nation of F/A ratio and serum HGF level offered a useful marker for predicting mortality from acute liver injury. However, these results may reflect both sides of the hepatocyte destruction and macrophage activation in FHF. Some limitations to the present study must be considered, such as the relatively small number of samples and the heterogeneous etiologies. Prospective studies are thus required to validate the predictive value of the markers identified.

In conclusion, our results suggest that activation of macrophages participates in the pathophysiology of acute liver injury, and that the combination of F/A ratio and serum HGF level offers a potent marker for predicting the severity and prognosis of acute liver injury.

Acknowledgments

The expert technical assistance of Mika Fukuda is greatly appreciated.

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JOURNAL OF CLINICAL MICROBIOLOGY, May 2011, p. 1853–1860 Vol. 49, No. 5
0095-1137/11/$12.00 doi:10.1128/JCM.02139-10
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The rs8099917 Polymorphism, When Determined by a Suitable Genotyping Method, Is a Better Predictor for Response to Pegylated Alpha Interferon/Ribavirin Therapy in Japanese Patients than Other Single Nucleotide Polymorphisms Associated with Interleukin-28B▽†

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Received 22 October 2010/Returned for modification 4 January 2011/Accepted 28 February 2011

We focused on determining the most accurate and convenient genotyping methods and most appropriate single nucleotide polymorphism (SNP) among four such polymorphisms associated with interleukin-28B (IL-28B) in order to design tailor-made therapy for patients with chronic hepatitis C virus (HCV) patients. First, five different methods (direct sequencing, high-resolution melting analysis [HRM], hybridization probe [HP], the InvaderPlus assay [Invader], and the TaqMan SNP genotyping assay [TaqMan]) were developed for genotyping four SNPs (rs11881222, rs8103142, rs8099917, and rs12979860) associated with IL-28B, and their accuracies were compared for 292 Japanese patients. Next, the four SNPs associated with IL-28B were genotyped by Invader for 416 additional Japanese patients, and the response to pegylated interferon/ribavirin (PEG-IFN/RBV) treatment was evaluated when the four SNPs were not in linkage disequilibrium (LD). HRM failed to genotype one of the four SNPs in five patients. In 2 of 287 patients, the results of genotyping rs8099917 by direct sequencing differed from the results of the other three methods. The HP, TaqMan, and Invader methods were accurate for determination of the SNPs associated with IL-28B. In 10 of the 708 (1.4%) patients, the four SNPs were not in LD. Eight of nine (88.9%) patients whose rs8099917 was homozygous for the major allele were virological responders, even though one or more of the other SNPs were heterozygous. The HP, TaqMan, and Invader methods were suitable to determine the SNPs associated with IL-28B. The rs8099917 polymorphism should be the best predictor for the response to the PEG-IFN/RBV treatment among Japanese chronic hepatitis C patients.

Hepatitis C virus (HCV) infection is a global health problem, with worldwide estimates of 120 to 130 million carriers (7). Chronic HCV infection can lead to progressive liver disease, resulting in cirrhosis and complications, including decompensated liver disease and hepatocellular carcinoma (25). The current standard of care treatment for suitable patients with chronic HCV infection consists of pegylated alpha 2a or 2b interferon (PEG-IFN) given by injection in combination with oral ribavirin (RBV), for 24 or 48 weeks, dependent on HCV genotype. Large-scale treatment programs in the United States and Europe showed that 42 to 52% of patients with HCV genotype 1 achieved a sustained virological response (SVR) (3, 8, 13), and similar results were found in Japan. This treatment is associated with well-described side effects (such as a flu-like syndrome, hematologic abnormalities, and neuropsychiatric events) resulting in reduced compliance and fewer patients completing treatment (2). It is valuable to predict an individual's response before treatment with PEG-IFN/RBV to avoid these side effects, as well as to reduce the treatment cost. The HCV genotype, in particular, is used to predict the response: patients with HCV genotype 2 or 3 have a relatively high rate of SVR (70 to 80%) with 24 weeks of treatment, whereas those infected with genotype 1 have a much lower rate of SVR despite 48 weeks of treatment (8).

Recently, we reported from genome-wide association stud-

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† Supplemental material for this article may be found at http://jcm.asm.org/.

▽ Published ahead of print on 9 March 2011.

TABLE 1. Characteristics of the patients examined

Parameter	Result for:	
	1st stage (n = 292)	2nd stage (n = 416)
Age (yr)	57.2 ± 10.2	56.6 ± 10.9
No. of patients male/female	145/147	194/222
No. (%) of patients in institution[a]:		
1	18 (6.2)	0 (0)
2	178 (61.0)	0 (0)
3	57 (19.5)	0 (0)
4	39 (13.3)	0 (0)
5	0 (0)	249 (59.9)
6	0 (0)	94 (22.6)
7	0 (0)	52 (12.5)
8	0 (0)	21 (5.0)

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ies (GWAS) that several highly correlated common single nucleotide polymorphisms (SNPs), located in the vicinity of the lambda 3 interferon (IFN-λ3), coded for by the interleukin-28B (IL-28B) gene on chromosome 19, are implicated in nonvirological response (NVR) to PEG-IFN/RBV among patients with HCV genotype 1 (21). At almost exactly the same time as our report, the association between response to PEG-IFN/RBV and SNPs associated with IL-28B was reported from the results of GWAS by two other groups (6, 19). Determination of these SNPs associated with IL-28B before PEG-IFN/RBV treatment will provide extremely valuable information, because the patients predicted as showing NVR to PEG-IFN/RBV treatment could avoid the treatment. There are two questions to be asked before using these SNPs in clinical practice: (i) which methods for genotyping these SNPs are efficient, and (ii) which SNP is most informative in cases where the SNPs are not in linkage disequilibrium (LD)? We have developed five different methods for detecting the SNPs associated with IL-28B and compared their accuracies to establish the most efficient genotyping method. The response to PEG-IFN/RBV treatment was evaluated, when the SNPs associated with IL-28B were not in LD, to determine the best SNP to predict the response to PEG-IFN/RBV treatment.

MATERIALS AND METHODS

Study population. Samples were obtained from 708 Japanese chronic hepatitis C patients and divided into groups of 292 patients (145 males and 147 females; mean age, 57.2 years) and 416 patients (194 males and 222 females; mean age, 56.6 years) for the first and second stages (Table 1). In the first stage, we focused on analyzing the effective methods for determining the genotypes of four SNPs (rs11881222, rs8103142, rs12979860, and rs8099917) associated with IL-28B (Fig. 1A). Figure 2 shows the locations of these four SNPs in chromosome 19; rs11881222 and rs8103142 are located in the IL-28B gene, and rs12979860 and rs8099917 are located downstream from the IL-28B gene. The results of genotyping the four SNPs by five different methods, described below, were compared and evaluated for consistency. For this first stage, the 292 chronic hepatitis C patients were recruited from the National Center for Global Health and Medicine, Hokkaido University Hospital, Tonami General Hospital, and Shin-Kokura Hospital in Japan (Table 1). From the results of the first stage, the InvaderPlus assay was chosen as one of the best methods to determine the genotypes of the four SNPs associated with IL-28B and was used for genotyping 416 patients (Fig.

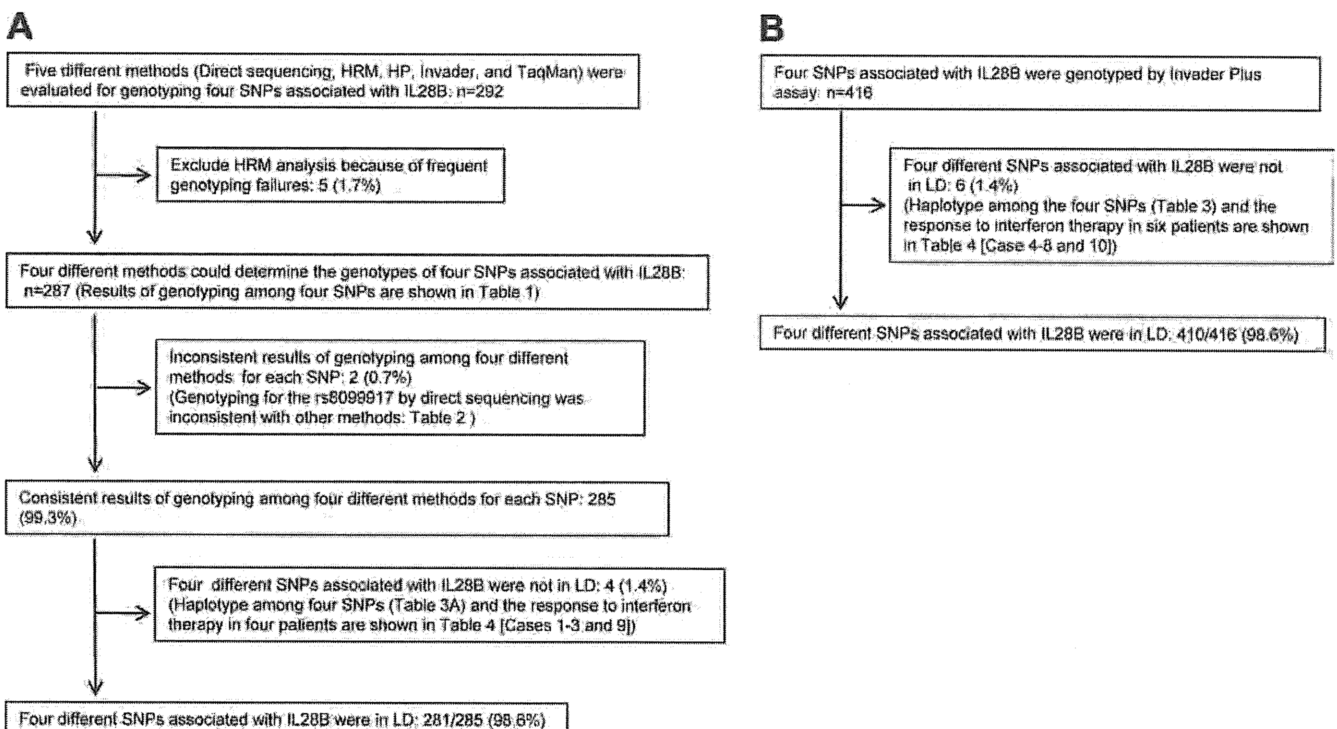

FIG. 1. Schema for the flowchart of the examinations.

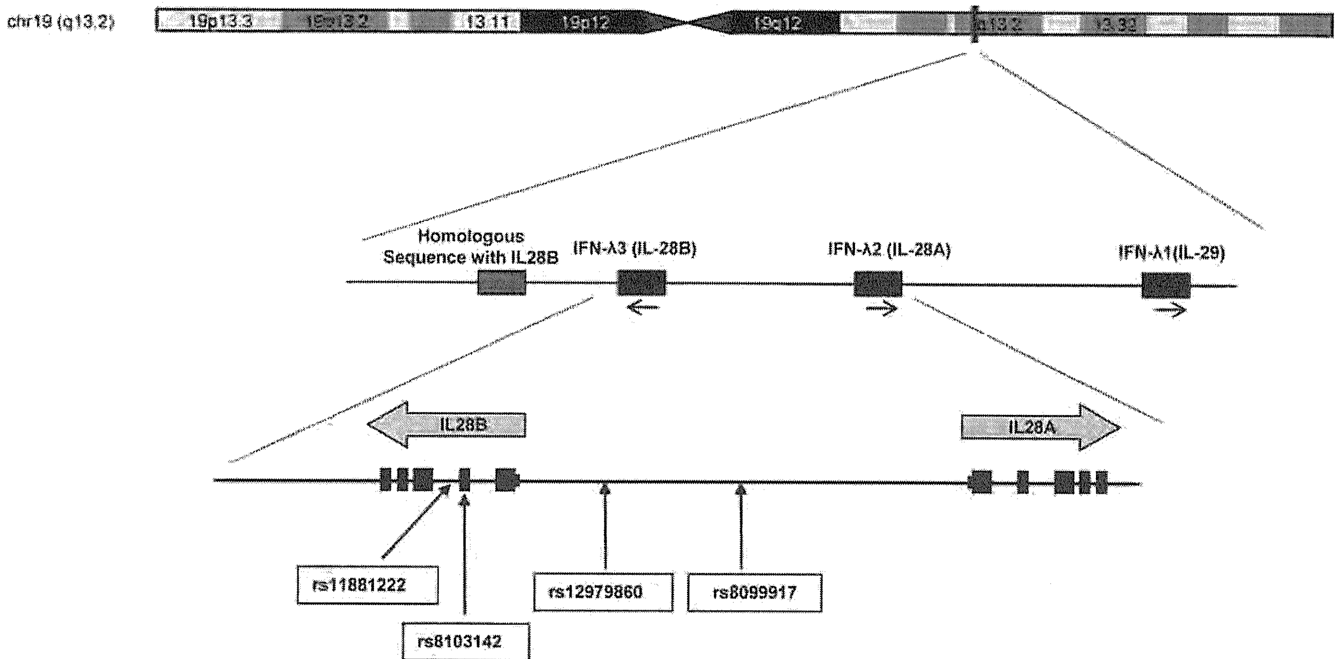

FIG. 2. Location of interferon lambda genes and the four SNPs (rs11881222, rs8103142, rs12979860, and rs8099917) associated with IL-28B. chr19, chromosome 19.

1B), recruited from NHO Nagasaki Medical Center, Nagoya City University Hospital, Nagoya Daini Red Cross Hospital, and Kawasaki Medical University Hospital in Japan, in the second stage (Table 1). We then focused on 10 patients whose four SNPs were found in the first and second stages not to be in LD and investigated the response to PEG-IFN/RBV treatment in detail for these patients. Informed consent was obtained from each patient who participated in the study. This study was conducted in accordance with provisions of the Declaration of Helsinki.

Definition of treatment responses. Nonvirological response (NVR) was defined as less than a 2-log-unit decline in the serum level of HCV RNA from the pretreatment baseline value within the first 12 weeks or detectable viremia 24 weeks after treatment. Virological response (VR) was defined in this study as the achievement of sustained VR (SVR) or transient VR (TVR); SVR was defined as undetectable HCV RNA in serum 6 months after the end of treatment, whereas TVR was defined as a reappearance of HCV RNA in serum after treatment was discontinued in a patient who had undetectable HCV RNA during the therapy or had achieved a more than 2-log-unit decline within the first 12 weeks after treatment.

DNA extraction. Whole blood was collected from all participants and centrifuged to separate the buffy coat. Genomic DNA was extracted from the buffy coat with Genomix (Talent SRL, Italy).

Five different genotyping methods. Four SNPs (rs11881222, rs8103142, rs12979860, and rs8099917) (Fig. 2) were determined in 292 patients by five different genotyping methods. We developed the five methods (direct sequencing, high-resolution melting analysis [HRM], hybridization probe (HP), InvaderPlus assay (Invader), and the TaqMan SNP genotyping assay (TaqMan) to determine the genotypes of the rs11881222 and rs8103142 polymorphisms. We also developed four different methods (direct sequencing, HRM, HP, and Invader) to determine the genotypes of the rs12979860 and rs8099917 polymorphisms. The genotype of rs12979860 was also determined by the TaqMan genotyping method developed by Duke University, and the genotype of rs8099917 was also determined with the TaqMan predesigned SNP genotyping assay. Figures 3,

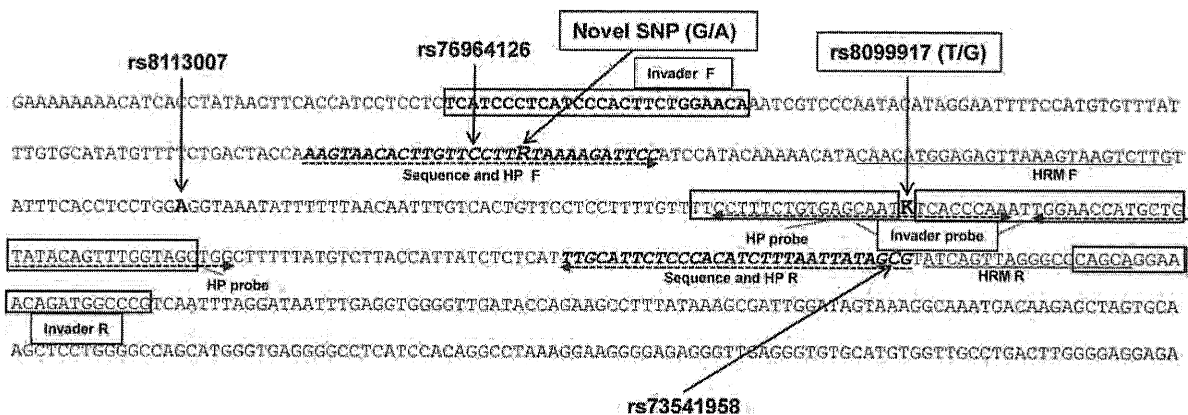

FIG. 3. The nucleotide sequence around rs8099917 is shown. Primers and probes for four different methods (Sequence, direct sequencing; HRM, high-resolution melting analysis; HP, hybridization probe; Invader, InvaderPlus assay) to determine rs8099917 polymorphism are shown. F, forward primer; R, reverse primer.

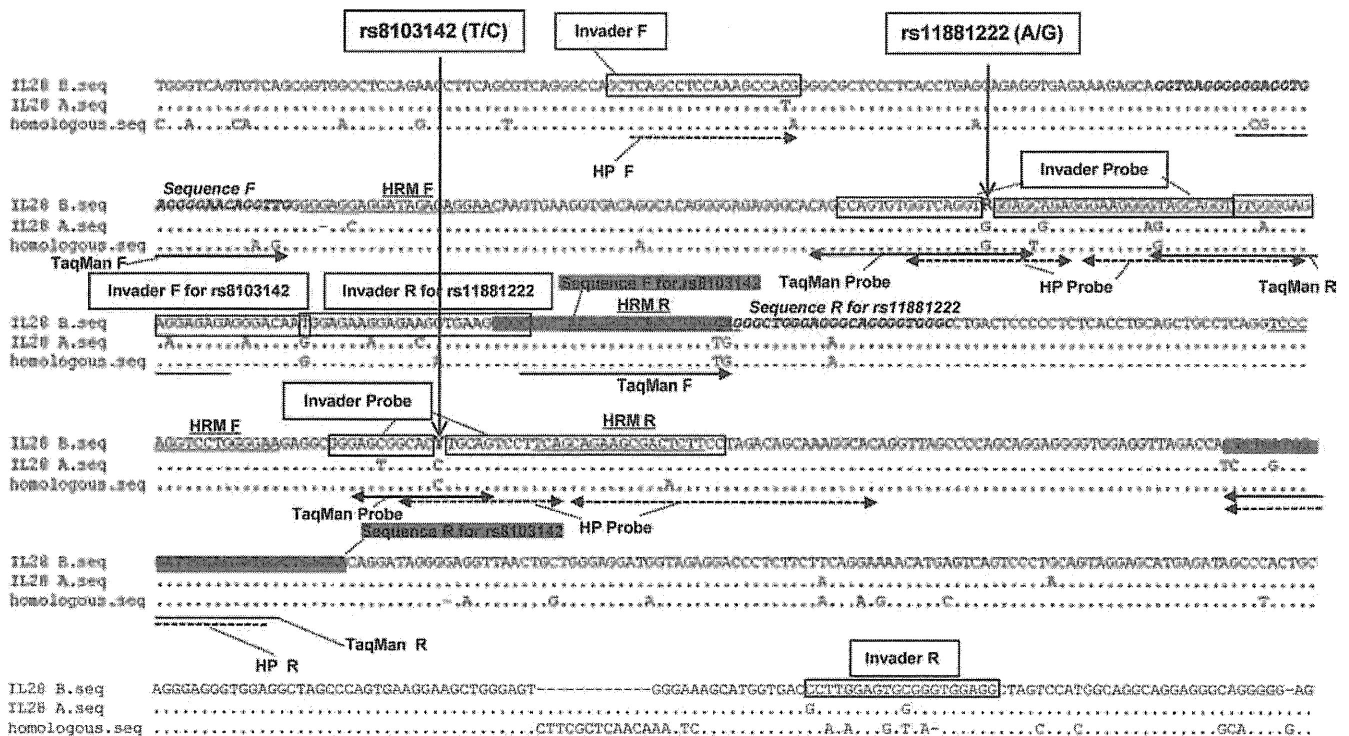

FIG. 4. The nucleotide sequence around rs11881222 and rs8103142 is shown. Primers and probes for five different methods (Sequence, direct sequencing; HRM, high-resolution melting analysis; HP, hybridization probe; Invader, InvaderPlus assay; TaqMan, TaqMan assay) to determine rs11881222 and rs8103142 polymorphisms are shown. F, forward primer; R, reverse primer.

4, and 5 show the primers and probes for each genotyping method. Because the sequence of IL-28B is very similar to those of IL-28A, IL-29, and a homologous sequence upstream of IL-28B, we had to design the primers and probe for each method to distinguish IL-28B from the other sequences. First, primers were designed with Visual OMP Nucleic Acid software, and then we confirmed that the candidate primers should not amplify sequences other than the target region by using UCSC Genome Browser. Next, we confirmed that the amplicon was resolved as a single band, when the PCR products amplified by the primers under evaluation were electrophoresed. Finally, we had to optimize each set of primers and probe for each method (Fig. 3 to 5; see the table in the supplemental material).

Direct sequencing. PCR was carried out with 12.5 μl AmpliTaq Gold 360 master mix (Applied Biosystems), 10 pmol of each primer, and 10 ng of genomic DNA under the following thermal cycler conditions: stage 1, 94°C for 5 min; stage 2, 94°C for 30 s, 65°C for 30 s, 72°C for 45 s, for a total of 35 cycles; and stage 3, 72°C for 7 min. For sequencing, 1.0 μl of the PCR products was incubated with the use of a BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems). After ethanol purification, the reaction products were applied to the Applied Biosystems 3130xl DNA analyzer.

HRM analysis. HRM analysis was performed on a LightCycler 480 (LC480; Roche Diagnostics) as described previously (5, 15, 24). We designed pairs of primers flanking each SNP (Fig. 3 to 5) to amplify DNA fragments shorter than 200 bp. PCR was performed in a 20-μl volume containing 10 μl LightCycler 480 high-resolution melting master mix (Roche Applied Science), 4 pmol of each primer, and 10 ng genomic DNA. The cycling conditions were as follows: SYBR green I detection format, 1 cycle of 95°C for 10 min and 50 cycles of 95°C for 5 s, 60°C for 10s, and 72°C for 20 s, followed by an HRM step of 95°C for 1 min, 40°C for 1 min, and 74°C for 5 s and continuous acquisition to 90°C at 25 acquisitions per 1°C. HRM data were analyzed with Gene Scanning software (Roche Diagnostics).

Hybridization probe. We designed oligonucleotide primers and hybridization probes for the four SNPs (Fig. 3 to 5). All assays were performed with the LC480 as described previously (4, 18). The amplification mixture consisted of 4 μl of 5× reaction mixture (LightCycler 480 genotyping master; Roche Diagnostics), 5 pmol of each oligonucleotide primer, 3.2 pmol of each oligonucleotide probe, and 10 ng of template DNA in a final volume of 20 μl. Samples were amplified as follows: 45 cycles of denaturation at 95°C for 10 s, annealing at 60°C for 10 s, and extension at 72°C for 20 s. The generation of target amplicons for each sample was monitored between the annealing and elongation steps at 610 and 640 nm. Samples positive for target genes were identified by the instrument at the cycle number where the fluorescence attributable to the target sequences exceeded that measured as background. Those scored as positive by the instrument were confirmed by visual inspection of the graphical plot (cycle number versus fluorescence value) generated by the instrument.

InvaderPlus assay. The InvaderPlus assay, which combines PCR and the Invader reaction (11, 12), was performed with the LC480. The enzymes used in InvaderPlus are native *Taq* polymerase (Promega Corporation, Madison, WI) and Cleavase enzyme (Third Wave Technologies, Madison, WI). The reaction is configured to use PCR primers with a melting temperature (T_m) of 72°C and Invader detection probe with a target-specific T_m of 63°C. The Invader oligonucleotide overlaps the probe by one nucleotide, forming at 63°C an overlap flap substrate for the Cleavase enzyme. The first step of InvaderPlus is PCR target amplification, in which the reaction is subjected to 18 cycles of a denaturation step (95°C for 15 s) and hybridization and extension steps (70°C for 1 min). At the end of PCR cycling, the reaction mixture is incubated at 99°C for 10 min to inactivate the *Taq* polymerase. Next, the reaction temperature is lowered to 63°C for 15 to 30 min to permit the hybridization of the probe oligonucleotide and the formation of the overlap flap structure. Data were analyzed by endpoint genotyping software (Roche Diagnostics).

TaqMan assay. The rs8099917 polymorphism was determined by using TaqMan predesigned SNP genotyping assays, as recommended by the manufacturer. The TaqMan assay for determination of the genotype of rs12979860 was kindly provided by David B. Goldstein at Duke University. We designed primers and probes for TaqMan genotyping assays for the other two SNPs. Each genomic DNA sample (20 ng) was amplified with TaqMan universal PCR master mix reagent (Applied Biosystems, Foster City, CA) combined with the specific TaqMan SNP genotyping assay mixture, corresponding to the SNP to be genotyped. The assays were carried out using the LC480 (Roche Applied Science) and the following conditions: 2 min at 50°C and 10 min at 95°C, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. Data were analyzed by endpoint genotyping software (Roche Diagnostics).

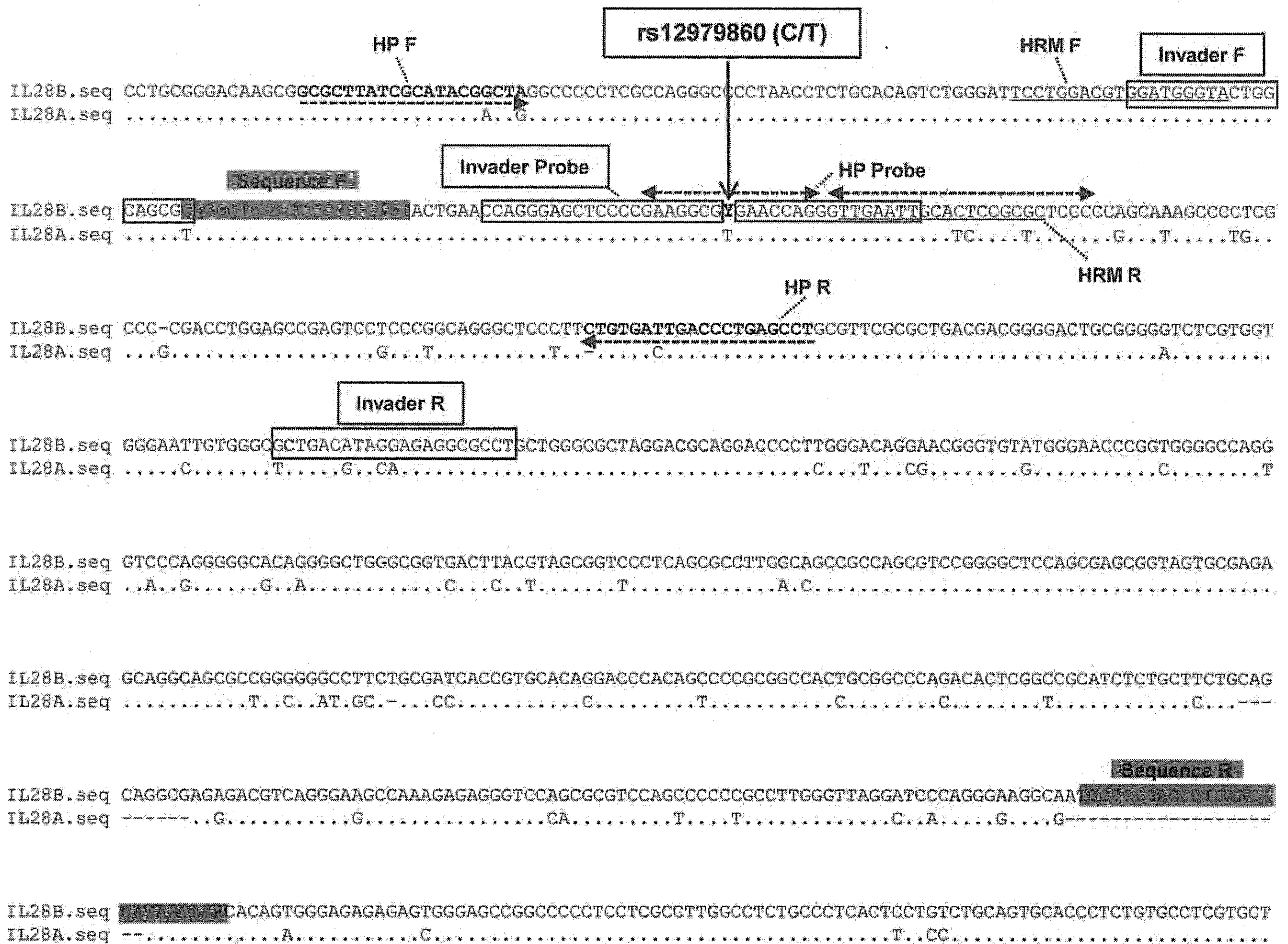

FIG. 5. The nucleotide sequence around rs12979860 is shown. Primers and probes for four different methods (Sequence, direct sequencing; HRM, high-resolution melting analysis; HP, hybridization probe; Invader, InvaderPlus assay) to determine rs12979860 are shown. F, forward primer; R, reverse primer.

RESULTS

Genotyping for four SNPs associated with IL-28B was unsuccessful by HRM in five cases. Figure 1A shows the patients' flowchart of the first stage. Genotyping of four SNPs (rs11881222, rs8103142, rs12979860, and rs8099917) was attempted by five different methods (direct sequencing, HRM, HP, Invader, and TaqMan) for 292 patients. In five cases, one of the four SNPs could not be genotyped by HRM. Therefore, we excluded the HRM method from further study. The genotyping failures by HRM involved two cases for rs11881222, two cases for rs8103142, and one case for rs8099917.

Consistencies of four different methods to determine genotypes for four SNPs associated with IL-28B. Consistencies among the results of genotyping by the remaining four methods were 100%, except for the results for rs8099917 (Table 2). For rs8099917, the results determined by direct sequencing were inconsistent with the other three methods in two cases (Tables 2 and 3). The HP, TaqMan, and Invader methods were accurate and reliable for genotyping the four SNPs associated with IL-28B. Invader was chosen for genotyping in the second stage, because the analysis time was the shortest and the sen-

TABLE 2. Determination of four SNPs associated with IL-28B by four different methods[a]

SNP	Genotype	No. (%) of cases with genotype by:			
		Direct sequencing	HP	Invader	TaqMan
rs11881222	AA	199 (69.3)	199 (69.3)	199 (69.3)	199 (69.3)
	AG	84 (29.3)	84 (29.3)	84 (29.3)	84 (29.3)
	GG	4 (1.4)	4 (1.4)	4 (1.4)	4 (1.4)
rs8103142	TT	199 (69.3)	199 (69.3)	199 (69.3)	199 (69.3)
	TC	84 (29.3)	84 (29.3)	84 (29.3)	84 (29.3)
	CC	4 (1.4)	4 (1.4)	4 (1.4)	4 (1.4)
rs12979860	CC	198 (69.0)	198 (69.0)	198 (69.0)	198 (69.0)
	CT	85 (29.6)	85 (29.6)	85 (29.6)	85 (29.6)
	TT	4 (1.4)	4 (1.4)	4 (1.4)	4 (1.4)
rs8099917	TT	204 (71.1)	202 (70.4)	202 (70.4)	202 (70.4)
	TG	79 (27.5)	81 (28.2)	81 (28.2)	81 (28.2)
	GG	4 (1.4)	4 (1.4)	4 (1.4)	4 (1.4)

[a] There was 100% consistency for rs11881222, rs8103142, and rs12979860, and there was 99.3% consistency for rs8099917.

TABLE 3. Inconsistency in two cases between rs8099917 genotyping by direct sequencing and three other methods

Case no.	rs8099917 genotype by[a]:			
	Direct sequencing	HP	Invader	TaqMan
1	**T/T**	T/G	T/G	T/G
2	**T/T**	T/G	T/G	T/G

[a] Homozygous genotypes are highlighted in boldface.

sitivity was the greatest of the three methods (HP, TaqMan, and Invader), as reported previously (20).

Genotyping error for rs8099917 by direct sequencing due to novel SNP. In two cases, the results of genotyping for rs8099917 by direct sequencing were inconsistent with the results by the other methods (Table 3). Direct sequencing determined the genotype for rs8099917 as T/T in cases 1 and 2; however, the other three genotyping methods (HP, Invader, and TaqMan) determined the genotypes for rs8099917 as T/G in both cases. Further study using alternative primers for direct sequencing revealed that the correct genotypes were T/G and revealed a novel minor SNP present in the forward primer binding site in these two cases (data on file) and which interfered with the PCR amplification step (Fig. 3).

Distribution of haplotypes among four SNPs associated with IL-28B. In the first stage, the four SNPs were in LD in 281 (98.6%) of 285 cases and not in LD in the remaining 4 (1.4%). The first stage revealed five different haplotypes (no. 1 to 5 in Table 4). In haplotypes 1 to 3, the four SNPs were in LD (haplotype 1, homozygous of the major allele among 4 SNPs; $n = 198$ [69.5%]; haplotype 2, heterozygous among 4 SNPs; $n = 79$ [27.7%]; and haplotype 3, homozygous of the minor allele among 4 SNPs; $n = 4$ [1.4%]). In haplotype 4 (3 cases) rs11881222, rs8103142, rs12979860, and rs8099917 were AG, TC, CT, and TT, respectively. In haplotype 5 (one case), rs11881222, rs8103142, rs12979860, and rs8099917 were AA, TT, CT, and TT, respectively. Genotyping by the Invader method of the four SNPs associated with IL-28B in 416 patients in the second stage revealed that the four SNPs were not in LD in 6 cases (1.4%) (Table 4). A total of 410 (98.6%) of 416 cases were in LD for the four different SNPs. The second stage showed six different haplotypes (haplotypes 1 to 4, 6, and 7). Haplotypes 1 to 4 were detected in the first stage, but haplotypes 6 and 7 were not. The distribution of haplotypes was such that haplotypes 1, 2, 3, and 4 were found in 294 (70.7%), 110 (26.5%), 6 (1.4%), and 4 (1.0%) cases, respectively. In haplotype 6 (one case), rs11881222, rs8103142, rs12979860, and rs8099917 were AG, TT, CC, and TT, respectively. In haplotype 7 (one case), rs11881222, rs8103142, rs12979860, and rs8099917 were AA, TT, CT, and TG, respectively.

Response to PEG-IFN/RBV treatment in 10 cases in which the four SNPs associated with IL-28B were not in LD. In 7 (cases 1 to 7 [70%]) of the 10 cases where the four SNPs were not in LD, the haplotype was such that rs11881222, rs8103142, rs12979860, and rs8099917 were AG, TC, CT, and TT, respectively (Table 5). In nine cases (cases 1 to 9), rs8099917 was homozygous for the major allele, while one or more of the other SNPs were heterozygous. Eight (cases 1 to 8) of these

TABLE 4. Distribution of haplotypes among four SNPs associated with IL-28B in stages 1 and 2

Stage	Haplotype no.	Genotype for SNP:				No. (%) of cases with haplotype shown
		rs11881222	rs8103142	rs12979860	rs8099917	
1	1	AA	TT	CC	TT	198 (69.5)
	2	AG	TC	CT	TG	79 (27.7)
	3	GG	CC	TT	GG	4 (1.4)
	4	AG	TC	CT	TT	3 (1.0)
	5	AA	TT	CT	TT	1 (0.4)
2	1	AA	TT	CC	TT	294 (70.7)
	2	AG	TC	CT	TG	110 (26.5)
	3	GG	CC	TT	GG	6 (1.4)
	4	AG	TC	CT	TT	4 (1.0)
	6	AG	TT	CC	TT	1 (0.2)
	7	AA	TT	CT	TG	1 (0.2)

nine cases were viral responders who met the following criteria: HCV had disappeared during therapy, or HCV RNA had decreased more than 2 log copies/ml before 12 weeks after beginning of therapy, although some cases were under treatment or before determination of the final response to PEG-IFN/RBV. Case 9 was NVR due to poor adherence of PEG-IFN (<50% dose), even though rs8099917 was homozygous of the major allele. The haplotype of case 9 showed that rs11881222, rs8103142, rs12979860, and rs8099917 were AA, TT, CT, and TG, respectively. NVR in case 10 was reasonable from the genotypes of rs8099917 and rs12979860, because they were heterozygous, although rs11881222 and rs8103142 were homozygous for the major allele.

DISCUSSION

The relationship between SNPs associated with IL-28B and the response to PEG-IFN/RBV therapy for chronic hepatitis C was found by SNP array, using GWAS technology, by three different groups throughout the world, including our own, in 2009 (6, 19, 21). Following these reports, many studies have confirmed the association between the response to PEG-IFN/RBV and SNPs associated with IL-28B (14, 16). Therefore, it is obvious that these SNPs may be valuable for predicting the response to PEG-IFN/RBV therapy. Recently, it was reported that various SNPs were associated with development of disease and response to therapy and correlated with adverse effects. Several SNPs, such as the UGT1A1 polymorphism for the treatment with irinotecan (1, 17), have already been exploited in clinical practice to avoid severe adverse effects. These tailor-made therapies are expected to become more common in clinical practice in the near future (9). The next step toward tailor-made therapy for PEG-IFN/RBV therapy against chronic hepatitis C involved the development of simple, accurate, and inexpensive methods to determine the genotype of SNPs and determination of the best SNP where the four SNPs associated with IL-28B were not in LD, so that they may be applied in clinical practice.

Genotyping of IL-28B SNPs is quite different from other SNPs, because the sequence of IL-28B is very similar to those of IL-28A, IL-29, and an additional homologous sequence upstream of IL-28B (Fig. 2). We had to design primers and probes for each method to distinguish IL-28B specifically. We

TABLE 5. Clinical characteristics of 10 cases in which the SNPs associated with IL-28B were not in LD

Case no.[a]	SNP of IL-28B[b]				Age (yr)	Gender	Genotype	Viral titer	Final response to PEG-IFN/RBV	VR or NVR	Period of disappearance of HCV
	rs11881222	rs8103142	rs12979860	rs8099917							
1	A/G	T/C	C/T	**T/T**	64	Female	1b	6.5	TR	VR	4 wk
2	A/G	T/C	C/T	**T/T**	72	Male	1b	2.9	SVR	VR	4 wk
3	A/G	T/C	C/T	**T/T**	64	Male	1b	7	ND[c]	VR	8 wk
4	A/G	T/C	C/T	**T/T**	51	Female	1b	7.2	Under treatment	VR	3.6 log units down after 12 wk
5	A/G	T/C	C/T	**T/T**	60	Female	2	5.8	Under treatment	VR	12 wk
6	A/G	T/C	C/T	**T/T**	56	Female	1b	5.9	Under treatment	VR	2.0 log units down after 2 wk
7	A/G	T/C	C/T	**T/T**	62	Male	1b	5.4	SVR	VR	4 wk
8	A/G	**T/T**	**C/C**	**T/T**	58	Male	1b	6.2	TR	VR	12 wk
9	**A/A**	**T/T**	C/T	**T/T**	68	Male	1b	7	NVR	NVR	—[d]
10	**A/A**	**T/T**	C/T	T/G	48	Female	1b	6	NVR	NVR	—

[a] All cases shown were treated with PEG-IFN/RBV.
[b] Homozygous genotypes are highlighted in boldface.
[c] ND, not determined. The final response to PEG-IFN/RBV was not determined in this patient because 6 months had not passed after the end of treatment.
[d] —, HCV did not disappear.

think that the results in this paper are especially applicable to IL-28B genotyping. In this study, only HRM failed to determine the genotype of SNPs associated with IL-28B. The reason HRM failed more frequently than the other genotyping methods is attributable to the characteristics of this specific method. Because HRM determines the genotype of each SNP by distinguishing the melting curve of an amplicon of around 200 bp, it may tend to be influenced by another SNP. As a matter of fact, minor SNPs around rs8099917 were found in cases of genotyping failure by HRM (data not shown). Although this specific characteristic of the HRM method is useful for detecting novel mutations or SNPs, it is not suitable for determination of the genotype of SNPs associated with IL-28B.

Direct sequencing erroneously reported the T/G genotype as T/T for the rs8099917 polymorphism. We found that the cause of this genotyping error was a novel rare SNP in the forward primer binding site used for amplification and direct sequencing (data on file). Because this novel SNP was not registered as an SNP in the NCBI database, the primer was designed at this site. Since the novel SNP correlated with the rs8099917 polymorphism in LD, adenine for the novel SNP is present on the same allele as guanine in the rs8099917 polymorphism. Therefore, the forward PCR primer (AAGTAACACTTGTTCCTT GTAAAAGATTCC) could not anneal to the binding site, which was changed from guanine (G) to adenine (A) at the underlined nucleotide position: only the allele which has T at the rs8099917 was amplified, the genotype was determined as T/T. Rare sequence variations not registered in the database, might be present in the primer binding sites for amplification and might be the cause of erroneous direct sequencing. Ikegawa et al. reported that annealing efficiency in direct sequencing led to the mistyping of an SNP (10). Although our results in this paper are especially applicable to IL-28B genotyping, it should be recognized that allele-dependent PCR amplification and erroneous typing can occur when SNPs are genotyped by a PCR-based approach. Should SNPs associated with IL-28B be found not to be in LD, it would be preferable to confirm the genotype by another method.

In 10 cases, four SNPs associated with IL-28B were not in LD. In seven (70%) of the 10 cases, the haplotype showed that rs11881222, rs8103142, rs12979860, and rs8099917 were AG, TC, CT, and TT, respectively. Only the rs8099917 polymorphism differed frequently from the other three SNPs. The reason for the high frequency of this haplotype is thought to be attributable to the location of these SNPs. The location of rs8099917 is downstream and quite far from the two SNPs (rs11881222 and rs8103142) in the IL-28B gene (Fig. 2). The SNPs rs11881222 and rs8103142 were almost perfectly in LD, because they are located close to each other.

It is well described that homozygosity for the major allele of SNPs associated with IL-28B is correlated with a better response to PEG-IFN/RBV treatment, and minor allele-positive patients are poor responders. However, the response to PEG-IFN/RBV remains unknown when several SNPs associated with IL-28B are not in LD. Because cases in which the SNPs are not in LD are quite rare, it was thought to be difficult to study such cases. In this study, 10 (1.4%) of 708 patients showed haplotypes in which the four SNPs were not in LD. We focused on the response to PEG-IFN/RBV therapy in these 10 cases (Table 5). We evaluated the response to PEG-IFN/RBV treatment from the viewpoint of virological response, because some patients had not completed their PEG-IFN/RBV treatment. (Case 3 was before determination for the final response after finishing the treatment, and cases 4 to 6 were under treatment.)

Thomas et al. reported that allele frequencies for rs12979860 varied among racial and ethnic groups (23). Indeed, the observation that the major allele is less frequent among individuals of African descent than those of European descent might explain the observed discrepancy in the frequencies of viral clearance in these two ethnic groups, where clearance occurs in 36.4% of HCV infections in individuals of non-African ancestry, but in only 9.3% of infections in individuals of African ancestry (22). We have recruited only Japanese chronic hepatitis C patients for this study. Since the distribution of haplotype and response to PEG-IFN/RBV treatment should vary among populations, further study will be necessary for any other populations except Japanese.

We have shown that the rs8099917 polymorphism determined by Invader assay should be the best predictor of the

response to PEG-IFN/RBV in Japanese chronic hepatitis C patients.

ACKNOWLEDGMENT

This study was supported by grants (22-302 and 21-113) from the National Center for Global Health and Medicine in Japan.

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JJCO Japanese Journal of Clinical Oncology

Jpn J Clin Oncol 2012;42(2)139–147
doi:10.1093/jjco/hyr184
Advance Access Publication 15 December 2011

Epidemiology Note

Cancer Incidence and Incidence Rates in Japan in 2006: Based on Data from 15 Population-based Cancer Registries in the Monitoring of Cancer Incidence in Japan (MCIJ) Project

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Received September 13, 2011; accepted November 14, 2011

The Japan Cancer Surveillance Research Group estimated the cancer incidence in 2006 as part of the Monitoring of Cancer Incidence in Japan (MCIJ) project, on the basis of data collected from 15 of 32 population-based cancer registries. The total number of incidences in Japan for 2006 was estimated as 664 398 (C00–C96). The leading cancer site was stomach for men and breast for women. Age-standardized incidence rates remained at almost the same level as for the previous 3 years.

Key words: cancer incidence – incidence estimates – cancer registry – Japan

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The Japan Cancer Surveillance Research Group is involved in cancer monitoring in Japan since 2000 (1–6). This group estimated the cancer incidence in 2006 as part of the Monitoring of Cancer Incidence in Japan (MCIJ) project, on the basis of data collected from 15 of 32 population-based cancer registries: Miyagi, Akita, Yamagata, Tochigi, Chiba, Kanagawa, Niigata, Fukui, Aichi, Tottori, Okayama, Hiroshima, Saga, Nagasaki and Kumamoto.

If data from all 32 registries were used, this would have led to a large underestimation of the national cancer incidence because of poor registration. The methods of registry selection, estimation of incidence and the limitations of these methods have been explained in previous studies (7–9). We maintained the same methodology since the MCIJ2003: (1) we invited all 32 population-based cancer registries in Japan to participate, and from these we selected the 15 cancer registries with high-quality data, which cover 33.7% of the total population in Japan; in order to estimate the national incidence, (2) we used 2006 data alone for the national estimation.

Through estimation, we consider those who meet the following standards as belonging to the 'high-quality' data area: DCO% (death certificate only: proportion of patients for whom the death certificate provides the only notification to the registry) <25% or DCN% (death certificate notification: proportion of patients for whom the death certificate provides the first notification to the registry) <30%, and IM ratio (incidence to mortality ratio) >1.5.

In estimating cancer incidence, in order to avoid a bias caused by the size of the registry population, an arithmetic mean of incidence rates of all the eligible registries (by primary site, sex and 5-year age group) was used, instead of dividing the total incidence by the total population. Cancer mortality in Japan was estimated by using the exact methods employed for the estimation of incidence, by taking mortality data from the vital statistics of the same eligible registries. Correction coefficients were then computed according to primary site and sex by using the observed cancer deaths divided by the number of estimated deaths. The estimated uncorrected cancer incidence

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according to primary site, sex and 5-year age group were multiplied by the correction coefficients to obtain corrected estimates. These corrected figures were then multiplied by the corresponding Japanese population to obtain the incidence figures for all Japan.

For this year, data from the Osaka prefecture, regularly considered as one of the registries with high quality, were not available for the MCIJ project. The other registries remained since the previous estimation in 2005, and Akita, Tochigi and Saga joined the registries for the national estimation.

The number of incidences, crude rates, age-standardized rates and completeness of registration in 2006 are shown in Table 1, and the age-specific number of incidences and the rates according to sex and primary site are shown in Tables 2 and 3. The total number of incidences in Japan for 2006 was estimated as 664 398 (C00–C96). The time trends of age-standardized incidence rates for the five major sites and male- and female-specific sites in 1975–2006 are shown in Fig. 1 (standard population: the world population) and in Fig. 2 (standard population: the 1985 Japanese model population). The leading cancer site according to the crude and age-standardized incidence rates was stomach followed by colon and rectum and lung for men and breast followed by colon and rectum and stomach for women since the research group took over national estimation of incidence, as shown in Table 1. Since the early 2000s, the age-standardized incidence rates in all cancer sites slightly increased, whereas they remained almost flat in the 1990s. The slight increase is thought to be due to the substantive increase of incidence in some primary sites, and partly due to improvement in the completeness of registry data in the 2000s. The estimated cancer incidence data in Japan by sex, site, 5-year age group and calendar year during the period 1975–2006 are available as a booklet, and as an electronic database on the website (only available in Japanese, http://ganjoho.jp/professional/statistics/monita.html).

Table 1. Incidence, completeness of reporting and accuracy of diagnosis in Japan according to sex and primary site, 2006

Primary sites	ICD-10th	Number of incidences	Crude rate[a]	Age-standardized rate[a]		Indices of data quality		
				World population	Japanese 1985 model population	DCO/I (%)	I/M	MV/I (%)
Male								
All sites (incl. CIS)	C00–C96, D00–D09	400 605	642.7	287.3	407.1	10.6	2.02	52.9
All sites	C00–C96	388 496	623.3	278.0	394.3	10.7	1.96	52.6
Lip, oral cavity and pharynx	C00–C14	9130	14.6	7.3	9.9	9.4	2.12	81.0
Esophagus	C15	15 818	25.4	11.7	16.2	10.9	1.64	80.8
Stomach	C16	79 437	127.4	56.9	80.8	11.3	2.43	81.9
Colon and rectum	C18–C20	62 116	99.7	45.7	64.1	9.8	2.77	84.5
Colon	C18	38 182	61.3	27.3	38.8	10.4	2.79	81.4
Rectum	C19	23 934	38.4	18.4	25.4	9.1	2.75	83.1
Liver	C22	28 872	46.3	20.9	29.5	16.6	1.28	44.3
Gallbladder, etc.	C23–C24	9740	15.6	6.1	9.2	20.7	1.23	43.4
Pancreas	C25	13 768	22.1	9.6	13.8	21.8	1.10	41.5
Larynx	C32	3447	5.5	2.5	3.5	9.5	3.66	74.7
Trachea, bronchus and lung	C33–C34	59 934	96.2	39.2	58.2	14.6	1.30	74.2
Melanoma of skin, etc.	C43–C44	4660	7.5	3.2	4.6	7.7	7.56	88.6
Prostate	C61	42 517	68.2	27.1	40.2	9.1	4.46	86.0
Bladder	C67	12 478	20.0	8.3	12.2	9.7	2.96	82.0
Kidney, renal pelvis, ureter, etc.	C64–C66, C68	9608	15.4	7.4	10.2	10.1	2.40	76.1
Brain and nervous system	C70–C72	2491	4.0	2.8	3.2	14.4	2.60	71.8
Thyroid	C73	2382	3.8	2.3	2.9	4.8	4.78	86.0
Malignant lymphoma	C81–C85, C96	9867	15.8	8.0	10.8	9.3	1.97	88.8
Multiple myeloma	C88, C90	2505	4.0	1.6	2.4	16.3	1.32	75.2
All leukaemias	C91–C95	5544	8.9	5.2	6.5	16.5	1.27	84.4

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Table 1. *Continued*

Primary sites	ICD-10th	Number of incidences	Crude rate[a]	Age-standardized rate[a]		Indices of data quality		
				World population	Japanese 1985 model population	DCO/I (%)	I/M	MV/I (%)
Female								
All sites (incl. CIS)	C00–C96, D00–D09	293 179	448.0	205.8	274.6	12.4	2.23	76.9
All site	C00–C96	275 902	421.6	187.3	251.8	13.2	2.10	75.6
Lip, oral cavity and pharynx	C00–C14	3496	5.3	2.2	3.1	12.4	2.05	78.7
Esophagus	C15	2905	4.4	1.6	2.3	14.1	1.71	78.9
Stomach	C16	37 474	57.3	21.2	29.7	15.9	2.12	78.4
Colon and rectum	C18–C20	44 788	68.4	25.9	36.1	12.5	2.40	81.9
Colon	C18	31 719	48.5	17.6	24.7	13.5	2.33	77.6
Rectum	C19	13 069	20.0	8.3	11.3	11.4	2.60	80.4
Liver	C22	14 021	21.4	6.8	10.0	21.7	1.26	40.1
Gallbladder, etc.	C23–C24	10 358	15.8	4.3	6.5	26.7	1.16	35.6
Pancreas	C25	11 722	17.9	5.6	8.2	27.3	1.08	33.0
Larynx	C32	278	0.4	0.2	0.2	11.6	4.56	73.3
Trachea, bronchus and lung	C33–C34	25 543	39.0	13.9	19.6	21.2	1.48	60.3
Melanoma of skin, etc.	C43–C44	3930	6.0	2.1	2.8	9.7	6.09	86.8
Breast (incl. CIS)	C50, D05	53 783	82.2	51.0	65.6	4.3	4.81	91.0
Breast (only invasive)	C50	49 772	76.1	46.8	60.3	4.6	4.45	90.2
Uterus (incl. CIS)	C53–C55, D06	25 859	39.5	28.0	34.7	4.9	4.69	89.9
Uterus (only invasive)	C53–C55	18 642	28.5	17.9	22.8	6.1	3.38	87.4
Cervix uteri	C53	8968	13.7	9.5	12.0	5.3	3.61	88.4
Corpus uteri	C54	8629	13.2	7.9	10.1	3.7	5.83	90.9
Ovary	C56	7913	12.1	7.2	9.1	10.3	1.78	80.0
Bladder	C67	4032	6.2	1.8	2.7	14.0	2.11	75.3
Kidney, renal pelvis, ureter, etc.	C64–C66, C68	5278	8.1	3.0	4.3	14.8	2.30	70.7
Brain and nervous system	C70–C72	2217	3.4	1.9	2.3	16.3	3.04	65.2
Thyroid	C73	7852	12.0	7.4	9.4	7.1	7.53	85.9
Malignant lymphoma	C81–C85, C96	8769	13.4	6.1	8.0	10.2	2.37	87.1
Multiple myeloma	C88, C90	2304	3.5	1.2	1.7	20.1	1.18	68.6
All leukaemias	C91–C95	3835	5.9	3.4	3.9	19.6	1.26	83.5

ICD-10th, *International Classification of Diseases*, 10th Revision; DCO/I, proportion of cases with the death certificate only to incident cases; I/M, number of incidences/number of deaths; MV/I, proportion of microscopically verified cases to incident cases; CIS, carcinoma *in situ*.
[a]Per 100 000 population.

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Acknowledgements

The survey on cancer incidence in Japan was conducted with contributions from the 32 registries: Hokkaido, Aomori, Iwate, Miyagi, Akita, Yamagata, Ibaraki, Tochigi, Gunma, Chiba, Kanagawa, Niigata, Toyama, Ishikawa, Fukui, Gifu, Aichi, Shiga, Kyoto, Tottori, Okayama, Hiroshima, Yamaguchi, Tokushima, Kagawa, Ehime, Kochi, Saga, Nagasaki, Kumamoto, Kagoshima and Okinawa.

Funding

The study was supported by the 3rd-term Comprehensive Ten-year Strategy for Cancer Control.

Conflict of interest statement

None declared.

Table 2. Age-specific incidence in Japan according to sex and primary site, 2006

Primary sites	ICD-10th	Age group (years)																	
		0–4	5–9	10–14	15–19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–79	80–84	85+
Male																			
All sites (incl. CIS)	C00–C96, D00–D09	310	178	232	250	682	831	1646	2780	4530	8088	15 994	37 893	45 699	60 319	74 748	70 466	44 689	31 270
All sites	C00–C96	310	178	232	250	682	819	1594	2703	4326	7762	15 304	36 250	44 007	58 233	72 418	68 673	43 861	30 894
Lip, oral cavity and pharynx	C00–C14	0	0	7	6	18	54	104	127	137	297	675	1307	1320	1512	1435	1175	599	357
Esophagus	C15	0	0	0	1	0	4	0	13	104	283	749	2042	2498	2929	2893	2304	1221	777
Stomach	C16	0	0	0	2	31	61	192	490	823	1818	3462	8381	9209	12 708	14 940	13 466	8247	5607
Colon and rectum	C18–C20	1	0	14	10	22	80	207	553	861	1600	3014	6717	7618	10 000	11 212	10 086	5784	4337
Colon	C18	1	0	14	10	6	59	101	357	469	775	1517	3546	4460	6051	7168	6711	3851	3086
Rectum	C19	0	0	0	0	16	21	106	196	392	825	1497	3171	3158	3949	4044	3375	1933	1251
Liver	C22	24	4	0	0	5	22	49	112	329	577	1327	3010	3647	4493	6286	4799	2602	1586
Gallbladder, etc.	C23–C24	0	0	0	0	0	16	2	37	27	70	225	541	772	1171	1704	2058	1554	1563
Pancreas	C25	0	0	1	0	0	1	13	42	105	324	602	1284	1681	2000	2215	2423	1693	1384
Larynx	C32	0	0	0	0	0	0	3	18	12	52	177	329	570	683	629	559	265	150
Trachea, bronchus and lung	C33–C34	5	0	0	1	30	26	59	179	365	809	1627	4490	5933	7781	11 021	12 384	9161	6063
Melanoma of skin, etc.	C43–C44	6	1	1	10	15	18	62	53	43	78	186	359	373	473	744	853	709	676
Prostate	C61	0	0	0	0	1	0	12	19	16	74	480	2079	4249	7079	10 252	9286	5337	3633
Bladder	C67	1	0	0	0	23	10	8	25	103	253	368	1057	1211	1490	2089	2474	1917	1449
Kidney, renal pelvis, ureter, etc.	C64–C66, C68	14	10	12	0	0	24	39	121	191	304	539	1165	1247	1364	1671	1396	956	555
Brain and nervous system	C70–C72	47	70	66	39	64	35	51	152	105	107	149	182	254	352	342	280	141	55
Thyroid	C73	0	0	0	5	32	55	103	121	140	129	163	299	334	304	312	204	84	97
Malignant lymphoma	C81–C85, C96	11	10	29	37	78	121	142	188	278	343	549	1133	992	1319	1456	1451	1079	651
Multiple myeloma	C88, C90	0	0	0	0	0	0	5	2	35	37	45	189	228	354	461	509	398	242
All leukaemias	C91–C95	89	60	50	65	114	79	126	131	218	172	269	498	501	660	779	830	545	358

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Female																			
All sites (incl. CIS)	C00–C96, D00–D09	215	167	177	346	1107	2706	5038	7522	10 598	14 515	18 482	28 369	26 894	30 850	37 506	36 649	31 881	40 157
All site	C00–C96	215	167	177	332	763	1659	3427	5650	9066	13 024	17 301	27 011	25 515	29 221	36 123	35 424	31 101	39 726
Lip, oral cavity and pharynx	C00–C14	0	0	2	12	5	24	42	90	111	174	161	308	231	303	528	471	452	582
Esophagus	C15	0	0	0	0	0	0	0	26	24	68	98	354	331	255	410	431	342	566
Stomach	C16	0	0	0	10	36	90	212	407	626	971	1572	3003	3038	4113	5553	5576	5422	6845
Colon and rectum	C18–C20	0	8	0	8	26	71	165	327	628	1158	2030	3825	4287	5490	6679	6834	5830	7422
Colon	C18	0	8	0	8	18	53	114	179	396	667	1272	2597	2834	3749	4766	4999	4261	5798
Rectum	C19	0	0	0	0	8	18	51	148	232	491	758	1228	1453	1741	1913	1835	1569	1624
Liver	C22	4	0	0	0	8	10	40	42	37	109	217	576	1044	1694	2822	2917	2241	2260
Gallbladder, etc.	C23–C24	0	0	0	0	0	4	2	18	5	98	151	384	554	888	1475	1705	2044	3030
Pancreas	C25	0	0	1	4	6	0	18	27	39	153	339	633	886	1211	1782	2021	1988	2614
Larynx	C32	0	0	0	0	0	0	0	0	1	0	13	26	58	25	42	32	40	41
Trachea, bronchus and lung	C33–C34	0	0	0	1	15	29	76	138	252	467	969	2162	2248	2915	4098	3974	3341	4858
Melanoma of skin, etc.	C43–C44	0	9	0	9	33	42	34	49	24	74	142	240	188	388	406	465	605	1222
Breast (incl. CIS)	C50, D05	0	0	0	3	12	277	987	2371	4652	6629	6479	7865	6576	5754	4685	3511	2159	1823
Breast (only invasive)	C50	0	0	0	3	12	243	932	2148	4245	5930	5880	7414	6112	5322	4405	3258	2065	1803
Uterus (incl. CIS)	C53–C55, D06	0	0	0	15	466	1448	2455	2841	2409	2075	2584	3140	2066	1720	1518	1195	921	1006
Uterus (only invasive)	C53–C55	0	0	0	1	160	444	944	1293	1450	1486	2306	2807	1830	1515	1400	1109	895	1002
Cervix uteri	C53	0	0	0	1	127	411	814	1039	1018	828	712	1010	554	531	616	430	443	434
Corpus uteri	C54	0	0	0	0	33	33	128	242	426	637	1540	1748	1221	901	696	529	265	230
Ovary	C56	1	1	36	56	97	93	166	241	433	672	992	1161	895	725	750	585	417	592
Bladder	C67	0	0	0	1	1	17	14	6	9	30	79	225	228	388	666	615	765	988
Kidney, renal pelvis, ureter, etc.	C64–C66, C68	9	0	0	0	0	9	26	49	89	135	225	444	357	595	960	895	680	805
Brain and nervous system	C70–C72	26	41	32	27	38	66	62	34	62	86	106	238	124	179	291	379	212	214
Thyroid	C73	0	0	0	41	103	210	374	412	518	627	750	1134	847	798	754	591	372	321
Malignant lymphoma	C81–C85, C96	37	25	2	20	71	111	78	131	172	318	533	835	967	867	1203	1121	1159	1119
Multiple myeloma	C88, C90	0	0	0	0	0	0	0	0	20	37	87	118	241	252	332	432	375	410
All leukaemias	C91–C95	92	66	23	71	71	86	70	77	113	103	152	347	265	436	410	591	390	472

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Table 3. Age-specific incidence rate per 100 000 population in Japan according to sex and primary site, 2006

Primary sites	ICD-10th	Age group (years)																	
		0–4	5–9	10–14	15–19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–79	80–84	85+
Male																			
All sites (incl. CIS)	C00–C96, D00–D09	11.0	5.9	7.5	7.6	18.2	20.4	33.7	59.4	112.7	209.6	380.9	706.8	1153.4	1655.3	2386.6	3019.1	3310.3	3678.8
All sites	C00–C96	11.0	5.9	7.5	7.6	18.2	20.1	32.6	57.7	107.6	201.2	364.5	676.2	1110.7	1598.1	2312.2	2942.3	3249.0	3634.6
Lip, oral cavity and pharynx	C00–C14	0.0	0.0	0.2	0.2	0.5	1.3	2.1	2.7	3.4	7.7	16.1	24.4	33.3	41.5	45.8	50.3	44.4	42.0
Esophagus	C15	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.3	2.6	7.3	17.8	38.1	63.0	80.4	92.4	98.7	90.4	91.4
Stomach	C16	0.0	0.0	0.0	0.1	0.8	1.5	3.9	10.5	20.5	47.1	82.4	156.3	232.4	348.7	477.0	576.9	610.9	659.6
Colon and rectum	C18–C20	0.0	0.0	0.5	0.3	0.6	2.0	4.2	11.8	21.4	41.5	71.8	125.3	192.3	274.4	358.0	432.1	428.4	510.2
Colon	C18	0.0	0.0	0.5	0.3	0.2	1.4	2.1	7.6	11.7	20.1	36.1	66.1	112.6	166.1	228.9	287.5	285.3	363.1
Rectum	C19	0.0	0.0	0.0	0.0	0.4	0.5	2.2	4.2	9.8	21.4	35.7	59.1	79.7	108.4	129.1	144.6	143.2	147.2
Liver	C22	0.9	0.1	0.0	0.0	0.1	0.5	1.0	2.4	8.2	15.0	31.6	56.1	92.0	123.3	200.7	205.6	192.7	186.6
Gallbladder, etc.	C23–C24	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.8	0.7	1.8	5.4	10.1	19.5	32.1	54.4	88.2	115.1	183.9
Pancreas	C25	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.9	2.6	8.4	14.3	24.0	42.4	54.9	70.7	103.8	125.4	162.8
Larynx	C32	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.4	0.3	1.3	4.2	6.1	14.4	18.7	20.1	24.0	19.6	17.6
Trachea, bronchus and lung	C33–C34	0.2	0.0	0.0	0.0	0.8	0.6	1.2	3.8	9.1	21.0	38.7	83.8	149.7	213.5	351.9	530.6	678.6	713.3
Melanoma of skin, etc.	C43–C44	0.2	0.0	0.0	0.3	0.4	0.4	1.3	1.1	1.1	2.0	4.4	6.7	9.4	13.0	23.8	36.5	52.5	79.5
Prostate	C61	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.4	0.4	1.9	11.4	38.8	107.2	194.3	327.3	397.9	395.3	427.4
Bladder	C67	0.0	0.0	0.0	0.0	0.6	0.2	0.2	0.5	2.6	6.6	8.8	19.7	30.6	40.9	66.7	106.0	142.0	170.5
Kidney, renal pelvis, ureter, etc.	C64–C66, C68	0.5	0.3	0.4	0.0	0.0	0.6	0.8	2.6	4.8	7.9	12.8	21.7	31.5	37.4	53.4	59.8	70.8	65.3
Brain and nervous system	C70–C72	1.7	2.3	2.1	1.2	1.7	0.9	1.0	3.2	2.6	2.8	3.5	3.4	6.4	9.7	10.9	12.0	10.4	6.5
Thyroid	C73	0.0	0.0	0.0	0.2	0.9	1.4	2.1	2.6	3.5	3.3	3.9	5.6	8.4	8.3	10.0	8.7	6.2	11.4
Malignant lymphoma	C81–C85, C96	0.4	0.3	0.9	1.1	2.1	3.0	2.9	4.0	6.9	8.9	13.1	21.1	25.0	36.2	46.5	62.2	79.9	76.6
Multiple myeloma	C88, C90	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.9	1.0	1.1	3.5	5.8	9.7	14.7	21.8	29.5	28.5
All leukaemias	C91–C95	3.2	2.0	1.6	2.0	3.0	1.9	2.6	2.8	5.4	4.5	6.4	9.3	12.6	18.1	24.9	35.6	40.4	42.1

Female																			
All sites (incl. CIS)	C00–C96, D00–D09	8.0	5.8	6.0	11.1	31.1	68.7	105.9	163.8	267.4	378.3	438.0	519.2	643.2	775.1	1018.6	1190.3	1381.3	1788.7
All site	C00–C96	8.0	5.8	6.0	10.6	21.4	42.1	72.0	123.0	228.8	339.4	410.0	494.3	610.3	734.2	981.1	1150.5	1347.5	1769.5
Lip, oral cavity and pharynx	C00–C14	0.0	0.0	0.1	0.4	0.1	0.6	0.9	2.0	2.8	4.5	3.8	5.6	5.5	7.6	14.3	15.3	19.6	25.9
Esophagus	C15	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.6	1.8	2.3	6.5	7.9	6.4	11.1	14.0	14.8	25.2
Stomach	C16	0.0	0.0	0.0	0.3	1.0	2.3	4.5	8.9	15.8	25.3	37.3	55.0	72.7	103.3	150.8	181.1	234.9	304.9
Colon and rectum	C18–C20	0.0	0.3	0.0	0.3	0.7	1.8	3.5	7.1	15.8	30.2	48.1	70.0	102.5	137.9	181.4	222.0	252.6	330.6
Colon	C18	0.0	0.3	0.0	0.3	0.5	1.3	2.4	3.9	10.0	17.4	30.1	47.5	67.8	94.2	129.4	162.4	184.6	258.3
Rectum	C19	0.0	0.0	0.0	0.0	0.2	0.5	1.1	3.2	5.9	12.8	18.0	22.5	34.8	43.7	52.0	59.6	68.0	72.3
Liver	C22	0.1	0.0	0.0	0.0	0.2	0.3	0.8	0.9	0.9	2.8	5.1	10.5	25.0	42.6	76.6	94.7	97.1	100.7
Gallbladder, etc.	C23–C24	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.4	0.1	2.6	3.6	7.0	13.3	22.3	40.1	55.4	88.6	135.0
Pancreas	C25	0.0	0.0	0.0	0.1	0.2	0.0	0.4	0.6	1.0	4.0	8.0	11.6	21.2	30.4	48.4	65.6	86.1	116.4
Larynx	C32	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.5	1.4	0.6	1.1	1.0	1.7	1.8
Trachea, bronchus and lung	C33–C34	0.0	0.0	0.0	0.0	0.4	0.7	1.6	3.0	6.4	12.2	23.0	39.6	53.8	73.2	111.3	129.1	144.8	216.4
Melanoma of skin, etc.	C43–C44	0.0	0.3	0.0	0.3	0.9	1.1	0.7	1.1	0.6	1.9	3.4	4.4	4.5	9.7	11.0	15.1	26.2	54.4
Breast (incl. CIS)	C50, D05	0.0	0.0	0.0	0.1	0.3	7.0	20.7	51.6	117.4	172.8	153.5	143.9	157.3	144.6	127.2	114.0	93.5	81.2
Breast (only invasive)	C50	0.0	0.0	0.0	0.1	0.3	6.2	19.6	46.8	107.1	154.5	139.3	135.7	146.2	133.7	119.6	105.8	89.5	80.3
Uterus (incl. CIS)	C53–C55, D06	0.0	0.0	0.0	0.5	13.1	36.7	51.6	61.9	60.8	54.1	61.2	57.5	49.4	43.2	41.2	38.8	39.9	44.8
Uterus (only invasive)	C53–C55	0.0	0.0	0.0	0.0	4.5	11.3	19.8	28.2	36.6	38.7	54.6	51.4	43.8	38.1	38.0	36.0	38.8	44.6
Cervix uteri	C53	0.0	0.0	0.0	0.0	3.6	10.4	17.1	22.6	25.7	21.6	16.9	18.5	13.3	13.3	16.7	14.0	19.2	19.3
Corpus uteri	C54	0.0	0.0	0.0	0.0	0.9	0.8	2.7	5.3	10.7	16.6	36.5	32.0	29.2	22.6	18.9	17.2	11.5	10.2
Ovary	C56	0.0	0.0	1.2	1.8	2.7	2.4	3.5	5.2	10.9	17.5	23.5	21.2	21.4	18.2	20.4	19.0	18.1	26.4
Bladder	C67	0.0	0.0	0.0	0.0	0.0	0.4	0.3	0.1	0.2	0.8	1.9	4.1	5.5	9.7	18.1	20.0	33.1	44.0
Kidney, renal pelvis, ureter, etc.	C64–C66, C68	0.3	0.0	0.0	0.0	0.0	0.2	0.5	1.1	2.2	3.5	5.3	8.1	8.5	14.9	26.1	29.1	29.5	35.9
Brain and nervous system	C70–C72	1.0	1.4	1.1	0.9	1.1	1.7	1.3	0.7	1.6	2.2	2.5	4.4	3.0	4.5	7.9	12.3	9.2	9.5
Thyroid	C73	0.0	0.0	0.0	1.3	2.9	5.3	7.9	9.0	13.1	16.3	17.8	20.8	20.3	20.1	20.5	19.2	16.1	14.3
Malignant lymphoma	C81–C85, C96	1.4	0.9	0.1	0.6	2.0	2.8	1.6	2.9	4.3	8.3	12.6	15.3	23.1	21.8	32.7	36.4	50.2	49.8
Multiple myeloma	C88, C90	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	1.0	2.1	2.2	5.8	6.3	9.0	14.0	16.2	18.3
All leukaemias	C91–C95	3.4	2.3	0.8	2.3	2.0	2.2	1.5	1.7	2.9	2.7	3.6	6.4	6.3	11.0	11.1	19.2	16.9	21.0

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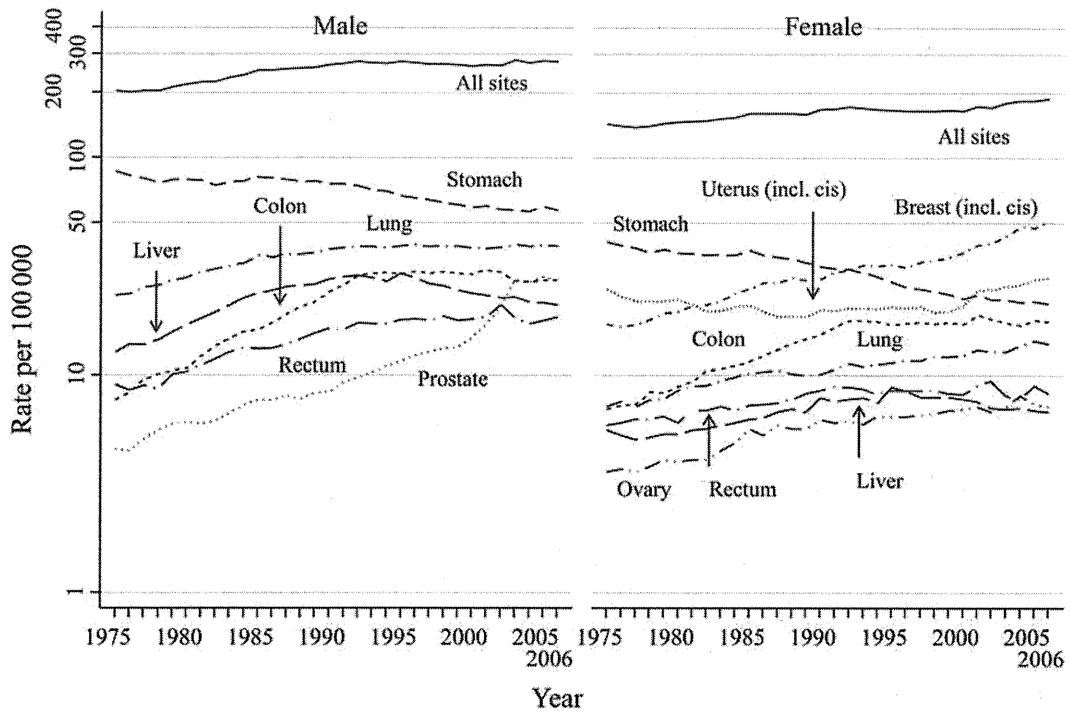

Figure 1. Trends of age-standardized cancer incidence rates for five major sites and specific sites for each sex (standard population: world population).

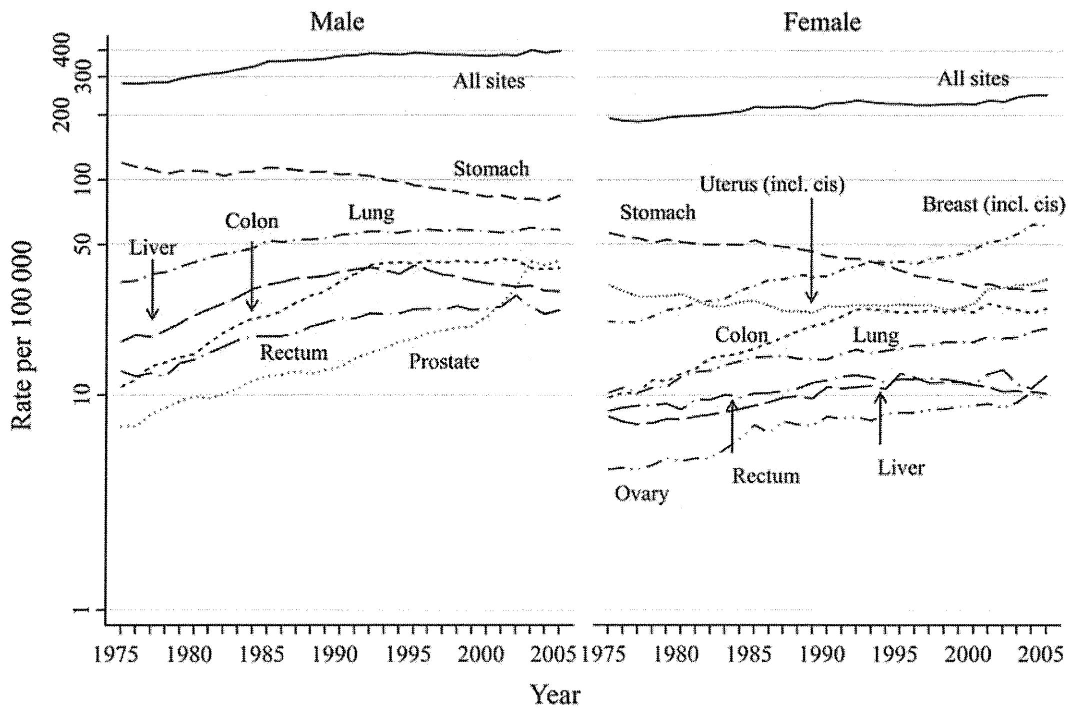

Figure 2. Trends of age-standardized cancer incidence rates for five major sites and specific sites for each sex (standard population: 1985 Japanese model population).

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Cancer Science
The official journal of the Japanese Cancer Association

Trend analysis of cancer incidence in Japan using data from selected population-based cancer registries

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(Received August 23, 2011/Revised October 14, 2011/Accepted October 21, 2011/Accepted manuscript online November 8, 2011/Article first published online December 15, 2011)

Population-based cancer registries are operated by over 80% of prefectures in Japan. However, only a limited proportion of the registries can provide long-term incidence data. Here, we aimed to establish a method for monitoring cancer incidence trends in Japan using data from selected prefectures. Based on the availability of long-term (≥20 years) high-quality data, we collected incidence data from five prefectures (Miyagi, Yamagata, Fukui, Osaka, and Nagasaki), which included an annual average of 54 539 primary cancer cases diagnosed between 1985 and 2004. Cancer mortality data for 1995–2004 were obtained from the vital statistics. Representativeness and homogeneity of the trends were examined by funnel plot analysis of log-linear regression coefficients calculated for the most recent 10 years of data (1995–2004) of age-standardized rates (ASR). The ASR of incidence for five prefectures in total (5-pref total) showed a significant decrease, with an annual percent change (APC) of −1.0 (95% confidence interval [CI] −1.4: −0.6) for males and −0.4 (95% CI −0.8: −0.1) for females. Excluding data from Osaka (4-pref total) reversed the decreasing trend; the corresponding APC was +0.4 (95% CI −0.2: +1.0) for males and +0.7 (95% CI +0.5: +0.9) for females. The APCs for the ASR of mortality for the 4-pref total (males, −1.5; females, −1.3) were more representative of nationwide data (males, −1.4 [95% CI −1.7: −1.2]; females, −1.1 [95% CI −1.4: −0.9]) than those for the 5-pref total (males, −1.7; females, −1.4). We conclude that using data from Miyagi, Yamagata, Fukui, and Nagasaki prefectures, with continuous monitoring of the representativeness of the data, is a provisionally relevant way to evaluate cancer incidence trends in Japan. (*Cancer Sci* 2012; 103: 360–368)

Assessing cancer burden is an essential part of national cancer control.[1,2] In Japan, cancer mortality data have been used for describing the secular trends of national cancer burden,[3–5] and the Japanese national cancer control plan, which was initiated in 2007, set a 10-year target of 20% mortality reduction based on national vital statistics data.[6]

With respect to cancer incidence, although annual national estimates of cancer incidence have been available in Japan since 1975,[7,8] there are several difficulties in interpreting the annual trends identified from these data.[9] First, different sets of cancer registries are used for the estimation of incidence between years.[10] Second, to reduce the effect of geographical variation, the estimated values are corrected for differences in cancer mortality between selected prefectures and the nationwide averages.[11] Third, the estimation method has been subjected to several methodological changes.[12,13] Thus, a need exists for establishing an alternative method for monitoring cancer incidence trends in Japan.

As of May 2011, population-based cancer registries are operated by over 80% of prefectures in Japan,[14] but only a limited number of the prefectural registries (Miyagi, Yamagata, Fukui, Osaka, and Nagasaki) can provide long-term incidence data fulfilling international quality standards.[15] Thus, the present study aimed to propose a method for monitoring and evaluating cancer incidence trends in Japan using data from these selected population-based cancer registries.

Materials and Methods

Study population. We selected five prefectures in Japan for the present analysis, Miyagi, Yamagata, Fukui, Osaka, and Nagasaki. The rationale of this selection was the availability of long-term (≥20 years), high-quality, population-based cancer incidence data. All of the selected prefectures, with the exception of Fukui, were included in the publication *Cancer Incidence in Five Continents* (CI5), volume VI (years at diagnosis, 1983–1987), and all subsequent volumes.[15] Although Fukui prefecture was not included in CI5 until volume IX, high-quality population-based incidence data in this prefecture have been available since 1984.[15,16] To ensure the stability of data quality and minimize the effect of reporting delay, we analyzed data collected from 1985 to 2004 (years at diagnosis).

Table 1 shows the demographic information of the five selected prefectures, which covered 11.7% of the entire population of Japan. Osaka prefecture alone accounted for nearly 60% of the total population of the five prefectures; when Osaka prefecture was excluded, the population coverage reduced from 11.7% to 4.7%. No major differences were detected in age distribution across the five prefectures, or between each of the five prefectures and all of Japan.

Data sources. We requested data for all primary tumor cases diagnosed between 1985 and 2004 from each prefectural cancer registry. Data collection was carried out from May to December 2010, according to the same protocol used for the Monitoring of Cancer Incidence in Japan (MCIJ) project.[7,17] Tumors were classified in all registries according to the International Classification of Diseases for Oncology (ICD-O)-2 or ICD-O-3 codes. The codes of collected data were checked and converted into ICD-10 codes according to the rules developed by the International Agency for Research on Cancer.[18] We analyzed the following malignant primary tumors defined according to ICD-10 codes: all cancers, C00–C96; stomach, C16; colon/rectum, C18–C21; liver, C22; lung, C33–C34; breast, C50 and D05; and prostate, C61. We included the codes C21 and D05 in colorectal and breast cancers, respectively, for comparability with the published data of national estimates of cancer incidence,[8] which were obtained from the website of the National Cancer Center, Japan.[7,8]

We also obtained the corresponding data for cancer mortality from published vital statistics. Due to the limitation of

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doi: 10.1111/j.1349-7006.2011.02145.x
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Table 1. Basic characteristics of five population-based cancer prefectural registries in Japan†

	Miyagi	Yamagata	Fukui	Osaka	Nagasaki	5-pref total	4-pref total (excl. Osaka)	All Japan
Population‡	2 299 360	1 251 115	824 868	8 770 472	1 543 002	14 688 817	5 918 345	125 032 909
% of national population	1.8	1.0	0.7	7.0	1.2	11.7	4.7	100.0
Age distribution (%)								
0–14 years	17.5	17.0	17.6	16.2	18.5	16.8	17.7	16.7
15–39 years	35.2	29.5	31.5	36.9	30.7	35.0	32.3	34.7
40–64 years	32.8	34.1	33.4	34.7	33.3	34.1	33.3	34.0
≥65 years	14.5	19.3	17.5	12.3	17.4	14.0	16.7	14.6
Incidence (*n*)	9277	5791	3334	28 941	7195	54 539	25 598	466 114§
% of national estimate	2.0	1.2	0.7	6.2	1.5	11.7	5.5	100.0
DCN%	12.5	22.7	16.7	31.5	9.4	23.5	14.5	22–26¶
DCO%	10.0	11.1	3.7	14.1	8.9	11.8	9.1	17–20¶
MV%	79.9	76.6	78.9	69.3	80.2	73.9	79.1	65–73¶
M/I ratio††	0.56	0.61	0.60	0.70	0.58	0.65	0.58	0.51–0.59¶
ASR of mortality††,‡‡								
Males	210.2	211.4	193.0	244.1	230.1	230.5	213.1	214.1
Females	100.0	100.4	95.4	115.9	106.1	109.2	101.1	103.5

†Annual average 1985–2004, unless otherwise specified. ‡Total population, including foreigners. §National estimate. ¶Range of annual average 1997–2004 for the pooled data of registries included in the national estimate. ††Annual average 1995–2004. ‡‡Standardized to the Japanese 1985 model population. 4-pref total, data from four cancer prefectural registries (Miyagi, Yamagata, Fukui, and Nagasaki); 5-pref total, data from five cancer prefectural registries (Miyagi, Yamagata, Fukui, Osaka, and Nagasaki); ASR, age-standardized rate; DCN%, proportion of death certificate notifications; DCO%, proportion of death certificate only; M/I ratio, mortality to incidence ratio; MV%, proportion of microscopically verified cases.

availability of prefectural data,[19] the years of deaths were analyzed between 1995 and 2004. For mortality data, all cancers were defined as C00–C97 (ICD-10). Data for the population were obtained from the website managed by the Surveillance Division, Center for Cancer Control and Information Services, National Cancer Center, Japan.[20]

Statistical analyses. To examine the data quality of each cancer registry, we calculated the proportions of death certificate notification (DCN%) and microscopically verified (MV%) cases, and the mortality to incidence ratio (M/I ratio). We also calculated the proportion of death certificate only (DCO%), as efficient follow-back procedures are carried out in many Japanese registries. The quality indexes for the annual national estimate of cancer incidence were obtained from published reports.[21–26] Annual age-standardized rates (ASR; standardized to the 1985 model Japanese population) of incidence and mortality were calculated for each prefecture and five prefectures in total (5-pref total). As Osaka prefecture accounted for greater than half of the total population of the five selected prefectures, we also analyzed four prefectures in total (4-pref total), by excluding Osaka. Trends in the ASR of incidence and mortality were examined for each of the 5-pref total, 4-pref total, and the whole of Japan. It should be noted that for incidence data, ''all Japan'' represents the national estimate.[7,8]

To examine the representativeness and homogeneity of the 5-pref total and 4-pref total data, we used a funnel plot method,[27] which is a 2-D plot of a given statistical estimate and its precision, represented by the inverse standard error (SE). As the

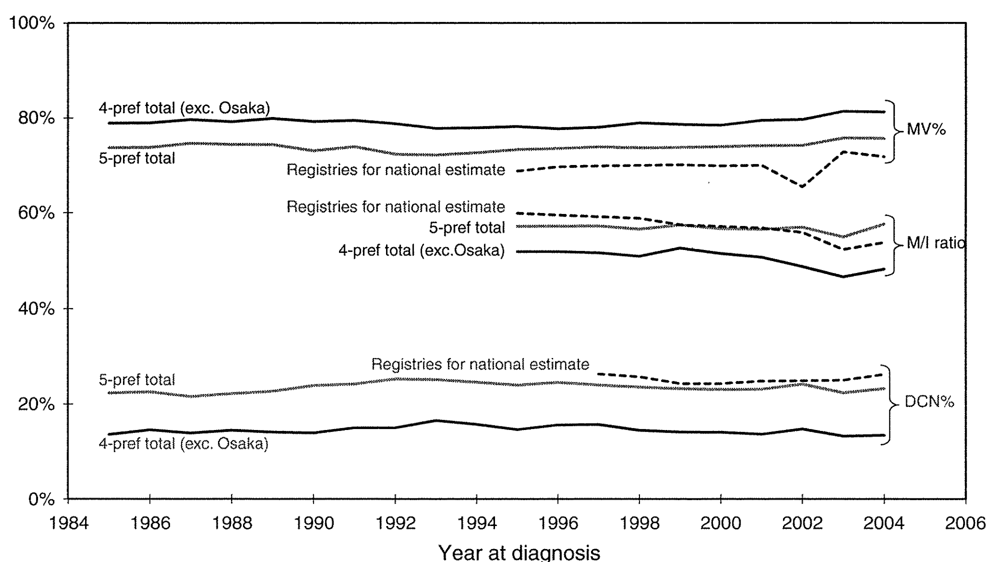

Fig. 1. Annual trend of quality indexes for Japanese cancer registry data from two sets of prefectures. 4-pref total, data from four cancer prefectural registries (Miyagi, Yamagata, Fukui, and Nagasaki); 5-pref total, data from five cancer prefectural registries (Miyagi, Yamagata, Fukui, Osaka, and Nagasaki); DCN%, proportion of death certificate notifications; M/I ratio, mortality to incidence ratio; MV%, proportion of microscopically verified cases.

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