($p=0.00024$, uncor-

rected). The undertransmission of this haplotype was observed in schizoaffective disorder, in North American white population (Hodgkinson et al., 2004). In contrast, we failed to observe association of two SNPs (SNPs 1 and 2) in this region of *DISC1* (from intron 1 to exon 2) with schizophrenia. There may be at least two possible reasons to account for the difference. First, difference in the ethnic groups may influence the result, suggesting that the *DISC1/TRAX* locus may not play a strong role in Japanese schizophrenia. Second, the limitation of the statistical power may be considered. Required sample size (for statistical power=80% at alpha=5%) to detect a difference in allele frequencies of SNP 1 and SNP 2 (with minor allele frequencies of approximately 30%) at the odds ratio of 1.5 is approximately 190. Although the present sample size is larger than the required size, it might be still insufficient to detect any smaller effect in the polymorphisms of the *DISC1/TRAX* locus.

In conclusion, we obtained no significant support for the association of the *DISC1/TRAX* locus with schizophrenia in the Japanese subjects, although a weak trend of the association of *TRAX* was observed. Further studies with denser polymorphisms and a larger Japanese sample set might provide more definitive conclusions.

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REVIEW ARTICLE

Genetic or epigenetic difference causing discordance between monozygotic twins as a clue to molecular basis of mental disorders

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Classical twin research focused on differentiating genetic factors from environmental factors by comparing the concordance rate between monozygotic (MZ) and dizygotic twins. On the other hand, recent twin research tries to identify genetic or epigenetic differences between MZ twins discordant for mental disorders. There are a number of reports of MZ twins discordant for genetic disorders caused by genetic or epigenetic differences of known pathogenic genes. In the case of mental disorder research, for which the causative gene has not been established yet, we are trying to identify the 'pathogenic gene' by comprehensive analysis of genetic or epigenetic difference between discordant MZ twins. To date, no compelling evidence suggesting such difference between MZ twins has been reported. However, if the genetic or epigenetic difference responsible for the discordant phenotype is found, it will have impact on the biology of mental disorder, in which few conclusive molecular genetic evidences have been obtained.
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Introduction

Evidence proving the etiological roles of genetic factors in schizophrenia and bipolar disorder was derived mainly from twin studies. While the monozygotic (MZ) twins have identical genotypes, dizygotic (DZ) twins share only 50% of their genotypes on average. Thus, higher concordance rate in MZ twins compared with DZ twins is a hallmark of the role of genetic factors in a disease. Using this classical approach, the concordance rate in MZ twins in schizophrenia and bipolar disorder was found to be significantly higher than that in DZ twins. However, in spite that many candidate loci and candidate genes were proposed and analyzed, these findings are not yet conclusive. *DISC1* (disrupted in schizophrenia 1), cloned from a break point of balanced chromosomal translocation linked with mental disorders in a large pedigree, may be only one exception. In this situation, an alternative or complementary approach to study the molecular basis of mental disorders has been pursued.

Since the MZ twins provide a valuable opportunity of studying the role of genetic factors, methodology used for the twin study has been continuously evolving. If we refer the classical twin research noted above as 'first-generation twin research', second-generation twin research may be the study to identify environmental risk factors causing discordance, or to identify endophenotypes associated with the disease. The former strategy was used, for example, to identify the role of birth complications in the etiology of schizophrenia.[1,2] Using the latter approach, decreased hippocampal volume in schizophrenia[3] was established as an intermediate phenotype.[4] These studies were based on an assumption that there is no difference of genomes between MZ twins.

The first study in which the presence of genetic or epigenetic difference was pre-assumed in MZ twins was reported by Polymeropoulos *et al.*[5] Since then, several groups also have tried to identify genetic or epigenetic difference between MZ twins.[6–10] These studies seem to have induced a paradigm shift to the third-generation twin research, from focusing on the higher concordance rate in MZ twins, to looking for the genetic or epigenetic difference between MZ discordant twins.[11]

In this review, the theoretical concept of such study to search for genetic or epigenetic difference between MZ discordant twins is explained and its application to schizophrenia and bipolar disorder is summarized.

What is epigenetics?

Epigenetics is defined as the study of mitotically or meiotically heritable variations in gene function that

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cannot be explained by changes in DNA sequence.[12] For such mechanisms, methylation of the cytosine residue in the DNA molecule, and acetylation, methylation, and other modifications of histones have been well described. These modifications stably affect gene expression through alteration of DNA–protein interaction. These events refer to the inheritance from a parental cell to daughter cells. With regard to the inheritance from parents to the offspring, DNA methylation status is once totally reprogrammed at the fertilization. However, the DNA methylation can be conserved throughout the process of fertilization in some special cases.[13,14]

As evidenced by the study of clone animals, a different phenotype can be produced from the genomes having completely the same DNA sequences. The phenotypic difference between cloned animals and donor should have arisen from epigenetic differences. Similarly, we can postulate that epigenetic difference is responsible for the discordance of phenotypes between MZ twins.

Tsujita *et al*[6] showed that electrophoresis patterns of the genomic DNA digested with a methylation-sensitive restriction enzyme were different between MZ twins discordant for schizophrenia. This finding raised a possibility that phenotypic discordance between MZ twins may be caused by some epigenetic difference rather than 'genetic' difference, that is difference of DNA sequence.[15] This does not always preclude a possible effect of environmental factors in discordant phenotypes of MZ twins, since environmental factors may affect the DNA methylation status.[16–18]

Cause of discordance between MZ twins

A number of case reports have revealed that phenotypic discordance between MZ twins can be arisen from several kinds of genetic or epigenetic differences, which are inter-correlated each other.[19] For example, expansion of triplet repeat can alter DNA methylation status, and reduced DNA methylation of transposon can cause transposition and finally causes disruption of a gene. Thus, dichotomy of 'genetic' and 'epigenetic' is difficult, and only a tentative classification is given below.

Genetic

Point mutation To our knowledge, there are only two cases of MZ twins, in which a point mutation causative for the discordance of a disease was identified. One case is the MZ twin pair discordant for Darier's disease. Darier's disease is an autosomal dominantly inherited dermatological disease caused by mutations in the *ATP2A2*, encoding endoplasmic reticulum Ca^{2+}-ATPase.[20] In this pair of MZ twins discordant for Darier's disease, a point mutation of *ATP2A2* was identified. This mutation, G23E, was not found in the healthy co-twin and their parents, suggesting that it was a *de novo* mutation. In the other report, a mutation, Glu92X, in interferon regulatory factor 6 (*IRF6*) was identified in a MZ twin having Van der Woude syndrome characterized by the cleft lip and palate with lip pits, whose healthy co-twin did not have this disease.[21] This mutation was not found in healthy co-twins and their parents. They reported this finding as one of the evidences to prove the causative role of *IRF6* in Van der Woude syndrome.

This kind of genetic difference may happen at or after the twinning. In these reports, however, it cannot be ruled out that both twins had the mosaic mutation whose percentage is different.

Chromosomal Abnormality Chromosomal abnormality is sometimes seen as mosaicism. Since the percentage and the tissue distribution may differ between the MZ twins, mosaicism of chromosomal abnormality can cause discordance between MZ twins. Machin[19] extensively reviewed the MZ twin cases with discordant phenotype caused by chromosomal abnormalities. They reviewed 16 pairs of MZ twins discordant for Turner syndrome. In addition, other discordant MZ twins, such as trisomy 21 and trisomy 13, were also reported. After that, a number of cases of MZ twins having discordant phenotypes arisen from *de novo* mosaic chromosomal abnormalities were reported. These include Turner syndrome,[22] skin pigmentation,[23] minor anomalies,[24] and sex phenotypes.[25,26]

Since there are a number of case reports of MZ discordant twins caused by mosaicism of chromosomal abnormality, it may be a frequent cause of discordance in MZ twins. However, it might be biased by methodology. While discordance of DNA sequence is difficult to identify, the methods to detect chromosomal abnormality are well established and can be tested in clinical settings. This might be the reason of higher number of case reports on discordance caused by chromosomal abnormality.

Phenotypic discordance is known in the MZ twins with chromosome 22q11 deletion. This could be explained by epigenetic mechanism,[27] rather than difference in chromosomal abnormality.

Mitochondrial DNA (mtDNA) heteroplasmy

In the mitochondrial encephalomyopathies, mutated mtDNA usually coexists with wild-type mtDNA. This phenomenon is referred to as heteroplasmy. Clinical phenotype alters with the ratio and tissue distribution of the mutation. *De novo* heteroplasmic 11778 mutation of mtDNA reportedly caused the discordance of Leber's disease in MZ twins.[28] An MZ twin pair discordant for chronic progressive external ophthalmoplegia was also reported. In this pair, both twins had a small amount of 4115 base pair deletion in muscles, but the affected twin had much higher amount of deletion.[29] Discordant phenotypes due to uneven amount of heteroplasmic mutations were also found in DZ twins with myopathy, encephalopathy,