J Hum Genet (2007) 52:179–190
DOI 10.1007/s10038-006-0092-3

ORIGINAL ARTICLE

A genomewide linkage analysis of Kawasaki disease: evidence for linkage to chromosome 12

Yoshihiro Onouchi · Mayumi Tamari · Atsushi Takahashi · Tatsuhiko Tsunoda · Mayumi Yashiro · Yoshikazu Nakamura · Hiroshi Yanagawa · Keiko Wakui · Yoshimitsu Fukushima · Tomisaku Kawasaki · Yusuke Nakamura · Akira Hata

Received: 12 September 2006 / Accepted: 10 November 2006 / Published online: 8 December 2006

Abstract Kawasaki disease (KD) is an acute systemic vasculitis syndrome that primarily affects infants and young children. The cause of KD is largely unknown, but its higher incidence in the Asian population and increased risk in patients' families suggests the existence of underlying genetic factors. To determine the loci of a susceptibility gene for KD, a genomewide linkage analysis with affected sib pairs was performed on 78 family samples collected from all over Japan. Multipoint linkage analysis using MAPMAKER/SIBS 2.0 identified evidence of linkage on 12q24 [maximum lod score (MLS) = 2.69]. Possible linkage (MLS > 1.0) was also found on 4q35, 5q34, 6q27, 7p15, 8q24, 18q23, 19q13, Xp22, and Xq27. This is the first large-scale study of the genetic susceptibility to KD, and our results, combined with the accumulated knowledge of the human genome, could greatly promote research on identification of the molecular pathogenesis of KD.

Keywords Kawasaki disease · MCLS · Complex disorders · Susceptibility genes · Affected sib pair analysis

Introduction

Kawasaki disease (KD; MIM300530), also known as mucocutaneous lymph node syndrome (MCLS), was first described in 1967 by the Japanese pediatrician

Y. Onouchi (✉) · A. Hata
Laboratory for Gastrointestinal Diseases,
SNP Research Center, RIKEN,
1-7-22 Suehiro-cho, Tsurumi-ku,
Yokohama, Kanagawa 230-0045, Japan
e-mail: onouchi@src.riken.jp

M. Tamari
Laboratory for Genetics of Allergic Diseases,
SNP Research Center, RIKEN, Yokohama, Japan

A. Takahashi
Laboratory for Statistical Analysis,
SNP Research Center, RIKEN, Tokyo, Japan

T. Tsunoda
Laboratory for Medical Informatics,
SNP Research Center, RIKEN, Yokohama, Japan

M. Yashiro · Y. Nakamura
Department of Public Health, Jichi Medical School,
Minamikawachi, Tochigi, Japan

H. Yanagawa
Saitama Prefectural University,
Koshigaya, Saitama, Japan

K. Wakui · Y. Fukushima
Department of Preventive Medicine,
Shinshu University School of Medicine,
Matsumoto, Japan

T. Kawasaki
Japan Kawasaki Disease Research Center,
Tokyo, Japan

Y. Nakamura
Laboratory of Molecular Medicine,
Human Genome Center, Institute of Medical Science,
The University of Tokyo, Tokyo, Japan

A. Hata
Department of Public Health,
Chiba University Graduate School of Medicine,
Chiba, Japan

Tomisaku Kawasaki. It is a self-limiting vasculitis syndrome that affects systemic small and medium-sized blood vessels. The symptoms include prolonged fever, nonsuppurative cervical lymphadenitis, and changes in the skin and mucous membranes, such as rash; edema; conjunctival injection; erythema of the oral cavity, lips, and palms; and desquamation of the fingertips (Kawasaki 1967; Burns 2002). Although the incidence has been dramatically lowered by introduction of high-dose immunoglobulin therapy, 15–20% of untreated patients and patients resistant to the therapy develop serious, sometimes life-threatening, cardiac sequelae associated with coronary artery aneurysms (Kato et al. 1975). In Japan, approximately 0.08% of patients die in the acute phase of the disease, mostly due to cardiac complications (Yanagawa et al. 1998). Furthermore, patients with cardiac sequelae have an increased risk of ischemic heart disease that may lead to myocardial infarction and sudden death (Kato et al. 1996). Currently, KD is the most common cause of acquired heart disease in childhood in the developed countries. Despite intensive study over more than 35 years, the etiology of the disease has not been clarified. In Japan, 18 nationwide epidemiologic surveys of KD have been carried out biennially from 1970 to the present. These surveys have revealed several important features, such as: (1) unidentified infectious agent(s) appear to play some role, (2) siblings and offspring of KD patients are at higher risk of the disease (Fujita et al. 1989; Uehara et al. 2003), and (3) male predominance of its occurrence (male:female ratio 1.4) and cardiac complications (Yanagawa et al. 1998). These features strongly indicate the existence of genetic factors that determine the susceptibility and severity of KD, in addition to any environmental factors. A possible mechanism of KD onset would be a genetically determined exaggerated immune response triggered by some unknown infection. Thus, identification of genetic factors would greatly facilitate understanding of the disease etiology and pathophysiology. We conducted a nonparametric genome-wide linkage analysis on 78 Japanese affected sib pairs (trios), and several candidate gene loci have been identified.

Materials and methods

Family samples

Siblings affected with KD were recruited nationwide for this study. All patients were diagnosed by pediatricians according to the criteria established by the Japan Kawasaki Disease Research Committee (http://www.kawasaki-disease.org/diagnostic/index.html). Genomic DNA was extracted from peripheral blood leukocytes or Epstein-Barr-virus-transformed lymphoblastoid cell lines. The Ethical Committee of the Institute of Medical Science, the University of Tokyo and RIKEN approved the study, and all parents gave written informed consent.

Markers and genotyping

Microsatellite markers from the ABI Linkage Mapping Set MD-10 (Applied Biosystems, Foster City, CA, USA) were used for screening, and genetic mapping information on these markers was obtained from the web site of the Whitehead Institute (http://www.broad.mit.edu/cgi-bin/contig/phys_map). The allele frequencies and heterozygosity of these markers in the Japanese population have already been described (Ikari et al. 2001). Markers whose heterozygosity has been determined as less than 0.60 in the Japanese population were substituted with alternative ones. To avoid the problem of population stratification, data analyses were conducted with allele frequencies of the markers in founders of the sib pairs. The total number of markers was 399 and the average interval 9.9 cM. Polymerase chain reactions (PCRs) for genotyping were performed by the method described previously (Ikari et al. 2001). Pooled PCR products were mixed with GeneScan 500 LIZ Size Standard, and electrophoresis was performed on ABI 3700 capillary DNA sequencers (Applied Biosystems) according to the manufacturer's protocol. Genotyping for each individual was performed using ABI GeneScan 3.5.2 and Genotyper 3.7 software (Applied Biosystems). Inconsistency within families was ruled out using the Checkfam program (http://www.genstat.net/checkfam/index.cgi?lang=ja).

Detailed mapping of a locus in 12q24

To obtain more information on a locus around the linkage peak in 12q24, we introduced three markers, one already known and two newly identified (Table 1). Primers used for amplification of these markers were as follows: D12S366F 5′-AAATACAGAGAATTG

Table 1 Markers used in high information content mapping

Markers	Position (cM)	Position (Mb)	Heterozygosity
D12S366	133.8	117.1	0.86
12qMS054	140.9	123.5	0.88
12qMS069	148.3	125.2	0.89

Table 2 Record of sample collection

Type of family samples	
Affected sib pair only	29
Affected sib pair and one parent	13
Affected sib pair and parents	23
Affected sib pair and unaffected sib and both parents	8
Affected sib pair and unaffected sib and one parent	2
Affected sib trio only	1
Affected sib trio and one parent	1
Affected sib trio and both parents	1
Affected half sib pair	1
Total	79
Age	
Average age of founders at affection	3.2[a]
Average age of siblings of founders at affection	2[b]
Over all average age at affection	2.5
Gender	
Brother/brother pairs	23
Sister/sister pairs	15
Mixed pairs	38
Mixed trios	3
Total	79

[a] Information was available in 34 patients

[b] Information was available in 50 patients

GCTCG-3′, D12S366R 5′-GGCCGATCACTTCTTG AATC-3′, 12qMS054F 5′-CAATCCAGGAACCCAA GTAG-3′, 12qMS054R 5′-ACAAGAGACTGCAA CCAGAG-3′, 12qMS069F 5′-ATCCAGGTATCCA GGAAAGG-3′, 12qMS069R 5′-GAAGGCAGATTT CCCTCAAC-3′.

Table 3 Power calculation of affected sib pair study ($N = 78$)

Locus-specific λs	Lod score	Power
5	3.6[a]	0.84
	2.2[a]	0.98
3	3.6	0.54
	2.2	0.85
1.5	3.6	0.02
	2.2	0.13

[a] Threshold for significant and suggestive evidence of linkage in genomewide analysis (Lander and Kruglyak 1995)

Statistical analysis

The power of affected sib pair test was calculated using the previously described method (Risch 1990). Both single and multipoint nonparametric linkage (NPL) analyses for all genotype data was performed using the MAPMAKER/SIBS 2.0 program (Kruglyak and Lander 1995). Calculations of the identity by descent (IBD) distribution were conducted every 1.0 cM in the multipoint analyses. In the analyses, family members who had no obvious history of KD were treated as unknown as to whether they were affected. The program was also used to estimate information content. NPL scores and P values were calculated with the GENEHUNTER 2.1 program (Kruglyak et al. 1996); the sex-linked mode of MAPMAKER/SIBS and GENEHUNTER 1.3 program were used for the analysis of chromosome X.

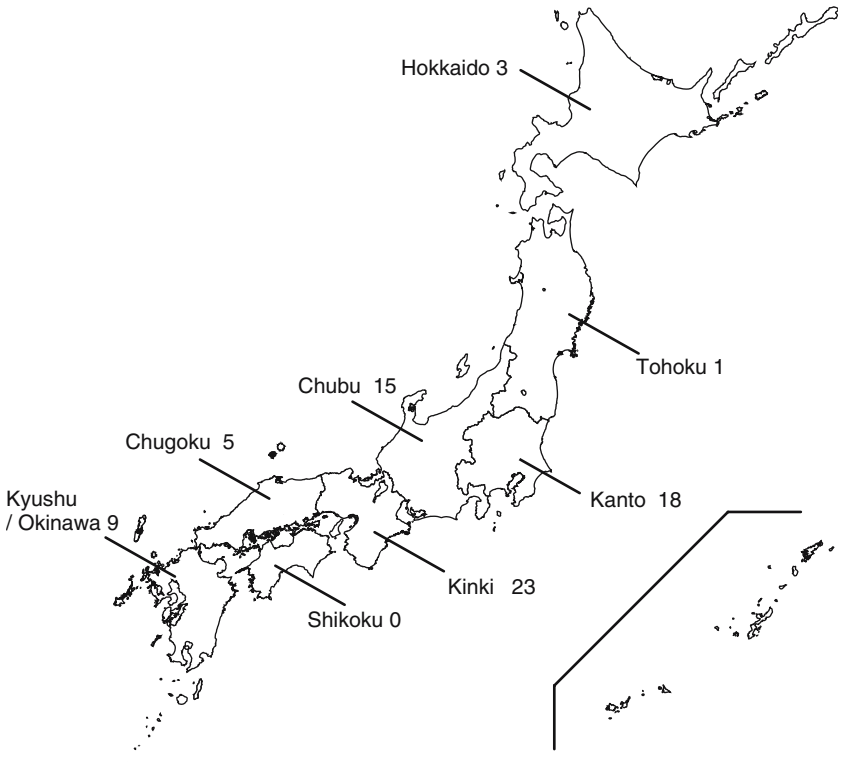

Fig. 1 Geographical distribution of pedigrees collected in eight districts of Japan

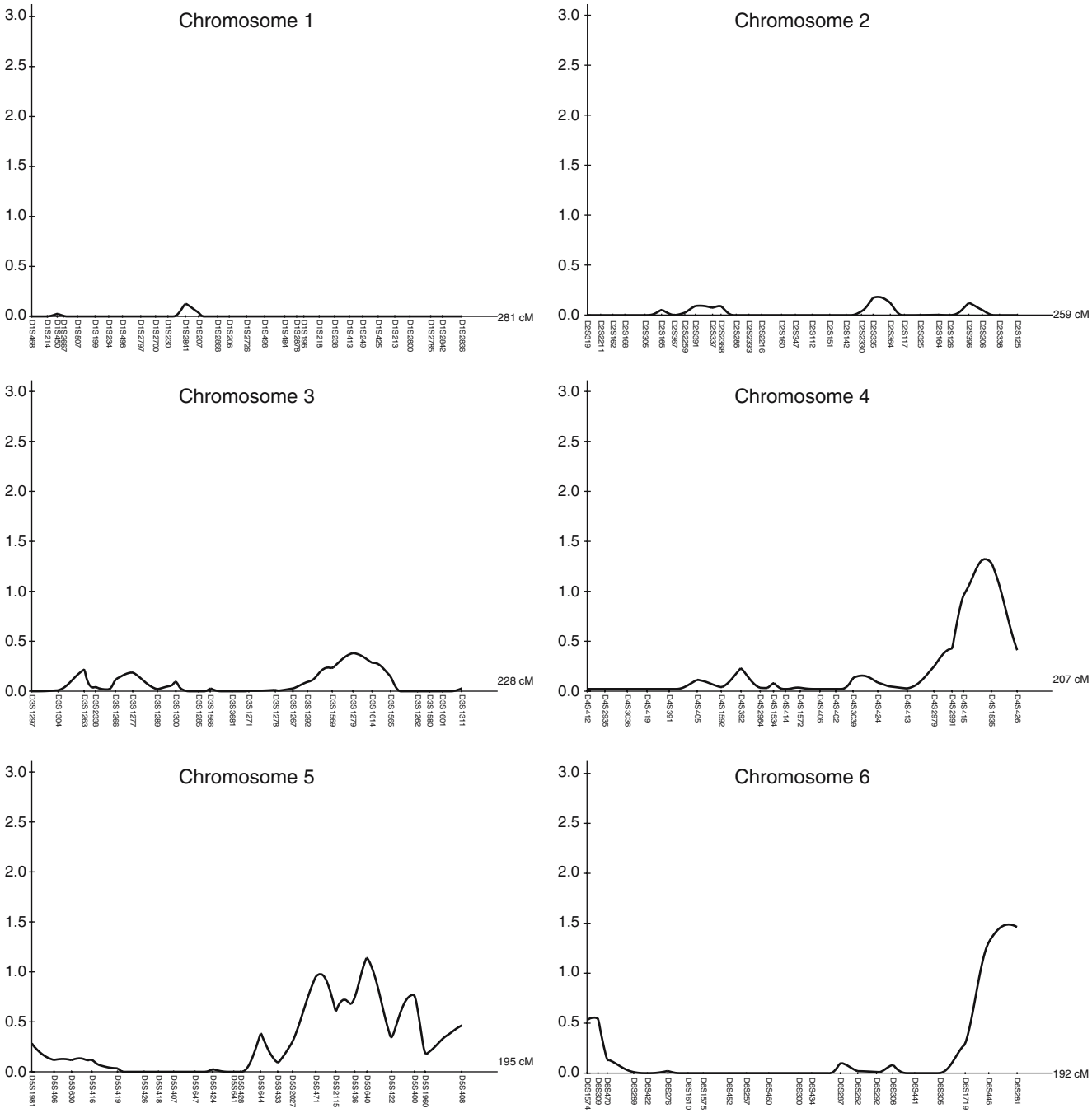

Fig. 2 Genomewide linkage analysis of Kawasaki disease (KD). Genotype data for 78 Japanese KD families were analyzed by MAPMAKER/SIBS. The *Y axis* indicates the maximum lod score (MLS) values, and the *X axis* indicates the relative position of the microsatellite markers in each chromosome. The *right side* of the X axis corresponds to the q terminal of the chromosome

Results

A total of 79 families including 75 full sib pairs, three sib trios, and one half sib pair were collected; samples from the half sib pair were not employed in this study. In 48 families, samples of either or both parents were available. Samples from unaffected siblings were also obtained in ten families (Table 2). The geographical distribution of patients recruited in this study as affected sib pairs is displayed in Fig. 1. The result of the power calculation of sib pair analysis is summarized in Table 3. According to this result, detection of suggestive linkage could be expected at loci with λs larger than 3.0 with an acceptable power (> 0.80). Average

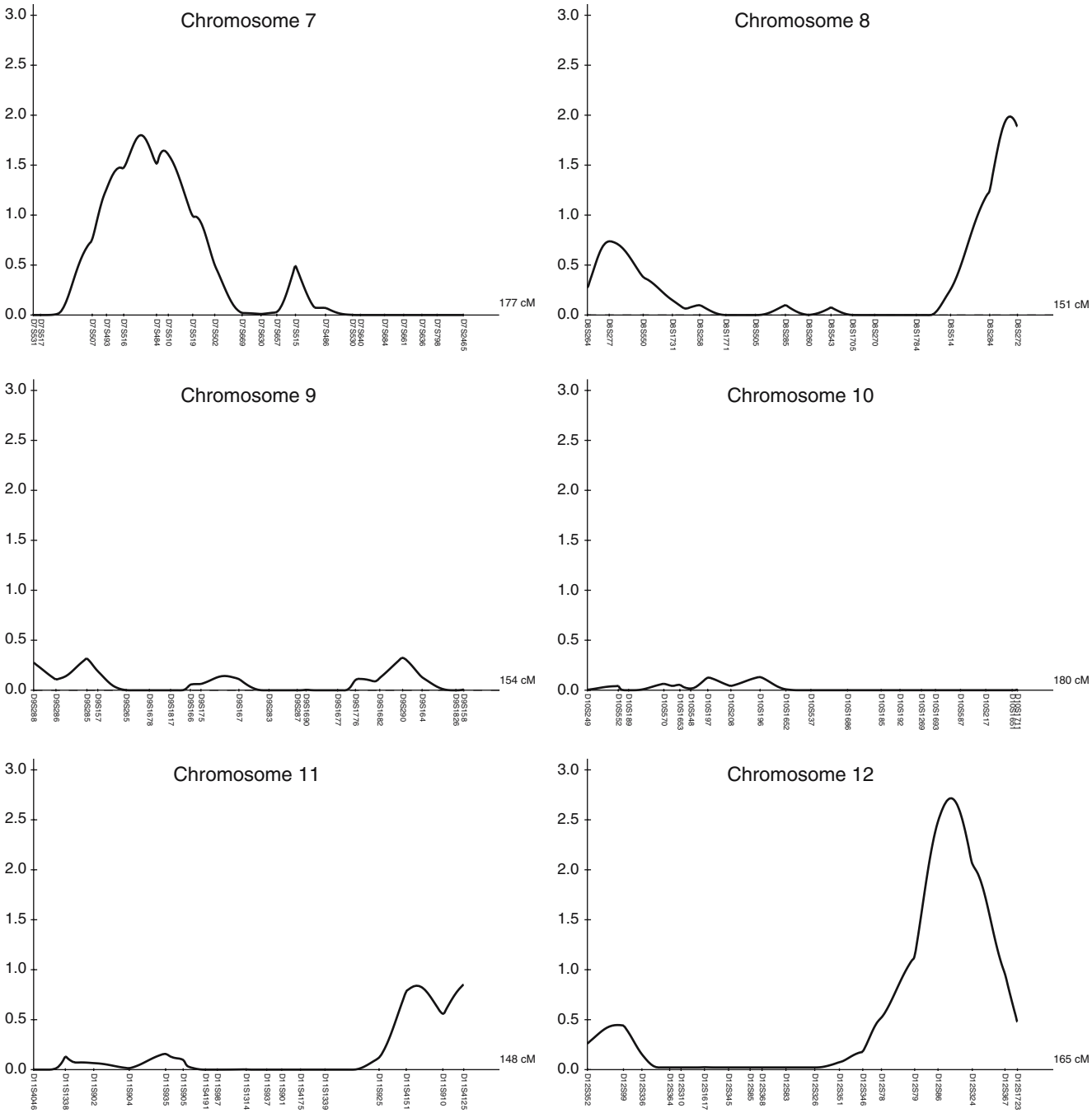

Fig. 2 continued

genotyping success rate was 98.4%, and the overall average of information content was 0.70.

The results of the genomewide linkage analysis are displayed in Fig. 2. Ten chromosomal regions, 4q35, 5q34, 6q27, 7p15, 8q24, 12q24, 18q23, 19q13, Xp22, and Xq27 showed maximum lod scores (MLS) greater than 1.0 (Fig. 2a–d; Table 4). Among them, the most significant region was 12q24 (MLS 2.69, NPL 2.64, $P = 0.0043$). The 1-lod support interval for this region spanned 25 cM (129 ~ 154 cM). One hundred and twenty-eight genes are currently mapped within this interval (Table 5). Among them, 90 genes are believed to be expressed in organs related to immune function. Replacement and addition of markers around the peak of chromosome 12 increased information content from 0.66 to 0.84. In this condition, MLS of the locus was 2.26 and was still above the threshold of suggestive linkage (Fig. 3).

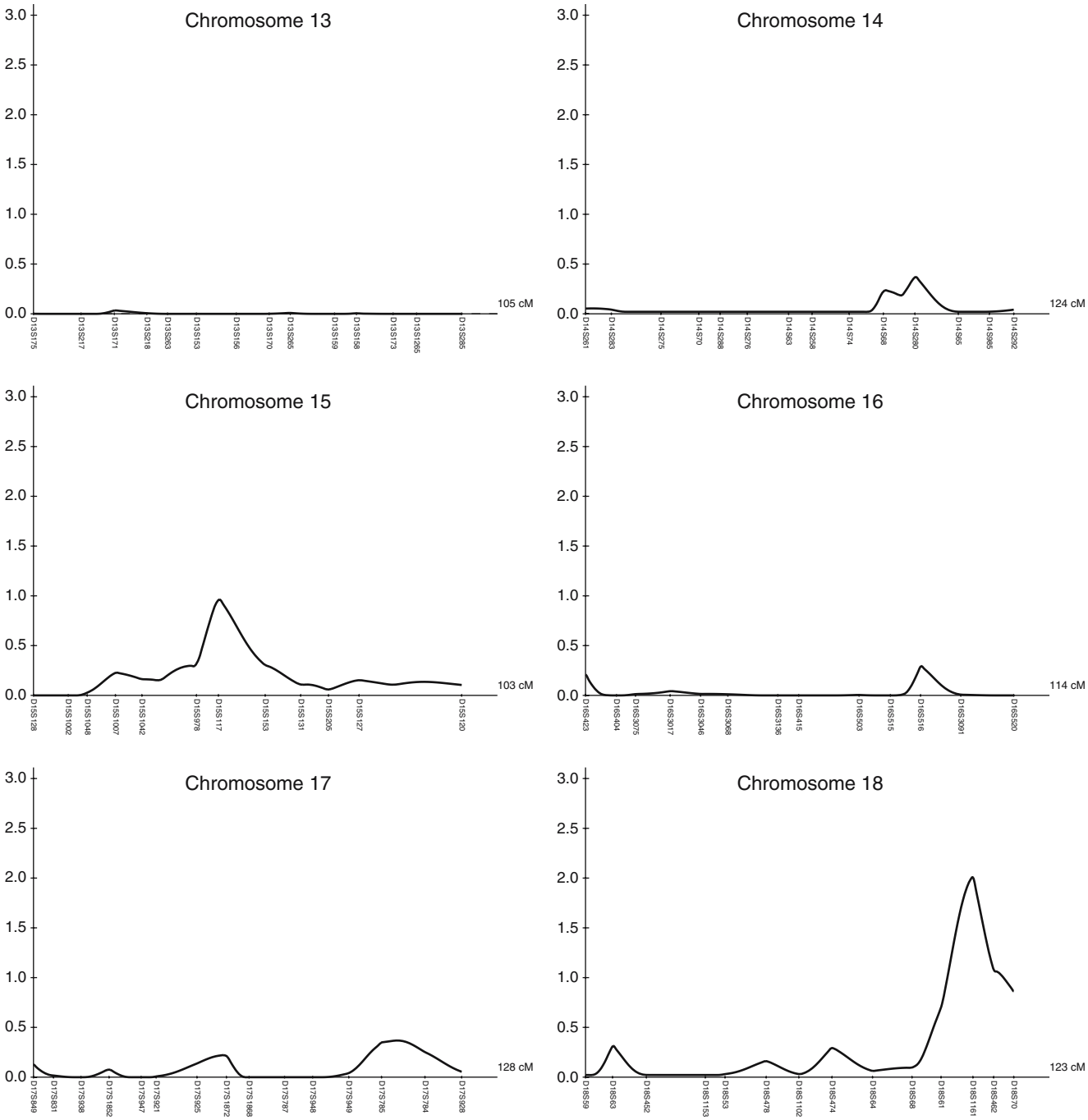

Fig. 2 continued

Discussion

KD is basically a self-limiting febrile illness that mainly affects infants and children. The most plausible trigger of KD, as determined from clinical features, epidemiologic profiles, and laboratory findings, would be some unknown infection(s). Several candidate infectious agents have been isolated from specimens of KD patients in the acute phase (Tsurumizu et al. 1991; Leung et al. 1993), but to date, no agents have been confirmed by the subsequent studies.

Since 1967, when KD was first reported by Kawasaki in Japan (Kawasaki 1967), much effort has been directed toward clarification of the pathogenesis and pathophysiology of the disease, but no therapeutic or preventive strategy based on etiological evidence has been developed. On the other hand, epidemiologic studies have shed light on the genetic aspects of KD as

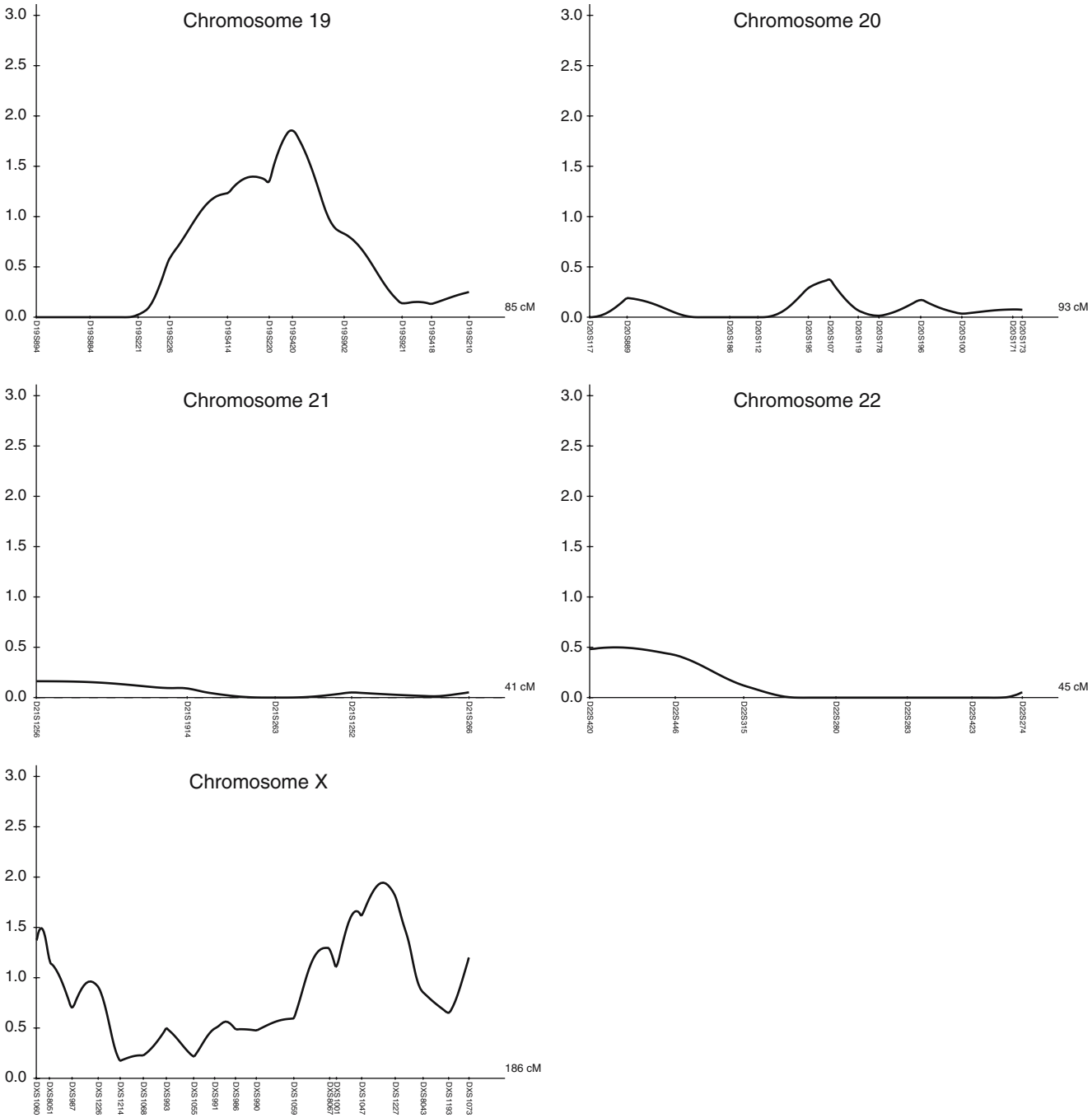

Fig. 2 continued

a result of two findings. One is an ethnic difference in disease prevalence: in Hawaii, increased prevalence in the Japanese American population has been observed (Dean et al. 1982). The other is familial aggregation of the disease: the relative risk of siblings (λs) for KD is about ten, and two-generation KD patients have been observed more frequently than expected (Fujita et al. 1989; Uehara et al. 2003). These findings clearly indicate the existence of some genetic factor(s) responsible for susceptibility to the disease. However, no multigenerational family suggesting a distinct inheritance pattern has been reported during the almost 40 years since KD was identified. Hence, we employed an NPL method in this study. As shown in Table 3, the observed linkage peaks were almost concordant using the two analysis methods. However, we considered that the utmost care was needed in the interpretation of NPL scores in this study. In contrast to the affected sib pair

Table 4 Chromosomal regions with multipoint maximum likelihood scores >1

Chromosome	Position (cM)[a]	Position (Mb)	Multipoint MLS	Corresponding markers	Two-point MLS	NPL score	*P* value	Position (cM)[b]
4	195.7	184.9	1.30	D4S1535	1.75	1.92	0.026	185.0
5	152.6	149.6	1.14	D5S640	0.76	1.64	0.054	148.7
6	197.7	169.9	1.49	D6S281	1.15	1.98	0.026	201.1
7	48.8	30.1	1.80	D7S510	1.54	2.47	0.0075	60.5
8	149.7	135.7	1.99	D8S272	1.39	1.85	0.034	152.5
12	140.0	120.6	2.69	D12S86	1.57	2.64	0.0043	135.1
18	112.1	70.4	1.99	D18S1161	1.49	2.12	0.018	112.0
19	65.4	47.9	1.86	D19S420	1.37	1.70	0.042	66.0
X	12.1	7.0	1.49	DXS1060	0.96	–[c]	–[c]	–[c]
X	159.1	139.3	1.94	DXS1227	1.57	1.47	0.075	164.7

[a] Genetic position of loci where maximum lod score (MLS) peaks were observed

[b] Genetic position of loci where nonparametric linkage (NPL) peaks were observed

[c] No NPL peak was observed

analysis, the affection status of all relatives analyzed is reflected in the NPL score. Although correct information from the parents of their history of KD in childhood is essential, no such record was available. Thus, it is possible that unrecognized patients exist in the parents and the NPL score was underestimated.

The strongest evidence of linkage was observed in the 12q24 region (MLS = 2.69, NPL = 2.64, $P = 0.0043$). The level of likelihood of the region surpassed the threshold of suggestive linkage for genomewide sib pair analysis of complex trait (lod = 2.2) (Lander and Kruglyak 1995). To achieve precise calculation of shared alleles identical by descent (IBD) in sib pair analysis, both collection of as many parents' samples as possible and usage of highly informative markers are important. In this study, we obtained samples of at least one parent from 48 pedigrees. In addition, the information content of the linkage peak on 12q24 was improved by addition and replacement of the markers.

Interestingly, positive linkage results have been reported in several studies on allergic disorders on the very same chromosomal region (Shao et al. 2004; Yokouchi et al. 2002; CSGA 1997). Together with the fact that children with KD history are at higher risk for allergy (Matsubara et al. 1998), it is plausible that the responsible gene(s) exists in this loci. As demonstrated in Table 5, more than a hundred known and predicted genes are located in the 1-lod support interval in this region. We are now searching for the gene variation for KD susceptibility by use of gene expression and linkage disequilibrium data available from public databases.

In terms of KD susceptibility, a significant association between a single nucleotide polymorphisms (SNP) in the *IL-4* gene and KD was reported by Burns et al. (2005). *IL-4* is known to be located in the 5q31.1 region, where the so-called cytokine gene cluster exists. Several diseases related to autoimmunity or allergy have already been mapped to this region (Rioux et al. 2001; Palmer et al. 2001). In this study, although not significant, nominal evidence of linkage was observed in the region (MLS = 0.98, data not shown). *IL-12B*, located on 5q33.3 region, is one of the most-studied genes as candidates for immune-mediated diseases. In our study, another linkage peak was observed in the 5q33-34 region (Table 3). It is possible that variation of the genes associated with such disorders is associated with marked activation of the immune system in acute-phase KD.

Another chromosomal region that has frequently been investigated in relation to immune disorders is 6p21, where human leukocyte antigen (HLA) genes are located. The association between HLA and development of KD remains controversial (Barron et al. 1992; Fildes et al. 1992; Kaslow et al. 1985; Krensky et al. 1981, 1983; Matsuda et al. 1977). No evidence of linkage was observed in the 6p21 region in the present study, which might indicate that HLA does not play a major role in terms of KD susceptibility.

Development of a prior prediction method of disease severity and outcome for individual KD patients in the acute phase would be a significant contribution to clinical practice. Responsiveness to immunoglobulin therapy and/or vascular wall fragility to immune insults could be also determined by genetic factors that could predict disease outcome, if clarified. Applying a positional candidate gene approach to the preliminary results of this linkage study, we have already identified an SNP within the CD40 ligand gene on chromosome X that is associated with coronary artery lesions in

Table 5 Genes loacted within 1-lod confidence interval of linkage position on chromosome 12

Start[a]	End[a]	Symbol	Orientation[b]	Description	Expression[c]
116065968	116112683	FBXO21	–	F-box protein 21	+
116135362	116283965	NOS1	–	Nitric oxide synthase 1 (neuronal)	–
116389387	116777724	KSR2	–	Kinase suppressor of ras 2	NA[d]
116938893	116954422	RFC5	+	Replication factor C (activator 1) 5, 36.5 kDa	+
116954875	116983334	WSB2	–	WD repeat and SOCS box-containing 2	+
116987864	117034763	FLJ20674	–	Hypothetical protein FLJ20674	+
117025878	117026516	LOC653276	–	Similar to hypothetical protein FLJ20674	NA
117058253	117067773	PEBP1	+	Phosphatidylethanolamine binding protein 1	+
117071989	117294934	TAOK3	–	TAO kinase 3	+
117293767	117294150	LOC728359	+	Hypothetical protein LOC728359	NA
117294521	117295201	LOC728363	+	Hypothetical protein LOC728363	NA
117298741	117340223	SUDS3	+	Suppressor of defective silencing 3 homolog (*Saccharomyces cerevisiae*)	+
117903779	118085240	KIAA1853	+	KIAA1853	–
118100978	118116934	HSPB8	+	Heat shock 22 kDa protein 8	+
118117019	118118290	LOC643737	+	Similar to 28 kDa heat- and acid-stable phosphoprotein (PDGF-associated protein) (PAP) (PDGFA-associated protein 1) (PAP1)	NA
118256900	118463235	CCDC60	+	Coiled-coil domain containing 60	–
118515614	118563001	LOC387890	+	Similar to tumor suppressor candidate 5	NA
118590144	118603812	PRKAB1	+	Protein kinase, AMP-activated, beta 1 non-catalytic subunit	+
118607981	118799475	CIT	–	Citron (rho-interacting, serine/threonine kinase 21)	+
118912031	119016680	CCDC64	+	Coiled-coil domain containing 64	+
119017286	119038982	RAB35	–	RAB35, member RAS oncogene family	+
119049397	119116896	GCN1L1	–	GCN1 general control of amino-acid synthesis 1-like 1 (yeast)	+
119118886	119123397	RPLP0	–	Ribosomal protein, large, P0	+
119132640	119187892	PXN	–	Paxillin	+
119140500	119143144	LOC728803	–	Similar to paxillin	NA
119204323	119205015	NME2P1	+	Nonmetastatic cells 2, protein (NM23B) expressed in, pseudogene 1	NA
119213946	119214089	RNU4B1	–	RNA, U4B1 small nuclear	NA
119224546	119235428	SIRT4	+	Sirtuin (silent mating type information regulation 2 homolog) 4 (*S. cerevisiae*)	–
119244297	119249975	PLA2G1B	–	Phospholipase A2, group IB (pancreas)	–
119263516	119291341	MSI1	–	Musashi homolog 1 (*Drosophila*)	–
119360287	119362915	COX6A1	+	Cytochrome c oxidase subunit VIa polypeptide 1	+
119366147	119368598	TRIAP1	–	TP53 regulated inhibitor of apoptosis 1	+
119368667	119382145	15E1.2	+	Hypothetical protein LOC283459	+
119383854	119391941	SFRS9	–	Splicing factor, arginine/serine–rich 9	+
119392043	119420681	DYNLL1	+	Dynein, light chain, LC8-type 1	+
119425467	119451336	COQ5	–	Coenzyme Q5 homolog, methyltransferase (*S. cerevisiae*)	+
119456538	119499740	RNF10	+	Ring finger protein 10	+
119501231	119503584	POP5	–	Processing of precursor 5, ribonuclease P/MRP subunit (*S. cerevisiae*)	+
119516198	119516734	LOC643499	–	Similar to 60S ribosomal protein L11	NA
119562805	119589510	CABP1	+	Calcium binding protein 1 (calbrain)	–
119609332	119624050	KIAA0152	+	KIAA0152	+
119632214	119645826	MGC5139	+	Hypothetical protein MGC5139	+
119648050	119662193	ACADS	+	Acyl-coenzyme A dehydrogenase, C-2 to C-3 short chain	+
119685418	119826534	SPPL3	–	Signal peptide peptidase 3	+
119826639	119837312	LOC390363	+	Similar to chloride intracellular channel 1	NA
119837402	119839284	LOC643550	+	Similar to 60S ribosomal protein L12	NA
119900932	119924698	TCF1	+	Transcription factor 1, hepatic; LF-B1, hepatic nuclear factor (HNF1), albumin proximal factor	+
119925231	119938683	C12orf43	–	Chromosome 12 open reading frame 43	+
119942478	119961163	OASL	–	2′-5′-oligoadenylate synthetase-like	+
120029367	120029654	LOC390364	+	Similar to ribosomal protein L10	NA
120055061	120108259	P2RX7	+	Purinergic receptor P2X, ligand-gated ion channel, 7	+
120132047	120156292	P2RX4	+	Purinergic receptor P2X, ligand-gated ion channel, 4	+
120159878	120220494	CAMKK2	–	Calcium/calmodulin-dependent protein kinase kinase 2, beta	+
120230543	120274585	ANAPC5	–	Anaphase promoting complex subunit 5	+
120322285	120346538	RNF34	+	Ring finger protein 34	+
120351282	120503272	FBXL10	–	F-box and leucine-rich repeat protein 10	+
120548858	120564322	TMEM142A	+	Transmembrane protein 142A	+
120573676	120591943	MORN3	–	MORN repeat containing 3	+

Table 5 Continued

Start[a]	End[a]	Symbol	Orientation[b]	Description	Expression[c]
120700043	120715977	RHOF	–	Ras homolog gene family, member F (in filopodia)	+
120717566	120720119	LOC338799	–	Hypothetical locus LOC338799	+
120726308	120754945	SETD1B	+	SET domain containing 1B	+
120761816	120781152	HPD	–	4-Hydroxyphenylpyruvate dioxygenase	–
120811029	120840154	PSMD9	+	Proteasome (prosome, macropain) 26S subunit, non-ATPase, 9	+
120840866	120926203	WDR66	+	WD repeat domain 66	+
120944244	120984333	BCL7A	+	B-cell CLL/lymphoma 7A	+
121001143	121001559	LOC728551	+	Similar to mondoA	NA
121177744	121192312	MLXIP	+	MLX interacting protein	+
121218219	121253970	LRRC43	+	Leucine rich repeat containing 43	+
121222530	121224699	IL31	–	Interleukin 31	+
121254181	121258037	B3GNT4	+	UDP-GlcNAc:betaGal beta-1,3-*N*-acetylglucosaminyltransferase 4	+
121258162	121276552	DIABLO	–	Diablo homolog (*Drosophila*)	+
121282047	121317021	VPS33A	–	Vacuolar protein sorting 33 homolog A (*S. cerevisiae*)	+
121321934	121473069	RSN	–	Restin (Reed-Steinberg cell-expressed intermediate filament-associated protein)	+
121415246	121416199	RPL21P1	+	Ribosomal protein L21 pseudogene 1	NA
121472222	121473785	LOC728569	+	Hypothetical protein LOC728569	NA
121523388	121551471	ZCCHC8	–	Zinc finger, CCHC domain containing 8	+
121555226	121577500	FLJ11021	–	Similar to splicing factor, arginine/serine-rich 4	+
121577762	121676900	KNTC1	+	Kinetochore associated 1	+
121751793	121753857	GPR109A	–	G protein-coupled receptor 109A	+
121765256	121767297	GPR109B	–	G protein-coupled receptor 109B	+
121778106	121781082	GPR81	–	G protein-coupled receptor 81	+
121803324	121821906	DENR	+	Density-regulated protein	+
121825026	121877884	CCDC62	+	Coiled-coil domain containing 62	+
121885992	121913460	HIP1R	+	Huntingtin interacting protein 1 related	+
121912215	121913461	LOC728014	+	Similar to huntingtin interacting protein 1 related	NA
121915835	121946665	VPS37B	–	Vacuolar protein sorting 37 homolog B (*S. cerevisiae*)	+
121979492	122025705	ABCB9	–	ATP-binding cassette, subfamily B (MDR/TAP), member 9	+
122025307	122030541	OGFOD2	+	2-Oxoglutarate and iron-dependent oxygenase domain containing 2	+
122030833	122033413	ARL6IP4	+	ADP-ribosylation-like factor 6 interacting protein 4	+
122033980	122160928	PITPNM2	–	Phosphatidylinositol transfer protein, membrane-associated 2	+
122199995	122202729	LOC728604	+	Hypothetical protein LOC728604	NA
122206899	122272394	MPHOSPH9	–	M-phase phosphoprotein 9	+
122283416	122308459	FLJ38663	+	Hypothetical protein FLJ38663	+
122311491	122322640	CDK2AP1	–	CDK2-associated protein 1	+
122346408	122400941	SBNO1	–	Sno, strawberry notch homolog 1 (Drosophila)	+
122414368	122416610	LOC728046	–	Similar to sno, strawberry notch homolog 1 (Drosophila)	NA
122434657	122459853	SETD8	+	SET domain containing (lysine methyltransferase) 8	+
122464088	122466421	LOC728069	+	Similar to T-box 1 isoform C	NA
122465889	122487217	MGC7036	–	hypothetical protein MGC7036	+
122508604	122516894	U1SNRNPBP	+	U11/U12 snRNP 35K	+
122522316	122584453	FLJ39378	–	Hypothetical protein FLJ39378	+
122584314	122584955	LOC728618	–	Hypothetical protein LOC728618	–
122635114	122648639	TMED2	+	Transmembrane emp24 domain trafficking protein 2	+
122652625	122671435	DDX55	+	DEAD (Asp-Glu-Ala-Asp) box polypeptide 55	+
122671523	122684200	EIF2B1	–	Eukaryotic translation initiation factor 2B, subunit 1 alpha, 26 kDa	+
122684334	122711287	GTF2H3	+	General transcription factor IIH, polypeptide 3, 34 kDa	+
122721644	122758901	C12orf38	+	Chromosome 12 open reading frame 38	+
122762818	122810394	ATP6V0A2	+	ATPase, H+ transporting, lysosomal V0 subunit a2	+
122831604	122864757	DNAH10	+	Dynein, axonemal, heavy polypeptide 10	+
122986908	123023116	CCDC92	–	Coiled-coil domain containing 92	+
123023623	123065922	ZNF664	+	Zinc finger protein 664	+
123339663	123366521	FAM101A	+	Family with sequence similarity 101, member A	–
123374914	123568793	NCOR2	–	Nuclear receptor co-repressor 2	+
123828129	123914287	SCARB1	–	Scavenger receptor class B, member 1	+
123962145	123965360	UBC	–	Ubiquitin C	+
123985979	123986364	LOC644022	–	Hypothetical protein LOC644022	NA
123997325	124039620	DHX37	–	DEAH (Asp-Glu-Ala-His) box polypeptide 37	+
124044147	124076302	BRI3BP	+	BRI3 binding protein	+
124115960	124193819	AACS	+	Acetoacetyl-CoA synthetase	+
124237299	124238021	LOC728696	+	Hypothetical protein LOC728696	–
124377115	124709542	TMEM132B	+	Transmembrane protein 132B	+

Table 5 Continued

Start[a]	End[a]	Symbol	Orientation[b]	Description	Expression[c]
124735720	124736028	LOC644152	–	Similar to CG32774-PA	NA
125218734	125222165	LOC728171	+	Hypothetical protein LOC728171	–
125476645	125484720	LOC728173	+	Hypothetical protein LOC728173	NA
125560617	125561774	PGBD3P3	+	PiggyBac transposable element derived 3 pseudogene 3	NA
125780624	125822726	LOC387895	–	Hypothetical gene supported by BC040060	+
125914962	125927273	LOC121296	+	Hypothetical LOC121296	NA
126681767	126686506	LOC644489	–	Hypothetical protein LOC644489	NA

[a] Annotation was done according to the recent National Center for Biotechnology Information (NCBI) build 36.2 chromosome 12 sequence

[b] Genes transcribed in the orientation from p ter to q ter are represented by '+', and vice versa

[c] Information of gene expression in immune-related organs (blood, bone marrow, lymph node, lymphocyte, tonsil, spleen, and thymus) was obtained from the web site of UniGene EST Profile Viewer. (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=unigene)

[d] No data was available

male KD patients (Onouchi et al. 2004). Although only a few concordant sib pair cases of coronary artery lesions were involved in the present study, other linkage peaks observed may also represent loci for susceptibility to cardiac complication.

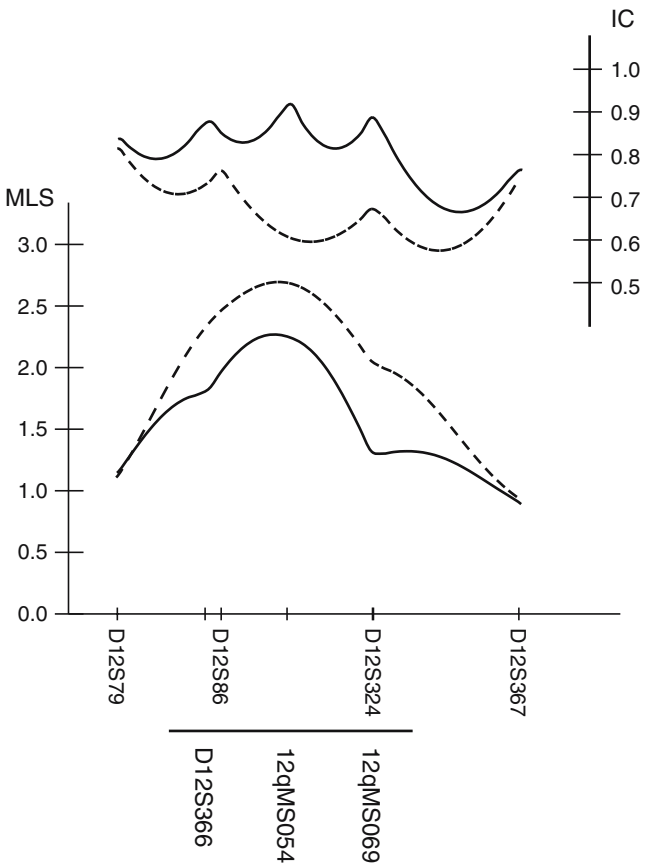

Fig. 3 Information content and lod score with alternative marker set in 12q region. Markers of the first row are the set used in the initial scan. Replaced or added markers are in the second row. Plots for information content (*IC*) and maximum lod score (MLS) are drawn on the *upper* and *lower* part of the chart, respectively. *Broken* and *continuous* lines indicate the results in the initial scan and alternative high information scan, respectively

The present study has revealed ten regions potentially involved in KD susceptibility. This is the first genome-wide genetic analysis for KD. Our findings will facilitate identification of genetic variations associated with KD susceptibility. The accumulation of knowledge on genetic factors should clarify etiology and pathogenesis of the disease and lead to the development of effective therapeutic measures.

Acknowledgments We thank patients and their families for participating in the project. We are grateful to Dr. Etsuko Tsuda, Dr. Shigeo Kojima, Dr. Marina Takenaka, Dr. Yutaka Izumi, Dr. Zenshiro Onouchi, Dr. Kaname Koyama, Dr. Atushi Ono, Dr. Fumie Furukawa, Dr. Takuji Otsuka, Dr. Takashi Hashimoto, Dr. Reiko Yabuta, Dr. Hirotoshi Iwahori, Dr. Yujiro Kimura, Dr. Yuji Murata, Dr. Ken Suzaki, Dr. Ken Takagawa, Dr. Michindo Ninomiya, Dr. Yukinobu Shibata, Dr. Minoru Kubo, Dr. Yuriko Sekine, Dr. Eikan Koh, Dr. Takeshi Terao, Dr. Mayumi Sugimori, Dr. Takashi Soga, Dr. Shigeki Terada, Dr. Nobuyuki Kiyosawa, Dr. Kunihiro Hamamoto, Dr. Noriko Baba, Dr. Hideo, Yamaguchi, Dr. Tatsuo Handa, Dr. Masashi Shiomi, Dr., Kensuke Harada, Dr. Yoshiyasu Yamada, Dr. Kazue IInuma, Dr. Sumio Hyodo, Dr. Masaru Miura, Dr. Haruo Terada, Dr. Mayumi Onda, Dr. Yoshiharu Suzuki, Dr. Shiro Tsuchiya, Dr. Naoto Tanaka, Dr. Masaki Nakagawa, Dr. Kisei Minami, Dr. Atsushi Toyoda, Dr. Chieko Udo, Dr. Kenji Yabuta, Dr. Tomio Kobayashi, Dr. Akira Kohno, Dr. Masumi Koujiro, Dr. Yasuhiko Tonegawa, Dr. Yuji Sakakura, Dr. Yutaka Yamada, Dr. Masanobu Ito, Dr. Fumiko Baba, Dr. Junji Suga, Dr. Kiyotaka Usui, Dr. Takashi Kawamura, Dr. Katsumi Takei, Dr. Seiji Noma, Dr. Mamie Watanabe, Kazunobu Ouchi, Dr. Tsutomu Saji, Dr. Tsuyoshi Isobe, and all their colleagues for cooperating in sample collection. The authors declare that they have no competing financial interests.

References

Barron KS, Silverman ED, Gonzales JC, St Clair M, Anderson K, Reveille JD (1992) Major histocompatibility complex class II alleles in Kawasaki syndrome—lack of consistent

correlation with disease or cardiac involvement. J Rheumatol 19:1790–1793
Burns JC (2002) Commentary: translation of Dr. Tomisaku Kawasaki's original report of fifty patients in 1967. Pediatr Infect Dis J 21:993–995
Burns JC, Shimizu C, Shike H, Newburger JW, Sundel RP, Baker AL, Matsubara T, Ishikawa Y, Brophy VA, Cheng S, Grow MA, Steiner LL, Kono N, Cantor RM. (2005) Family-based association analysis implicates IL-4 in susceptibility to Kawasaki disease. Genes Immun 6:438–444
Dean AG., Melish ME, Hicks R, Palumbo NE (1982) An epidemic of Kawasaki syndrome in Hawaii. J Pediatr 100:552–557
Fildes N, Burns JC, Newburger JW, Klitz W, Begovich AB (1992) The HLA class II region and susceptibility to Kawasaki disease. Tissue Antigens 39:99–101
Fujita Y, Nakamura Y, Sakata K, Hara N, Kobayashi M, Nagai M, Yanagawa H, Kawasaki T (1989) Kawasaki disease in families. Pediatrics 84:666–669
Ikari K, Onda H, Furushima K, Maeda S, Harata S, Takeda J (2001) Establishment of an optimized set of 406 microsatellite markers covering the whole genome for the Japanese population. J Hum Genet 46:207–210
Kaslow RA, Bailowitz A, Lin FY, Koslowe P, Simonis T, Israel E (1985) Association of epidemic Kawasaki syndrome with the HLA-A2, B44, Cw5 antigen combination. Arthritis Rheum 28:938–940
Kato H, Koike S, Yamamoto M, Ito Y, Yano E (1975) Coronary aneurysms in infants and young children with acute febrile mucocutaneous lymph node syndrome. J Pediatr 86:892–898
Kato H, Sugimura T, Akagi T, Sato N, Hashino K, Maeno Y, Kazue T, Eto G, Yamakawa R (1996) Long-term consequences of Kawasaki disease. A 10- to 21-year follow-up study of 594 patients. Circulation 94:1379–1385
Kawasaki T (1967) Acute febrile mucocutaneous syndrome with lymphoid involvement with specific desquamation of the fingers and toes: my clinical observation (in Japanese). Jpn J Allergy 16:178–222
Krensky AM, Berenberg W, Shanley K, Yunis EJ (1981) HLA antigens in mucocutaneous lymph node syndrome in New England. Pediatrics 67:741–743
Krensky AM, Grady S, Shanley KM, Berenberg W, Yunis EJ (1983) Epidemic and endemic HLA-B and DR associations in mucocutaneous lymph node syndrome. Hum Immunol 6:75–77
Kruglyak L, Lander ES (1995) Complete multipoint sib-pair analysis of qualitative and quantitative traits. Am J Hum Genet 57:439–454
Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES (1996) Parametric and nonparametric linkage analysis: a unified multipoint approach. Am J Hum Genet 58:1347–1363
Lander E, Kruglyak L (1995) Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat Genet 11:241–247
Leung DY, Meissner HC, Fulton DR, Murray DL, Kotzin BL, Schlievert PM (1993) Toxic shock syndrome toxin-secreting *Staphylococcus aureus* in Kawasaki syndrome. Lancet 342:1385–1388
Matsubara T, Fujita Y, Sato T, Sasai K, Furukawa S (1998) The prevalence of allergy in Kawasaki disease. Allergy 53:815–816
Matsuda I, Hattori S, Nagata N, Fruse A, Nambu H (1977) HLA antigens in mucocutaneous lymph node syndrome. Am J Dis Child 131:1417–1418
Onouchi Y, Onoue S, Tamari M, Wakui K, Fukushima Y, Yashiro M, Nakamura Y, Yanagawa H, Kishi F, Ouchi K, Terai M, Hamamoto K, Kudo F, Aotsuka H, Sato Y, Nariai A, Kaburagi Y, Miura M, Saji T, Kawasaki T, Nakamura Y, Hata A (2004) CD40 ligand gene and Kawasaki disease. Eur J Hum Genet 12:1062–1068
Palmer LJ, Barnes KC, Burton PR, Chen H, Cookson WO, Collaborative Study on the Genetics of Asthma, Deichmann KA, Elston RC, Holloway JW, Jacobs KB, Laitinen T, Wjst M (2001) Meta-analysis for linkage to asthma and atopy in the chromosome 5q31–33 candidate region. Hum Mol Genet 10:891–899
Rioux JD, Daly MJ, Silverberg MS, Lindblad K, Steinhart H, Cohen Z, Delmonte T, Kocher K, Miller K, Guschwan S. Kulbokas EJ, O'Leary S, Winchester E, Dewar K, Green T, Stone V, Chow C, Cohen A, Langelier D, Lapointe G, Gaudet D, Faith J, Branco N, Bull SB, McLeod RS, Griffiths AM, Bitton A, Greenberg GR, Lander ES, Siminovitch KA, Hudson TJ (2001) Genetic variation in the 5q31 cytokine gene cluster confers susceptibility to Crohn disease. Nat Genet 29:223–228
Risch N (1990) Linkage strategies for genetically complex traits. II. The power of affected relative pairs. Am J Hum Genet 46:229–241
Shao C, Suzuki Y, Kamada F, Kanno K, Tamari M, Hasegawa K, Aoki Y, Kure S, Yang X, Endo H, Takayanagi R, Nakazawa C, Morikawa T, Morikawa M, Miyabayashi S, Chiba Y, Karahashi M, Saito S, Tamura G, Shirakawa T, Matsubara Y (2004) Linkage and association of childhood asthma with the chromosome 12 genes. J Hum Genet 49:115–122
The Collaborative Study on the Genetics of Asthma (CSGA) (1997) A genome-wide search for asthma susceptibility loci in ethnically diverse populations. Nat Genet 15:389–392
Tsurumizu T, Okonogi H, Shibusawa T, Hashimoto T, Makino M, Ota H, Kurosaki T, Toba T (1991) A case of Kawasaki's disease combined with septicemia–isolation of *Streptococcus sanguis* (MCLS-1) and *Streptococcus* pyogenes from blood at the acute stage. Kansenshogaku Zasshi (Jpn) 65:124–128
Uehara R, Yashiro M, Nakamura Y, Yanagawa H (2003) Kawasaki disease in parents and children. Acta Paediatr 92:694–697
Yanagawa H, Nakamura Y, Yashiro M, Ojima T, Tanihara S, Oki I, Zhang T (1998) Results of the nationwide epidemiologic survey of Kawasaki disease in 1995 and 1996 in Japan. Pediatrics 102:E65
Yokouchi Y, Shibasaki M, Noguchi E, Nakayama J, Ohtsuki T, Kamioka M, Yamakawa-Kobayashi K, Ito S, Takeda K, Ichikawa K, Nukaga Y, Matsui A, Hamaguchi H, Arinami T (2002) A genome-wide linkage analysis of orchard grass-sensitive childhood seasonal allergic rhinitis in Japanese families. Genes Immun 3:9–13