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Original Article

Descriptive Epidemiology of Kawasaki Disease in Japan, 2011–2012: From the Results of the 22nd Nationwide Survey

Nobuko Makino, Yosikazu Nakamura, Mayumi Yashiro, Ryusuke Ae, Satoshi Tsuboi, Yasuko Aoyama, Takao Kojo, Ritei Uehara, Kazuhiko Kotani, and Hiroshi Yanagawa

Department of Public Health, Jichi Medical University, Shimotsuke, Tochigi, Japan

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ABSTRACT

Background: The number of patients and incidence rate of Kawasaki disease (KD) are increasing in Japan. We have therefore characterized the latest epidemiological information on KD.

Methods: The 22nd nationwide survey of KD, which targeted patients diagnosed with KD in 2011 and 2012, was conducted in 2013 and included a total of 1983 departments and hospitals. In order to report on all patients with KD during the 2 survey years, we targeted hospitals of 100 beds or more with pediatric departments, or specialized pediatric hospitals.

Results: From a total of 1420 hospitals and departments (71.6% response rate), 26 691 KD patients were reported (12 774 in 2011 and 13 917 in 2012; 15 442 males and 11 249 females). The annual incidence rates were 243.1 per 100 000 population aged 0 to 4 years in 2011 and 264.8 in 2012. The number of cases of KD recorded in 2012 was the highest ever reported in Japan. The incidence rate of complete cases was also the highest ever reported in Japan and contributed to the increase in the rate of total cases in recent years. The number of patients diagnosed per month peaked in January, and additional peaks were noted during summer months, although these peaks were lower than those seen in winter. Age-specific incidence rate showed a monomodal distribution with a peak in the latter half of the year in which patients were born.

Conclusions: The number of patients and the incidence rate of KD in Japan continue to increase. A similar trend has also been seen for patients with complete KD.

Key words: mucocutaneous lymph node syndrome; incidence; cardiovascular disease; intravenous immunoglobulin; epidemiology

INTRODUCTION

Almost half a century has passed since Dr. Kawasaki first reported on 50 patients with Kawasaki disease (KD) in 1967 (http://www.jskd.jp/info/pdf/kawasaki.pdf), and since then, approximately 300 000 patients have been registered in Japan.[1] However, the etiology of KD is still unknown. This disease affects infants and toddlers, and it causes systemic vasculitis.[2,3] The vasculitis mainly attacks coronary arteries, and cardiac sequelae, such as coronary aneurysms and coronary insufficiencies, are some of the more serious outcomes of this disease.[4–8] Fortunately, the proportion of patients with cardiac sequelae has decreased to less than 5%, down from 15%–20% approximately two decades ago. However, KD is still the leading cause of acquired heart disease in children, not only in Japan but also in European countries and the United States.[9,10]

Up to 2010, we conducted 21 nationwide epidemiologic surveys of KD.[1] Previous nationwide surveys have revealed that the number of patients and incidence rate of this disease have been increasing yearly. As the etiology of this disease remains unknown, the reason for this increase is also unclear. Thus, surveillance of trends related to disease occurrence should be conducted.

Here, we describe the results of the 22nd nationwide survey of KD in Japan, which targeted KD diagnosed in 2011 and 2012.

Address for correspondence. Nobuko Makino, Department of Public Health, Jichi Medical University, 3311-1 Yakushiji, Shimotsuke, Tochigi 329-0498, Japan (e-mail: n-makino@jichi.ac.jp).

METHODS

We conducted a retrospective survey of patients with KD who visited target hospitals for treatment of acute KD during the two-year period from January 2011 through December 2012. This survey was conducted in almost the same manner as the 21st nationwide survey of KD.[1]

Participating medical facilities were hospitals specializing in pediatrics and hospitals with a total of 100 or more beds and a pediatric department. These criteria have been used since the first nationwide survey in 1970.[11] Questionnaires (http://www.jichi.ac.jp/dph/kawasakibyou/20100715/kawasaki21final20100715.pdf, in Japanese) and diagnostic guidelines prepared by the Japan Kawasaki Disease Research Committee[12] were sent by mail to administrators in charge of the pediatric department of their respective hospitals in January 2013. The prepared list of hospitals for the survey was based on the Listing of Hospitals 2003–2004 compiled by the Committee on Studies of Health Policies of Japan's Ministry of Health, Labour and Welfare, and was revised using newly received information. A total of 2006 facilities met the conditions for inclusion. Patient information requested on the questionnaire was: name (initials only); address (municipality); sex; date of birth; date and day of first hospital visit for illness; diagnosis (typical definite, atypical definite, or incomplete); receipt of intravenous immunoglobulin (IVIG) therapy; IVIG resistance status; receipt of additional therapy, if conducted (additional IVIG therapy, steroids, infliximab, immunosuppressive agents, and plasmapheresis); recurrences; history of KD among patient's siblings and parents; cardiac lesions (at the first visit, acute phase, and sequelae); and blood tests (white blood cell count, platelet count, albumin level, and C-reactive protein level). We classified typical definite cases (patients with five or six of the six symptoms specified in guidelines for the diagnosis of KD) and atypical definite cases (patients with four of the six diagnostic symptoms who had a coronary artery aneurysm, as confirmed by coronary angiography or two-dimensional echocardiography in the course of treatment) as complete cases. We classified patients who did not satisfy the diagnostic criteria for typical definite cases or atypical definite cases but were suspected of having KD by the pediatricians reporting the cases as incomplete type cases. Acute cardiac lesions were defined as those that developed within one month of the onset (acute lesions); cardiac sequelae were defined as those that persisted beyond one month after the onset. Almost all patients were diagnosed on the basis of two-dimensional echocardiography. After checking for possible inconsistencies on the questionnaires, the forms were sent back to the respondents to correct any errors. The incidence rates (reported as rate per 100 000 children aged 0 to 4 years) were based on the population data used in the vital statistics of Japan.[13] The Bioethics Committee for Epidemiologic Research at Jichi Medical University approved this survey in advance (August 22, 2012; No. 12-18).

RESULTS

Of the 2006 hospitals asked to participate in the survey, 23 reported that the pediatric department had been discontinued or that the hospital itself had fallen into disuse. Of the 1983 eligible hospitals receiving the invitation letter, 1420 (71.6%) returned the questionnaire. A total of 26 691 patients (12 774 in 2011 and 13 917 in 2012) were reported, including 15 442 male patients (7406 in 2011 and 8036 in 2012) and 11 249 female patients (5368 in 2011 and 5881 in 2012). The average annual incidence rate for the 2-year observation was 254.0 per 100 000 children aged 0 to 4 years (286.9 for males and 219.4 for females). The average annual incidence was 243.1 in 2011 and 264.8 in 2012, the highest ever reported in Japan.

The annual number of patients with KD and the incidence rates are shown in Figures 1 and 2. In 1979, 1982, and 1986, there were 3 large nationwide epidemics of the disease in Japan, as previously reported. Since then, no national epidemics have been observed; however, the number of patients with KD began to increase in the mid-1990s. The incidence rate has increased continuously since then, reaching 264.8 in 2012. This was the first time that the annual incidence rate has been higher than 250, exceeding the rates observed in 1979, 1982, and 1986, when nationwide epidemics occurred. Figure 3 shows the incidence rate of complete KD in the 22 nationwide surveys. It revealed that rate of complete KD has increased year by year (reaching 241.2 for males and 184.9 for females in 2012). Figure 4 shows the number of patients by month for the 6 most recent national surveys (17th to 22nd). Seasonal variations in the 21st survey differed slightly from those seen in previous surveys. Within the basic pattern of seasonal variation, there is a maximum peak in winter, and a slightly lower peak in summer. The peaks for the winters of 2009 and 2010 were lower than usual. However, seasonal patterns for 2012 were similar to those seen for the 17th–20th surveys. The peak in January 2012 was the highest ever reported in Japan. Figure 5 shows the age-specific incidence rates by sex. The incidence rate was highest in children aged 9 to 11 months. After this period, it decreased gradually with age.

There were no regional differences in the incidence rate of KD. Of the 26 691 patients who were reported, 20 915 (78.4%) were typical definite cases, and 486 (1.8%) were atypical definite cases. Therefore, 21 401 (80.2%) were complete cases, while 5274 (19.8%) were incomplete cases and 16 were unknown. Of the 5274 incomplete cases, 3575 (67.8%) had 4 of the 6 principal symptoms, 1321 (25.0%) had 3 symptoms, 311 (5.9%) had 2 symptoms, 41 (0.8%) had 1 symptom, and 26 were unknown. The number of patients with 1 or more siblings affected by KD was 408 (1.5%), and 273 (0.89%) patients had at least 1 parent with a history of KD. There were 946 (3.5%) cases of recurrence. Of the 26 691 patients reported, 4 patients (all male; two aged 0 to 5 months, one aged 1 year, and one aged 5 years) died.

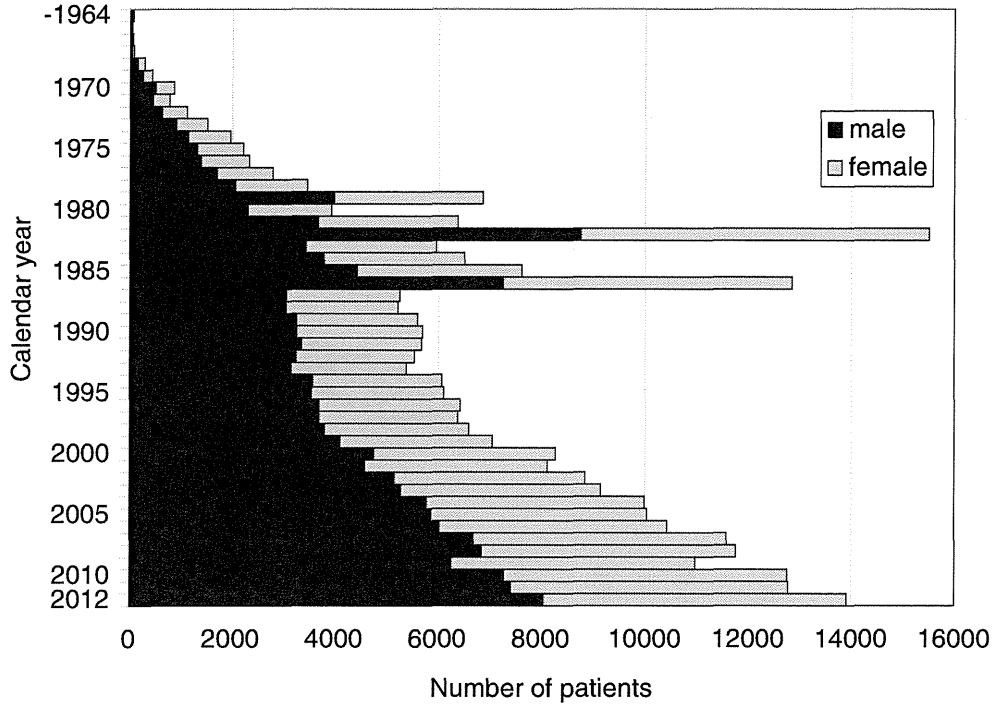

Figure 1. Number of patients with Kawasaki disease in Japan, by calendar year and sex

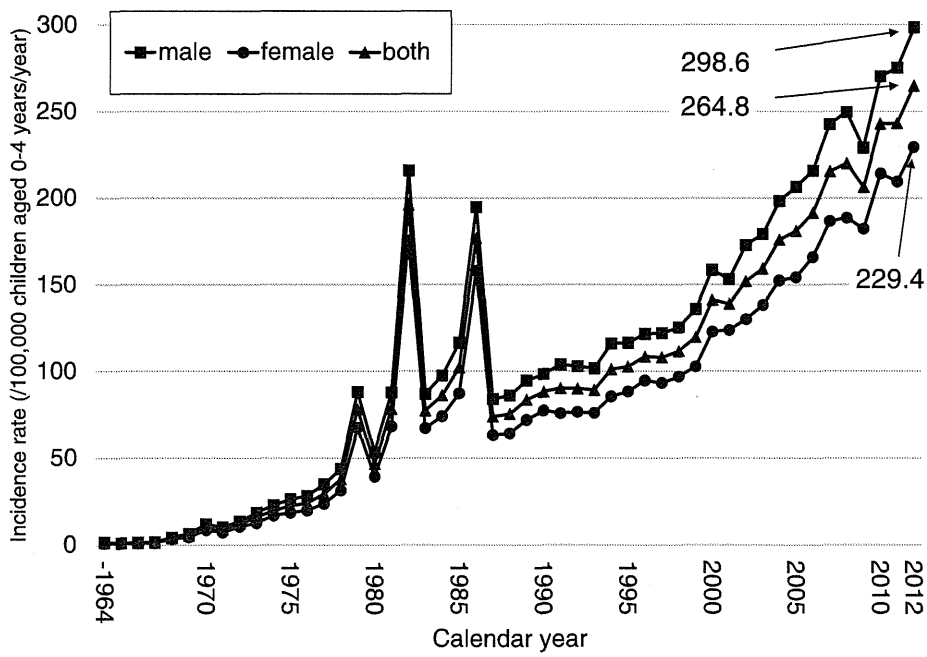

Figure 2. Incidence rate of Kawasaki disease in Japan, by calendar year and sex

At their first visit, 1241 (4.6%) patients had 1 or more cardiac lesions: 11 (0.04%) had giant coronary aneurysms, 66 (0.25%) had coronary aneurysms less than 8 mm in diameter, 957 (3.6%) had coronary dilatations, 3 (0.01%) had coronary stenoses, and 244 (0.91%) had valvular lesions (the sum of patients exceeds the total number of patients with cardiac lesions as some patients had more than one of these symptoms). During the acute phase, 2487 (9.3%) patients had 1 or more cardiac lesions: 47 (0.18%) had giant coronary aneurysms, 244 (0.91%) had coronary aneurysms less than 8 mm in diameter, 1866 (6.99%) had coronary dilatations, 5 (0.02%) had coronary stenoses, 1 (0.004%) had a myocardial infarction, and 443 (1.7%) had valvular lesions (the sum of patients exceeds the total number of patients with cardiac

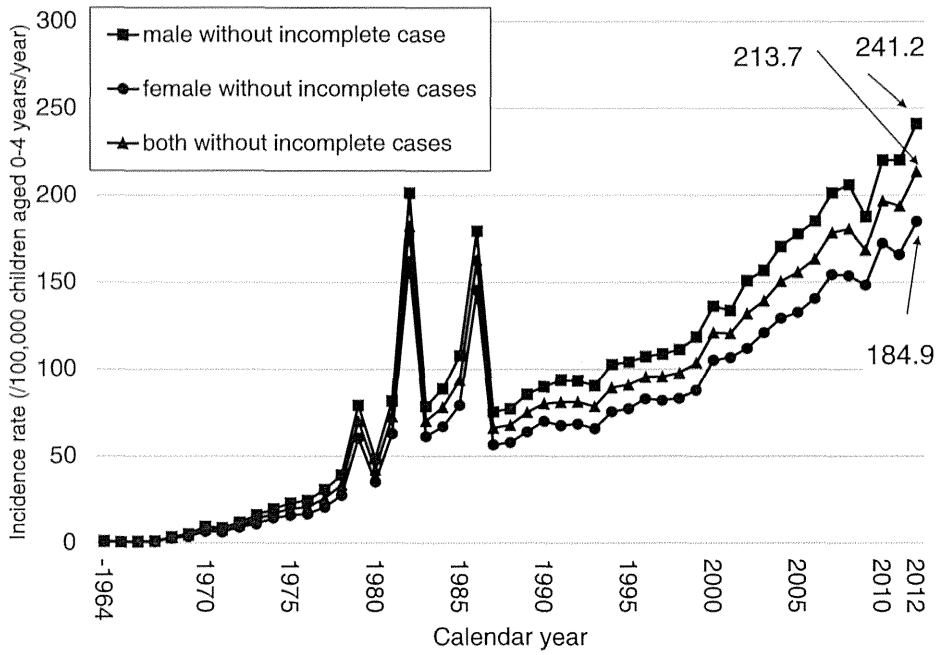

Figure 3. Incidence rate of complete Kawasaki disease in Japan, by calendar year and sex

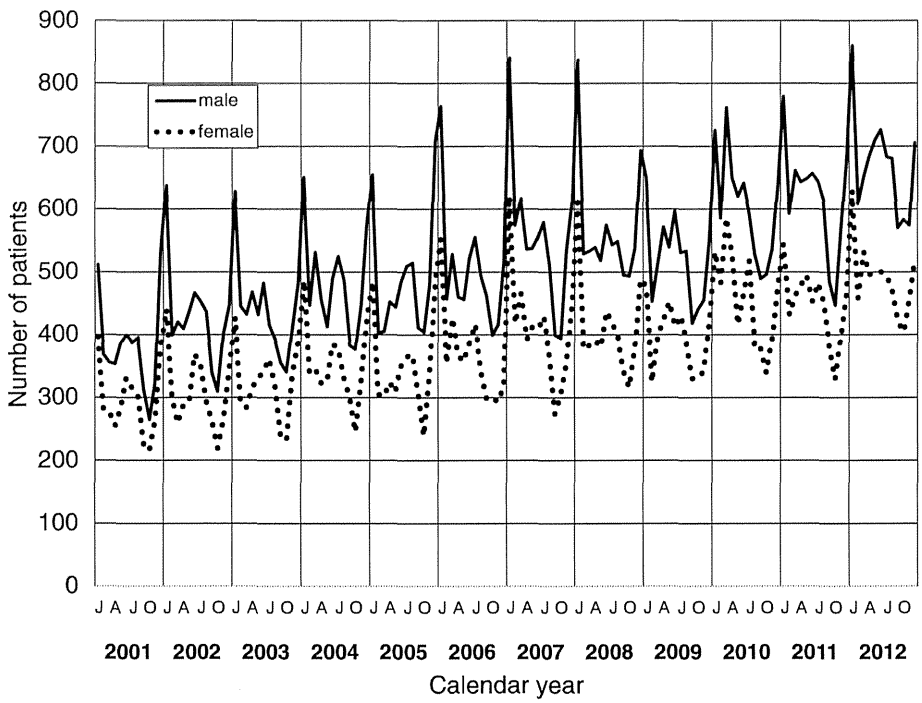

Figure 4. Number of patients with Kawasaki disease in Japan from 2001 through 2012, by month and sex

lesions as some patients had more than one of these symptoms). A total of 754 patients (2.8%) had cardiac sequelae 1 month after the onset of KD: 47 (0.18%) had giant coronary aneurysms, 191 (0.72%) had coronary aneurysms less than 8 mm in diameter, 466 (1.8%) had coronary dilatations, 6 (0.02%) had coronary stenoses, 1 (0.004%) had a myocardial infarction, and 98 (0.37%) had valvular lesions (the sum of percentages exceeds 100% as some patients underwent more than one treatment type). Furthermore, cardiac abnormalities were more prevalent in males (878 at their first visit, 1768 during the acute phase, and 571 with sequelae) than in females (403 at their first visit, 838 during the acute phase, and 238 with sequelae).

Of all patients, 24 346 (91.2%) received IVIG therapy. Of these, 20 954 (86.1%) underwent IVIG therapy in the 6 days after the onset of disease and 1279 (5.3%) received steroid

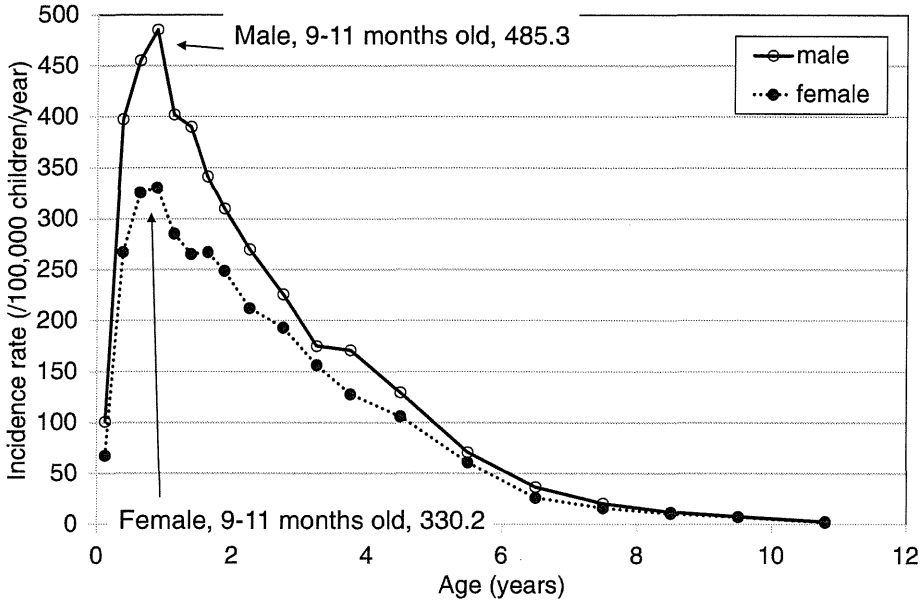

Figure 5. Age-specific incidence rate of Kawasaki disease from 2011 through 2012, by sex

therapy with IVIG therapy. However, 4150 (17.0%) of the 24 346 patients treated with IVIG did not respond to treatment (ie, fever lasted despite treatment), and 3798 of these (91.5% of non-responders) received additional IVIG therapy: 1245 (30.0%) with steroids, 179 (4.31%) with infliximab, 155 (3.73%) with immunosuppressants, and 93 (2.24%) with plasmapheresis (the sum of percentages exceeds 100% as some patients underwent more than one treatment type).

Upon analysis of the distribution of leukocyte levels by age at the first visit, the proportion of patients showing values greater than 10 000/μL tended to increase with age. In addition, the proportion of patients with platelet counts lower than 250 000/μL at first visit tended to increase with age, and those with counts higher than 400 000/μL tended to decrease with age. Regarding C-reactive protein levels by age at the first visit, the proportion of patients with levels in the range of 1–5 mg/dL and 5–10 mg/dL tended to decrease with age, while those showing a level higher than 15 mg/dL tended to increase with age. For these three factors and albumin level, there were no apparent differences observed between sexes.

DISCUSSION

We have described the most recent epidemiological features of KD using our analysis of the results of the 22nd nationwide survey of KD in Japan. Since 1970, nationwide surveys have been carried out almost every 2 years.[1,14–19] The number of patients and the incidence rate of this disease have increased rapidly since the mid-1990s. Due to the declining birth rate in Japan, the number of patients has increased less rapidly than the incidence rate of patients. However, the annual incidence rates in 2011 and 2012 were higher than those for years of nationwide epidemics (1979, 1982, and 1986), reaching 264.8 in 2012. The etiology of KD is unknown, and therefore, the reasons for these increases are also unclear. However these findings are very important, and attention should be paid to them. We need continued observation of the ongoing epidemiological features of KD in Japan.

After two reminders were sent, the response rate of the survey was 71.6%. Therefore, the actual number of patients with KD might be higher than that described by this study. Survey response rates have remained at approximately 70% over the past 10 years. In addition, we must remember that using the national population to calculate the incidence may result in underestimation of the true incidence, since patients may have received treatment in medical settings other than the 'target hospitals', such as small clinics not covered by the studied facilities. However, data suggest that the actual number of cases is likely to be within 10% of the value calculated from the survey, as discussed in a previous report.[20]

The seasonal epidemiologic features of KD in Japan observed in the latest survey are very similar to those seen before 2008 (Figure 4 and previous report[15]). In 2009, a new influenza strain (H1N1) was prevalent worldwide, including in Japan.[21] It is possible that the influenza epidemic may have led to changes in the pattern of KD in 2009 and 2010, as the patterns observed were specific to these years. We compared the incidence rate of total cases and complete cases and found that the increase in total cases was due to the increase in complete cases in recent years.

One of the most serious problems in KD is cardiac sequelae, including coronary aneurysms, myocardial infarctions, and giant coronary aneurysms. Fortunately, the proportion of patients with coronary aneurysms and myocardial infarctions has decreased, from 6.0% for the 16th nationwide survey (1999–2000)[11] to 2.8% for this

survey. Furthermore, the proportion of patients with giant coronary artery aneurysms, the most serious sequela associated with KD, was also reduced substantially (0.40% in the 16th survey vs 0.18% in this survey). Advances in patient management during the acute phase are likely to have contributed to this improvement. Of the long-term outcomes of KD, the mortality rate with cardiac sequelae due to KD is the most important factor in KD management.[22]

A major feature of KD is seasonal variation in the number of patients affected. In Japan there is a maximum peak in January and a smaller peak in summer. The presence of seasonal variation supports the hypothesis that this disease is caused by an infectious agent. If KD is stimulated by several infectious agents of varying incidence in the peak season, there is the possibility that a number of different peaks are generated per year. In addition, the seasonality of KD differs by country and region,[23,24] which might be due to the presence of different causative agents among these countries/areas. No geographic movement of KD was evident in data from the most recent nationwide survey. However, epidemiologic data from epidemic years (1979, 1982, and 1986) also supports the infectious agent hypothesis.[19] On the other hand, increased likelihood of KD between parents and childen[25] and siblings[26] indicates a genetic cause. Therefore, we hypothesize that the onset of KD is involved with factors on the host side and infectious agents.

In conclusion, we have reported the result of the 22nd nationwide survey of KD in Japan. The number of patients and the incidence rate of KD continued to increase in 2011–2012. The incidence rate of complete KD also continued to increase in this period.

ONLINE ONLY MATERIAL

Abstract in Japanese.

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Conflicts of interest: None declared.

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Tropospheric winds from northeastern China carry the etiologic agent of Kawasaki disease from its source to Japan

Xavier Rodó[a,b,1], Roger Curcoll[b], Marguerite Robinson[b], Joan Ballester[b,c], Jane C. Burns[d], Daniel R. Cayan[e,f], W. Ian Lipkin[g], Brent L. Williams[g], Mara Couto-Rodriguez[g], Yosikazu Nakamura[h], Ritei Uehara[h], Hiroshi Tanimoto[i], and Josep-Anton Morgui[b]

[a]Institució Catalana de Recerca i Estudis Avançats, 08010 Barcelona, Catalonia, Spain; [b]Unitat de Dinàmica i Impacte Climàtic (UDIC), Institut Català de Ciències del Clima, 08005 Barcelona, Catalonia, Spain; [c]Geological and Planetary Sciences, California Institute of Technology, Pasadena, CA 91125; [d]Department of Pediatrics, Rady Children's Hospital-San Diego and University of California, San Diego, La Jolla, CA 92093; [e]Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA 92037; [f]US Geological Survey, La Jolla, CA 92037; [g]Center for Infection and Immunity, Mailman School of Public Health of Columbia University, New York, NY 10032; [h]Department of Public Health, Jichi Medical Hospital, Togichi 108-8639, Japan; and [i]Center for Global Environmental Research, National Institute for Environmental Studies, Tsukuba 305-8506, Japan

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Evidence indicates that the densely cultivated region of northeastern China acts as a source for the wind-borne agent of Kawasaki disease (KD). KD is an acute, coronary artery vasculitis of young children, and still a medical mystery after more than 40 y. We used residence times from simulations with the flexible particle dispersion model to pinpoint the source region for KD. Simulations were generated from locations spanning Japan from days with either high or low KD incidence. The postepidemic interval (1987–2010) and the extreme epidemics (1979, 1982, and 1986) pointed to the same source region. Results suggest a very short incubation period (<24 h) from exposure, thus making an infectious agent unlikely. Sampling campaigns over Japan during the KD season detected major differences in the microbiota of the tropospheric aerosols compared with ground aerosols, with the unexpected finding of the *Candida* species as the dominant fungus from aloft samples (54% of all fungal strains). These results, consistent with the *Candida* animal model for KD, provide support for the concept and feasibility of a windborne pathogen. A fungal toxin could be pursued as a possible etiologic agent of KD, consistent with an agricultural source, a short incubation time and synchronized outbreaks. Our study suggests that the causative agent of KD is a preformed toxin or environmental agent rather than an organism requiring replication. We propose a new paradigm whereby an idiosyncratic immune response, influenced by host genetics triggered by an environmental exposure carried on winds, results in the clinical syndrome known as acute KD.

northeastern China source | agriculture | heart disease | FLEXPART | cereal croplands

After more than four decades of intensive research on Kawasaki disease (KD) (1, 2), no agreement regarding its cause has yet emerged (3). In fact, it is not even clear whether KD should be considered an infectious disease or an abnormal host response in genetically susceptible children to one or more noninfectious environmental triggers (4). Although progress has been made in defining the genetic influence on KD susceptibility and disease outcome, the complex genetic variants that are responsible have only partially been identified. Based on both the clinical presentation and histopathology of tissues, it has been postulated that the trigger enters through the mucosa of the upper respiratory tract (5). A recent study demonstrated a role for both regional winds and large-scale atmospheric circulation in transporting an etiologic agent responsible for the seasonal and nonseasonal anomalous variation in the number of KD patients in both Japan and the United States (6). Certain patterns of winds in the troposphere above the earth's surface flowing from Asia were associated with the times of the annual peak in KD cases and with days having anomalously high numbers of KD patients. It was demonstrated that these regional wind patterns first impact Japan and, when large-scale conditions are favorable, extend across the North Pacific to the West Coast of the United States (6, 7). In advance of peak KD occurrences, a regional pattern with decreased atmospheric pressure forms over Japan that pumps winds to the archipelago (Fig. S1). Further, this same study (7) demonstrated capabilities of predicting anomalous KD activity in Japan and in the West Coast of the United States with time leads of a few months, based on coupled ocean and atmosphere processes developing in regions of the tropical and north Pacific oceans. However, the nature of the etiologic agent, the source region from which the putative KD agent is lofted into the atmosphere, and the incubation time between exposure and onset of fever remained unknown. Previous studies of KD dynamics suggested a potential source region in northeastern Asia (6, 7). In the present study, we accurately define this region, with the sharpened temporal resolution afforded by the best record of KD available worldwide, that of daily case occurrences in individual prefectures in Japan. The exposure mechanism and incubation time are addressed using two different approaches: an air parcel trajectory analysis of the time taken by wind to travel from the inferred source region and

Significance

Kawasaki disease (KD), the leading cause of acquired heart disease in children worldwide, has remained a mystery for more than 40 y. No etiological agent has yet been identified. By using simulations with the flexible particle dispersion model from different Japanese cities from each single high (low) KD incidence day, the source region KD is retrieved in cereal croplands in northeastern China. We infer the incubation time for KD ranges from 6 h to 2 d, thus favoring an antigenic or toxic exposure as the trigger. *Candida* sp. is reported as the dominant fungal species collected aloft (54% of all fungal DNA clones) demonstrating the potential for human disease in aerosols transported by wind currents traveling long distances.

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[1]To whom correspondence should be addressed. E-mail: xavier.rodo@ic3.cat.

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an analysis of the population dynamics of the disease using a simple ordinary differential equation model. Finally, toward a detailed understanding of KD etiology, we also investigate possible KD agent(s) by means of direct airborne sampling conducted over Japan, followed by a detailed analysis of nucleic acids extracted from the aerosolized atmospheric samples trapped on filters collected at selected altitudes during the 2011 KD season.

Results

Inference of the Source Region. A core element in addressing these questions is the high-resolution daily KD case record for the 47 Japanese prefectures (8). The Japanese Epidemiologic Surveillance System for KD provided the KD onset dates for all cases during the interval 1970–2010 (*Data and Methods*; see also the clinical definition of KD cases in ref. 6). Dates contained solely within the three KD epidemics in Japan in 1979, 1982 and 1986 were selected for the first analysis, to capitalize on the larger signal-to-noise ratios due to large bursts of KD cases that occurred during those epidemics. For each epidemic, days having a number of cases at least equal to the 95% value of the distribution of KD cases within that epidemic were selected as peak (p95) days. Similarly, for assessing possible differences with the rising phase in each epidemic (*Data and Methods*), days above the 70th percentile of KD cases within each epidemic that were also separated in time at least 12 d before the identified sequence of p95 d, were selected as rising (p70) KD days. We then generated ensembles of backward (in time) air parcel trajectories with the flexible particle dispersion model (FLEXPART) Version 8.23 (9) at a sequence of 3-h intervals before each of these peak and rising dates. An ensemble of such trajectories was generated for each of a number of selected locations in Japan (*Data and Methods*). Each backward trajectory traced the geographical and vertical location of an air parcel in its upstream transit 10 d before it crossed over the selected location. Within the air parcel trajectories associated with peak KD events, we postulated that there is a common precursory source region where the air preferentially resided (higher-residence time regions) that should show up in the analyses. It is in these high-residence time regions that there is the greatest chance that the potential agent would be lofted from the land surface into the air mass; this air parcel would then be subsequently borne via the wind currents to Japan. Therefore, we searched the set of peak KD-associated back trajectories to identify common upstream regions having maximal residence times within the swarm of trajectories at all times in a season when both abnormal and extreme numbers of KD cases occurred in Japan.

Fig. 1 displays the largest residence times over land (>95%) inferred from simulations for the composite average of all peak days within the three epidemics for Tokyo, Japan's most populated city. Potential source areas associated with epidemic peak events (orange areas in Fig. 1 denote grid points with largest residence times; see *SI Data and Methods* for details) reproducibly pointed to the same constrained region, between northeastern China and southwestern Russia, as the most likely source region for the KD agent. The remarkable stability in the location of the overlapping region obtained at all lag times analyzed strongly supports the hypothesis that the northeastern winds pick up the etiological agent of epidemic KD in that region. Furthermore, when simulations were performed including all peak days calculated from the period excluding the epidemic years (hereafter p95 for the whole 1987–2010 interval), the same area over land with maximum residence times was identified, indicating the historical stability of the inferred results (Fig. S2). Both the high resolution and calibration of the reanalysis used to run the FLEXPART model, the stability of the region obtained when using different extreme percentiles for KD (ranging from 1% to 5%), and the fact that the simulations are obtained in a hindcast mode, adds strong confidence to the inferred region. Because epidemics occurred in different months (March to May in 1979, May 1982, and March 1986) (6), the comparison was made between the residence times of the upstream wind trajectories associated with epidemic peaks and others similarly derived from trajectories for nonepidemic peaks, the latter therefore outside of the epidemic months but contained within the winter calendar months (October–March, Fig. S2). Confining the sampling to these calendar months and comparing the two situations effectively removed the potentially confounding effects of seasonality in air mass exposure from either the same or different months.

In an attempt to assess whether there were differences in regions where the air resided preferentially during the initial stages of the epidemics (rising, green in Fig. S3*A*) in comparison with those at the peak of the epidemic (peak, blue in Fig. S3*A*), we compared residence times from ensembles of similar backward simulations for the two situations. Results therein clearly indicate that the southernmost regions in NE and East China, from Fig. 1, also contribute during the rising stages compared with the more northern areas in northeastern China, which dominate during the epidemic peaks indicating that most of the etiologic agent load must come from this region. When the temporal difference between the two situations, rising and peak phases are maximized (e.g., for ensembles at lag 15 d), the source regions strongly differ further clarifying this separation (Fig. S3*B*).

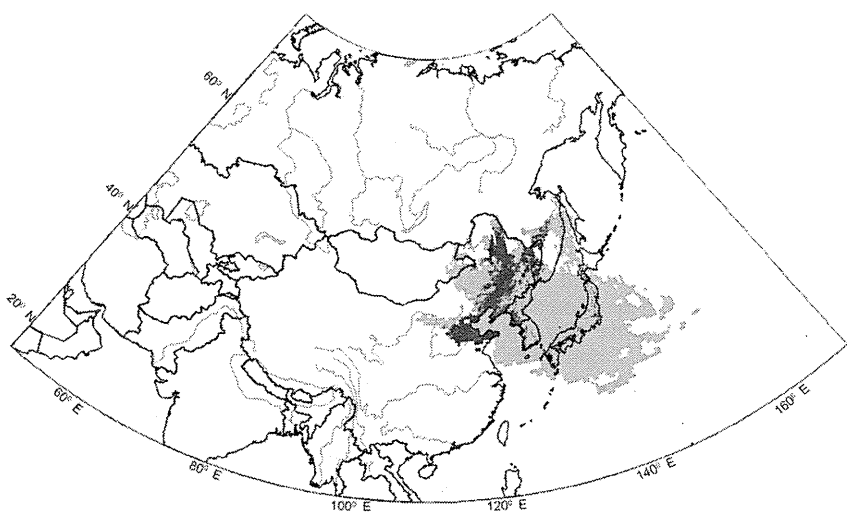

Fig. 1. Upstream grid cell locations registering residence times over 30 s for the ensemble of FLEXPART 10-d backward simulations (light brown) for dates within the three epidemics (1979, 1982, and 1986) when KD cases were at or above the 95% threshold of cases (threshold calculated for the entire timespan, 1977–2010). The ensemble represents a total of 257 dates. A 0.5° grid scale was used (latitude, longitude). Brown dots denote crops according to the land cover type yearly climate modeling grid (CMG) datasets with 0.05° resolution from the NASA Land Processes Distributed Active Archive Center (LP DAAC, Sioux Falls, SD), ASTER L1B (32). Grid cells with dots have at least 50% or more subgrids as crops or 100% subgrids as mosaic (mosaic representing crops + natural vegetation).

The large number of simulated days involved provides consistency to these results (902 in total, corresponding to KD days in Fig. 1 and in Figs. S2–S4).

Following the assertion that the KD agent is carried by the wind, it is expected that cases would occur simultaneously in neighboring locations because wind patterns operate over regional scales. In fact, for the largest KD epidemics shown in Fig. 2 (1982; 1986 in Fig. S4), a striking covariation was seen in the daily number of cases in the two major cities in Japan (Tokyo and Yokohama, 28 km apart). Such covariation between the anomalous KD occurrences in these same two prefectures and between Tokyo and Saitama also during the nonepidemic period from 1987 to 2010 is shown in Fig. S5. Delving further, a scale-dependent correlation (SDC) (10, 11) analysis was used to investigate possible commonalities in KD behavior over short time scales (only a few days) among the most populated cities of the Tokyo metropolitan area (Greater Tokyo, comprising Tokyo, Yokohama, Saitama, and Chiba; *SI Data and Methods* and Table S1). SDC analysis revealed surprisingly high in-phase correlations during epidemics occurring in the same year (see also Table S1). Results demonstrate that maximum numbers of KD cases occurred in these two cities on exactly the same days [Monte Carlo randomization test (MCR), $P < 0.001$]. Applying the same analysis to all major cities in the Tokyo metropolitan area resulted in similar temporal coherence. Table S1 reports the results of two-way SDC analyses among Tokyo, Kanagawa, Saitama, and Chiba, showing that in all city pairwise comparisons, maximum correlations consistently occurred at zero lag times, attaining correlation values between 0.56 and 0.86 (MCR, $0.04 < P < 0.000$). The total variability accounted for by the daily covariation among cases between cities is indeed remarkable (on average above 50%, in a range between 31% and 75%), as it represents a portion of variability not fully resolved in the seasonal–interannual scales addressed in previous studies (6, 7). The present analysis reveals that the two cities had their KD cases fully synchronized for a period of at least 3 wk during the 1982 epidemic. Furthermore, results from an SDC analysis of wind and Greater Tokyo KD fluctuations attained values above 0.6 (MCR, $P < 0.05$) during the same intervals and with maxima around lags of 2–3 d, indicating that this is the approximate time between the agent leaving the source, traveling for 2 d, arriving in Japan, and rapidly causing the onset of fever in a KD patient. Previous studies of KD examined temporal scales ranging from seasonality to the interannual variability (6, 7), whereas here we focus on the daily variation. In the present analysis, fluctuations in daily KD cases are shown to be coherently associated with regional winds and their previous upstream history, an unprecedented result even compared with other well-known infectious diseases (12). The fact that exactly the same dates are associated with maximal numbers of KD cases in different locations and that the peak in KD cases is rapid after the arrival of the wind suggests that there is a short incubation time for KD.

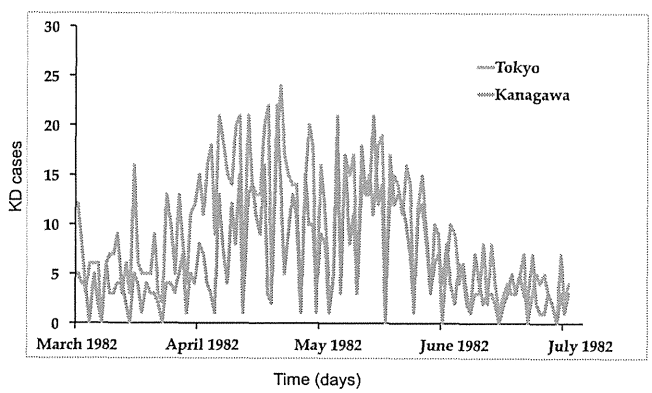

Fig. 2. Daily time series of KD date of onset for patients in Tokyo (blue) and Kanagawa (red) during the epidemics of 1982 (see Fig. S4 for 1986). Axes display cases (Y) and day since epidemic onset (X). See SDC (11) analysis between the KD datasets for Tokyo and Kanagawa, as well as for all cities in the Greater Tokyo Area in *Data and Methods* and Table S1. Maximum overall correlation is attained in this 1982 epidemic, with over 75% of the total KD variability synchronized between Tokyo and Kanagawa during at least 3 wk.

Incubation Time for KD. To obtain an independent estimate of the incubation time (here time between exposure to the presumed agent and the onset of fever) from that suggested by the SDC analysis, an experiment was conducted using the ensemble of 10-d backward FLEXPART simulations for the 532 d cataloged as high KD occurrences (Movie S1). For comparison, we ran a second experiment with the ensemble of FLEXPART simulations for all 3,206 d with low KD occurrences (having 0 to 1 cases per day) in the postepidemic interval (1987–2010; Fig. S6*A*). As for Movie S1, average residence times for simulations shown in Fig. S6*B* mark the time between the moment a particle leaves the source area and the onset of fever in patients to be in the range of 6 h to 2.5 d (see *SI Data and Methods* for details). This value matches the predicted incubation time inferred from the SDC analysis above to likely be less than 1 d, beyond the 2-d time necessary for the wind to traverse the distance from northeastern China to Japan. Although the confidence interval extends this incubation time to around 2 d more, this longer duration is less likely, and therefore these results imply that there is rapid host response following exposure to the KD agent. Similar results were obtained when days were selected for either Tokyo or the composite of cities in the entire Greater Tokyo Area (Tokyo, Kanagawa, Saitama, and Chiba, which includes over 35 million people).

Apart from the three main KD epidemics in Japan, there are seasonal peaks and troughs in disease occurrence (8). We therefore investigated coincidence in potential source regions for KD during periods out of epidemics, and conducted backward trajectory analyses also for high KD activity after the last big epidemic (in the postepidemic interval, 1987–2010). In this case, dates were selected with individual days displaying high (≥95%) numbers of KD cases (around 2 SDs above the mean of the normalized distribution) in locations spanning Japan from north to south (Sapporo, Tokyo, and Nagoya). We used data for the entire 1970–2010 records (epidemic values were included also but only used to set the threshold for the selection of high KD days) and compared with days when KD occurrences were unusually low (days displaying zero KD cases having no high KD event in the preceding 10 d; *SI Data and Methods*). To properly characterize the time lag between an air parcel leaving the presumed source region and having an influence on KD in the selected location, the air parcel crossing times were varied from the same day to 1 mo in advance of the date associated with the peak in KD cases. We allowed such a variety of lag times to give equal chance to any value in the range of the suspected lag times for the potential KD incubation time, both according to previous hypotheses and known times for airway diseases (13–16). Average residence times were therefore computed for high and low KD dates and areas with residence times greater than the threshold for a high-residence time of 30 s in the preliminary high–low maps retained for further analyses (this threshold corresponds to 95% of the average simulated residence time; *Data and Methods*). Fig. 3*A* shows the average residence time map composited for days 1–3 to conform to results obtained above from the trajectory mapping and the SDC analysis of the KD epidemics. Furthermore, we sought for residence times maxima within the nonepidemic trajectories between 1 d and 1 mo, but, similar to the epidemic trajectory results, maximum residence times were centered around 1–2 d. Residence times were retrieved at each grid point for each of the three Japanese locations, namely Sapporo (green), Tokyo (blue), and Nagoya (yellow). Red areas in Fig. 3*A* indicate the overlap between

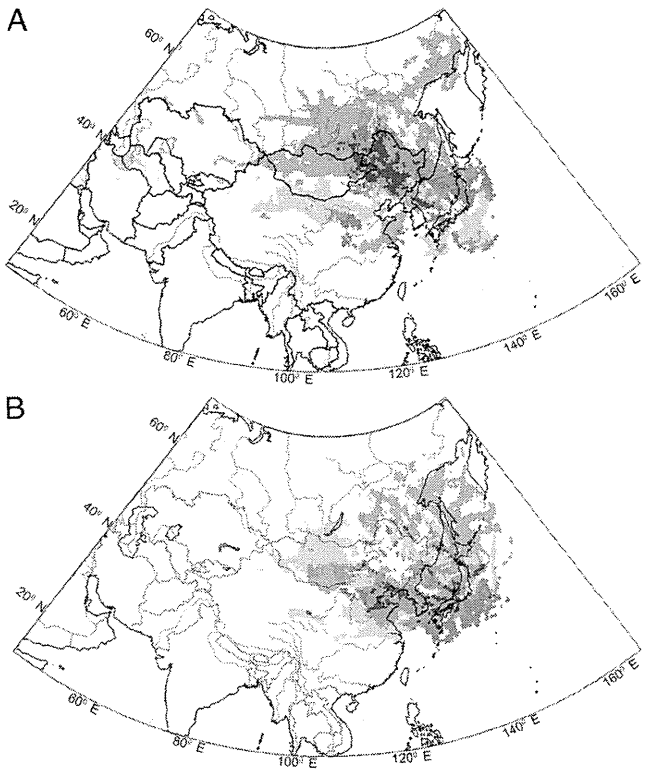

Fig. 3. Areas of (*A*) high–low or (*B*) low–high differences in the residence time between sets of cases with high KD and low KD occurrences in the postepidemic interval. Residence time differences higher than 30 s are shown for Tokyo (blue), Sapporo (green), and Nagoya (yellow). The ensembles included have been generated averaging residence time data corresponding to lags of 1–3 d, which appears to capture all of the potential range of values for the KD incubation time. The total number of dates used in the simulations was 1,344. Red areas indicate overlapping high-residence time differences from two cities (light) or three cities (dark) residence time anomaly areas.

plumes of ensemble FLEXPART simulations for two cities (light) or three cities (dark). These areas largely coincide with the ones identified in Fig. 1 and Fig. S2 in northeastern China that were associated with the source for the three KD epidemics in Japan. Remarkably, other smaller areas highlighted by the same analysis were associated with positive high–low residence times at lag times from 10 to 30 d but were instead residual and not coherently positioned in the spatial analysis when percentiles and intervals for data selection were varied. Therefore, striking similarities exist between Figs. 1 and 3 that identify the same region as being linked to high KD case numbers in and out of epidemic periods.

As a comparison, a similar analysis to determine upstream regions with the high-residence time from trajectories associated with anomalously low numbers of KD cases among the three cities was conducted. In contrast to the preferred upstream regions associated with peak KD events, the high-residence time regions associated with low KD incidence appear to lie mostly in the sea between China and Japan (Fig. 3*B*). Importantly, this observation demonstrates that there are other preferential air mass sources that supply wind flow when KD occurrence is low in Japan, and reinforces our conclusion that there is a KD source region over northeastern China. Also, any local influence on KD is at most small compared with the northeastern China region. This result underscores the need to identify possible KD agent(s) in land regions and appears to eliminate the possibility of a sea-related origin.

A surprisingly short interval between exposure and onset of fever is indicated by the trajectory analysis. In searching for candidate KD agents, such a short interval makes many infectious agents unlikely, which leads us to consider that the trigger may be a plant or microbial toxin or an inhaled antigen that would, therefore, not replicate inside the host. Under this model, a dose-dependent reaction may account for variance in disease severity among patients. As an alternative hypothesis, we explored the relationship of daily time series of atmospheric pollutants and pollen species with KD cases in Tokyo. Time series for SO_2, O_X, NO, NO_2, NO_X, CO, and NMHC from three stations in the Tokyo Metropolitan area and pollen counts measured in Tokyo were compared with local wind and KD records, but no consistent relationship emerged. Neither seasonality nor trends in emissions of atmospheric mercury were consistent either with fluctuations in KD case occurrences (*SI Data and Methods*).

To investigate whether person-to-person spread of an infectious agent could fit the observed data, a simple susceptible–exposed–infected–recovered (SEIR) population disease model was built (*SI Data and Methods*). Results show that not even an extremely rapidly replicating infectious disease could propagate that quickly (even diseases with idealized incubation times of only 2 h, e.g., 10 times shorter than the fastest respiratory viruses known, e.g., influenza B and rhinovirus) (15, 16). No known infectious agent would be able to produce synchronous (same day) infections over distances between cities, such as those in the Tokyo metropolitan area on the basis of secondary infections only (*SI Data and Methods* and Fig. S7). In fact, at least 5.6 d would be needed for an infectious agent with only a 2-h incubation time and with the current city populations in the Greater Tokyo metropolitan area to cover the distance between any two cities using only secondary infections, even if there were up to 1% asymptomatic individuals in the population (Fig. 4). In this experiment, children younger than 6 mo were considered not susceptible due to protection from maternal antibodies (17). Indeed, the rarity of KD in very young infants suggests that there may be the acquisition of a transplacental antibody that is protective (18). The possibility that a known infectious agent is responsible for these dynamics is therefore remote. Instead, an immediate response in the form of an idiosyncratic immune reaction in genetically susceptible children that takes place within 24 h after inhalation of the etiologic trigger is further reinforced by our results.

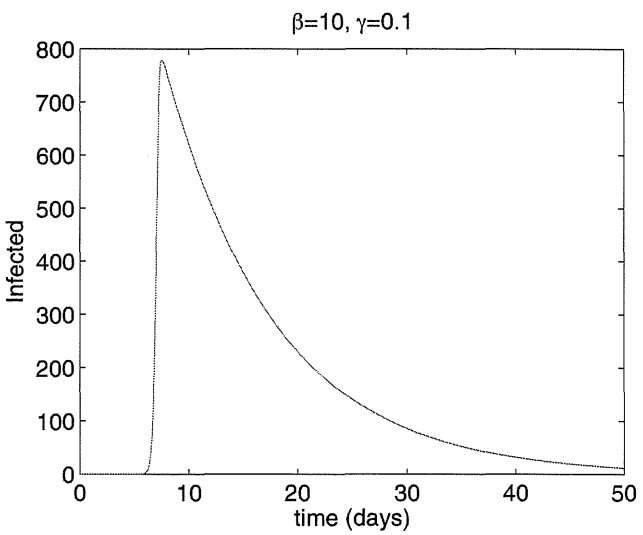

Fig. 4. Epidemic evolution 30 km from the initial site of infection, for $\beta = 10\ d^{-1}$ and $\gamma = 0.1\ d^{-1}$. The maximum number of infected denotes the time at which the disease reaches the next town (on average after 5.66 d).

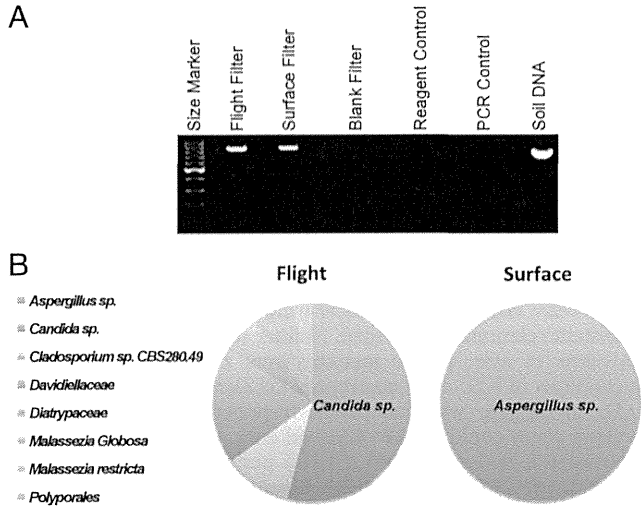

Fig. 5. Differences in the mycobiome distribution from tropospheric and surface-level aerosols. (*A*) Fungal 18S rRNA gene PCR demonstrates amplification products in flight and surface filters. DNA extracted from soil was used as a positive control for fungal amplification. (*B*) Pie charts demonstrating the percent abundance of fungal taxa identified from clone library sequencing (100 clones per filter) of 18S rRNA gene amplification products from flight and surface filters. *Candida* sp. sequences accounted for 54% of total flight filter sequences, whereas *Aspergillus* sp. sequences accounted for 100% of surface filter sequences.

Tropospheric Microbiome Campaign. To investigate the microbial diversity of aerosols carried by these tropospheric winds, an air sampling campaign was conducted during a period of high KD activity in the first week of March 2011 over Japan, and aerosol samples were collected at heights between 2,000 and 3,000 m only. The research aircraft flew in a northwesterly direction into the winds toward northeastern China (see Fig. S8 and *SI Data and Methods* for an explanation of the aircraft campaigns conditions). Flight direction was designed to optimally filter air far from any surface contamination and on selected days when the air came exclusively from the region suspected as a source for the KD agent (compare Fig. 1 with Fig. S8). Major differences in the mycobiome of the tropospheric aerosols were detected compared with ground aerosols with the intriguing finding of *Candida* species as the dominant fungus aloft (Fig. 5). Filters collected on the same day of the flight included the collection of aerosols at ground level (Fig. 5 *A* and *B*, surface filter) and a negative control filter placed in the air sampling device and removed without filtering air (Fig. 5*A*, blank filter). Quartz filters were handled in a sterile manner to avoid contamination with extraneous nucleic acid and shipped on dry ice for analysis. Methods for profiling microbes were optimized using nucleic acids extracted from the filters. Approximately 2 ng of DNA were obtained from one-fourth of a filter and used as template in 16S rRNA and 18S rRNA gene-specific PCR amplification to identify bacterial and fungal signatures, respectively. The 16S rRNA products were detected only inconsistently on flight and surface filters. In contrast, 18S rRNA gene products were amplified in both the flight filter sample (Fig. 5 *A* and *B*, flight filter) and the surface filter sample, whereas the unsampled filter (blank filter), extraction reagents, and PCR controls were negative (Fig. 5*A*). Differences in the consistency of bacterial vs. fungal signal in these assays may be a consequence of differences in the size of fungal spores (1–40 μm) vs. bacterial cells (1–5 μm) that influences the distribution of fungi and bacteria in the troposphere or entrapment in the filter matrix.

Large differences in the distribution of fungi amplified with primers for the 18s rRNA gene were obtained from the surface and flight filters based on the sequencing of 100 clones containing PCR product from each filter. Whereas *Candida* species sequences were abundant in the flight filter sample, accounting for 54% of all fungal sequences, only *Aspergillus* species sequences were identified from the surface filter sample (Fig. 5*B*). Although the 723-nt *Candida* sequence matched several different species with only a single mismatch and 99% identity, including *Candida tropicalis*, we were unable to pursue further species discrimination due to the limited yield of nucleic acid from the filter. Despite the abundance of marine and agricultural soil *Candida* species,[†] to our knowledge, *Candida* has not been previously reported from surveys of microbes collected from aircraft sampling over Asia, the Mediterranean, or the Caribbean (19, 20). The presence of *Candida* sequences is intriguing in light of murine models of coronary arteritis based on exposure to *Candida* glucans comprising mannose, β-1,3-glucan and β-1,6-glucan. Vulnerability to disease varies by mouse strain and is associated with elevated levels of IL-6, TNF-α, and IFN-γ (21, 22). Similar to KD, vasculitis can be abrogated by use of i.v. Ig (23) and immunosuppressive drugs including the imidazole nucleoside mizoribine (24) and the TNF-α antagonist etanercept (25). However, the finding of *Candida* sp. aloft can only be reported here as providing support for the concept and feasibility of a windborne pathogen rather than implicating any particular organism.

Discussion

A closer inspection of the geographic area identified by the FLEXPART air parcel trajectory residence times from both nonepidemic intervals and large epidemics of KD in Japan reveals a strikingly uniform source landscape with intensively cultivated croplands and farms. Cropland coverage is denoted by dark dots in Fig. 1 (also Figs. S2 and S3). In contrast to the preferred upstream regions associated with peak KD events, the high-residence time regions associated with low KD incidence appear to lie mostly in the sea between China and Japan (Fig. 3*B*). The principle crops in the region uncovered include cereals such as corn, rice, and spring wheat in a region known to be the main grain area of China (26) [Fig. 1, with brown dots in denoting at least 50% total coverage by crops according to the land cover type yearly climate modeling grid (CMG) datasets with 0.05° resolution from the NASA Land Processes Distributed Active Archive Center (LP DAAC).]. Among other crops, the Heilongjiang province is the principal corn-growing region and accounts for over 29% of total Chinese corn grain production (27). Given the clear link to croplands/agricultural soils indicated by a variety of our results at a time and in a region where the ground is frozen throughout the high KD season, a whole range of new possibilities emerges regarding the nature of the KD etiological agent. Possibilities include environmental toxins linked to crops or to plant decay by-products (e.g., bacterial/fungal toxins), as well as a combination of the former with agricultural practices, atmospheric chemistry, and an idiosyncratic immune reaction in genetically susceptible children (28, 29). The lack of an industrial signature in the inferred region and the lack of correlation with pollutant levels in the winds associated with increased KD activity argue against a chemical pollutant as the relevant causative agent. A new calculation of the impact of KD in northeastern China when the population density is taken into account might also point to an underestimation of the impact of this disease in the region (*SI Data and Methods*).

Many crop species harbor fungal spores and inhalation of spore-associated mycotoxins is a common route of exposure. Fungal toxins (mycotoxins) are a diverse group of molecules that are difficult to classify because they are synthesized through diverse biochemical pathways and cause a diverse array of

[†]Kang H-Y, Ryu Y-W, Xylitol production by flocculating yeast, *Candida* sp. HY200. 25th Symposium on Biotechnology for fuels and chemicals, National Renewable Energy Laboratory, May 4–7, 2003, Breckenridge, CO, poster presentation 2-23.

symptoms in humans with the liver, kidneys, and brain being prominent targets. What mycotoxins share is a rapid onset of symptoms following exposure (30). Some notable examples of crop-associated mycotoxins are the aflatoxins, difuranocoumarin derivatives synthesized by a polyketide pathway by many strains of *Aspergillus*, the citrinins produced by *Penicillium* and *Aspergillus* species, the fumonisins produced by *Fusarium* species, and the ochratoxins produced by various *Aspergillus* species. None of the known mycotoxins, however, produce a vasculitis similar to the clinical picture in KD.

Results in this study on source regions and incubation times, as well as detailed analysis of nucleic acids in atmospheric samples provide important clues regarding both the potential source regions and the nature of the etiologic agent for KD in Japan. Avenues for future research should include detailed characterization of the organic and inorganic compounds and microbiome of tropospheric winds collected aloft over northeastern China and Japan during the annual peak in disease activity in Japan. These air sampling campaigns will need to be further expanded in the future in close connection with a simultaneous detailed surveillance of the occurrence of KD in Japan to further explore the role that microorganisms or their toxins may play in triggering KD. Study of the transport and circulation patterns for air masses before peaks and troughs in KD activity in other regions across the globe with substantial numbers of KD cases may further inform on their sources and the role that tropospheric winds and their chemistry play in the transport of the KD agent. Finally, future studies will be needed to directly test the capacity for microbes, antigens, or toxins contained in those aerosol samples to elicit a humoral and cellular immune response in KD patients.

Data and Methods

KD Datasets. Approved written consent was obtained from all participants. Human population epidemiology studies were approved by the Bioethics Committee for Epidemiologic Research, Jichi Medical University. Data used in this study for Japan are total daily counts of KD patients admitted to hospitals for each of the 47 prefectures of Japan. To account for possible effects of different population sizes in cities, incidence was also calculated for comparison in the case of major Japanese cities from population censuses interpolated from 10-y national demographic records and only small differences emerged with results obtained for cases. KD cases were then weighed against the total pediatric population in that prefecture, with data covering the interval 1977–2010 (see KD case ascertainment protocol for Japan sites) (6). KD dates used in this study were in all case onset dates. The Japanese datasets were derived from 21 separate questionnaire surveys of hospitals in Japan spanning the period 1970–2010. For subjects with multiple admissions, only the first hospitalization date was used.

Backtrajectory Ensemble Simulations. Trajectories traced back in time 10 d for each dataset and location were generated using the FLEXPART Lagrangian particle dispersion model Version 8.23 in backward mode. FLEXPART, a Lagrangian model suitable for the simulation of a large range of atmospheric transport processes (9), was originally designed for calculating the long-range and mesoscale dispersion of air pollutants from point sources but has evolved into a comprehensive tool for atmospheric transport modeling and analysis. FLEXPART can be run in backward or forward mode. FLEXPART uses 3D atmospheric wind data, in this case supplied from the European Centre for Medium-Range Weather Forecasts (ECMWF) Re-Analysis (ERA) 40 (31) for dates until June 1989 and after that to December 2010 from the ECMWF global ERA-Interim reanalysis at 1° resolution, 60 vertical levels and time resolution of 3 h. The particles modeled were air tracers, with 10,000 particles used on each model run. Residence time is the collective amount of time that a particular area upstream of, or including, the selected location was overlain by any of the air parcels from the trajectories in the sample set. Model output used was residence time, with an output grid of 0.5° latitude × longitude and a time resolution of 3 h (*SI Data and Methods* for further description).

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LETTER TO THE EDITOR

Kawasaki disease patients homozygous for the rs12252-C variant of interferon-induced transmembrane protein-3 are significantly more likely to develop coronary artery lesions

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Kawasaki disease (KD) is the most common systemic vasculitis syndrome, primarily affecting small- to medium-sized arteries, more particularly the coronary arteries (Kato et al. 1996). KD was first described in 1967 and is now identified as the leading cause of acquired heart disease among children in developed countries (Wang et al. 2005). The annual incidence of KD in children of Japanese descent is about 218 per 100,000 children less than 5 years of age (Nakamura et al. 2012) as compared to about 20 per 100,000 in the United States (Holman et al. 2010a). Timely treatment with high-dose intravenous γ globulin (IVIG) reduces the duration of fever and incidence of coronary artery lesions (CAL). However, even after IVIG treatment ~5–7% of patients develop aneurysms (Ogata et al. 2013).

It is widely believed that KD is induced by one or more infectious agents that evoke an abnormal immunological response in genetically susceptible individuals (Burgner and Harnden 2005). However, since the initial description of KD, identification of a definitive infectious agent has been elusive. Several lines of evidence support the infection hypothesis including the acute onset of a self-limited illness, increased susceptibility at younger age, and geographic clustering of outbreaks with a seasonal predominance (later winter and early spring) (Wang et al. 2005).

There is a higher incidence of KD in Japan as well as among Japanese descendants residing in the United States than in any other ethnic populations (Holman et al. 2010b), suggesting that a genetic predisposition also plays an important role in susceptibility to the disease. In addition, there is evidence that the incidence of KD in parents and siblings of an affected patient is higher than in the general population (Onouchi 2012). For example, it has been reported that siblings of affected children are at 10- to 30-fold greater risk of developing KD than children in the general population (Fujita et al. 1989). In addition, offspring of individuals diagnosed with KD are more likely to develop KD (Uehara et al. 2004). More recently there have been a large number of genetic linkage and genome-wide association studies (GWAS) that have reported genetic loci associated with risk and outcomes, see Onouchi (2012) for a comprehensive review. Among the loci that have been implicated in large GWAS studies and have been replicated by separate studies are *FCGR2A* (Khor et al. 2011; Onouchi et al. 2012), *CASP3* (Onouchi et al. 2010; Kuo et al. 2013), and *BLK* (Onouchi et al. 2012; Chang et al. 2013).

HLA-B haplotypes have also been linked to KD with one study identifying KD-associated polymorphisms in *ABHD16A* (abhydrolase domain containing 16A; also known as BAT5: HLA-B associated transcript 5) (Hsieh et al. 2010), this association has not been replicated by other studies. *ABHD16A* encodes a highly conserved, widely expressed lipase of unknown specificity although it has been proposed to function as a palmitoylthioesterase (Martin et al. 2012). ABHD16A binds to IFITM1 (interferon-induced transmembrane protein 1) (Lehner et al. 2004). Another member of the family, IFITM3 (OMIM: 605579), is transcriptionally induced by type I and II interferons and serves to block cellular infection by viruses (such as influenza and dengue) that require endosomal entry into the cytoplasm for replication (Brass et al. 2009; Jiang et al. 2010; Weidner et al. 2010; Lu et al. 2011).

An allelic variant in the human *IFITM3* gene (SNP rs12252: NM_021034.2:c.42T>C; p.Ser14=) truncates the first 20 amino acids of the protein by introducing an alternative splice site and results in the loss of its "antiviral" function (Everitt et al. 2012). Everitt and colleagues also showed that for European Caucasian patients infected with influenza A H1N1/09 virus, those homozygous for the C allele were significantly more likely to develop severe infections requiring hospitalization. More recently, Zhang et al. (2013) made a similar observation in Chinese patients infected with H1N1/09 influenza. The objectives of the study were (1) to evaluate for differences in *IFITM3* genotype frequencies between KD and control cohorts, (2) to assess whether there are differences in the incidences of CAL among the three KD genotypes, and (3) to assess for differences in the distributions of demographic factors (age, gender), IVIG treatment, laboratory data (C-reactive protein [CRP] levels and numbers of white blood cells [WBC]), and duration of fever.

In this study, we genotyped 140 KD patients recruited at three centers, the University of Toyama (n = 89),

Kanazawa Medical University (n = 10), and the University of Utah (n = 41), for the rs12252 SNP. Patients were diagnosed with KD according to standard diagnostic criteria (Kawasaki et al. 1974; Kawasaki 1979). All patients were treated with IVIG and oral aspirin at the time of diagnosis. Echocardiography was used to determine whether the patients had developed CAL, defined as a coronary artery with a diameter of 3 mm or more (4 mm if the subject was over the age of 5 year) at ≥1 month after the onset of KD (Shulman et al. 1995).

With informed consent, venous blood samples or buccal swabs were obtained at the time of diagnosis and DNA isolated and stored at −20°C. For genotyping, both coding exons of *IFITM3* were amplified from 10 ng of genomic DNA using Platinum *Taq* polymerase (Life Technologies, Carlsbad, CA) (Arrington et al. 2012) and the oligonucleotide primers, IFITM3_1_F: 5′-CAAATGCCAGGAAAAGGAAA-3′ and IFITM3_2_R: 5′-CGAGGAATGGAAGTTGGAGT-3′. The 1158 bp PCR product was analyzed by agarose gel electrophoresis, purified by treating with Exo-SAP-IT (Affymetrix, Santa Clara, CA), and then submitted to the University of Utah DNA sequencing core for analysis (Arrington et al. 2012). The study was approved by the Ethics Committees of the University of Toyama and the Kanazawa Medical University, and the Institutional Review Board of the University of Utah.

Corresponding to the three objectives stated above, we carried out the analyses and summarized the results in three tables. In the first analysis (Table 1), we reported the distribution of KD allele and genotype frequency for the control and the KD (case) cohort. The percentage was the conditional probability of having the specific allele or genotype category. These conditional probabilities were compared between the control and case cohort, stratified by race (white, Japanese), by using the chi-square test, or the Fisher's Exact test when the frequency count was less than 5 in at least one cell in the contingency table. In the second analysis (Table 2), the association between CAL incidence and genotype was assessed using either chi-square test or Fisher's exact test. We performed four different contingency table analyses for the overall KD cohort (genotype, allele, dominant, recessive) and thus have used an adjusted type-I error by the Bonferroni method (by dividing the level of significance 0.05 by 4 which yield 0.0125). Thus, the P-value was considered significant if it was less than 0.0125 instead of 0.05. Similarly, we performed this analysis for the stratified cohort of Asian, and white patients. In the third analysis (Table 3), we first assessed the shape of the distribution of the continuous variable of age, CRP, WBC, and fever duration and learned using the normality test of Shapiro–Wilk and examined the histograms that these variables did not follow near normal distribution. Thus, we also reported the median in addition to the mean and standard deviation, overall, and for each of the three genotype categories. We used the nonparametric Wilcoxon rank-sum test to compare among the three groups of genotype. For gender, and treatment response to IVIG, we used either chi-square or Fisher's exact test. For this table, since all the comparisons were preplanned, and no pairwise comparisons were done, we maintained the type-I error at 0.05. All of our analyses were carried out using the SAS/STAT software version 9.3 (Cary, NC) (procedure FREQ for chi-square or Fisher's exact test, and procedure NPAR1WAY for the nonparametric Wilcoxon rank-sum test). Allelic and genotype frequencies were assessed for Hardy–Weinberg equilibrium using the online calculator at http://www.oege.org/software/hwe-mr-calc.shtml (Rodriguez et al. 2009).

All 99 patients recruited in Toyama and Kanazawa were of Japanese descent. Of the 41 patients recruited in Utah, 37 were Caucasian (five with Hispanic ethnicity), 1 Asian, 1 Pacific Islander, and 2 Alaskan Native/Native American. Comparing the allelic frequencies and genotype distribution for rs12252 in the KD Caucasian/non-Hispanic and Japanese patients with 1000 genome (1000g) data from 170 Caucasian/non-Hispanic and 178 Japanese patients who did not have KD (control), did not reveal a

Table 1. Allele and genotype frequencies of the SNP rs12252 (NM_021034.2:c.42T>C) in Utah Caucasian/non-Hispanic and Japanese patients with KD, compared with 1000 genome data for Utah and Japanese controls.

Genotype	Controls (1000g CEU: n = 170)	Utah White-non Hispanic cases (n = 32)	P-value	Controls (1000g JPT: n = 178)	Japanese cases (n = 99)	P-value
Allele C	16 (5%)	5 (8%)	0.352	210 (63%)	121 (65%)	0.625
Allele T	324 (95%)	59 (92%)		146 (37%)	77 (35%)	
CC	0 (0%)	0 (0%)	0.358	68 (38%)	38 (38%)	0.683
CT	16 (9%)	5 (16%)		74 (42%)	45 (46%)	
TT	154 (91%)	27 (84%)		36 (20%)	16 (16%)	

P values were obtained by chi-square test or Fisher's exact test.

Table 2. The C allele and CC genotype for rs12252 (NM_021034.2:c.42T>C) are significantly associated with the development of CAL in KD patients.

			Contingency table		*P* value
All patients					
Genotype		CC	CT	TT	
	CAL	21 (51%)	13 (26%)	10 (20%)	0.004
	No CAL	20 (49%)	37 (74%)	39 (80%)	
Allelic frequency		C	T		
	CAL	55 (42%)	33 (22%)		0.0004
	No CAL	76 (58%)	116 (78%)		
Genetic model					
Dominant		CC + CT	TT		
	CAL	34 (37%)	10 (20%)		0.039
	No CAL	57 (63%)	39 (80%)		
Recessive		CC	CT + TT		
	CAL	21 (51%)	23 (23%)		0.001
	No CAL	20 (49%)	76 (77%)		
Asian patients					
Genotype		CC	CT	TT	
	CAL	20 (51%)	12 (27%)	3 (19%)	0.025
	No CAL	19 (49%)	33 (77%)	13 (81%)	
Allelic frequency		C	T		
	CAL	52 (42%)	18 (23%)		0.006
	No CAL	71 (58%)	59 (77%)		
Genetic model					
Dominant		CC + CT	TT		
	CAL	32 (38%)	3 (19%)		0.164
	No CAL	52 (62%)	13 (81%)		
Recessive		CC	CT + TT		
	CAL	20 (51%)	15 (25%)		0.009
	No CAL	19 (49%)	46 (75%)		
Caucasian patients					
Genotype		CC	CT	TT	
	CAL	0 (0%)	1 (20%)	7 (23%)	1.000
	No CAL	1 (100%)	4 (80%)	24 (77%)	
Allelic frequency		C	T		
	CAL	1 (14%)	15 (22%)		1.000
	No CAL	6 (86%)	52 (78%)		
Genetic model					
Dominant		CC + CT	TT		
	CAL	1 (17%)	7 (23%)		1.000
	No CAL	5 (83%)	24 (77%)		
Recessive		CC	CT + TT		
	CAL	0 (0%)	8 (22%)		1.000
	No CAL	1 (100%)	28 (78%)		

P values were obtained by chi-square analysis.

significant difference in either (Table 1), all genotypes were in Hardy–Weinberg equilibrium. Three patients from Utah were homozygous CC, the Asian and Pacific Islander patients as well as one of the Caucasian/Hispanic patients.

Further analysis of the allelic frequencies and genotype distribution for rs12252 identified a significant association with outcome. Patients who developed CAL were significantly more likely to carry the C allele ($P = 0.0004$) and the distribution of genotypes was significantly different ($P = 0.004$) (Table 2). In addition, significantly more patients homozygous for the SNP developed CAL than patients with the other genotypes (51.2% vs. 23.2%: $P = 0.001$), supporting a recessive model for the effect of this SNP (Table 2). There was not a significant association with a dominant model ($P = 0.039$). These associations were also true when comparing outcomes in Asian patients (Table 2). There was not a significant association for Caucasians, possibly because the minor allele is very

Table 3. Comparison of clinical and laboratory data in KD patients with different rs12252 (NM_021034.2:c.42T>C) genotypes.

Demographic	All KD (*N* = 140)	CC (*N* = 41)	CT (*N* = 50)	TT (*N* = 49)	*P* value
All patients					
Age at Dx (years)	2.73 ± 2.38 (2.37)	2.63 ± 2.58 (1.80)	2.76 ± 2.16 (2.00)	2.78 ± 2.45 (2.50)	0.718[1]
Gender (M/F)	94/46	29/12	29/21	36/13	0.221[2]
Second dose of IVIG required	31/139[3]	9/40	11/50[3]	11/49	0.968[2]
Laboratory data[4]					
CRP (mg/dL)	10.23 ± 7.61 (8.40)	8.71 ± 7.11 (6.37)	9.99 ± 5.82 (8.55)	11.67 ± 9.24 (8.85)	0.289[1]
WBC/μL	15,103 ± 4974 (14,510)	15,347 ± 5466 (15,570)	14,174 ± 4559 (13,400)	15,752 ± 4922 (14,555)	0.303[1]
Duration of fever (days)	9.35 ± 4.90 (8.00)	10.47 ± 5.72 (8.50)	9.60 ± 4.42 (9.00)	8.27 ± 4.52 (6.00)	0.055[1]
Asian patients	All KD (*N* = 100)	CC (*N* = 39)	CT (*N* = 45)	TT (*N* = 16)	*P* value
Age at Dx (years)	2.62 ± 2.10 (1.90)	2.42 ± 2.07 (1.80)	2.74 ± 2.16 (2.00)	2.72 ± 2.09 (2.70)	0.607[1]
Gender (M/F)	64/36	27/12	26/19	11/5	0.503[2]
Second dose of IVIG required	23/99	8/38	11/45[3]	4/16	0.956[2]
Laboratory data					
CRP (mg/dL)	9.21 ± 6.39 (7.80)	8.28 ± 6.63 (6.37)	9.71 ± 5.84 (8.50)	9.92 ± 7.21 (6.75)	0.303[1]
WBC/μL	14,427 ± 4847 (13,900)	15,047 ± 5459 (15,000)	14,116 ± 4848 (13,500)	13,869 ± 3521 (13,130)	0.566[1]
Duration of fever (days)	10.02 ± 5.22 (9.00)	10.81 ± 5.72 (9.00)	9.69 ± 4.40 (9.00)	9.20 ± 6.05 (6.00)	0.323[1]
Caucasian patients	All KD (*N* = 37)	CC (*N* = 1)	CT (*N* = 5)	TT (*N* = 31)	*P* value
Age at Dx (years)	2.75 ± 2.63 (2.00)	1.1 ± NA (1.1)	2.9 ± 2.44 (2.00)	2.78 ± 2.72 (2.40)	0.853[1]
Gender (M/F)	27/10	1/0	3/2	23/8	0.711[2]
Second dose of IVIG required	7/37	1/0	0/5	6/31	0.455[2]
Laboratory data					
CRP (mg/dL)	12.56 ± 9.74 (12.20)	5.10 ± NA (5.10)	11.86 ± 5.92 (13.50)	13.03 ± 10.56 (12.20)	0.747[1]
WBC/μL	16,443 ± 5010 (15,250)	17,800 ± NA (17,800)	14,560 ± 2152 (13,300)	16,779 ± 5466 (15,250)	0.530[1]
Duration of fever (days)	7.76 ± 3.66 (6.00)	5.00 ± NA (5.00)	9.00 ± 5.05 (6.00)	7.64 ± 3.47 (6.00)	0.43[1]

Mean ± SD and median values (in parentheses) are reported. Dx, diagnosis; SD, standard deviation; M, male; F, female; IVIG, intravenous γ globulin; CRP, C-reactive protein; WBC, white blood cells; μL, microliter; NA, not applicable (one patient).

[1]*P* values obtained by nonparametric Wilcoxon rank-sum test.

[2]*P* values obtained by chi-square test and Fisher's exact test when cell counts <5.

[3]Data for one patient incomplete.

[4]Laboratory data incomplete for 31 of the 140 patients; 9 CC, 12 CT, and 10 TT.

rare in this population limiting the power of the comparison in this small cohort. There were no significant differences in other clinical and laboratory data between genotypes (Table 3), including the duration of fever and the response to IVIG.

The IFITM proteins restrict the cellular entry of various viruses, including influenza A, flaviviruses, dengue virus, West Nile virus, and severe acute respiratory syndrome coronavirus (Brass et al. 2009; Huang et al. 2011). These viruses share common characteristics in that they are enveloped and enter cells via membrane fusion in endosomal compartments. It has been shown that IFITM3 prevents emergence of viral genomes from the endosomal pathway, although this may be restricted to late endosomes or lysosomes (Feeley et al. 2011). Since many enveloped viruses enter host cells through the late endocytic pathway, it is possible that enveloped viruses are an important etiologic agent in KD, particularly in patients that develop CAL. The symptoms of KD suggest that tissue damage may also occur from an over-reaction of the immune response characterized by the elevated expression of inflammatory cytokines (Saji and Kemmotsu 2006). The IFITM proteins of man and mouse have also been shown to be associated with membrane signaling complexes (Smith et al. 2006), consequently the loss of functional IFITM3 in KD patients may predispose to enhanced inflammatory responses and tissue damage.

Among the Japanese cohort, 19 (50%) of 38 patients carrying the CC genotype developed CAL. In the Utah cohort, 2 (66.7%) of 3 patients homozygous for rs12252-C developed CAL. At least in the Asian population, where the frequency of the C allele is high, screening for this SNP may be a relatively cost effective way to identify patients at higher risk of developing CAL.

In conclusion, our data reveal a novel association between the *IFITM3* rs12252 CC genotype and the development of CAL in patients with KD, particularly in Asian patients. This association did not extend to the susceptibility to develop KD but it is noteworthy that the frequency of this allele is much higher in the Asian

population, as is the frequency of KD. Since this variant leads to production of a truncated protein with reduced ability to block viral release from the endocytic pathway, these data suggest enveloped viruses may be an important etiologic agent for KD and/or the development of CAL.

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Conflict of Interest

None declared.

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Neil E. Bowles[1], Cammon B. Arrington[1], Keiichi Hirono[2], Tsuneyuki Nakamura[3], Long Ngo[4], Yin Shen Wee[5], Fukiko Ichida[2] and John H. Weis[5]

[1]*Division of Cardiology, Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, Utah*

[2]*Department of Pediatrics, Faculty of Medicine, University of Toyama, Toyama, Japan*

[3]*Department of Pediatrics, Kanazawa Medical University, Kanazawa, Japan*

[4]*Department of Medicine, Harvard Medical School, Beth Israel Deaconess Medical Center, Brookline, Massachusetts*

[5]*Department of Pathology, University of Utah School of Medicine, Salt Lake City, Utah*

Correspondence

Neil E. Bowles, Department of Pediatrics (Cardiology), University of Utah School of Medicine, Eccles Institute of Human Genetics, 15 North 2030 East, Room 7110B, Salt Lake City, UT 84112. Tel: 801-585-7574; Fax: 801-581-7404; E-mail: neil.bowles@hsc.utah.edu

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