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

The Harvard community has made this article openly available. Please share how this access benefits you. Your story matters.

Citation: Bowles, Neil E, Cammon B Arrington, Keiichi Hirono, Tsuneyuki Nakamura, Long Ngo, Yin Shen Wee, Fukiko Ichida, and John H Weis. 2014. “Kawasaki disease patients homozygous for the rs12252-C variant of interferon-induced transmembrane protein-3 are significantly more likely to develop coronary artery lesions.” Molecular Genetics & Genomic Medicine 2 (4): 356-361. doi:10.1002/mgg3.79. http://dx.doi.org/10.1002/mgg3.79.

Published Version: doi:10.1002/mgg3.79

Citable link: http://nrs.harvard.edu/urn-3:HUL.InstRepos:12717563

Terms of Use: This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA
Kawasaki disease patients homozygous for the rs12252-C variant of interferon-induced transmembrane protein-3 are significantly more likely to develop coronary artery lesions

doi: 10.1002/mgg3.79

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 “anti-viral” 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 \(\text{Kawasaki et al. 1974; Kawasaki 1979}\). 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 \((4 \text{ mm if the subject was over the age of 5 year})\) at \(\geq 1\) month after the onset of KD \(\text{Shulman et al. 1995}\).

With informed consent, venous blood samples or buccal swabs were obtained at the time of diagnosis and DNA isolated and stored at \(-20^\circ\text{C}\). For genotyping, both coding exons of \text{IFITM3}\ were amplified from 10 ng of genomic DNA using Platinum \text{Taq}\ polymerase \(\text{Life Technologies, Carlsbad, CA}\) \(\text{Arrington et al. 2012}\) and the oligonucleotide primers, \text{IFITM3}_1\_F: 5'-CAAATGCGACAGGAAAGGA AA-3' and \text{IFITM3}_2\_R: 5'-CGAGAATGGAAGTTGGA GT-3'. The 1158 bp PCR product was analyzed by agarose gel electrophoresis, purified by treating with Exo-SAP-IT \(\text{Affymetrix, Santa Clara, CA}\), and then submitted to the University of Utah DNA sequencing core for analysis \(\text{Arrington et al. 2012}\). 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 \(\text{white, Japanese}\), 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 \(\text{genotype, allele, dominant, recessive}\) and thus have used an adjusted type-I error by the Bonferroni method \(\text{by dividing the level of significance 0.05 by 4 which yield 0.0125}\). 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 \(\text{Table 3}\), 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 \text{SAS/STAT} software version 9.3 \(\text{Cary, NC}\) \(\text{procedure FREQ for chi-square or Fisher’s exact test, and procedure NPAR1WAY for the nonparametric Wilcoxon rank-sum test}\). Allelic and genotype frequencies were assessed for Hardy–Weinberg equilibrium using the online calculator at http://www.oegge.org/software/hwe-mr-calculator.html \(\text{Rodriguez et al. 2009}\).

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

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

| Genotype | Controls \(n = 170\) | Utah White-non Hispanic cases \(n = 32\) | Controls \(n = 178\) | Japanese cases \(n = 99\) | \(P\)-value |
|----------|-----------------------|-----------------------------------------|-----------------------|-----------------------------------------|-----------|
| Allele C | 16 (5%)               | 5 (8%)                                  | 210 (63%)             | 121 (65%)                               | 0.625     |
| Allele T | 324 (95%)             | 59 (92%)                                | 146 (37%)             | 77 (35%)                                |           |
| CC       | 0 (0%)                | 0 (0%)                                  | 68 (18%)              | 38 (19%)                                | 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.
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 \( (51.2\% \text{ vs. } 23.2\%): \ P = 0.001 \), 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 rare.

### 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.
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

### 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 |
| Gender (M/F) | 94/46 | 29/12 | 29/21 | 36/13 | 0.221 |
| Second dose of IVIG required | 31/139 | 9/40 | 11/50 | 11/49 | 0.968 |
| Laboratory data* |            |            |            |            |         |
| 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 |
| WBC/µL | 15,103 ± 4974 (14,510) | 15,347 ± 5466 (15,570) | 14,174 ± 4559 (13,400) | 15,752 ± 4922 (14,555) | 0.303 |
| 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 |
| **Asian patients** |                  |            |            |            |         |
| 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 |
| Gender (M/F) | 64/36 | 27/12 | 26/19 | 11/5 | 0.503 |
| Second dose of IVIG required | 23/99 | 8/38 | 11/45 | 4/16 | 0.956 |
| 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 |
| WBC/µL | 14,427 ± 4847 (13,900) | 15,047 ± 5459 (15,000) | 14,116 ± 4848 (13,500) | 13,869 ± 3521 (13,130) | 0.566 |
| 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 |
| **Caucasian patients** |                  |            |            |            |         |
| 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 |
| Gender (M/F) | 27/10 | 1/0 | 3/2 | 23/8 | 0.711 |
| Second dose of IVIG required | 7/37 | 1/0 | 0/5 | 6/31 | 0.453 |
| Laboratory data |            |            |            |            |         |
| CRP (mg/dL) | 12.56 ± 9.74 (12.20) | 5.10 ± NA (5.10) | 11.86 ± 5.92 (12.50) | 13.03 ± 10.56 (12.20) | 0.747 |
| WBC/µL | 16,443 ± 5010 (15,250) | 17,800 ± NA (17,800) | 14,560 ± 2152 (13,300) | 16,779 ± 5466 (15,250) | 0.530 |
| 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 |

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).
1P values obtained by nonparametric Wilcoxon rank-sum test.
2P values obtained by chi-square test and Fisher’s exact test when cell counts <5.
3Data for one patient incomplete.
4Laboratory data incomplete for 31 of the 140 patients; 9 CC, 12 CT, and 10 TT.
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.

Acknowledgments

This work was supported by funds to N. E. B from the Division of Cardiology, Department of Pediatrics, University of Utah. DNA extractions were performed in the University of Utah Center for Clinical and Translational Science, which is funded by Public Health Services research grant #M01-RR00064 from the National Center for Research Resources, the Children’s Health Research Center at the University of Utah, and the Clinical Genetics Research Program at the University of Utah. This work was supported by funds to J. H. W. from the Department of Pathology, the Weber Presidential Endowed Chair for Immunology and the National Institutes of Health (AI088451).

Conflict of Interest

None declared.

References

Arrington, C. B., S. B. Bleyl, N. Matsunami, G. D. Bonnell, B. E. Otterud, D. C. Nielsen, et al. 2012. Exome analysis of a family with pleiotropic congenital heart disease. Circ. Cardiovasc. Genet. 5:175–182.

Brass, A. L., I. C. Huang, Y. Benita, S. P. John, M. N. Krishnan, E. M. Feeley, et al. 2009. The IFITM proteins mediate cellular resistance to influenza A H1N1 virus, West Nile virus, and dengue virus. Cell 139:1243–1254.

Burgner, D., and A. Harnden. 2005. Kawasaki disease: what is the epidemiology telling us about the etiology? Int. J. Infect. Dis. 9:185–194.

Chang, C. J., H. C. Kuo, J. S. Chang, J. K. Lee, F. J. Tsai, C. C. Khor, et al. 2013. Replication and meta-analysis of GWAS identified susceptibility loci in Kawasaki disease confirm the importance of B lymphoid tyrosine kinase (BLK) in disease susceptibility. PLoS One 8:e72037.

Everitt, A. R., S. Clare, T. Pertel, S. P. John, R. S. Wash, S. E. Smith, et al. 2012. IFITM3 restricts the morbidity and mortality associated with influenza. Nature 484:519–523.

Feeley, E. M., J. S. Sims, S. P. John, C. R. Chin, T. Pertel, L. M. Chen, et al. 2011. IFITM3 inhibits influenza A virus infection by preventing cytosolic entry. PLoS Pathog. 7:e1002337.

Fujita, Y., Y. Nakamura, K. Sakata, N. Hara, M. Kobayashi, M. Nagai, et al. 1989. Kawasaki disease in families. Pediatrics 84:666–669.

Holman, R. C., E. D. Belay, K. Y. Christensen, A. M. Folkema, C. A. Steiner, and L. B. Schonberger. 2010a. Hospitalizations for Kawasaki syndrome among children in the United States, 1997–2007. Pediatr. Infect. Dis. J. 29:483–488.

Holman, R. C., K. Y. Christensen, E. D. Belay, C. A. Steiner, P. V. Effler, J. Miyamura, et al. 2010b. Racial/ethnic differences in the incidence of Kawasaki syndrome among children in Hawaii. Hawaii Med. J. 69:194–197.

Hsieh, Y. Y., Y. J. Lin, C. C. Chang, D. Y. Chen, C. M. Hsu, Y. K. Wang, et al. 2010. Human lymphocyte antigen B-associated transcript 2, 3, and 5 polymorphisms and haplotypes are associated with susceptibility of Kawasaki disease and coronary artery aneurysm. J. Clin. Lab. Anal. 24:262–268.

Huang, I. C., C. C. Bailey, J. L. Weyer, S. R. Radoshitzky, M. M. Becker, J. J. Chiang, et al. 2011. Distinct patterns of IFITM-mediated restriction of filoviruses, SARS coronavirus, and influenza A virus. PLoS Pathog. 7:e1001258.

Jiang, D., J. M. Weidner, M. Qing, X. B. Pan, H. Guo, C. Xu, et al. 2010. Identification of five interferon-induced cellular proteins that inhibit west nile virus and dengue virus infections. J. Virol. 84:8332–8341.

Kato, H., T. Sugimura, T. Akagi, N. Sato, K. Hashino, Y. Maeno, et al. 1996. Long-term consequences of Kawasaki disease. A 10- to 21-year follow-up study of 594 patients. Circulation 94:1379–1385.

Kawasaki, T. 1979. Clinical signs and symptoms of mucocutaneous lymph node syndrome (Kawasaki disease). Jpn. J. Med. Sci. Biol. 32:237–238.

Kawasaki, T., F. Kosaki, S. Okawa, I. Shigematsu, and H. Yanagawa. 1974. A new infantile acute febrile mucocutaneous lymph node syndrome (MLNS) prevailing in Japan. Pediatrics 54:271–276.

Khor, C. C., S. Davila, W. B. Breunis, Y. C. Lee, C. Shimizu, V. J. Wright, et al. 2011. Genome-wide association study identifies FCGR2A as a susceptibility locus for Kawasaki disease. Nat. Genet. 43:1241–1246.

Kuo, H. C., Y. W. Hsu, C. M. Wu, S. H. Chen, K. S. Hung, W. P. Chang, et al. 2013. A replication study for association of ITPKC and CASP3 two-locus analysis in IVIG unresponsiveness and coronary artery lesion in Kawasaki disease. PLoS One 8:e69685.

Lehner, B., J. I. Semple, S. E. Brown, D. Counsell, R. D. Campbell, and C. M. Sanderson. 2004. Analysis of a high-throughput yeast two-hybrid system and its use to predict the function of intracellular proteins encoded within the human MHC class III region. Genomics 83:153–167.

Lu, J., Q. Pan, L. Rong, S. L. Liu, and C. Liang. 2011. The IFITM proteins inhibit HIV-1 infection. J. Virol. 85:2126–2137.

Martin, B. R., C. Wang, A. Adibekian, S. E. Tully, and B. F. Cravatt. 2012. Global profiling of dynamic protein palmitoylation. Nat. Methods 9:84–99.

Nakamura, Y., M. Yashiro, R. Uehara, A. Sadakane, S. Tsuboi, Y. Aoyama, et al. 2012. Epidemiologic features of Kawasaki disease.
disease in Japan: results of the 2009–2010 nationwide survey. J. Epidemiol. 22:216–221.

Ogata, S., A. H. Tremoulet, Y. Sato, K. Ueda, C. Shimizu, X. Sun, et al. 2013. Coronary artery outcomes among children with Kawasaki disease in the United States and Japan. Int. J. Cardiol. 168:3825–3828.

Onouchi, Y. 2012. Genetics of Kawasaki disease: what we know and don’t know. Circ. J. 76:1581–1586.

Onouchi, Y., K. Ozaki, J. C. Burns, C. Shimizu, H. Hamada, T. Honda, et al. 2010. Common variants in CASP3 confer susceptibility to Kawasaki disease. Hum. Mol. Genet. 19:2898–2906.

Onouchi, Y., K. Ozaki, J. C. Burns, C. Shimizu, M. Terai, H. Hamada, et al. 2012. A genome-wide association study identifies three new risk loci for Kawasaki disease. Nat. Genet. 44:517–521.

Rodriguez, S., T. R. Gaunt, and I. N. Day. 2009. Hardy-Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. Am. J. Epidemiol. 169:505–514.

Saji, T., and Y. Kemmotsu. 2006. Infliximab for Kawasaki syndrome. J. Pediatr. 149:426.

Shulman, S. T., J. De Inocencio, and R. Hirsch. 1995. Kawasaki disease. Pediatr. Clin. North Am. 42:1205–1222.

Smith, R. A., J. Young, J. J. Weis, and J. H. Weis. 2006. Expression of the mouse fragilis gene products in immune cells and association with receptor signaling complexes. Genes Immun. 7:113–121.

Uehara, R., M. Yashiro, Y. Nakamura, and H. Yanagawa. 2004. Clinical features of patients with Kawasaki disease whose parents had the same disease. Arch. Pediatr. Adolesc. Med. 158:1166–1169.

Wang, C. L., Y. T. Wu, C. A. Liu, H. C. Kuo, and K. D. Yang. 2005. Kawasaki disease: infection, immunity and genetics. Pediatr. Infect. Dis. J. 24:998–1004.

Weidner, J. M., D. Jiang, X. B. Pan, J. Chang, T. M. Block, and J. T. Guo. 2010. Interferon-induced cell membrane proteins, IFITM3 and tetherin, inhibit vesicular stomatitis virus infection via distinct mechanisms. J. Virol. 84:12646–12657.

Zhang, Y. H., Y. Zhao, N. Li, Y. C. Peng. E. Giannoulatou, R. H. Jin, et al. 2013. Interferon-induced transmembrane protein-3 genetic variant rs12252-C is associated with severe influenza in Chinese individuals. Nat. Commun. 4:1418.

Neil E. Bowles1, Cammon B. Arrington1, Keiichi Hirono2, Tsuneyuki Nakamura3, Long Ngo4, Yin Shen Wee5, Fukiko Ichida2 and John H. Weis5

1Division of Cardiology, Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, Utah
2Department of Pediatrics, Faculty of Medicine, University of Toyama, Toyama, Japan
3Department of Pediatrics, Kanazawa Medical University, Kanazawa, Japan
4Department of Medicine, Harvard Medical School, Beth Israel Deaconess Medical Center, Brookline, Massachusetts
5Department 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

Funding Information
This work was supported by funds to N. E. B from the Division of Cardiology, Department of Pediatrics, University of Utah. DNA extractions were performed in the University of Utah Center for Clinical and Translational Science, which is funded by Public Health Services research grant #M01-RR00064 from the National Center for Research Resources, the Children’s Health Research Center at the University of Utah, and the Clinical Genetics Research Program at the University of Utah. This work was supported by funds to J. H. W. from the Department of Pathology, the Weber Presidential Endowed Chair for Immunology and the National Institutes of Health (AI088451).