Table 3. Continued

Affi ID	Gene abbreviation	Fold change CB 1	CB 2	PB 1	PB 2	Gene name
Cytokines and chemokines						
207533_at	*CCL1*	1·67	1·48	0·52	0·49	Chemokine (C-C motif) ligand 1
205099_s_at	*CCR1*	4·70	1·21	0·61	0·79	Chemokine (C-C motif) receptor 1
207681_at	*CXCR3*	1·51	1·33	0·41	0·67	Chemokine (C-X-C motif) receptor 3
211469_s_at	*CXCR6*	1·58	1·95	0·32	0·42	Chemokine (C-X-C motif) receptor 6
206613_at	*IL-18R1*	2·32	1·38	0·61	0·62	Interleukin-18 receptor 1
207072_at	*IL-18RAP*	2·16	1·44	0·46	0·56	Interleukin-18 receptor accessory protein
212657_s_at	*IL-1RN*	1·44	3·12	0·56	0·37	Interleukin 1 receptor
206341_at	*IL-2RA*	1·52	1·27	0·73	0·66	Interleukin-2 receptor alpha
202859_x_at	*IL-8*	1·31	3·75	0·38	0·69	Interleukin-8
202643_s_at	*TNFAIP3*	1·61	1·25	0·67	0·75	Tumour necrosis factor, alpha-induced protein 3
202687_s_at	*TNFSF10*	2·83	1·23	0·67	0·77	Tumour necrosis factor (ligand) superfamily member 10
205599_at	*TRAF1*	2·25	1·32	0·68	0·61	Tumour necrosis factor receptor-associated factor 1
202871_at	*TRAF4*	1·43	1·58	0·57	0·48	Tumour necrosis factor receptor-associated factor 4
206366_x_at	*XCL1*	1·24	2·66	0·46	0·76	Chemokine (C motif) ligand 1
Signal transduction						
210538_s_at	*AIP1*	1·35	1·54	0·65	0·61	Baculoviral IAP repeat-containing 3
209369_at	*ANXA3*	1·39	6·82	0·61	0·05	Annexin A3
1554343_a_at	*BRDG1*	1·45	1·67	0·52	0·55	BCR downstream signalling 1
225946_at	*C12orf2*	3·20	1·77	0·23	0·23	Ras association (RalGDS/AF-6) domain family 8
204392_at	*CAMK1*	1·26	1·62	0·74	0·54	Calcium/calmodulin-dependent protein kinase I
231042_s_at	*CAMK2D*	1·31	1·63	0·25	0·69	Calcium/calmodulin-dependent protein kinase (CaM kinase) II delta
205692_s_at	*CD38*	1·37	1·29	0·71	0·48	CD38 antigen (p45)
231747_at	*CYSLTR1*	3·16	1·45	0·55	0·43	Cysteinyl leukotriene receptor 1
211272_s_at	*DGKA*	1·43	1·23	0·77	0·54	Diacylglycerol kinase alpha 80 kDa
200762_at	*DPYSL2*	1·35	1·40	0·37	0·65	Dihydropyrimtdinase-like 2
208370_s_at	*DSCR1*	1·23	1·90	0·63	0·77	Down syndrome critical region gene 1
204794_at	*DUSP2*	1·55	2·57	0·39	0·45	Dual specificity phosphatase 2
204015_s_at	*DUSP4*	1·35	2·66	0·65	0·39	Dual specificity phosphatase 4
211333_s_at	*FASLG*	1·20	1·37	0·49	0·80	Fas ligand (TNF superfamily, member 6)
211535_s_at	*FGFR1*	1·23	2·79	0·70	0·77	Fibroblast growth factor receptor 1
224148_at	*FYB*	1·50	1·21	0·45	0·79	FYN binding protein (FYB-120/130)
209304_x_at	*GADD45B*	1·55	1·29	0·65	0·71	Growth arrest and DNA-damage-inducible beta
234284_at	*GNG8*	1·50	3·16	0·50	0·35	Guanine nucleotide binding protein (G protein), gamma 8
224285_at	*GPR174*	1·91	1·42	0·56	0·58	G protein-coupled receptor 174
223767_at	*GPR84*	4·41	1·44	0·05	0·56	G protein-coupled receptor 84
211555_s_at	*GUCY1B3*	1·66	1·73	0·34	0·03	Guanylate cyclase 1, soluble, beta 3
38037_at	*HBEGF*	1·54	1·36	0·55	0·64	Heparin-binding EGF-like growth factor
203820_s_at	*IMP-3*	1·83	2·18	0·17	0·17	IGF-II-mRNA-binding protein 3
203006_at	*INPP5A*	1·40	1·86	0·60	0·52	Inositol polyphosphate-5-phosphatase, 40 kDa
231779_at	*IRAK2*	1·93	1·46	0·46	0·54	Interleukin-1 receptor associated kinase 2
32137_at	*JAG2*	1·58	1·29	0·71	0·64	Jagged 2
203904_x_at	*KAI1*	1·65	1·59	0·41	0·25	CD82 antigen
235252_at	*KSR*	1·72	1·56	0·43	0·44	Kinase suppressor of ras 1
210948_s_at	*LEF1*	1·21	1·64	0·41	0·79	Hypothetical protein LOC641518
203236_s_at	*LGALS9*	1·48	1·27	0·73	0·51	Lectin, galactoside-binding, soluble, 9 (galectin 9)
220253_s_at	*LRP12*	1·27	1·30	0·31	0·73	Low-density lipoprotein-related protein 12
206637_at	*P2RY14*	1·32	1·48	0·39	0·68	Purinergic receptor P2Y, G-protein coupled, 14
210837_s_at	*PDE4D*	1·35	1·31	0·62	0·69	Phosphodiesterase 4D, cAMP-specific
206726_at	*PGDS*	6·45	1·40	0·60	0·43	Prostaglandin D2 synthase, haematopoietic
210617_at	*PHEX*	1·53	4·08	0·21	0·47	Phosphate regulating endopeptidase homologue, X-linked
206370_at	*PIK3CG*	1·23	1·32	0·50	0·77	Phosphoinositide-3-kinase, catalytic, gamma polypeptide
205632_s_at	*PIP5K1B*	1·32	1·42	0·64	0·68	Phosphalidylinositol-4-phosphate 5-kinase, type 1 beta

Table 3. Continued

Affi ID	Gene abbreviation	Fold change CB 1	CB 2	PB 1	PB 2	Gene name
215195_at	*PRKCA*	2·17	1·36	0·64	0·61	Protein kinase C, alpha
210832_x_at	*PTGER3*	4·44	1·47	0·07	0·53	Prostaglandin E receptor 3 (subtype EP3)
1553535_a_at	*RANGAP1*	1·58	1·39	0·58	0·61	Ran GTPase activating protein 1
234344_at	*RAP2C*	1·75	1·26	0·46	0·74	RAP2C, member of RAS oncogene family
223809_at	*RGS18*	2·12	1·67	0·15	0·33	Regulator of G-protein signalling 18
209882_at	*RIT1*	1·74	1·32	0·63	0·68	Ras-like without CAAX 1
209451_at	*TANK*	1·34	1·20	0·42	0·80	TRAF family member-associated NFKB activator
204924_at	*TLR2*	1·60	2·52	0·36	0·40	Toll-like receptor 2
217979_at	*TM4SF13*	1·21	2·47	0·30	0·79	Tetraspanin 13
209263_x_at	*TM4SF7*	2·05	1·41	0·58	0·59	Tetraspanin 4
Transcription						
1566989_at	*ARID1B*	1·42	1·27	0·09	0·73	AT-rich interactive domain 1B (SWI1-like)
203973_s_at	*CEBPD*	3·06	1·51	0·33	0·49	CCAAT/enhancer binding protein (C/EBP), delta
221598_s_at	*CRSP8*	1·60	1·29	0·71	0·68	Cofactor required for Spl transcriptional activation, subunit 8, 34 kDa
205249_at	*EGR2*	1·33	4·27	0·67	0·60	Early growth response 2 (Krox-20 homologue, *Drosophila*)
206115_at	*EGR3*	1·31	6·15	0·69	0·48	Early growth response 3
201328_at	*ETS2*	1·57	1·72	0·43	0·40	V-ets erythroblastosis virus E26 oncogene homologue 2 (avian)
218810_at	*FLJ23231*	2·13	1·37	0·63	0·63	Zinc finger CCCH-type containing 12A
209189_at	*FOS*	21·56	1·31	0·13	0·69	V-fos FBJ murine osteosarcoma viral oncogene homologue
223408_s_at	*FOXK2*	2·26	1·22	0·48	0·78	Forkhead box K2
202723_s_at	*FOXO1A*	1·47	1·27	0·57	0·73	Forkhead box O1A (rhabdomyosarcoma)
224211_at	*FOXP3*	1·62	1·41	0·59	0·23	Forkhead box P3
207156_at	*HIST1H2AG*	1·73	1·30	0·41	0·70	Histone 1, H2ag
220042_x_at	*HIVEP3*	1·26	1·65	0·74	0·56	Human immunodeficiency virus type I enhancer binding protein 3
207826_s_at	*ID3*	1·34	8·64	0·60	0·66	Inhibitor of DNA binding 3, dominant negative helix-loop-hetix protein
204549_at	*IKBKE*	2·33	1·29	0·71	0·66	Inhibitor of kappa light polypeptide gene enhancer in B cells
219878_s_at	*KLF13*	1·89	1·26	0·34	0·74	Kruppel-like factor 13
207667_s_at	*MAP2K3*	1·33	1·28	0·72	0·57	Mitogen-activated protein kinase kinase 3
201502_s_at	*NFKBIA*	2·31	1·29	0·71	0·57	Nuclear factor of κ light polypeptide gene enhancer in B cells inhibitor
222105_s_at	*NKIRAS2*	1·84	1·21	0·69	0·79	NFKB inhibitor interacting Ras-like 2
204622_x_at	*NR4A2*	1·35	4·31	0·65	0·63	Nuclear receptor subfamily 4, group A, member 2
207978_s_at	*NR4A3*	1·33	3·53	0·62	0·67	Nuclear receptor subfamily 4, group A, member 3
202600_s_at	*NRIP1*	1·86	1·39	0·26	0·61	Nuclear receptor interacting protein 1
216841_s_at	*SOD2*	1·25	1·73	0·36	0·75	Superoxide dismutase 2, mitochondrial
201416_at	*SOX4*	1·53	2·21	0·47	0·38	SRY (sex determining region Y)-box 4
223635_s_at	*SSBP3*	2·12	1·25	0·75	0·62	Single-stranded DNA binding protein 3
206506_s_at	*SUPT3H*	1·47	1·31	0·57	0·69	Suppressor of Ty 3 homologue (*S. cerevisiae*)
221618_s_at	*TAF9L*	1·25	1·49	0·47	0·75	TAF9-like RNA polymerase II
203177_x_at	*TFAM*	1·63	1·23	0·77	0·57	Transcription factor A, mitochondrial
213943_at	*TWIST1*	1·89	3·14	0·04	0·11	Twist homologue 1 (acrocephalosyndactyly 3; Saethre-Chotzen syndrome)
219836_at	*ZBED2*	1·33	4·76	0·67	0·21	Zinc finger, BED-type containing 2
211965_at	*ZFP36L1*	2·02	1·47	0·29	0·53	Zinc finger protein 36, C3H type-like 1
230760_at	*ZFY*	1·41	1·25	0·75	0·02	Zinc finger protein, Y-linked
228854_at	*ZNF145*	3·26	1·21	0·40	0·79	Transcribed locus
235121_at	*ZNF542*	2·68	1·33	0·63	0·67	Zinc finger protein 542

To investigate whether increased expression of the *IL-17* gene is a general feature of PB-derived $CD4^+$ T cells, we also tested *IL-17* gene expression in the above-described additional samples by real-time RT-PCR analysis. As shown in Fig. 6, all of four PB-derived $CD4^+$ T-cell samples revealed significantly increased gene expression of *IL-17* when compared with the CB-derived samples at 1 week. At 2 weeks, however, *IL-17* gene expression in PB-derived $CD4^+$ T cells was diminished while some of the CB-derived $CD4^+$ T cells (such as sample CB 4) exhibited increased *IL-17* gene expression. When the data were analysed statistically, expression of the *IL-17* gene was found to be

Table 4. Genes up-regulated in $CD4^+$ T cells from peripheral blood (PB)

Affi ID	Gene abbreviation	Fold change CB 1	CB 2	PB 1	PB 2	Gene name
Apoptosis						
1553681_a_at	*PRF1*	0·66	0·51	1·41	1·34	Perforin 1 (pore-forming protein)
B- and T-cell development						
224499_s_at	*AICDA*	0·06	0·44	1·56	3·47	Activation-induced cytidine deaminase
205495_s_at	*GNLY*	0·40	0·51	1·49	6·34	Granulysin
217478_s_at	*HLA-DMA*	0·67	0·39	1·33	1·35	Major histocompatibility complex, class II, DM alpha
203932_at	*HLA-DMB*	0·64	0·31	2·02	1·36	Major histocompatibility complex, class II, DM beta
211991_s_at	*HLA-DPA1*	0·50	0·14	1·54	1·50	Major histocompatibility complex, class II, DP alpha 1
212671_s_at	*HLA-DQA1*	0·44	0·23	1·56	2·56	Major histocompatibility complex, class II, DQ alpha 1
211656_x_at	*HLA-DQB1*	0·63	0·48	1·37	7·07	Major histocompatibility complex, class II, DQ beta 1
210982_s_at	*HLA-DRA*	0·58	0·37	1·50	1·42	Major histocompatibility complex, class II, DR alpha
208306_x_at	*HLA-DRB1*	0·51	0·24	1·49	1·61	Major histocompatibility complex, class II, DR beta 3
204670_x_at	*HLA-DRB5*	0·63	0·22	1·47	1·37	Major histocompatibility complex, class II, DR beta 5
211634_x_at	*IGHV1-69*	0·69	0·77	1·23	1·99	Immunoglobulin heavy variable 1–69
211645_x_at	*IgK*	0·15	0·49	1·51	6·62	Immunoglobulin kappa light chain (IGKV)
221651_x_at	*IGKC*	0·46	0·68	1·32	5·57	Immunoglobulin kappa constant
215379_x_at	*IGLC2*	0·62	0·41	1·38	4·26	Immunoglobulin lambda joining 2
209031_at	*IGSF4*	0·50	0·03	2·33	1·50	Immunoglobulin superfamily, member 4
205686_s_at	*CD86*	0·70	0·23	1·30	1·39	CD86 antigen (CD28 antigen ligand 2, B7-2 antigen)
204698_at	*ISG20*	0·68	0·49	1·32	1·64	Interferon stimulated exonuclease gene, 20 kDa
213915_at	*NKG7*	0·72	0·42	1·28	2·31	Natural killer cell group 7 sequence
Cell growth and maintenance						
201334_s_at	*ARHGEF12*	0·74	0·50	1·26	1·96	Rho guanine nucleotide exchange factor (GEF) 12
230292_at	*CHC1L*	0·70	0·56	1·30	2·02	Regulator of chromosome condensation (RCC1)
205081_at	*CRIP1*	0·56	0·73	1·27	1·75	Cysteine-rich protein 1 (intestinal)
31874_at	*GAS2L1*	0·77	0·52	1·23	2·35	Growth arrest-specific 2 like 1
202364_at	*MXI1*	0·43	0·73	1·27	1·44	MAX interactor 1
219304_s_at	*PDGFD*	0·65	0·71	1·29	3·68	Platelet-derived growth factor D
213397_x_at	*RNASE4*	0·64	0·46	1·36	2·21	Ribonuclease, RNase A family, 4
213566_at	*RNASE6*	0·69	0·39	1·49	1·31	Ribonuclease, RNase A family, k6
219077_s_at	*WWOX*	0·40	0·78	1·25	1·22	WW domain containing oxidoreductase
Cytokine and chemokine						
207861_at	*CCL22*	0·76	0·52	1·24	2·47	Chemokine (C–C motif) ligand 22
238750_at	*CCL28*	0·74	0·45	1·26	1·41	Chemokine (C–C motif) ligand 28
1555759_a_at	*CCL5*	0·71	0·23	1·29	1·92	Chemokine (C–C motif) ligand 5
208304_at	*CCR3*	0·50	0·12	1·50	2·35	Chemokine (C–C motif) receptor 3
205898_at	*CX3CR1*	0·30	0·20	1·70	4·16	Chemokine (C–X3–C motif) receptor 1
204533_at	*CXCL10*	0·80	0·16	1·20	2·53	Chemokine (C–X–C motif) ligand 10
219255_x_at	*IL-17RB*	0·73	0·04	1·27	1·29	Interleukin 17 receptor B
206148_at	*IL-3RA*	0·60	0·54	2·46	1·40	Interleukin 3 receptor, alpha (low affnity)
226333_at	*IL-6R*	0·22	0·79	1·21	2·43	Interleukin-6 receptor
206693_at	*IL-7*	0·09	0·54	1·46	5·86	Interleukin-7
Signal transduction						
204497_at	*ADCY9*	0·76	0·40	1·24	2·40	Adenylate cyclase 9
206170_at	*ADRB2*	0·58	0·35	1·42	3·97	Adrenergic, beta-2-, receptor, surface
202096_s_at	*BZRP*	0·50	0·54	1·59	1·46	Benzodiazapine receptor (peripheral)
230464_at	*EDG8*	0·04	0·09	1·91	2·42	Endothelial differentiation, sphingolipid G-protein-coupled receptor 8
223423_at	*GPR160*	0·54	0·68	1·40	1·32	G protein-coupled receptor 160
227769_at	*GPR27*	0·07	0·08	1·92	244	G protein in-coupled receptor 27
210095_s_at	*IGFBP3*	0·27	0·20	1·73	5·25	Insulin-like growth factor binding protein 3
38671_at	*PLXND1*	0·08	0·65	1·35	2·57	Plexin D1
226101_at	*PRKCE*	0·56	0·43	1·72	1·44	Protein kinase C. epsilon
232629_at	*PROK2*	0·01	0·13	1·87	2·09	Prokineticin 2

Table 4. Continued

Affi ID	Gene abbreviation	Fold change CB 1	CB 2	PB 1	PB 2	Gene name
203329_at	*PTPRM*	0·36	0·62	1·38	1·93	Protein tyrosine phosphatase, receptor type, M
204731_at	*TGFBR3*	0·78	0·55	1·22	2·04	Transforming growth factor, beta receptor III (betaglycan, 300 kDa)
Transcription						
203129_s_at	*KIF5C*	0·67	0·09	1·33	3·43	Kinesin family member 5C
213906_at	*MYBL1*	0·75	0·51	1·25	3·63	V-myb myeloblastosis viral oncogene homologue (avian)-like 1
209815_at	*PTCH*	0·59	0·27	1·41	4·17	Patched homologue (*Drosophila*)
213891_s_at	*TCF4*	0·74	0·65	2·06	1·26	Transcription factor 4
238520_at	*TRERF1*	0·70	0·77	1·23	2·30	Transcriptional regulating factor 1
203603_s_at	*ZFHX1B*	0·74	0·61	1·26	3·63	Zinc finger homobox 1b
213218_at	*ZNF187*	0·74	0·69	1·26	1·76	Zinc finger protein 187
221123_x_at	*ZNF395*	0·38	0·71	1·63	1·29	Zinc finger protein 395

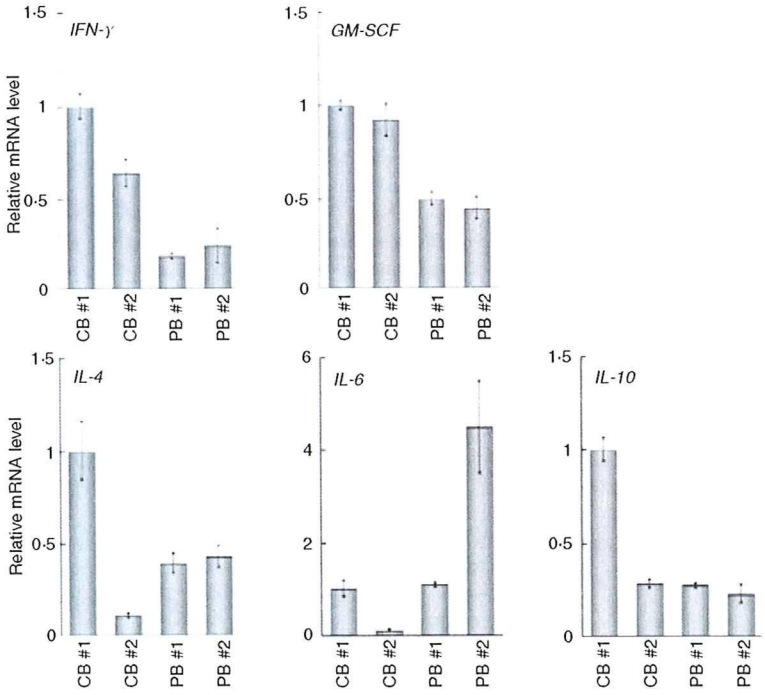

Figure 2. Quantitative polymerase chain reaction (PCR) analysis of the genes related to the T helper type 1 (Th1) and Th2 phenotypes. The expression of the genes indicated was examined by real-time reverse transcriptase (RT)-PCR using the same sample specimens as in Fig 1. Data are normalized to the mRNA level in PB 1 which is arbitrarily set to 1. The signal intensity was normalized using that of a control housekeeping gene [the human glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene]. Data are relative values with the standard deviation (SD) for triplicate wells.

significantly higher in PB-derived $CD4^+$ T cells in comparison with equivalent CB-derived $CD4^+$ T cells at 1 week ($P < 0{\cdot}05$) but not at 2 weeks (Fig. 6).

Discussion

Although it is generally believed that there are functional differences between CB and PB lymphocytes, the details are obscure. For instance, Azuma *et al.*[13] reported that the phenotype and function of expanded CB lymphocytes were essentially equivalent to those of expanded PB lymphocytes when evaluated in *in vitro* experiments. In the present study, however, we have shown that CB-derived $CD4^+$ T cells revealed a distinct expression profile of genes important for the function of particular T-cell subsets compared with PB-derived $CD4^+$ T cells.

$CD4^+$ T cells can be classified into distinct subsets, including effector $CD4^+$ cells and Tregs, according to their functional characteristics as well as differentiation profiles.[14–16] Typically, effector $CD4^+$ T cells have been further divided into two distinct lineages on the basis of their cytokine production profiles, namely Th1 and Th2. Th1 cells producing cytokines such as IL-2, IFN-γ and GM-CSF have evolved to enhance the eradication of intracellular pathogens and are thought to be potent activators of cell-mediated immunity. In contrast, Th2

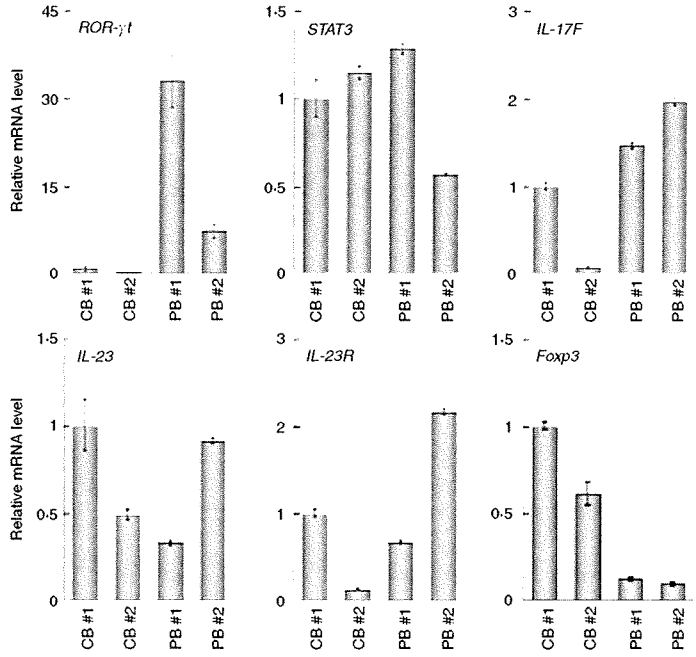

Figure 3. Quantitative polymerase chain reaction (PCR) analysis of the forkhead box protein 3 gene (*FOXP3*) and the genes related to the secretion of interleukin (IL)-17. The expression of the genes indicated was examined as in Fig 2. Data are normalized to the mRNA level in peripheral blood sample 1 (PB 1) as in Fig.2. The signal intensity was normalized using that of a control housekeeping gene [the human glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene]. Data are relative values with the standard deviation for triplicate wells.

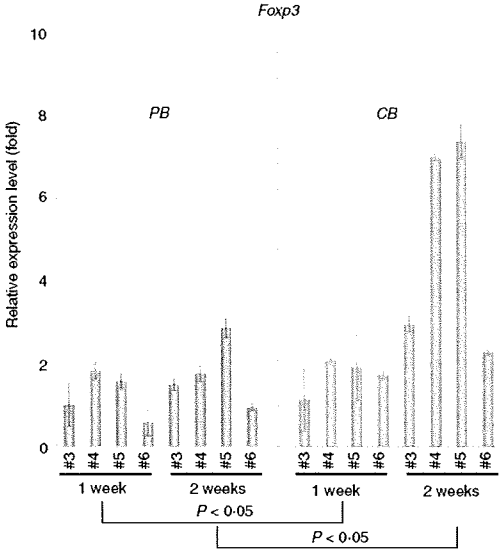

Figure 4. Quantitative polymerase chain reaction (PCR) analysis of the forkhead box protein 3 gene (*FOXP3*) in additional samples. Additional peripheral blood (PB) and cord blood (CB) samples were prepared and RNAs were extracted at 1 and 2 weeks. The expression of the *FOXP3* gene was examined as in Fig. 2. Data are normalized to the mRNA level in the sample of PB 3 at 1 week, which is arbitrarily set to 1. The signal intensity was normalized using that of a control housekeeping gene (the human β-actin gene). Data are relative values with the standard deviation for triplicate wells. The data were analysed statistically and *FOXP3* gene expression in CB-derived $CD4^+$ T cells was found to be significantly higher in comparison with equivalent PB-derived $CD4^+$ T cells at both 1 week ($P < 0{\cdot}05$) and 2 weeks ($P < 0{\cdot}05$).

cells secreting cytokines such as IL-4, IL-5, IL-6, IL-9 and IL-13 have evolved to enhance the elimination of parasitic infections and are thought to be potent activators of B-cell immunoglobulin E production, eosinophil recruitment, and mucosal expulsion. Th1-type responses to self or commensal floral antigens can promote tissue destruction and chronic inflammation, whereas dysregulated Th2-type responses can cause allergy and asthma. The development of Th1 is specified by the transcription factor T-bet (also known as Tbx-21) and master regulators of Th2 differentiation are GATA-3 and c-maf.

As shown in Fig. 2 and Table 2, the gene expression profiles of CB- and PB-derived $CD4^+$ T cells revealed no significant differences regarding cytokines related to the definition of Th1 and Th2, with the exceptions of IFN-γ and GM-CSF. The mRNA levels of IFN-γ and GM-CSF tended to be higher in CB-derived $CD4^+$ T cells than in PB-derived $CD4^+$ T cells. The mRNA expression of the transcription factors T-bet, GATA-3 and c-maf, which regulate Th1 and Th2 cell differentiation, did not differ significantly between CB- and PB-derived $CD4^+$ T cells.

In addition to Th1 and Th2 cells, IL-17 (also known as IL-17A)-producing T lymphocytes have been recently shown to comprise a distinct third subset of T helper cells, termed Th17 cells, in the mouse immune system. Th17 cells exhibit pro-inflammatory characteristics and act as major contributors to autoimmune disease. A number of experiments using animal models support a significant role for IL-17 in the response to allografts.[14,16,17] There is as yet no direct evidence for the existence of discrete Th17 cells in humans, although

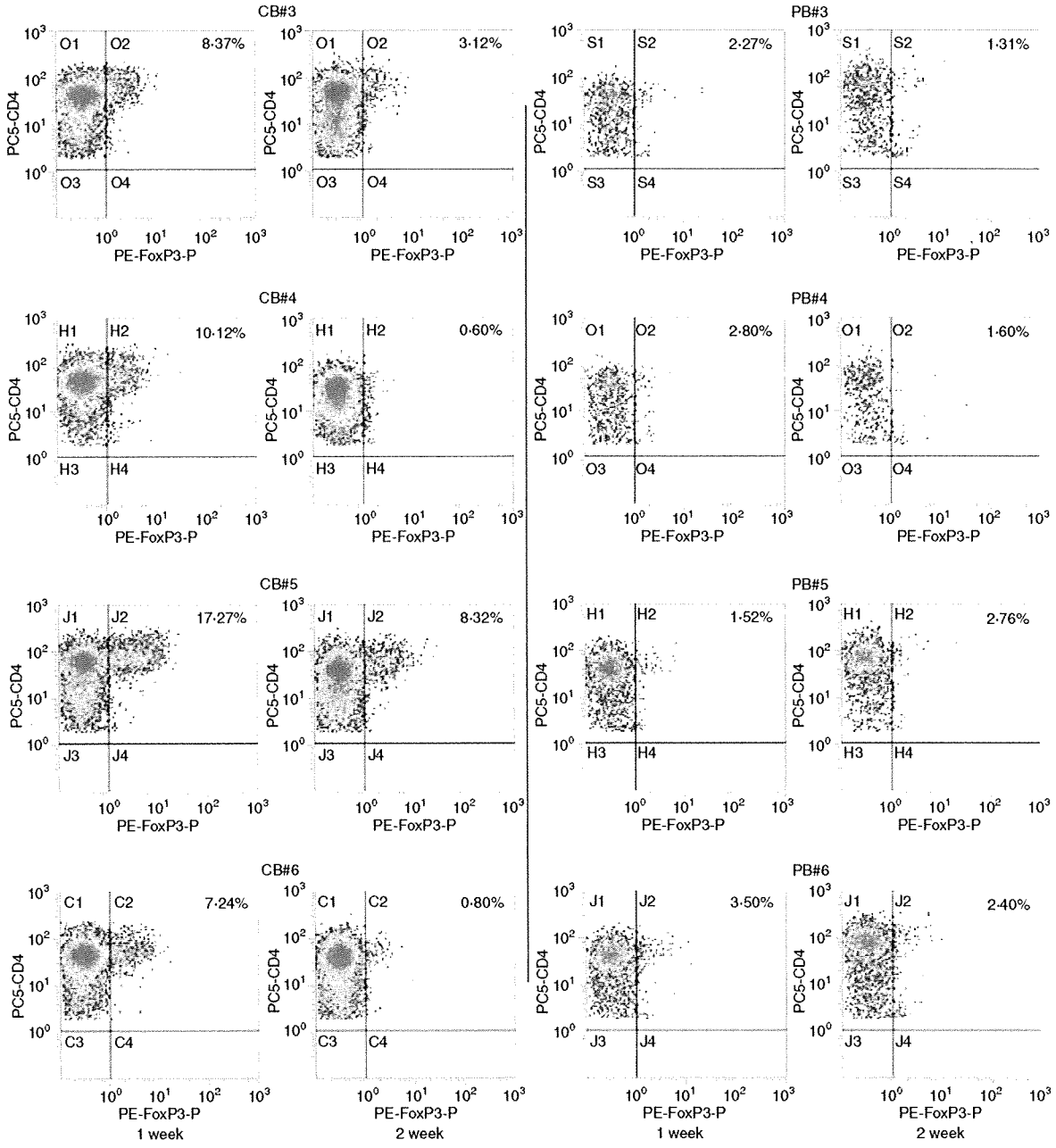

Figure 5. Protein expression of forkhead box protein 3 (Foxp3) in activated $CD4^+$ T cells. The protein expression of Foxp3 in same sample specimens as in Fig. 4 was examined by flow cytometry. The CD4 versus Foxp3 cytogram of the population gated with $CD3^+$ and $CD4^+$ in each sample is presented.

helper T cells secreting IL-17 have clearly been detected in the human immune system.[18] Several studies have shown a correlation between allograft rejection and IL-17. For example, IL-17 levels are elevated in human renal allografts during subclinical rejection and there are detectable mRNA levels in the urinary mononuclear cell sediments of these patients.[19,20] In human lung organ transplantation, IL-17 levels have also been reported to be elevated during acute rejection.[21] Interestingly, in this study, most of the PB-derived $CD4^+$ T-cell samples expressed higher levels of IL-17 mRNA than the CB-derived $CD4^+$ T-cell samples, suggesting that PB-derived $CD4^+$ T cells frequently include potent IL-17-secreting T cells.

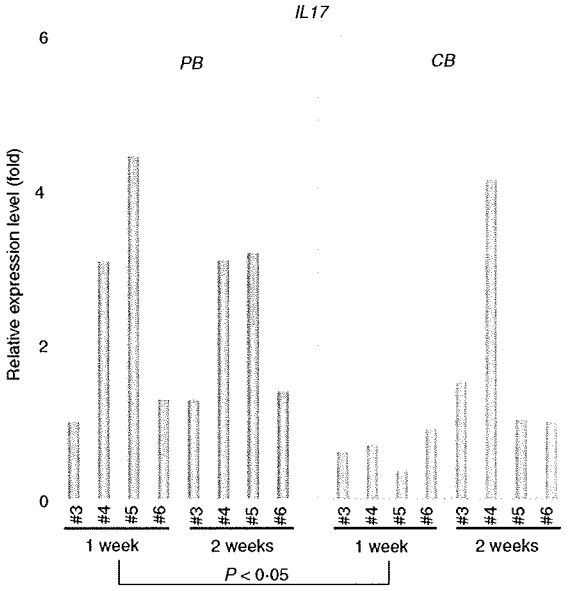

Figure 6. Quantitative polymerase chain reaction (PCR) analysis of interleukin (IL)-17 in additional samples. The expression of the *IL-17* gene in the same sample specimens as in Fig. 4 was examined and presented as in Fig 2. The data were analysed statistically and *IL-17* gene expression in peripheral blood (PB)-derived $CD4^+$ T cells was found to be significantly higher in comparison with equivalent CB-derived $CD4^+$ T cells at 1 week ($P < 0{\cdot}05$) but not at 2 weeks.

Th17 cells expand independently of T-bet or STAT-1. Ivanov *et al.*[22] have shown that the orphan nuclear receptor RORγt is the key transcription factor orchestrating the differentiation of the effector lineage. RORγt induces transcription of the gene encoding IL-17 in naïve $CD4^+$ T helper cells and is required for its expression in response to IL-6 and transforming growth factor (TGF)-β, the cytokines known to induce IL-17 expression. IL-23 is also involved in Th17 cell differentiation, but naïve T cells do not have the IL-23 receptor and are relatively refractory to IL-23 stimulation.[23,24] Although IL-23 seems to be an essential survival factor for Th17 cells, it is not required during their differentiation. It has been suggested that IL-23R expression is up-regulated on $RORγt^+$ Th17 cells in an IL-6-dependent manner. IL-23 may therefore function subsequent to IL-6/TGF-β-induced commitment to the Th17 lineage to promote cell survival and expansion and, potentially, the continued expression of IL-17 and other cytokines that characterize the Th17 phenotype. As presented in Fig. 3, the expression of the *RORγt* gene was significantly weaker in CB-derived $CD4^+$ T cells, whereas the expression of genes encoding IL-23 and the IL-23 receptor did not differ significantly between the $CD4^+$ T cells. Based on the above findings of others, it is possible that the low-level expression of the *RORγt* gene in CB-derived $CD4^+$ T cells is responsible for the absence of *IL-17* mRNA expression in those cells.

Tregs are another functional subset of T cells having anti-inflammatory properties and can cause quiescence of autoimmune diseases and prolongation of transplant function. *In vitro*, Tregs have the ability to inhibit the proliferation and production of cytokines by responder ($CD4^+$ $CD25^-$ and $CD8^+$) T cells subjected to polyclonal stimuli, as well as to down-regulate the responses of $CD8^+$ T cells, NK cells and $CD4^+$ cells to specific antigens.[25,26] These predicates translate *in vivo* to a great number of functions other than the maintenance of tolerance to self-components (prevention of autoimmune disease), such as the ability to prevent transplant rejection. Indeed, donor-specific Tregs can prevent allograft rejection in some models of murine transplant tolerance through a predominant effect on indirect alloresponses.

Foxp3 is thought to be responsible for the development of the Treg population and can act as a phenotypic marker of this fraction.[27] Tregs constitutively express CTLA-4 and there are suggestions that signalling through this pathway may be important for their function, as antibodies to CTLA-4 can inhibit Treg-mediated suppression.[28] As shown above, most of the CB-derived $CD4^+$ T cells were found to express either the *FOXP3* gene or the Foxp3 protein at higher levels compared with PB-derived $CD4^+$ T cells, suggesting that CB-derived $CD4^+$ T cells frequently include a potent Treg population.

As described above, *IL-17* mRNA was more detectable in PB-derived $CD4^+$ cells while *FOXP3* mRNA expression was higher in CB-derived $CD4^+$ cells. Post-transcriptional regulation, as well as differences in mRNA and protein turnover rates, can cause discrepancies between mRNA and protein expression and thus the differences observed in the mRNA expression do not necessary directly indicate those in protein expression.[29] Indeed, we observed some discrepancy between the levels of mRNA and protein with regard to Foxp3 expression in CB-derived $CD4^+$ T cells, as presented above. Nevertheless, changes in mRNA expression are mediated by the alteration of transcriptional regulation, and thus should indicate the differentiation ability of the cells. Therefore, our data indicate that CB-derived $CD4^+$ T cells tend frequently to include potent Tregs, while PB-derived $CD4^+$ T cells tend to include potent IL-17-secreting cells. As described above, DLI with donor CB-derived activated $CD4^+$ T cells is currently becoming established as a routine therapeutic strategy in Japan. It has been proposed that the skewing of responses towards Th17 or Th1 cells and away from Tregs may be responsible for the development and/or progression of autoimmune diseases or acute transplant rejection, and it may thus also be speculated that CB-derived $CD4^+$ T cells are more appropriate for DLI than PB-derived $CD4^+$ T cells.

However, our data also indicate the presence of individual, donor-dependent variations in the characteristics of activated $CD4^+$ T cells derived from CB and PB. More-

over, activated $CD4^+$ T cells do not consist of a single population and should include several distinct functional subsets of $CD4^+$ T cells. Therefore, it is important to clarify the characteristics of activated $CD4^+$ T cells in each preparation to predict the therapeutic effect of DLI in each clinical case.

In summary, our findings demonstrate a difference in gene expression between activated $CD4^+$ T cells derived from CB and those derived from PB. The higher level of *FOXP3* gene expression and the lower level of *IL-17* gene expression in CB-derived $CD4^+$ T cells may indicate that these cells have potential as immunomodulators in DLI therapy. Further detailed analysis should reveal the advantages of activated $CD4^+$ T cells from CB in DLI.

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Disclosures

No competing personal or financial interests exist for any of the authors in relation to this manuscript.

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original article

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Trends in cancer mortality in Japanese adolescents and young adults aged 15–29 years, 1970–2006

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Objective: The primary objective of this study is to describe cancer mortality rates and trends among Japanese adolescents and young adults aged 15–29 years for the period 1970–2006.

Materials and methods: Age-standardized mortality rates were calculated by the direct method using age-specific mortality rates at 5-year age intervals and weights based on the age distribution of the standard world population. The joinpoint regression model was used to describe changes in trends.

Results: For all cancers combined, the mortality rate at age 15–29 years during 2000–2006 was 4.41 (per 100 000 population) for males and 3.81 (per 100 000) for females. Trends of mortality from cancer in Japan were similar to that in other developed countries. A notable exception was cervical cancer, for which Japanese young women showed a significant increase, on average 4.0% per year throughout the period.

Conclusion: This report presents updated figures and trends in cancer mortality among adolescents and young adults aged 15–29 years in Japan and other developed countries. We hope this study will raise public awareness on cancer in this age group and provide the impetus for further research to improve the survival and quality of life of the young people in Japan.

Key words: adolescent, cancer, epidemiology, mortality, time trends, young adult

introduction

Cancer is the leading cause of disease-related deaths in adolescents and young adults (AYAs) in Japan. Nearly 7000 adolescents and young adults aged 15–29 died from cancer in Japan in the 7-year period of 2000–2006. Overall cancer patterns reflect cancers that are most prevalent in middle and old age, especially breast, prostate, lung and colorectal. However, the spectrum of malignant diseases among younger ages (childhood, adolescence and young adulthood) differs from patterns at older ages and even among themselves. A prior monograph from the Surveillance, Epidemiology and End Result (SEER) program indicated that malignant disease in persons aged 15–29 years is unique in the distribution of types that occur, with Hodgkin's lymphoma, melanoma, testis cancer, female genital tract malignancies, thyroid cancer, soft tissue sarcomas, and nongonadal germ-cell tumors accounting for 95% of the cancers in this age group. The vast majority of cancers diagnosed before age 30 appears to be spontaneous and unrelated to either carcinogens in the environment or inherited factors [1].

Compared with younger and older age groups, adolescents and young adults have experienced little or no improvement in cancer survival rates in more than two decades. The SEER reports have addressed concerns about the deficit in survival improvement in this population and attributed it to a lack of awareness of the cancer problem in this age group, lack of health-care insurance coverage and access to health care [1–4]. During recent years, more attention has been drawn to the need for further development of treatment services available to adolescents and young adults with cancer. Some developed countries have also reported descriptive epidemiologic results based on population data for AYAs with cancer [5–14]. However, little attention and few resources have been devoted to studying the incidence, risk factors, survival and mortality in this age group in Japan.

To provide a comprehensive picture of the cancer mortality and trend analysis in individuals aged 15–29 years in Japan, we analyzed the occurrence of death from cancer among AYAs at the population level over the period of 1970–2006, using official death certificates, which record 100% of deaths in Japan.

materials and methods

The number of deaths by cause, stratified for sex and by 5-year age group for cancer for the period 1970–2006, was derived from vital statistics compiled by the Ministry of Health, Labor and Welfare of Japan.

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Population figures were obtained from census data and intercensus estimates, by calendar year, age and gender. Population censuses of Japan are conducted every 5 years by the Statistics Bureau, Ministry of Internal Affair and Communications.

For comparison, we also calculated the cancer mortality rate in other developed countries, including Canada (2000–2004), the United States (2000–2005) and UK, England and Wales (2000–2005). Deaths at age 15–19, 20–24 and 25–29 years were derived from the World Health Organization (WHO) mortality database. Estimates of the residential population, based on official censuses, were obtained from the same WHO database.

During 1970–2006, three different revisions of the International Classification of Disease (ICD) were used. In Japan, this included ICD-8 from 1970 to 1978, ICD-9 from 1979 to 1994 and ICD-10 from 1995 onward. Since the differences were minor in various revisions, we recorded cancer sites, including all cancer combined (ICD-10: C00–97), lip, oral and cavity and pharynx (ICD10: C00–14), digestive organs (ICD-10: C15–26), colorectal (ICD-10:C18–21), respiratory and intrathoracic organs (ICD-10: C30–C39), bone and articular cartilage (ICD-10: C40–C41), melanoma of skin (ICD-10: C43–C44), mesothelial and soft tissue (ICD10:C45–C49), genitourinary organs (ICD-10:C51–C68), testis (ICD-10:C62), cervix (ICD-10: C53), ovary (ICD-10:C56), central nervous system (CNS) tumors (ICD-10: C70–C72), thyroid and other endocrine glands (ICD-10:C73–C75), leukemia (ICD10: C91–C95), lymphoid leukemia (ICD-10: C91), myeloid leukemia (ICD-10: C92), lymphomas (ICD-10: C81–85), Hodgkin's disease (ICD-10: C81) and non-Hodgkin's lymphoma (ICD-10: C82–85). Age-standardized mortality rates at age 15–29 years were calculated by the direct method using age-specific mortality rates for 5-year age intervals and weights based on the age distribution of the standard world population.

Joinpoint software 3.3.1 from the Surveillance Research Program of the US National Cancer Institute was used for trend analysis [15, 16]. We allowed up to four joinpoints for each model. Mortality rates and their standard errors were calculated using SAS 9.0. Time trends were assessed by site and sex. Mortality trends for Canada (1970–2004), the United States (1970–2005) and UK, England and Wales (1970–2005) were included for comparison.

The standardized mortality ratio (SMR) by sex was calculated for 47 prefectures in Japan by taking the ratio of the observed to expected deaths. The z value was computed for each SMR, based on the assumption that observed deaths follow a Poisson distribution. The maps were developed using SMR by gender.

results

Table 1 gives age-adjusted 15–29 years mortality rates from all malignant tumors and the main types of cancer in Japan and other developed countries. Age-adjusted mortality rates in Japan for six successive 5-year calendar periods, i.e. 1970–1974, 1975–1979, 1980–1984, 1985–1989, 1990–1994 and 1995–1999, and also for the 7-year period of 2000–2006 were calculated. For all cancers combined, the mortality rate at age 15–29 years during 2000–2006 was 4.41 (per 100 000 population) for males and 3.81 (per 100 000) for females. Trends of age-standardized mortality from cancer are shown in Figures 1 and 2 and Table 2. The mortality for all cancers combined has declined since 1970s for both sexes. The average annual percent change (AAPC) in the last 10 years was -3.1% ($P < 0.05$) for males and -1.6% ($P < 0.05$) for females.

malignant neoplasm of lip, oral cavity and pharynx

Mortality rates from lip, oral cavity and pharynx cancer remained stable at a low level for both genders throughout the period. Death rate was 0.13 (per 100 000) for males and 0.08 (per 100 000) for females during 2000–2006.

colorectal cancer

Since 1970, colorectal cancer mortality has declined among AYA population aged 15–29 years. The reduction during the past 10 years averaged 2.6% per year in males and 3.0% per year in females.

malignant neoplasm of respiratory and intrathoracic organs

Mortality rates from respiratory organs cancer in adolescents and young men increased in the 1970s and fell significantly from 0.20 per 100 000 in 1979 to 0.09 per 100 000 in 2006. For females, the death rates declined throughout the period. The AAPC in the last 10 years was -2.7% for males and -2.1% for females.

malignant neoplasm of bone, connective tissue, skin and breast

Mortality for malignant bone tumors during 2000–2006 was 0.38 (per 100 000) for males and 0.22 (per 100 000) for females. Reduction was observed throughout the period for both sexes (1.4% per year in males and 2.1% per year in females). Figures 1 and 2 show increases in mortality for mesothelial and soft tissue tumors of 3.2% per year in males and 3.0% per year in females in the period 1970–2006. Mortality for melanoma of skin remained low and stable in females from 1970 to 2006. For males, a decline trend has been observed in the last 6 years with an average of 23.8% per year. The rate for both sexes was ~0.05 (per 100 000) during 2000–2006. Female breast cancer mortality rates peaked in 1980 and then fell significantly from 0.35 (per 100 000) to 0.24 (per 100 000) with 1.9% per year during 1980–2006.

genitourinary organ cancer

Mortality rates from testicular cancer increased by 7.1% per year from 1970 to 1977 and fell significantly from 0.54 (per 100 000) in 1977 to 0.16 (per 100 000) in 2006. Relative to cervical cancer, mortality significant increased by an average of 4.0% per year throughout the period. For ovary cancer, the rates have remained stable for the past decade.

CNS tumors

CNS tumors mortality among AYA population was 0.30 (per 100 000) for females and 0.42 (per 100 000) for males from 2000 to 2006. Mortality for males increased significantly by 7.1% per year until 1981 at which point there was a slight and nonsignificant rise. For females, mortality increased by 1.4% per year in the whole period.

malignant neoplasm of thyroid and other endocrine glands

Mortality rates were stable throughout the period for both sexes, with the rate ~0.07 (per 100 000) for males and 0.04 (per 100 000) for females from 2000 to 2006.

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Table 1. Age-adjusted mortality rate (per 100 000) by sex and diagnostic group at age 15–29 years in Japan and other developed countries

Tumor	Japan							Canada	United States	England and Wales
	1970–1974	1975–1979	1980–1984	1985–1989	1990–1994	1995–1999	2000–2006	2000–2004	2000–2005	2000–2005
Males										
Total malignant tumors	8.76	8.39	7.36	6.61	5.92	5.46	4.41	4.91	5.53	5.77
Lip, oral cavity and pharynx	0.12	0.14	0.12	0.09	0.15	0.15	0.13	0.08	0.09	0.13
Digestive organs	2.98	2.39	1.83	1.40	1.12	0.97	0.73	0.43	0.55	0.52
Colorectal	0.78	0.51	0.41	0.37	0.31	0.27	0.24	0.20	0.25	0.20
Respiratory and intrathoracic organs	0.42	0.45	0.41	0.38	0.31	0.27	0.24	0.11	0.17	0.11
Bone and articular cartilage	0.64	0.53	0.46	0.50	0.45	0.44	0.38	0.56	0.51	0.64
Meothelial and soft tissue	0.11	0.15	0.18	0.25	0.22	0.34	0.31	0.29	0.42	0.39
Melanoma of skin	0.06	0.06	0.03	0.04	0.04	0.05	0.05	0.21	0.22	0.28
Genitourinary organs	0.47	0.61	0.51	0.46	0.42	0.28	0.23	0.35	0.35	0.32
Testis	0.38	0.50	0.42	0.35	0.31	0.21	0.16	0.24	0.26	0.21
Central nervous system	0.22	0.29	0.43	0.38	0.39	0.43	0.42	0.72	0.72	0.94
Thyroid and other endocrine glands	0.05	0.10	0.06	0.06	0.06	0.08	0.07	0.06	0.07	0.09
Leukemia	2.56	2.54	2.25	2.12	1.86	1.70	1.23	1.04	1.31	1.18
Lymphoid leukemia	0.25	0.38	0.62	0.74	0.74	0.72	0.53	0.49	0.56	0.59
Myeloid leukemia	1.59	1.46	1.12	1.00	0.84	0.80	0.59	0.36	0.50	0.52
Lymphoma	0.89	0.95	0.85	0.78	0.76	0.58	0.49	0.76	0.76	0.82
Hodgkin's lymphoma	0.13	0.10	0.07	0.06	0.05	0.03	0.04	0.24	0.23	0.26
Non-Hodgkin's lymphoma	0.76	0.85	0.78	0.72	0.71	0.55	0.45	0.51	0.53	0.55
Females										
Total malignant tumors	8.46	7.59	6.74	5.63	4.62	4.32	3.81	4.19	4.45	5.08
Lip, oral cavity and pharynx	0.06	0.07	0.07	0.06	0.06	0.08	0.08	0.08	0.06	0.05
Digestive organs	3.26	2.68	2.10	1.61	1.10	0.90	0.71	0.38	0.42	0.41
Colorectal	0.57	0.39	0.34	0.31	0.25	0.23	0.22	0.10	0.19	0.17
Respiratory and intrathoracic organs	0.28	0.24	0.21	0.20	0.17	0.16	0.14	0.13	0.10	0.10
Bone and articular cartilage	0.33	0.28	0.27	0.24	0.22	0.25	0.22	0.28	0.30	0.46
Meothelial and soft tissue	0.10	0.13	0.20	0.19	0.20	0.29	0.28	0.31	0.32	0.27
Melanoma of skin	0.05	0.06	0.04	0.04	0.03	0.04	0.05	0.12	0.16	0.25
Breast	0.21	0.25	0.31	0.27	0.20	0.21	0.20	0.22	0.28	0.42
Genitourinary organs	1.29	1.02	0.87	0.69	0.59	0.56	0.55	0.54	0.55	0.67
Cervix	0.06	0.05	0.06	0.09	0.13	0.15	0.16	0.26	0.24	0.35
Ovary	0.79	0.71	0.62	0.43	0.31	0.30	0.26	0.19	0.20	0.23
Central nervous system	0.18	0.24	0.22	0.22	0.23	0.30	0.30	0.50	0.51	0.72
Thyroid and other endocrine glands	0.05	0.07	0.07	0.05	0.06	0.04	0.04	0.07	0.07	0.06
Leukemia	2.00	1.90	1.68	1.50	1.28	1.07	0.86	0.78	0.88	0.85
Lymphoid leukemia	0.17	0.24	0.45	0.45	0.48	0.42	0.34	0.18	0.29	0.34
Myeloid leukemia	1.20	1.16	0.84	0.73	0.60	0.53	0.44	0.36	0.43	0.47
Lymphoma	0.40	0.46	0.49	0.41	0.38	0.30	0.27	0.58	0.54	0.59
Hodgkin's lymphoma	0.05	0.06	0.06	0.03	0.02	0.03	0.03	0.25	0.22	0.24
Non-Hodgkin's lymphoma	0.35	0.39	0.44	0.39	0.35	0.27	0.23	0.33	0.32	0.35

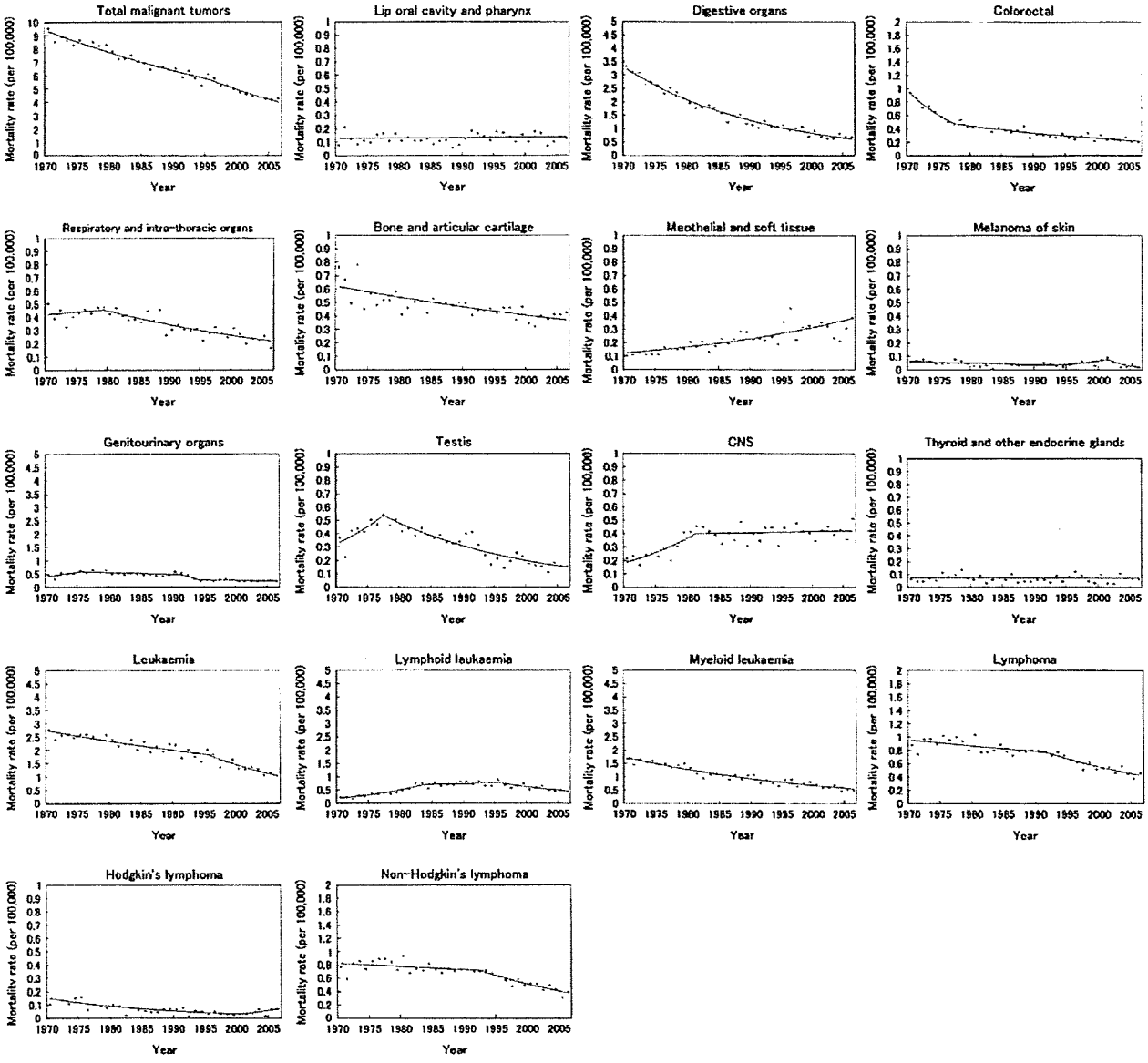

Figure 1. Trends on age-adjusted mortality for cancer among adolescents and young adults aged 15–29 years, Males, Japan, 1970–2006.

malignant neoplasm of lymphoid, hematopoietic and related tissue

Death rate from leukemia during 2000–2006 was 1.23 (per 100 000) for males and 0.86 (per 100 000) for females. Mortality showed continued improvement over time for both sexes. For lymphoma, rate among young people aged 15–29 years was 0.49 (per 100 000) in males and 0.27 (per 100 000) in females during 2000–2006. Significant decline was observed in both sexes (by 3.9% per year in males and 2.8% per year in females in recent decade).

Mortality rates varied among prefectures in Japan. A map of SMR by gender is shown in Figure 3. The SMR was significantly highest among AYAs aged 15–29 years in Hokkaido, Iwate, Akita, Fukushima, Niigata and Miyazaki prefectures for males and Akita, Fukushima, Tochigi, Kochi and Miyazaki prefectures for females.

discussion

This study has presented detailed analysis on the trends of cancer mortality in AYAs aged 15–29 years in Japan. There were 60 959 adolescents and young adults who died from cancer during 1970–2006. Approximately 900 deaths were attributed to cancer in AYAs aged 15–29 years in 2006, which is two times more common than mortality during the first 15 years of life.

Mortality rates decreased during the study period in both sexes. It is unlikely that the observed time trends in the mortality rate are due to variations in the completeness and accuracy of the population data, because the data we used were provided by official sources, which were founded on the population census. The AAPC was also reported in this study. The AAPC can be used to characterize a short segment based on a joinpoint model fit over a much longer series. This is

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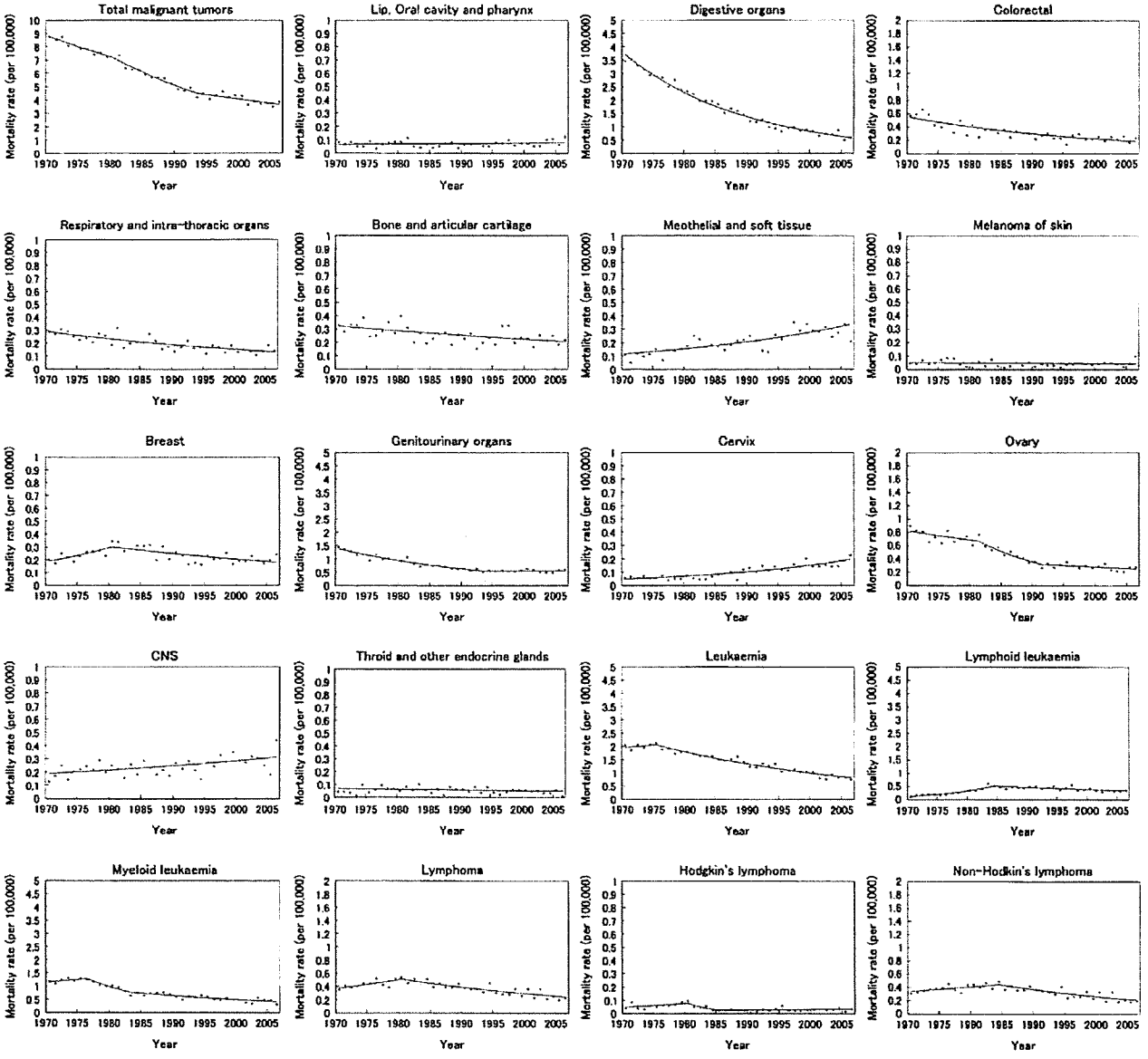

Figure 2. Trends on age-adjusted mortality for cancer among adolescents and young adults aged 15–29 years, Females, Japan, 1970–2006.

especially advantageous when data are sparse (e.g. a rare cancer or data from a small geographic area) [17]. According to trend analysis, marked mortality reductions occurred for cancer of digestive and respiratory organs, testicular cancer, leukemia and lymphoma. Similar decline trends were also observed in the United States, Canada and England and Wales in recent decade. These declines have been attributable to improving treatment for leukemia, lymphoma and testicular cancer.

Compared with the childhood cancers we reported previously [18], the reduction in the cancer mortality rate among AYAs aged 15–29 years has lagged behind the reduction in children. The AAPC in the last 10 years for pediatric cancer were −3.8% per year for boys and −1.9% per year for girls, while comparable declines for AYA population were −3.1% per year for males and −1.6% per years for females in the same period. Similar patterns have also been reported in the United States. A recent monograph published by SEER revealed that cancer mortality rates among this population have mysteriously flattened, while those of children and older adults have steadily improved [19]. The different distribution of cancer type between children and young people might partly explain this lowered reduction in mortality rate among AYA population. Furthermore, survival studies focused on AYAs have also found that some cancers common in adolescents, such as acute myeloid leukemia, acute lymphoblastic leukemia, rhabdomyosarcomam, osteogenic sarcoma and Ewing's sarcoma, are associated with lower 5-year disease-free survival rates in adolescents and young people [12], likely contributing to this deficit in mortality reduction between young adults and children. A report from the United States also attributes this discrepancy in part to the widespread gap in clinical trial participants and health-care insurance coverage between

Table 2. Joinpoint analysis for all cancer combined and selected diagnostic group of cancers, at ages 15–29 years in Japan, 1970–2006

Tumor	Japan								AAPC[a]			
	Trend 1		Trend 2		Trend 3		Trend 4		Japan	Canada	United States	England and Wales
	Years	APC	Years	APC	Years	APC	Years	APC	1997–2006	1995–2004	1996–2005	1996–2005
Male												
Total malignant tumors	1970–1995	−1.9*	1995–2006	−3.1*					−3.1*	−2.2*	−1.7*	−2.2*
Lip, oral cavity and pharynx	1970–2006	0.2							0.2	−1.0	−1.0*	0.4
Digestive organs	1970–2006	−4.5*							−4.5*	−2.9*	−0.9*	−2.0*
Colorectal	1970–1977	−9.2*	1977–2006	−2.6*					−2.6*	7.5	−0.4	−0.7
Respiratory and intrathoracic organs	1970–1979	0.9	1979–2006	−2.7*					−2.7*	−1.9*	−2.5*	−5.5*
Bone and articular cartilage	1970–2006	−1.4*							−1.4*	−1.6*	1.0*	−0.7*
Meothelial and soft tissue	1970–2006	3.2*							3.2*	−1.7	0.1	1.0*
Melanoma of skin	1970–1994	−2.5*	1994–2001	11.9	2001–2006	−23.8*			−9.6	−1.5*	−3.2*	−0.1
Genitourinary organs	1970–1975	7.0	1975–1991	−1.2	1991–1994	−17.2	1994–2006	−1.4	−1.4	−3.9*	−2.3*	−5.5*
Testis	1970–1977	7.1*	1977–2006	−4.3*					−4.3*	−4.7*	−2.3*	−6.5*
Central nervous system	1970–1981	7.1*	1981–2006	0.2					0.2	−1.3*	−0.9*	−1.4*
Thyroid and other endocrine glands	1970–2006	−0.1							−0.1	0.7	−1.9*	0.4
Leukemia	1970–1995	−1.5*	1995–2006	−5.0*					−5.0*	−2.7*	−1.6*	−3.3*
Lymphoid leukemia	1970–1983	9.8*	1983–1995	0.8	1995–2006	−4.6*			−4.6*	−1.7*	−0.7*	−3.7*
Myeloid leukemia	1970–2006	−3.1*							−3.1*	−2.6*	−3.5*	−2.6*
Lymphoma	1970–1991	−1.0*	1991–2006	−3.9*					−3.9*	−2.8*	−4.8*	−3.3*
Hodgkin's lymphoma	1970–2000	−5.0*	2000–2006	14.0					7.3	−4.2*	−4.5*	−5.2*
Non-Hodgkin's lymphoma	1970–1992	−0.6	1992–2006	−4.4*					−4.4*	−1.1*	−4.7*	−1.5*
Female												
Total malignant tumors	1970–1980	−2.0*	1980–1993	−3.5*	1993–2006	−1.6*			−1.6*	−1.7*	−2.1*	−1.6*
Lip, oral cavity and pharynx	1970–2006	0.5							0.5	−0.1	−0.4	−1.3
Digestive organs	1970–2006	−5.0*							−5.0*	−1.6*	−0.6*	−1.6*
Colorectal	1970–2006	−3.0*							−3.0*	−3.2*	−1.3*	−1.2*
Respiratory and intrathoracic organs	1970–2006	−2.1*							−2.1*	−2.1*	−2.4*	1.6
Bone and articular cartilage	1970–2006	−1.2*							−1.2*	−0.7	0.8	−0.6
Meothelial and soft tissue	1970–2006	3.0*							3.0*	1.3	−0.1	−2.6
Melanoma of skin	1970–2006	−0.6							−0.6	−2.7*	−2.9*	−1.4*
Breast	1970–1980	4.7*	1980–2006	−1.9*					−1.9*	−2.4*	−4.6*	−1.6*
Genitourinary organs	1970–1993	−3.9*	1993–2006	0.1					0.1	−2.2*	−1.6*	−3.8*
Cervix	1970–2006	4.0*							4.0*	−1.1*	−1.2*	−4.5*
Ovary	1970–1981	−1.8*	1981–1991	−7.0*	1991–2006	−1.6			−1.6	−2.7*	0.7	−3.0*
Central nervous system	1970–2006	1.4*							1.4*	−1.6*	−1.1*	−1.5*
Thyroid and other endocrine glands	1970–2006	−0.9							−0.9	0.6	−1.3*	−0.1
Leukemia	1970–1975	1.0	1975–2006	−2.9*					−2.9*	−2.3*	−1.7*	−1.9*
Lymphoid leukemia	1970–1984	10.7*	1984–2006	−1.9*					−1.9*	−6.2*	−0.8*	−0.3

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Table 2. (*Continued*)

Tumor	Japan							AAPC[a]				
	Trend 1			Trend 2		Trend 3		Japan	Canada	United States	England and Wales
	Years	APC	Years	APC	Years	APC	Years	1997–2006	1995–2004	1996–2005	1996–2005
Myeloid leukemia	1970–1976	1.7	1976–1983	−7.0*	1983–2006	−2.6*		−2.6*	−2.1*	−2.3*	−2.4*
Lymphoma	1970–1980	3.2*	1980–2006	−2.8*				−2.8*	−1.6*	−1.3*	−2.4*
Hodgkin's lymphoma	1970–1980	5.2	1980–1985	−19.9	1985–2006	1.3		1.3	−1.7*	−3.2*	−3.6*
Non-Hodgkin's lymphoma	1970–1984	1.9*	1984–2006	−3.3*				−3.3*	−0.1	−5.0*	−1.0*

[a]Last 10 observations.

*$P < 0.05$.

APC, annual percent change; AAPC, average annual percent change.

pediatric and young adults [20]. The proportion of Japanese teenagers with cancer who are included in national and international clinical trials and studies is still unknown. Further studies focused on health-care services and survival outcome among adolescents and young adults in Japan need to be conducted.

There was no decline in mortality from CNS tumors among AYAs in Japan. This implied an unfavorable trend in the incidence of CNS tumors in these populations, although improved diagnosis and certification could not be completely ruled out as contributing factors. The etiology of CNS tumors is complicated and remains largely unknown. Environmental factors are suggested to have a relationship with brain tumors. Increased incidences among children based on local population-based cancer registry data in Japan have been reported previously [21]. Investigations of the incidence of CNS tumors among adolescents and young people are necessary.

The increase in mortality from cervical cancer in AYAs suggests an increased incidence in these populations. This hypothesis can be proved by data from 11 regional population-based cancer registries in Japan [22]. The incidence rate increased from 0.7 (per 100 000) in 1975–1979 to 2.1 (per 100 000) in 2000–2002 among the AYA population aged 15–29 years. The reason for this increase trend among young women is complicated. Some research has attributed it to changes in sexual habits, oral contraceptive use, tobacco smoking, sexually transmitted diseases (papillomavirus) and the extension and distribution of screening among adolescents and young women. The cervical cancer-screening program in Japan was only offered to women aged >30 until 2004, and the coverage rate fell behind that of other developed countries. According to the Organization for Economic Co-operation and Development health data 2008, the coverage of cervical cancer screening was 23% among Japanese women aged 20–69 years, compared with 83.5% in the United States, and 72.4% in Germany [23].

Although Levi et al. [24] reported mortality trends in AYAs aged 15–24 years in Europe, including Japan for comparison previously, they primarily mentioned about seven diagnostic groups (bone sarcoma, soft tissue sarcoma, vary, testis, non-Hodgkin's lymphomas, Hodgkin's disease and leukemia) and only presented data up to 1998. Our report provides updated mortality rates and reliable time trend analysis for AYAs aged 15–29 years in Japan. Trends of mortality from cancer in Japan were generally comparable with other developed countries, but different patterns among countries have been found in this study. For example, the mortality rate from CNS tumors has decreased in the United States, Canada and UK in recent decades; however, no evidence of decline was found in Japan. Unfavorable mortality trends from cervical cancer in young Japanese women throughout the period were not observed in the United States, Canada and UK. Differences in the distribution of the histology pattern among different countries might play a role. Meanwhile, as mentioned above, the lag time in intervention in Japan might contribute to unfavorable trends in cancer mortality.

There are some limitations of this study. The low number of deaths from rare cancers may have biased the result. Some

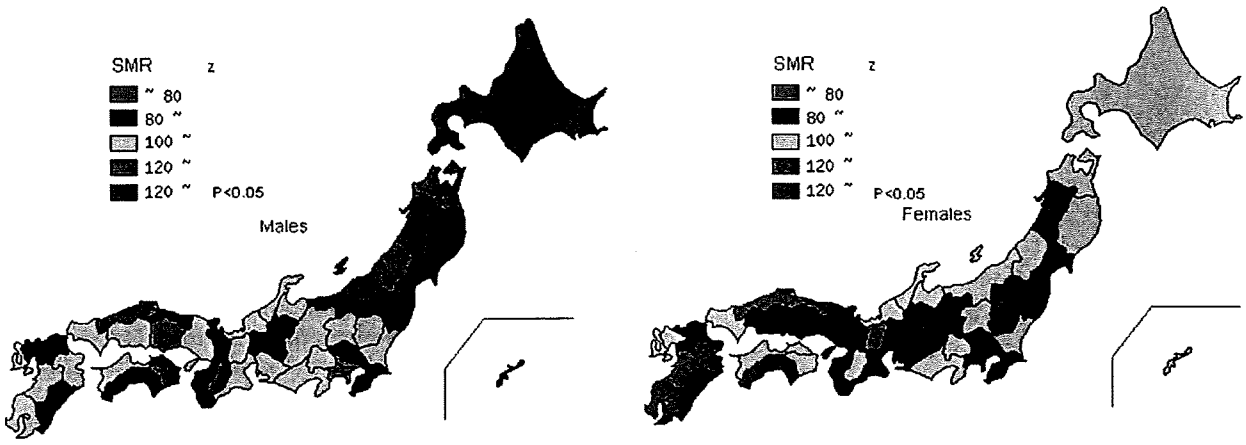

Figure 3. Standardized mortality ratios for cancer among adolescents and young adults aged 15–29 years in Japan, 2000–2006 by prefecture.

stable trends in the present study, such as mortality in thyroid cancer and Hodgkin's lymphoma, are more difficult to explain because of the small absolute number and substantial random variation. On the other hand, mortality rates are not a good substitute for incidence, because treatment for cancers has improved rapidly over time, and survival varies between age groups and populations. Furthermore, deaths occurring in adolescents and young adults relate to cancer diagnosed several years earlier, at younger ages.

Despite these limitations, this report is the first national large-scale study on mortality trends among individuals aged 15–29 years in Japan, which covered 100% AYA deaths from cancer. Moreover, there is no national-level cancer registry system in Japan. Against this background, the analysis of mortality trends over several decades remains an important method to provide additional insight into the cancer burden among AYA population. We believe that these analyses and observations will help to estimate care needs, to plan cancer prevention strategies and to provide reasonable health services for this group of patients.

conclusions

This report presents updated figures and trends in cancer mortality among adolescents and young adults aged 15–29 years in Japan and other developed countries. Mortality has improved for AYAs over the 37-year study period; however, the improvement lags behind that for children. The increased cervical cancer mortality presented here implies an increase in incidence among young Japanese women and draws attention to the need to strengthen 'cervical cancer screening in Japan'. We hope that this study will raise public awareness about cancer in this age group and provide the impetus for further research to improve the survival and quality of life of the young people in Japan.

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LY designed and carried out analyses and drafted the paper; DQ prepared data and created the figure and JF and N.S. edited the paper and commented on the interpretation of the results. All authors read and approved the final draft of the paper.

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LETTERS

Gain-of-function of mutated *C-CBL* tumour suppressor in myeloid neoplasms

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Acquired uniparental disomy (aUPD) is a common feature of cancer genomes, leading to loss of heterozygosity. aUPD is associated not only with loss-of-function mutations of tumour suppressor genes[1], but also with gain-of-function mutations of proto-oncogenes[2]. Here we show unique gain-of-function mutations of the *C-CBL* (also known as *CBL*) tumour suppressor that are tightly associated with aUPD of the 11q arm in myeloid neoplasms showing myeloproliferative features. The *C-CBL* proto-oncogene, a cellular homologue of *v-Cbl*, encodes an E3 ubiquitin ligase and negatively regulates signal transduction of tyrosine kinases[3–6]. Homozygous *C-CBL* mutations were found in most 11q-aUPD-positive myeloid malignancies. Although the *C-CBL* mutations were oncogenic in NIH3T3 cells, c-Cbl was shown to functionally and genetically act as a tumour suppressor. C-CBL mutants did not have E3 ubiquitin ligase activity, but inhibited that of wild-type C-CBL and CBL-B (also known as CBLB), leading to prolonged activation of tyrosine kinases after cytokine stimulation. *c-Cbl*$^{-/-}$ haematopoietic stem/progenitor cells (HSPCs) showed enhanced sensitivity to a variety of cytokines compared to *c-Cbl*$^{+/+}$ HSPCs, and transduction of *C-CBL* mutants into *c-Cbl*$^{-/-}$ HSPCs further augmented their sensitivities to a broader spectrum of cytokines, including stem-cell factor (SCF, also known as KITLG), thrombopoietin (TPO, also known as THPO), IL3 and FLT3 ligand (FLT3LG), indicating the presence of a gain-of-function that could not be attributed to a simple loss-of-function. The gain-of-function effects of C-CBL mutants on cytokine sensitivity of HSPCs largely disappeared in a *c-Cbl*$^{+/+}$ background or by co-transduction of wild-type *C-CBL*, which suggests the pathogenic importance of loss of wild-type *C-CBL* alleles found in most cases of *C-CBL*-mutated myeloid neoplasms. Our findings provide a new insight into a role of gain-of-function mutations of a tumour suppressor associated with aUPD in the pathogenesis of some myeloid cancer subsets.

Myelodysplastic syndromes (MDS) are heterogeneous groups of blood cancers originating from haematopoietic precursors. They are characterized by deregulated haematopoiesis showing a high propensity to acute myeloid leukaemia (AML)[7]. Some MDS cases have overlapping clinico-pathological features with myeloproliferative disorders, and are now classified into myelodysplasia/myeloproliferative neoplasms (MDS/MPN) by the World Health Organization (WHO) classification[8]. To obtain a comprehensive profile of allelic imbalances in these myeloid neoplasms, we performed allele-specific copy number analyses of bone marrow samples obtained from 222 patients with MDS, MDS/MPN, or other related myeloid neoplasms (Supplementary Tables 1 and 2) using high-density single nucleotide polymorphism (SNP) arrays combined with CNAG/AsCNAR software[9,10].

Genomic profiles of MDS and MDS/MPN showed characteristic unbalanced genetic changes, as reported in previous cytogenetic studies[11] (Supplementary Fig. 1a); however, they were detected more sensitively by SNP array analyses (Supplementary Table 3). aUPD was detected in 70 samples (31.5%) on the basis of the allele-specific copy number analyses, which substantially exceeded the detection rate obtained using a SNP call-based detection algorithm (20.7%) (Supplementary Figs 2 and 4, and Supplementary Tables 4 and 5). Long stretches of homozygous SNP calls caused by shared identical-by-descent alleles in parents were empirically predicted and excluded (Supplementary Fig. 3). aUPDs were more common in MDS/MPN than in MDS. They preferentially affected several chromosomal arms (1p, 1q, 4q, 7q, 11p, 11q, 14q, 17p and 21q) in distinct subsets of patients, and frequently associated with mutated oncogenes and tumour suppressor genes (Supplementary Figs 1b and 5). Among these, the most common aUPDs were those involving 11q ($n = 17$), which defined a unique subset of myeloid neoplasms that were clinically characterized by frequent diagnosis of chronic myelomonocytic leukaemia (CMML) with normal karyotypes (13 cases) (Fig. 1a and Supplementary Table 6). We identified a minimum overlapping aUPD segment of approximately 1.4 megabases (Mb) in 11q, which contained a mutated *C-CBL* proto-oncogene (Fig. 1b).

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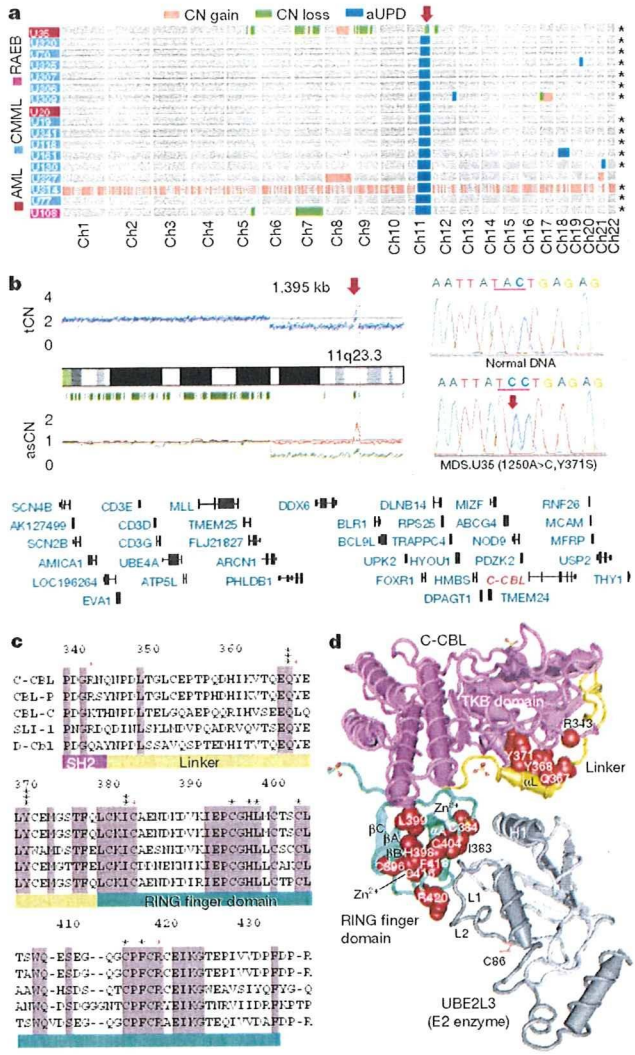

Figure 1 | Common UPD on the 11q arm and *C-CBL* mutations in myeloid neoplasms. a, Copy number profiles of 17 cases with myeloid neoplasms showing 11qUPD. Regions of copy number (CN) gains, losses and aUPD are depicted in different colours. Histologies are shown by coloured boxes. Asterisks denote *C-CBL*-mutated cases. Ch, chromosome; RAEB, refractory anaemia with excess blasts. **b**, CNAG output for MDS.U35. Total copy number (tCN) and allele-specific copy number (asCN) plots show a focal copy number gain spanning a 1.4-Mb segment within 3 Mb of an 11q-aUPD region (left), which contained mutated *C-CBL* in MDS.U35 (right). **c**, Alignments of amino acid sequences for human CBL family proteins and their homologues in *Caenorhabditis elegans* (SLI-1) and *Drosophila* (D-Cbl). Amino acid numbering is on the basis of human *C-CBL*. Conserved amino acids are highlighted. Positions of mutated amino acids are indicated by asterisks. Heterozygous mutations are shown in red. **d**, Mutated amino acid positions in the three-dimensional structure of a human C-CBL–UBE2L3 complex. TKB, tyrosine kinase binding domain.

C-CBL is the cellular homologue of the *v-Cbl* transforming gene of the Cas NS-1 murine leukaemia virus[5,12]. It was recently found to be mutated in human AML cases[13–15]. Together with its close homologue, CBL-B, C-CBL is thought to be involved in the negative modulation of tyrosine kinase signalling, primarily through their E3 ubiquitin ligase activity that is responsible for the downregulation of activated tyrosine kinases[3–5]. By sequencing all *C-CBL* exons in all 222 samples, we found *C-CBL* mutations in 15 of the 17 cases with 11q-aUPD, whereas only 3 out of 205 cases without 11q-aUPD had *C-CBL* mutations, showing a strong association of *C-CBL* mutations with 11q-aUPD ($P = 1.46 \times 10^{-18}$) (Supplementary Fig. 6 and Supplementary Tables 6 and 7), as also indicated in a recent report[16]. Thus, *C-CBL* was thought to be the major, if not the only, target of 11q-aUPD in myeloid neoplasms. Two different *C-CBL* mutations co-existed in three cases (Supplementary Fig. 6b). Somatic origins of the mutations were confirmed in three evaluable cases (Supplementary Fig. 6c).

In most cases, *C-CBL* mutations were missense, involving the evolutionarily conserved amino acids within the linker-RING finger domain that is central to the E3 ubiquitin ligase activity[17] (Fig. 1c). Another case with a predominant Cys384Tyr mutation also contained a nonsense mutation (Arg343X) in a minor subclone, which resulted in a v-Cbl-like truncated protein (Supplementary Fig. 6b). In the remaining two cases, mutations led to amino acid deletions (Δ369−371 and Δ368−382) involving the highly conserved α-helix (αL) of the linker domain and the first loop of the RING finger. According to the published crystal structure of *C-CBL*[17], most of the mutated or deleted amino acids were positioned on the interface for the binding to the E2 enzyme (Fig. 1d), making contact with either the tyrosine kinase binding domain (Tyr 368 and Tyr 371) or E2 ubiquitin-conjugating enzymes (Ile 383, Cys 404 and Phe 418). Especially, all seven linker-domain mutations selectively involved just three amino acids (Gln 367, Tyr 368 and Tyr 371) within the conserved αL helix (Fig. 1d). Mutations were clearly homozygous in nine cases, and the apparently heterozygous chromatograms in the other six cases could also be compatible with homozygous mutations affecting the aUPD-positive tumour clones, given the presence of substantial normal cell components within these samples. Mutations in the remaining three cases were considered to be heterozygous. About half of the *C-CBL*-mutated cases carried coexisting mutations of *RUNX1* (four cases), *TP53* (one case), *FLT3* internal tandem duplication (1 case) or *JAK2* (3 cases). *NRAS* and *KRAS* mutations were prevalent among CMML (15.1%) but occurred within discrete clusters from *C-CBL*-mutated cases (Supplementary Tables 2 and 6 and Supplementary Fig. 5). The mutation status of *C-CBL* did not substantially affect the clinical outcome (Supplementary Fig. 7).

All tested *C-CBL* mutants induced clear oncogenic phenotypes in NIH3T3 fibroblasts, as demonstrated by enhanced colony formation in soft agar and tumour generation in nude mice (Supplementary Fig. 8). Transformed NIH3T3 cells showed PI3 kinase-dependent activation of Akt and the transformed phenotype was reverted by treatment with the PI3 kinase inhibitor Ly294002 (Supplementary Fig. 9). When introduced into $Lin^-Sca1^+c\text{-}Kit^+$ (LSK) HSPCs, C-CBL mutants (C-CBL(Gln367Pro) and C-CBL(Tyr371Ser)), as well as a mouse lymphoma-derived oncogenic mutant (C-CBL(70Z)), significantly promoted the replating capacity of these progenitors (Fig. 2a). Because c-Cbl negatively modulates tyrosine kinase signalling, and all *C-CBL* mutations, including those previously reported[13–16], affected the critical domains for its enzymatic activity involved in this modulation, C-CBL was postulated to have a tumour suppressor function; loss-of-function could be a mechanism for the oncogenicity of these C-CBL mutants[3,5]. To assess this possibility and to clarify further the role of *C-CBL* mutations in the pathogenesis of myeloid neoplasms, we generated *c-Cbl*$^{-/-}$ mice and examined their haematological phenotypes (Supplementary Fig. 10).

In agreement with previous reports[18–20], *c-Cbl*$^{-/-}$ mice exhibited splenomegaly and an augmented haematopoietic progenitor pool, as was evident from the increased colony formation of bone marrow cells in methylcellulose culture and higher numbers of LSK and CD34-negative LSK cells in bone marrow and/or spleen compared to their wild-type littermates (Fig. 2b–d and Supplementary Fig. 11). Furthermore, when introduced into a *BCR-ABL* transgenic background[21], the *c-Cbl*$^{-/-}$ allele accelerated blastic crisis depending on the allele dosage (Fig. 2e, f). These observations supported the notion that wild-type *C-CBL* has tumour suppressor functions, whereas 'mutant' *C-CBL* acts as an oncogene; *C-CBL* can therefore be both a proto-oncogene and a tumour suppressor gene.