

表1 ITPKC 遺伝子の SNP (rs28493229) と川崎病との相関

日本人 (ケースコントロール相関解析) *				米国人 (伝達不平衡試験) ***			
C アレル頻度		オッズ比	P 値 **	伝達	非伝達	オッズ比	P 値
川崎病	対照	(95% 信頼区間) **		C アレル	C アレル	(95% 信頼区間)	
0.23	0.15	1.89	$2.2 \times 10^{-9}$	64	30	2.13	0.00045
						(1.38-3.29)	

\*川崎病 637 人, 対照 1,034 人, 優性遺伝モデル \*\*優性遺伝モデル \*\*\*川崎病患者とその両親 209 組

アレルは RNA のスプライシングの効率を低下させ, ITPKC 産物の減少を来す機能的遺伝子多型であり, 日本人及び米国人に共通して川崎病の罹患感受性と強く相関する (表1)<sup>2)</sup>. 細胞内で ITPKC の発現をロックダウンすると NFAT 活性が上昇し, ITPKC を強制発現させると逆に低下することから ITPKC は  $Ca^{2+}$ /NFAT 経路の負の制御因子として作用していると考えられ, 川崎病の病態への  $Ca^{2+}$ /NFAT 経路の関連が示唆される. また rs28493229 は川崎病に罹患した際の IVIG

治療への抵抗性や冠動脈病変 (CAL) のリスクとの相関もみられることから, 重症化を予測する遺伝マーカーとなる可能性も秘めている.

## 2. CASP3

4 番染色体上の候補領域からは *Caspase-3* (CASP3) 遺伝子が感受性遺伝子として同定されている. Caspase (cysteine-aspartic proteases) とはシステインプロテアーゼに分類されるタンパク群であり, 細胞のアポトーシスにおいて重要な

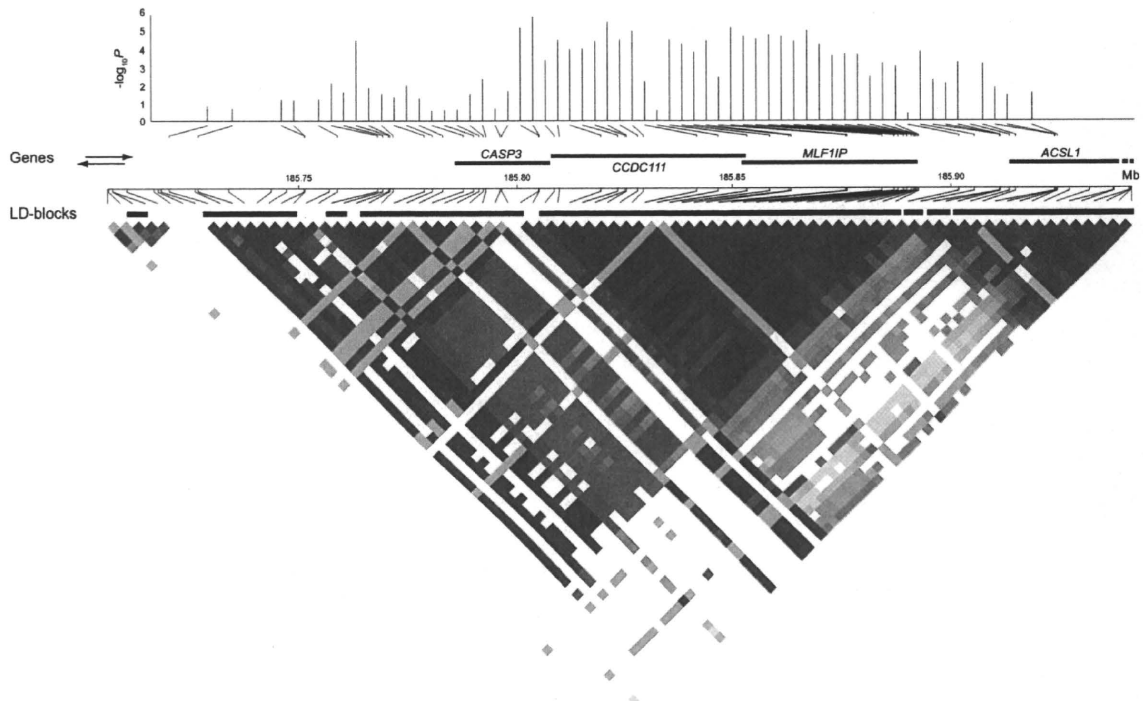


図3 CASP3 遺伝子近傍の連鎖不平衡ブロックと SNP の相関

4 番染色体長腕上の領域 (185.8 メガ塩基近傍) の SNP 間の連鎖不平衡 ( $D'$ /LOD) と各 SNP と川崎病との相関を p 値の対数のマイナス値を示す. 赤色が濃いほど連鎖不平衡が強いことを意味する.

表2 CASP3遺伝子のSNP (rs72689236) と川崎病との相関

日本人 (ケースコントロール相関解析) *				米国人 (伝達不平衡試験) **			
A アレル頻度		オッズ比	P値	伝達	非伝達	オッズ比	P値
川崎病	対照	(95%信頼区間)		A アレル	A アレル	(95%信頼区間)	
0.45	0.37	1.40	$4.2 \times 10^{-8}$	120	79	1.54	0.0037
			(1.24-1.57)				(1.16-2.05)

\*川崎病920人, 対照1,409人, アレル頻度比較 \*\*川崎病患児とその両親249組

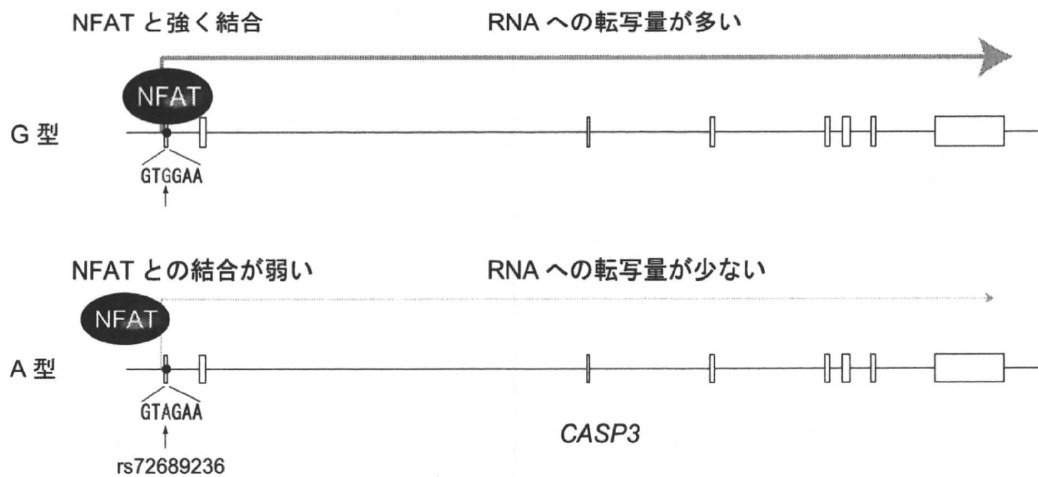


図4 CASP3遺伝子のSNP (rs72689236) の機能

川崎病に罹患しやすいタイプのA型では、転写因子NFATとDNAの結合が弱くなるためCASP3遺伝子のRNAへの転写量が低下する。

役割を演じている。哺乳類ではCASP1-14が知られ、ヒトではCASP11,12を除く12種類が見つかった。イニシエーターカスパーゼと呼ばれるCASP2, 8, 9, 10は、エフェクターカスパーゼと呼ばれるCASP3, 6, 7を分解、不活性型から活性型へと変換し、活性型エフェクターカスパーゼが細胞内にて種々の基質タンパクを分解することによりアポトーシスが進行する。中でもCASP3はT細胞においてT細胞受容体からのシグナル伝達によりその発現が選択的に亢進することや<sup>3)</sup>、ノックアウトマウスでT細胞、B細胞および好中球のアポトーシスが低下することが知られており<sup>4,5)</sup>、炎症細胞のアポトーシスや免疫応答に重要であると考えられる。CASP3遺伝子は筆者らが2007年に報告した川崎病感受性遺伝子の候補

領域の一つである4q35に位置し<sup>1)</sup>、遺伝子近傍に日本人及び白人に共通に川崎病の罹患感受性と相関する複数の多型が存在する(図3, 表2)<sup>6)</sup>。これらの多型はともに強い連鎖不平衡にあり、責任多型であるエクソン1内のSNP(rs72689236; G/A)の近傍には末梢血単核球やT細胞においてエンハンサー活性があることが確かめられており、感受性アレル(A型)では転写因子NFATc2の結合が低下する(図4)<sup>6)</sup>。末梢血単核球においてはG型と比較してA型ではCASP3のmRNA発現が約30%減少していることも確かめられた(図5)。CASP3とNFATc2との遺伝子間相互作用についてはNFATc2によるCASP3の発現制御<sup>7)</sup>の他に、CASP3がNFATc2を基質として分解するという関係が報告されている<sup>8)</sup>。我々が最近行った検討

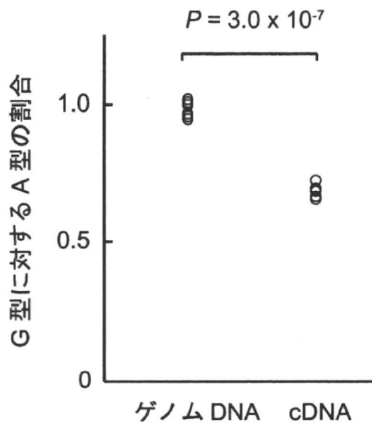


図5 *CASP3*遺伝子のSNP (rs72689236) と転写量との関係

rs72689236の遺伝子型がヘテロ (G/A) である被験者について、末梢血単核球中に発現したG型とA型の*CASP3*遺伝子の量 (cDNA) を比較すると、A型がG型よりも約30%少ない。

ではITPKCの発現にもNFATc2が直接関与していることを示すデータが得られている (未発表データ)。両遺伝子産物がともに直接もしくは間接的にNFATc2の活性を調節するネガティブフィードバック因子として機能しており、SNPによるその機能低下が川崎病の発症や重症化に結びついているとすれば (図6)、病態や治療法を考える上で興味深い。

## B. 海外の genome-wide 研究

近年、詳細なSNP地図およびSNP間の連鎖不平衡に関する情報を利用した大規模なゲノムワイド関連解析 genome-wide association study (GWAS) が様々な多因子遺伝性疾患について行われ成果の報告が相次いでいる。2009年、Burgnerらは世界初となる川崎病のGWASの結果を報告した<sup>9)</sup>。白人集団を対象としたGWASを実施し、その結果を伝達不平衡試験 (TDT) により検証する手法で、*N-acetylated alpha-linked acidic dipeptidase-like 2 (NAALADL2)*

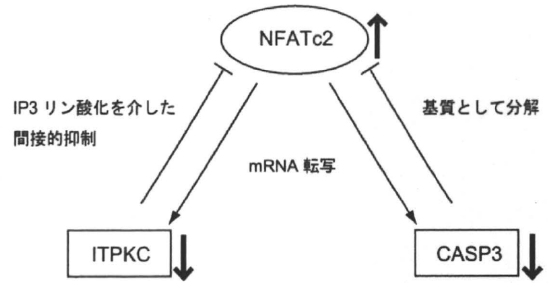


図6 NFATc2とITPKCおよびCASP3との関係のモデル

SNPによる影響を太い矢印で示す。

遺伝子、*AT-binding transcription factor 1 (ZFHX3)* 遺伝子、*Angiopoietin 1 (ANGPT1)* 遺伝子上に関連を示すSNPを見出している。またこの論文では、関連の認められた遺伝子の川崎病急性期および回復期の末梢血中における発現変動の検討や、遺伝子間に存在する相互作用を文献上の報告から検索し、病態と関連のあるgene networkを予測する試みを行っている。本研究はGWASに用いられた検体数が少ない (川崎病107人、対照134人) 等、検出力の問題があり、結果も統計学的に有意といえるレベルではないため、これらの遺伝子が川崎病の感受性遺伝子であると結論するにはさらなる再現性の検証が必要である。著者らは国際共同研究によりさらに規模を拡大したGWASを予定しているのでその結果に期待したい。

## C. 今後の展望

患者試料や臨床データの効率よい収集及び共同研究の円滑化をめざした日本川崎病遺伝コンソーシアム (JKDGC) が2010年2月に発足し、我々も参加している。日本人集団におけるGWASも進行中でありITPKC、CASP3に続く感受性遺伝子が見つかりつつある。今後病態に深く関わる pathway や gene network の解明も加速し、治療

法の開発や病原体の特定に寄与すること、様々な人種で遺伝的背景の解明が進み罹患率の人種差についてその理由が明らかとなることが期待される。次世代シーケンサーによるパーソナルゲノム解析は巨大冠動脈瘤合併例や血漿交換を要す超重症例、頻回罹患例等の解析に有効であると思われる、普及が待たれる。

### むすび

これまで候補遺伝子として検討されてこなかった複数の遺伝子のなかから感受性遺伝子、重症化関連遺伝子が見出され、genome-wideな検索の有用性が示された。今後は川崎病の遺伝的背景の全貌解明と得られた知見の臨床への還元が課題となろう。

### 文献

- 1) Onouchi Y, Tamari M, Takahashi A, et al. A genomewide linkage analysis of Kawasaki disease: evidence for linkage to chromosome 12. *J Hum Genet.* 2007; 52: 179-90.
- 2) Onouchi Y, Gunji T, Burns JC, et al. ITPKC functional polymorphism associated with Kawasaki disease susceptibility and formation of coronary artery aneurysms. *Nat Genet.* 2008; 40: 35-42.
- 3) Sabbagh L, Bourbonnière M, Sékaly RP, et al. Selective up-regulation of caspase-3 gene expression following TCR engagement. *Mol Immunol.* 2005; 42: 1345-54.
- 4) Woo M, Hakem R, Soengas MS, et al. Essential contribution of caspase 3/ CPP32 to apoptosis and its associated nuclear changes. *Genes Dev.* 1998; 12: 806-19.
- 5) Woo M, Hakem R, Furlonger C, et al. Caspase-3 regulates cell cycle in B cells: a consequence of substrate specificity. *Nat Immunol.* 2003; 4: 1016-22.
- 6) Onouchi Y, Ozaki K, Buns JC, et al. Common variants in CASP3 confer susceptibility to Kawasaki disease. *Hum Mol Genet.* 2010; 19: 2898-906.
- 7) Macián F, García-Cózar F, Im SH, et al. Transcriptional mechanisms underlying lymphocyte tolerance. *Cell.* 2002; 109: 719-31.
- 8) Wu W, Misra RS, Russell JQ, et al. Proteolytic regulation of nuclear factor of activated T (NFAT) c2 cells and NFAT activity by caspase-3. *J Biol Chem.* 2006; 281: 10682-90.
- 9) Burgner D, Davila S, Breunis WB, et al. A genome-wide association study identifies novel and functionally related susceptibility Loci for Kawasaki disease. *PLoS Genet.* 2009; 5: e1000319.

## Chapter Number

# Responsible genetic factors for vasculitis in Kawasaki disease

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### 1. Introduction

Kawasaki disease (KD) is an acute febrile illness of early childhood that is characterized by high fever, bilateral conjunctivitis, redness of the oral mucosa, polymorphous skin rash, indurative edema of the hands and feet, and cervical lymphadenopathy (Kawasaki, 1967). The major pathological lesion of KD is vasculitis of small and medium-sized arteries (Amano et al., 1979). The coronary arteries are the most severely affected and coronary artery lesions (CALs) occur in 15–25% of untreated patients (Kato et al., 1975), making KD a leading cause of childhood acquired heart disease in developed countries. The fact that the peak incidence of KD is at 9–11 months of age, which coincides with the waning of maternal immunity, indicates that infections could contribute to the pathogenesis of KD. However, despite more than 40 years of intensive research, the causative microorganism of KD remains unknown. On the other hand, epidemiological studies have revealed a significant role of genetic components in host susceptibility to KD pathogenesis.

### 2. Epidemiological features of KD suggesting a genetic predisposition

Since the first description of KD (Kawasaki, 1967) more than four decades ago, biannual epidemiological surveys conducted in Japan and epidemiological studies performed in almost all ethnic groups during this period have highlighted the contribution of genetic factors in the pathogenesis of KD.

#### 2.1 Ethnic differences in the incidence of KD

KD is much more prevalent in East Asia than in any other countries of the world. In Japan, its incidence was 218.6 per 100,000 children younger than 4 years old in 2008 and continues

to rise (Nakamura et al., 2010). Its incidence in Korea and Taiwan, the neighboring countries to Japan, are the second (113.1) and third (69.0) highest, respectively (Huang et al., 2009; Park et al., 2011). The incidence of KD in Western countries is 10–20 times lower than in Japan. Recent surveillance of KD in Hawaii revealed that Asian children, especially those of Japanese ancestry, had the highest incidence (210.5), and that the incidence among Caucasian children in Hawaii (13.7) was similar to that of the continental United States (Holman et al., 2010). These facts indicate that the high incidence of KD in East Asian countries is due to the racial/ethnic genetic background rather than to geographic factors.

## 2.2 Individual susceptibility to KD

In Japan, a total of 20 nationwide biannual continued surveillances of KD have been carried out since 1970. Epidemiological evidence collected from these surveys suggested individual susceptibility to KD, which was mainly composed of multiple genetic factors. For example, sibling cases of KD have a  $\geq 10$  times higher incidence than expected (Fujita et al., 1989). In addition, parents of KD patients, who were affected by KD during their childhood, were observed 2 times more often than expected (Uehara et al., 2003).

## 3. Genetic studies of KD

KD is considered to be a multifactorial disease that is caused by the interplay of external and personal factors (Fig. 1). Thus, identification of the responsible genetic factors, which presumably determine an individual's susceptibility to KD, should provide clues to the pathogenesis of the disease. Furthermore, it could contribute to the development of novel clinical applications, such as a severity prediction method and new therapeutic measures.

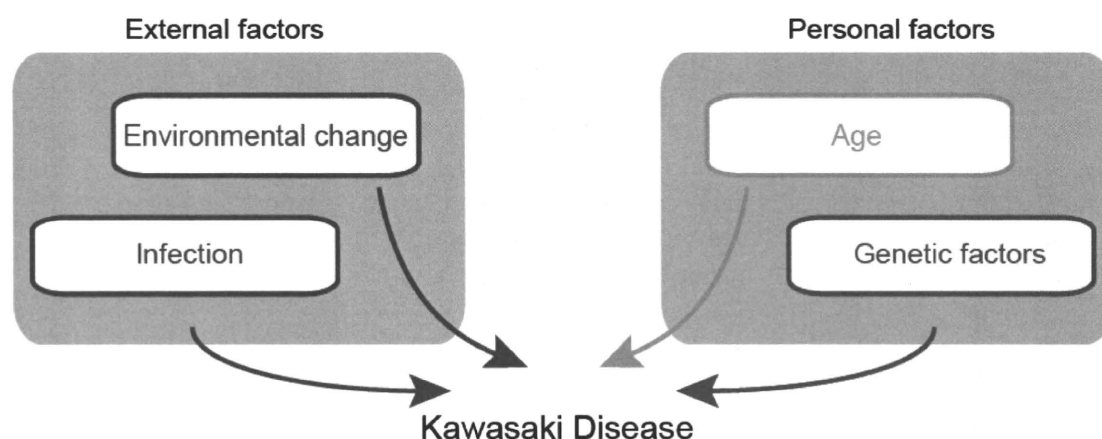


Fig. 1. Multiple factors that are linked to KD.

### 3.1 Candidate gene studies

KD is an immune-mediated vasculitis syndrome; therefore, genes encoding proteins related to innate and acquired immune function or to vascular remodeling could be involved in its pathogenesis, response to treatment, and prognosis. Variations within such “candidate genes” have been extensively studied.

### 3.1.1 Human leukocyte antigen (HLA) genes

Genetic studies of KD were initially conducted by focusing on the HLA class I genes, and several serotypes of the HLA-B locus have been associated with KD in different ethnic groups (Table 1).

Locus	Haplotype /SNP	Method	Ethnicity (cases/controls)	Reference
HLA-B	Bw22J	Serotyping	Japanese (32/76)	Matsuda et al., 1977
HLA-B	Bw22J2	Serotyping	Japanese (205/500)	Kato et al., 1978
HLA-B	Bw15			
HLA-B	Bw51	Serotyping	Caucasian (23/244)	Krensky et al., 1981
HLA-B	Bw51	Serotyping	Jewish (12/90)	Keren et al., 1982
HLA-B	Bw44	Serotyping	Caucasian (23/246)	Krensky et al., 1983
HLA-B	Bw44	Serotyping	Caucasian (16/608)	Kaslow et al., 1985
HLA-B	B35	Genotyping	Korean (74/159)	Oh et al., 2008
HLA-B	B75			
HLA-C	Cw09			
HLA-DRB3	DRB3*0301	Genotyping	Caucasian (21/200)	Barron et al., 1992
HLA-G	rs12722477 C/A A allele	Genotyping	Korean (92/90)	J.J. Kim et al., 2008
HLA-E	rs2844724 C/T C allele	Genotyping	Taiwanese (680/3312)	Y.J. Lin et al., 2009

Table 1. Association studies between HLA genes and KD.

Recently, significant associations of single nucleotide polymorphisms (SNPs) within the HLA-G and HLA-E genes were reported (J.J. Kim et al., 2008; Y.J. Lin et al., 2009). However, most of the previous studies, including those describing negative association results, were conducted by analyzing a relatively small number of cases and controls, while replication studies with larger cohorts have not been performed yet.

### 3.1.2 Non-HLA genes

Advances from the Human Genome and Hapmap projects have dramatically reduced the effort and cost of conducting genetic association studies of complex diseases, leading to a

recent increase in the number of candidate gene studies, especially since 2005 (Table 2). Among the genes studied, tumor necrosis factor (TNF), which is a proinflammatory cytokine deeply related to the pathogenesis of KD, has been most frequently analyzed. Although many of these studies have failed to identify a statistically significant association, a systematic meta-analysis revealed a trend of association between the G allele of rs180629, which is located 308 bases upstream of the TNF gene, and KD (Ari-Ong et al., 2010).

Gene	Chromosomal region	Reference	
		Associated	Not associated
<b>Cytokines, chemokines, and their receptors</b>			
<i>IL-10</i>	1q31-q32	Hsueh et al., 2009	Jin et al., 2007 Weng et al., 2010a
<i>TGFB2</i>	1q41	Shimizu et al., 2011	
<i>IL-1<math>\alpha</math></i>	2q14		Weng et al., 2010b S.K. Kim et al., 2011
<i>IL-1<math>\beta</math></i>	2q14		S.F. Wu et al., 2005 Weng et al., 2010b S.K. Kim et al., 2011
<i>IL-1Ra</i>	2q14.2	S.F. Wu et al., 2005	Weng et al., 2010b S.K. Kim et al., 2011
<i>CXCR2</i>	2q35		Breunis et al., 2007
<i>CXCR1</i>	2q35		Breunis et al., 2007
<i>TGFBR2</i>	3p22	Shimizu et al., 2011	
<i>CX3CR1</i>	3p21.3		Breunis et al., 2007
<i>CCR3</i>	3p21	Breunis et al., 2007	
<i>CCR2</i>	3p21	Breunis et al., 2007	
<i>CCR5</i>	3p21	Burns et al., 2005 Jhang et al., 2009 Breunis et al., 2007	Mamtani et al., 2010 Chaudhuri et al., 2011
<i>IL-8</i>	4q13-q21		Weng et al., 2010b
<i>IL-4</i>	5q31.1	Burns et al., 2005	S.F. Wu et al., 2005 F.Y. Huang et al., 2008a Weng et al., 2010b
<i>LTA</i>	6p21.3	Quasney et al., 2001	
<i>TNF-<math>\alpha</math></i>	6p21.3	Cheung et al., 2008	Kamizono et al., 1999 Quasney et al., 2001 Ahn et al., 2003 Chien et al., 2003 Weng et al., 2010b
<i>IL-6</i>	7p21		Sohn et al., 2001 Weng et al., 2010b
<i>IL-18</i>	11q22.2-q22.3	Hsueh et al., 2008a Chen et al., 2009	
<i>TNFRSF1A</i>	12p13.2	Wang et al., 2011	



<i>MCP1</i>	17q11.2-q12		Jibiki et al., 2001
<i>CCL3L1</i>	17q11.2	Burns et al., 2005 Mamtani et al., 2010	
<i>TGFB1</i>	19q13.1		Weng et al., 2010b
<i>MIF</i>	22q11.2		Simonini et al., 2009
<b>Vasoactive molecules or molecules related to vascular remodeling</b>			
<i>AGTR1</i>	3q21-q25		Fukazawa et al., 2004
<i>TIMP4</i>	3p25		Ban et al., 2009
<i>VEGFR2</i>	4q12		Kariyazono et al., 2004
<i>VEGFA</i>	6p12	Breunis et al., 2006 Hsueh et al., 2008b	Kariyazono et al., 2004 F.Y. Huang et al., 2008b
<i>eNOS</i>	7q36		Khajooe et al., 2003
<i>MMP26</i>	11p15		Ban et al., 2010
<i>MMP7</i>	11q21-22		Ban et al., 2010
<i>MMP3</i>	11q22.3		J.A. Park et al., 2005 Ikeda et al., 2008 Hong et al., 2008
<i>MMP12</i>	11q22.3		Ikeda et al., 2008 Ban et al., 2010
<i>MMP13</i>	11q22.3		Ikeda et al., 2008
<i>MMP2</i>	16q13-q21		Ikeda et al., 2008
<i>iNOS</i>	17q11.2-q12		Khajooe et al., 2003
<i>ACE</i>	17q23	S.F. Wu et al., 2004 Shim et al., 2006	Fukazawa et al., 2004
<i>TIMP2</i>	17q25		Furuno et al., 2007
<i>MMP9</i>	20q11.2-q13		J.A. Park et al., 2005 Ikeda et al., 2008
<i>MMP11</i>	22q11.2	Ban et al., 2010	
<b>Molecules related to innate immune functions</b>			
<i>CRP</i>	1q21-q23	Cheung et al., 2008	
<i>SLC11A1</i>	2q35	Ouchi et al., 2003	
<i>CD14</i>	5q31.1		Nishimura et al., 2003
<i>MBL</i>	10q11.2-q21	Biezeveld et al., 2003	Cheung et al., 2004
<b>Molecules related to acquired immune functions</b>			
<i>FCGR2A</i>	1q23		Taniuchi et al., 2005 Biezeveld et al., 2007
<i>FCGR2B</i>	1q23		Biezeveld et al., 2007
<i>FCGR3A</i>	1q23	Taniuchi et al., 2005	Biezeveld et al., 2007
<i>FCGR3B</i>	1q23		Taniuchi et al., 2005 Biezeveld et al., 2007

<i>CTLA4</i>	2q33		Kuo et al., 2010
<i>PD-1</i>	2q37.3	Chun et al., 2010	
<i>MICA</i>	6p21.3	F.Y. Huang et al., 2000	
<i>BTNL2</i>	6p21.3	Hsueh et al., 2010	
<i>CD40L</i>	Xq26		Y. Onouchi et al., 2004 F.Y. Huang et al., 2008c
<b>Others</b>			
<i>MTHFR</i>	1p36.3	Tsukahara et al., 2000	
<i>UGT1A1</i>	2q37		Kanai et al., 2003
<i>BAT2, 3, 5</i>	6p21.3	Hsieh et al., 2010	
<i>NOTCH4</i>	6p21.3		Kang et al., 2011
<i>COL11A2</i>	6p21.3	Shue et al., 2010	
<i>ITPR3</i>			Y.C. Huang et al., 2010
<i>PAFAH</i>	6p21.2-p12		Minami et al., 2005
<i>TPH2</i>	12q21.1		S.W. Park et al., 2010
<i>SMAD3</i>	15q22.33	Shimizu et al., 2011	
<i>MEFV</i>	16p13		Yamaguchi et al., 2009
<i>HMOX1</i>	22q12		Kanai et al., 2003

Table 2. Association studies between polymorphisms of candidate genes and KD.

The association of these candidate SNPs and patient response to intravenous immunoglobulin (IVIG) therapy and the development of CALs has also been studied (Table 3).

Gene	Phenotype	Reference	
		Association	No association
<i>MTHFR</i>	CAL	Tsukahara et al., 2000	
<i>CRP</i>	CAL		Cheung et al., 2008
	Intima-media thickness	Cheung et al., 2008	
	Arterial stiffness	Cheung et al., 2008	
<i>IL-10</i>	CAL	Jin et al., 2007	Hseuh et al., 2009
	Serum albumin	Jin et al., 2007	
<i>FCGR2A</i>	CAL	Taniuchi et al., 2005	Biezeveld et al., 2007
<i>FCGR2B</i>	CAL		Biezeveld et al., 2007
<i>FCGR3A</i>	CAL		Taniuchi et al., 2005 Biezeveld et al., 2007
<i>FCGR3B</i>	CAL		Taniuchi et al., 2005

			Biezeveld et al., 2007
TGFB2	CAL/ coronary z score	Shimizu et al., 2011	
	Diameter of aortic root	Shimizu et al., 2011	
	Response to IVIG	Shimizu et al., 2011	
IL-1 $\alpha$	CAL		S.K. Kim et al., 2011
IL-1 $\beta$	Response to IVIG	Weng et al., 2010	S.K. Kim et al., 2011
IL-1Ra	CAL		S.K. Kim et al., 2011
CTLA4	CAL	Kuo et al., 2010	
PD-1	CAL		Chun et al., 2010
TIMP4	CAL	Ban et al., 2009	
TGFB2	CAL/ coronary z score	Shimizu et al., 2011	
	Diameter of aortic root	Shimizu et al., 2011	
	Response to IVIG	Shimizu et al., 2011	
CCR5	CAL	Mamtani et al., 2010 Chaudhuri et al., 2011	Jhang et al., 2009
AGTR1	Coronary stenosis	Fukazawa et al., 2004	
VEGFR2	CAL	Kariyazono et al., 2003	
IL-4	CAL		Burns et al., 2005 F.Y.Huang et al., 2008a
CD14	CAL	Nishimura et al., 2003	
VEGFA	CAL	Kariyazono et al., 2003	Hsueh et al., 2008b F.Y.Huang et al., 2008b
MICA	CAL	F.Y. Huang et al., 2000	
LTA	CAL		Quasney et al., 2001
TNF- $\alpha$	CAL	Quasney et al., 2001	Cheung et al., 2008
	Arterial stiffness	Cheung et al., 2008	
BAT2, 3, 5	CAL	Hsieh et al., 2010	
NOTCH4	CAL		Kang et al., 2011
BTNL2	CAL	Hsueh et al., 2010	
COL11A2	CAL	Shue et al., 2010	
ITPR3	CAL	Y.C. Huang et al., 2010	
	CRP	Y.C. Huang et al., 2010	
PAFAH	CAL		Minami et al., 2005
PAFAH	Response to IVIG	Minami et al., 2005	
eNOS	CAL		Khajooe et al., 2003
MBL	CAL	Biezeveld et al., 2003 <sup>c</sup> Biezeveld et al., 2006 <sup>c</sup>	Cheung et al., 2004

	Arterial stiffness	Cheung et al., 2004	
<i>MMP26</i>	CAL		Ban et al., 2010
<i>IL-18</i>	CAL		Hsueh et al., 2008a
<i>MMP7</i>	CAL		Ban et al., 2010
<i>MMP3</i>	CAL	J.A. Park et al., 2005	Ikeda et al., 2008 Hong et al., 2008
<i>MMP12</i>	CAL		Ikeda et al., 2008 Ban et al., 2010
<i>MMP13</i>	CAL	Ikeda et al., 2008	
<i>TPH2</i>	CAL	S.W. Park et al., 2010	
<i>SMAD3</i>	CAL/ coronary z score	Shimizu et al., 2010	
	Diameter of aortic root	Shimizu et al., 2010	
	Response to IVIG	Shimizu et al., 2010	
<i>MEFV</i>	CAL		Yamaguchi et al., 2009
<i>MMP2</i>	CAL		Ikeda et al., 2008
<i>iNOS</i>	CAL		Khajoe et al., 2003
<i>CCL3L1</i>	CAL		Mamtani et al., 2010
	Response to IVIG	Mamtani et al., 2010	
<i>ACE</i>	CAL	Takeuchi et al., 1997	S.F. Wu et al., 2004 Shim et al., 2006
	Coronary stenosis	Fukazawa et al., 2004	
<i>TIMP2</i>	CAL	Furuno et al., 2007	
<i>MMP9</i>	CAL		J.A. Park et al., 2005 Ikeda et al., 2008
<i>MIF</i>	CAL	Simonini et al., 2009	
<i>MMP11</i>	CAL		Ban et al., 2010
<i>CD40L</i>	CAL	Y. Onouchi et al., 2004	Huang et al., 2008c

Table 3. Association studies between candidate gene polymorphisms and KD-related phenotypes.

### 3.2 Genome-wide studies

In contrast to candidate gene studies, which are based on an assumption of the underlying cause of the disease, a strategy to identify disease-causing mutations or variations from the whole genome relies solely on positional information and was originally developed to map and identify the genes for Mendelian disorders. This genome-wide strategy has been adapted for complex diseases and has become the most reliable tool to identify disease-related genes following the completion of the Human Genome Project.

#### 3.2.1 Linkage study

The first genome-wide study for KD was conducted by our group (Y. Onouchi et al., 2007). In this study, 399 microsatellite marker alleles that were shared identical by descent between 78 affected KD sib-pairs were analyzed, and 10 chromosomal regions linked with the disease were identified (Fig. 2).

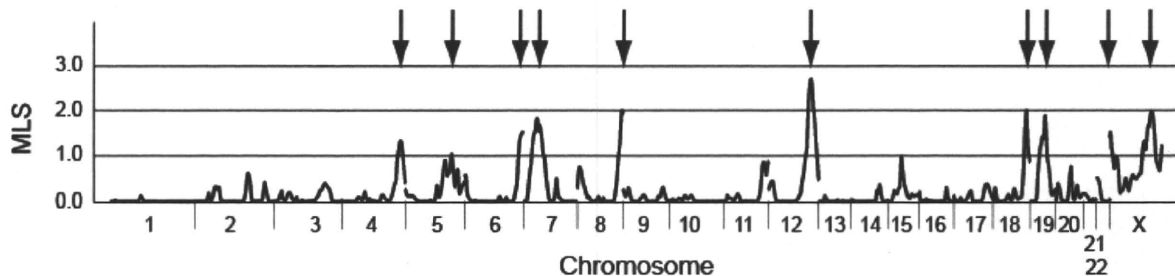


Fig. 2. Results from an affected sib-pair study (Y. Onouchi et al., 2007). Arrows indicate those chromosomal regions with a maximum LOD score (MLS) >1.0.

### 3.2.1.1 Linkage disequilibrium mapping

We narrowed down the candidate regions identified in the sib-pair study with a case-control association study using “tagging” SNPs. Chunks of genomic regions containing the associated tagging SNPs were extensively analyzed by re-sequencing and a further association study. We identified a SNP that was associated with KD in both the Japanese and US populations (Y. Onouchi et al., 2008) (Table 4). The SNP, rs28493229, is located in intron 1 of the inositol 1,4,5-trisphosphate 3-kinase C (ITPKC) gene, which catalyzes the phosphorylation of inositol 1,4,5-trisphosphate (IP3).

Alleles <sup>1</sup>	Japanese Case-control study			USA TDT		
	Risk allele frequency		OR (95% CI) <sup>2</sup>	No. of families	T:U <sup>3</sup>	OR (95% CI)
	KD n=637	Control n=1034	P value <sup>2</sup>			P value
G/ <u>C</u>	0.23	0.15	1.89 (1.53 - 2.33) 2.2x10 <sup>-9</sup>	209	64:30	2.13 (1.38 - 3.29) 0.00045

Table 4. Association of rs28493229 with KD. <sup>1</sup>Risk allele is underlined. <sup>2</sup>Dominant inheritance model. <sup>3</sup>Transmitted:untransmitted ratio. TDT: transmission disequilibrium test, OR: odds ratio, CI: confidence interval.

The at-risk allele of rs28493229 (C) reduces the splicing efficiency of ITPKC (Fig. 3). Transcripts with an unspliced intron are not properly translated because of premature termination. An increase in the number of such immature transcripts might lead to reduced ITPKC activity. IP3 is a second messenger molecule of the Ca<sup>2+</sup>/NFAT pathway in a wide variety of cells and, in mammals, 3 iso-enzymes (ITPKA, ITPKB, and ITPKC) have been identified with the same enzyme activity.

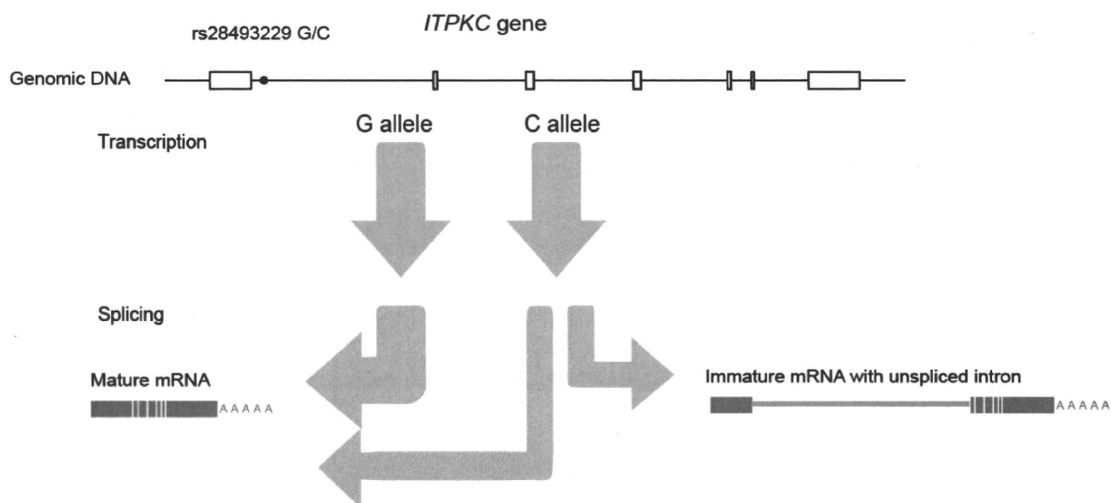


Fig. 3. Functional significance of rs28493229 on ITPKC mRNA.

Knockdown and overexpression experiments of ITPKC in the Jurkat cell line result in increased and decreased NFAT activity, respectively, as well as the expression of interleukin 2 mRNA. These findings highlighted the importance of the  $Ca^{2+}$ /NFAT pathway in the pathogenesis of KD. The association with the at-risk SNP allele was higher in KD patients with CAL than in those without CAL in both the Japanese and US populations. The same trend was also observed in KD patients in the US who responded poorly to intravenous immunoglobulin (IVIG) therapy. Two replication studies for the association of this SNP to KD, one negative and one positive, have been reported in the Taiwanese population (Chi et al., 2010; M.T. Lin et al., 2011). In the positive report, the SNP was also associated with the reactivation of previous BCG inoculation sites (M.T. Lin et al., 2011).

### 3.2.1.2 Positional candidate gene analysis

From the candidate region of chromosome 4, we identified the susceptibility gene via a different approach. The caspase-3 gene, which is located at 185.8 Mb, close to the linkage peak at 184.9 Mb, was focused on and studied as a positional candidate gene (Y. Onouchi et al. 2010). Multiple SNPs around the gene in linkage disequilibrium were associated with KD in the Japanese and US Caucasian populations. The functional SNP (rs72689236 G/A) was located in the 5'-untranslated region of the gene, and the risk allele (A) reduces the enhancer activity around the SNP to which NFATc2 is related (Table 5, Fig. 4).

Alleles <sup>1</sup>	Japanese case-control study			United States TDT		
	Risk allele frequency		OR (95% CI) <sup>2</sup>	No. of families	T:U <sup>3</sup>	OR (95% CI)
	KD n=920	Control n=1,409	P value <sup>2</sup>			P value
G/ <u>A</u>	0.45	0.37	1.40 (1.24-1.57) 4.2 × 10 <sup>-9</sup>	249	120:79	1.54 (1.16-2.05) 0.0037

Table 5. Association of rs72689236 with KD. <sup>1</sup>Risk allele is underlined. <sup>2</sup>Allelic model.

<sup>3</sup>Transmitted:untransmitted ratio. TDT: transmission disequilibrium test, OR: odds ratio, CI: confidence interval.

Caspase-3 is an effector caspase with a central role in apoptosis. T cells from caspase-3-deficient mice have a reduced susceptibility to activation-induced cell death (Woo et al, 1998). It was also reported that caspase-3 cleaves Nfatc2 as a substrate (W. Wu et al, 2006). Transient anergy of peripheral T cells in the convalescent phase of KD, which has been documented in a couple of reports, is suggestive because the NFAT-driven expression of caspase-3 in T cells is related to T cell anergy (Macián et al., 2002). Currently, only one replication study has examined the association between rs72689236 and KD (Kuo et al, 2011). Although not statistically significant, the same trend of association was observed in the Taiwanese population. Notably, in this report, the SNP was associated with increased risk for IVIG resistance and CAL formation.

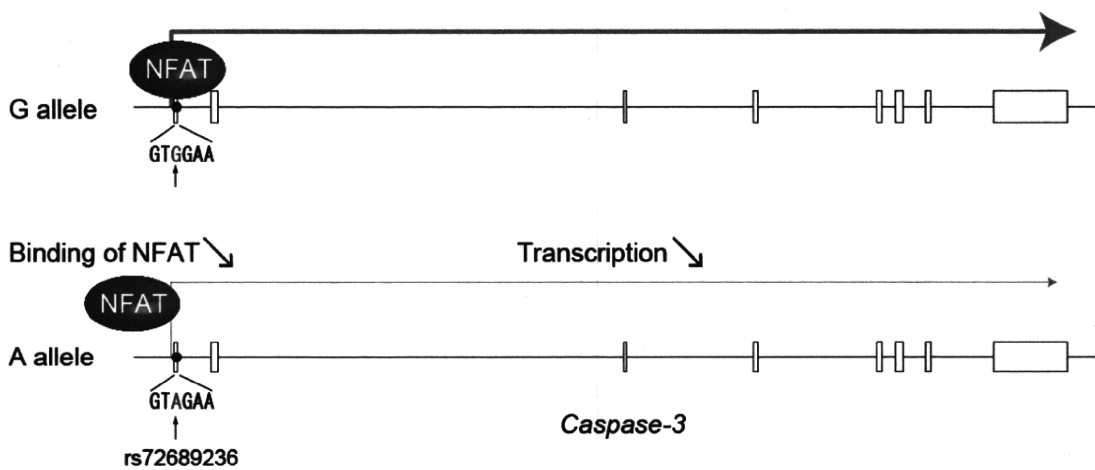


Fig. 4. Functional significance of rs72689236 on caspase-3 mRNA.

### 3.2.2 Genome-wide association studies (GWAS)

Today, GWAS using platforms by which  $5.0 \times 10^5$  to  $1 \times 10^6$  SNPs can be genotyped at a time have become commonplace for the analysis of complex disorders. GWAS for KD have been performed in 3 different ethnic groups: Caucasian, Korean, and Taiwanese (Burgner et al., 2009; J.J. Kim et al., 2011; Tsai et al., 2011). The number of subjects and SNPs analyzed in each study are summarized in Table 6.

Ethnic group	Number of subjects KD/controls		No. of SNPs	Reference
	GWAS	Follow-up		
Caucasian	119/135	583/1357 <sup>1</sup>	223,922	Burgner et al., 2009
Korean	186/600	266/600	641,760	J.J. Kim et al., 2011
Korean	63/600 <sup>2</sup>	86/600 <sup>2</sup>	641,760	J.J. Kim et al., 2011
Taiwanese	250/446	208/366	723,638	Tsai et al., 2011

Table 6. Summary of GWAS for KD (I). <sup>1</sup>1583 KD patients and their unaffected siblings and biological parents. <sup>2</sup>KD patients with coronary artery lesions and healthy controls.

Although many candidate SNPs were identified, no locus was repeatedly associated with KD in these studies (Table 7). Considering that none of these associations fulfilled the genome-wide level significance threshold, further validation of the association at each locus within the same populations is essential.

SNP	Chr	Position	P value	Gene	Reference
rs527409	1	58,757,915	$1.5 \times 10^{-6}$	<i>DAB1</i>	J.J. Kim et al., 2011
rs952354	1	63,549,282	$3.1 \times 10^{-5}$	-*	J.J. Kim et al., 2011
rs7604693	2	64,349,202	$2.0 \times 10^{-6}$	<i>PELI1</i> *	J.J. Kim et al., 2011
rs10183521	2	123,762,542	$9.5 \times 10^{-5}$	-	Burgner et al., 2009
rs16849083	3	139,184,279	$2.2 \times 10^{-5}$	<i>MRPS22,</i> <i>COPB2,</i> <i>RBP2</i>	Tsai et al., 2011
rs9834548	3	165,139,947	$9.8 \times 10^{-6}$	-	Burgner et al., 2009
rs17531088	3	174,893,775	$1.1 \times 10^{-6}$	<i>NAALADL2</i>	Burgner et al., 2009
rs3773986	3	190,278,915	$6.2 \times 10^{-5}$	<i>IL1RAP</i> *	J.J. Kim et al., 2011
rs4864471	4	54,426,184	$3.4 \times 10^{-5}$	<i>LNK1,</i> <i>LOC441016</i>	Burgner et al., 2009
rs13128867	4	138,840,995	$2.2 \times 10^{-5}$	<i>SLC7A11</i>	Tsai et al., 2011
rs149481	5	96,114,346	$4.6 \times 10^{-5}$	<i>ERAP1</i>	Tsai et al., 2011
rs9392158	6	7,427,350	$8.5 \times 10^{-5}$	<i>RIOK1</i>	Burgner et al., 2009
rs9364166	6	72,106,622	$9.8 \times 10^{-5}$	<i>OGFRL1,</i> <i>C6orf155</i>	J.J. Kim et al., 2011
rs362794	7	103,201,263	$3.0 \times 10^{-5}$	<i>RELN</i>	Tsai et al., 2011
rs6469101	8	108,252,238	$5.4 \times 10^{-5}$	<i>ANGPT1</i>	Burgner et al., 2009
rs328879	9	107,789,252	$1.2 \times 10^{-5}$	-	Burgner et al., 2009
rs10984642	9	122,450,340	$2.6 \times 10^{-5}$	-	Burgner et al., 2009
rs10984642	9	122,450,340	$5.8 \times 10^{-5}$	-	Burgner et al., 2009
rs4918458	10	111,505,407	$9.8 \times 10^{-5}$	-*	J.J. Kim et al., 2011
rs285032	13	98,786,532	$1.7 \times 10^{-5}$	<i>FARP1</i>	Burgner et al., 2009
rs34246750	14	52,868,757	$4.8 \times 10^{-5}$	<i>PTGER2,</i> <i>TXNDC16</i>	J.J. Kim et al., 2011
rs10129255	14	107,176,213	$6.8 \times 10^{-6}$	<i>IGHV</i>	Tsai et al., 2011
rs1568657	15	83,726,179	$6.6 \times 10^{-6}$	<i>BTBD1</i>	Tsai et al., 2011
rs7199343	16	73,009,024	$2.4 \times 10^{-6}$	<i>ZFH3</i>	Burgner et al., 2009
rs8059315	16	74,506,447	$8.5 \times 10^{-5}$	<i>GLG1</i>	Burgner et al., 2009



rs2270133	17	61,473,325	$4.6 \times 10^{-5}$	TANC2*	J.J. Kim et al., 2011
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Table 7. Summary of GWAS for KD (II). \* Association was observed between KD patients with CAL and healthy controls. Chr: chromosome.

#### 4. Clinical implementation of genetic findings

The standard treatment for KD is a combination of oral aspirin and high-dose IVIG (Z. Onouchi & Kawasaki, 1999). While the majority of patients respond to this therapy, around 15% are resistant and require additional IVIG or alternative drugs to prevent the development of CAL. As the etiology and pathophysiology of KD are largely unknown, the mechanism of action of these therapies on the disease is not fully understood. The identification of genetic factors that influence patient response to therapy might provide an insight to this problem.

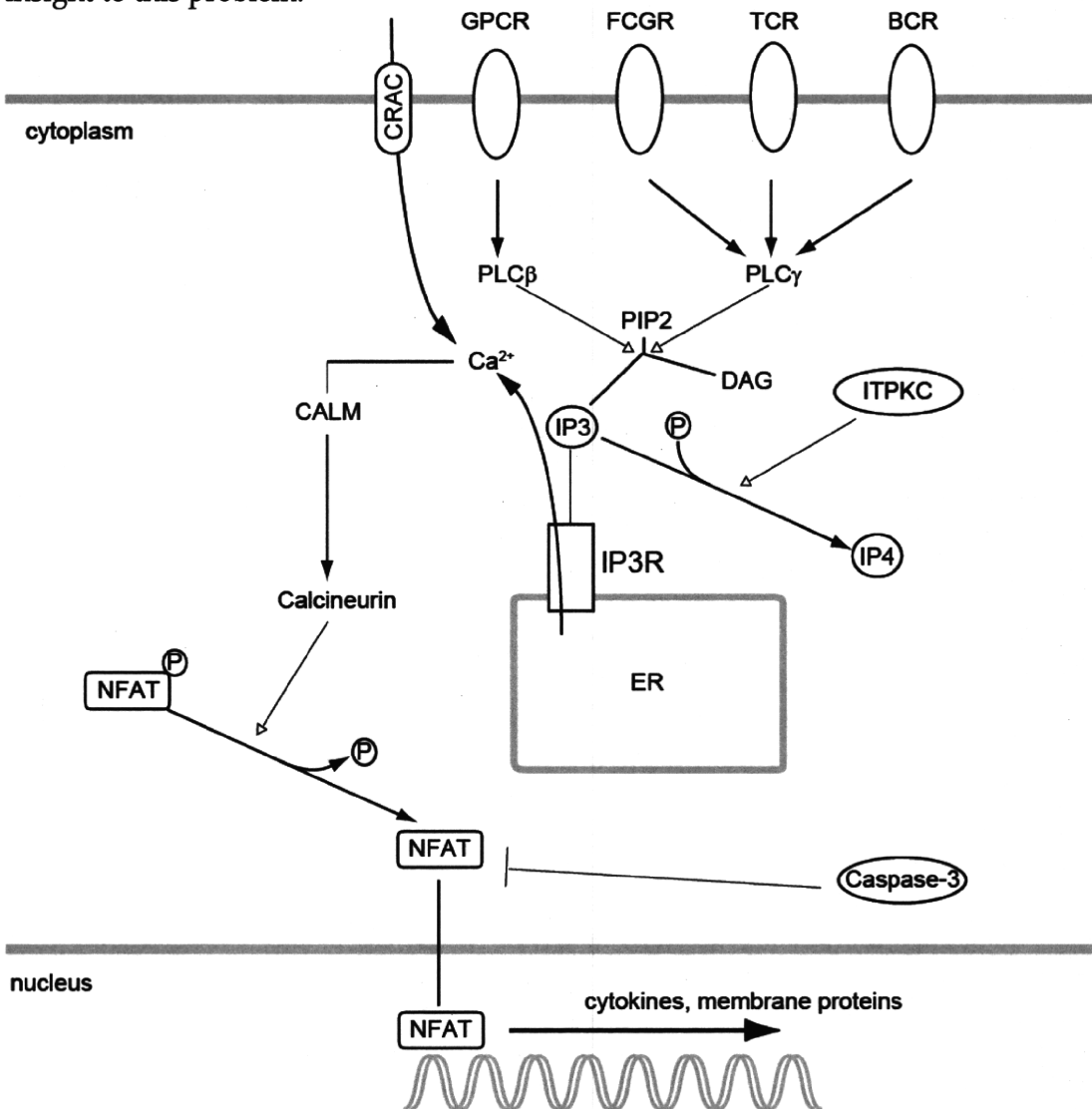


Fig. 5. Possible role of ITPKC and caspase-3 in the immune response. CRAC: calcium release-activated calcium channel, GPCR: G-protein-coupled receptor,

FCGR: Fc gamma receptor, BCR: B cell receptor, TCR: T cell receptor, NFAT: nuclear factor of activated T cells, PLC: phospholipase, PIP2: phosphatidylinositol 4,5-bisphosphate, DAG: diacylglycerol, CALM: calmodulin, IP3R: IP3 receptor, ER: endoplasmic reticulum.

We investigated the association between the functional SNPs in the ITPKC and caspase-3 genes with IVIG unresponsiveness and CAL formation, and found that patients with at least 1 susceptible allele at both SNPs had 2.7–2.9 times higher risk for these unfavorable events (manuscript submitted). Considering that ITPKC and caspase-3 are possibly negative regulators of the Ca<sup>2+</sup>/NFAT pathway (Fig. 5), hyperactivation of the pathway might underlie a more severe clinical manifestation of the disease.

From this point of view, Cyclosporine A, an immunosuppressant drug which potently suppresses the activity of T cells by targeting calcineurin, a key molecule of the Ca<sup>2+</sup>/NFAT pathway (Fig. 5), may be a good option for refractory cases of KD. In Japan, a study to investigate the tolerability, safety, and efficacy of Cyclosporine A for KD has been started.

## 5. Conclusion

Many candidate variations for susceptibility to KD have been reported from candidate gene and genome-wide studies; however, most of the findings from these studies are not robust and have yet to be confirmed by replication studies. Considering the modest odds ratios observed in recent GWAS for complex disorders, discovery studies should be conducted with much larger cohorts. Replication studies in different ethnic groups should be designed with careful attention to their power to detect a significant association and, most importantly, to the difference in the linkage disequilibrium structure. As the majority of KD patients are infants and children and the disease incidence is low, especially in countries outside East Asia, there are limitations in collecting subjects. In an attempt to overcome this difficulty, several consortia have been formed. Those engaged in KD research hope to unravel the mystery of this vasculitis and save these children from damage to their heart. We wish to contribute to this important mission by identifying the underlying genetic components of this disease.

## 6. References

- Ahn, SY.; Jang, GC.; Shin, JS.; Shin, KM. & Kim DS. (2003). Tumor necrosis factor- $\alpha$  levels and promoter polymorphism in patients with Kawasaki disease in Korea. *Yonsei Medical Journal*, Vol.44, No.6, pp. 1021-1026, (December 2003). ISSN 0513-5796
- Amano, S.; Hazama, F. & Hamashima Y. (1979). Pathology of Kawasaki disease: II. Distribution and incidence of the vascular lesions. *Japanese Circulation Journal*, Vol.43, No.8, pp. 741-748, (August 1979), ISSN 0047-1828
- Ari-Ong, S.; Thakkinstian, A.; McEvoy, M. & Attia, J. (2010). A systematic review and meta-analysis of tumor necrosis factor  $\alpha$ -308 polymorphism and Kawasaki disease. *Pediatrics International*, Vol.52, No.4, pp. 527-532, (February 2010), ISSN 1328-8067
- Ban, JY.; Yoon, KL.; Kim, SK.; Kang, S. & Chung, JH. (2009). Promoter polymorphism (rs3755724, -55C/T) of tissue inhibitor of metalloproteinase 4 (TIMP4) as a risk factor for Kawasaki disease with coronary artery lesions in a Korean population. *Pediatric Cardiology*, Vol.30, No.3, pp. 331-335, (April 2009). ISSN 0172-0643
- Ban, JY.; Kim, SK.; Kang, SW.; Yoon, KL. & Chung, JH. (2010). Association between

- polymorphisms of matrix metalloproteinase 11 (MMP-11) and Kawasaki disease in the Korean population. *Life Sciences*, Vol.86, No.19-20, pp. 756-759, (May 2010). ISSN 0024-3205
- Biezeveld, MH.; Kuipers, IM.; Geissler, J.; Lam, J.; Ottenkamp, JJ.; Hack, CE. & Kuijpers, TW. (2003). Association of mannose-binding lectin genotype with cardiovascular abnormalities in Kawasaki disease. *The Lancet*, Vol.361, No.9365, pp. 1268-1270, (April 2003). ISSN 0140-6736
- Biezeveld, MH.; Geissler, J.; Weverling, GJ.; Kuipers, IM.; Lam, J.; Ottenkamp, J. & Kuijpers, TW. (2006). Polymorphisms in the mannose-binding lectin gene as determinants of age-defined risk of coronary artery lesions in Kawasaki disease. *Arthritis & Rheumatism*, Vol.54, No.1, pp. 369-376, (January 2006). ISSN 0004-3591
- Biezeveld, M.; Geissler, J.; Merkus, M.; Kuipers, IM.; Ottenkamp, J. & Kuijpers, T. (2007). The involvement of Fc gamma receptor gene polymorphisms in Kawasaki disease. *Clinical & Experimental Immunology*, Vol.147, No.1, pp. 106-111, (January 2007). ISSN 0009-9104
- Breunis, WB.; Biezeveld, MH.; Geissler, J.; Ottenkamp, J.; Kuipers, IM.; Lam, J.; Hutchinson, A.; Welch, R.; Chanock, SJ. & Kuijpers, TW. (2006). Vascular endothelial growth factor gene haplotypes in Kawasaki disease. *Arthritis & Rheumatism*, Vol.54, No.5, pp. 1588-1594, (May 2006). ISSN 0004-3591
- Breunis, WB.; Biezeveld, MH.; Geissler, J.; Kuipers, IM.; Lam, J.; Ottenkamp, J.; Hutchinson, A.; Welch, R.; Chanock, SJ. & Kuijpers, TW. (2007). Polymorphisms in chemokine receptor genes and susceptibility to Kawasaki disease. *Clinical & Experimental Immunology*, Vol.150, No.1, pp. 83-90, (October 2007). ISSN 0009-9104
- Burgner, D.; Davila, S.; Breunis, WB.; Ng, SB.; Li, Y.; Bonnard, C.; Ling, L.; Wright, VJ.; Thalamuthu, A.; Odam, M.; Shimizu, C.; Burns, JC.; Levin, M.; Kuijpers, TW. & Hibberd, ML; International Kawasaki Disease Genetics Consortium. (2009). A genome-wide association study identifies novel and functionally related susceptibility loci for Kawasaki disease. *PLoS Genetics*, Vol.5, No.1, pp. e1000319, (January 2009). ISSN 1553-7404
- Burns, JC.; Shimizu, C.; Gonzalez, E.; Kulkarni, H.; Patel, S.; Shike, H.; Sundel, RS.; Newburger, JW. & Ahuja SK. (2005). Genetic variations in the receptor-ligand pair CCR5 and CCL3L1 are important determinants of susceptibility to Kawasaki disease. *The Journal of Infectious Diseases*, Vol.192, No.2, pp. 344-349. (July 2005). ISSN 0022-1899
- Burns, JC.; Shimizu, C.; Shike, H.; Newburger, JW.; Sundel, RP.; Baker, AL.; Matsubara, T.; Ishikawa, Y.; Brophy, VA.; Cheng, S.; Grow, MA.; Steiner, LL.; Kono, N. & Cantor RM. (2005). Family-based association analysis implicates IL-4 in susceptibility to Kawasaki disease. *Genes & Immunity*, Vol.6, No.5, pp. 438-444, (August 2005). ISSN 1466-4879
- Chaudhuri, K.; Singh, Ahluwalia, T.; Singh, S.; Binopal, G. & Khullar, M. (2011). Polymorphism in the promoter of the CCL5 gene (CCL5G-403A) in a cohort of North Indian children with Kawasaki disease. A preliminary study. *Clinical & Experimental Rheumatology*, in press. ISSN 0392-856X
- Chen, SY.; Wan, L.; Huang, YC.; Sheu, JJ.; Lan, YC.; Lai, CH.; Lin, CW.; Chang, JS.; Tsai, Y.; Liu, SP.; Lin, YJ. & Tsai, FJ. (2009). Interleukin-18 gene 105A/C genetic polymorphism is associated with the susceptibility of Kawasaki disease. *Journal of*

- Clinical Laboratory Analysis*, Vol.23, No.2, pp. 71-76. (n.d. 2009). ISSN 0887-8013
- Cheung, YF.; Ho, MH.; Ip, WK.; Fok, SF.; Yung, TC. & Lau, YL. (2004). Modulating effects of mannose binding lectin genotype on arterial stiffness in children after Kawasaki disease. *Pediatric Research*, Vol.56, No.4, pp. 591-596, (October 2004).ISSN 0031-3998
- Cheung, YF.; Huang, GY.; Chen, SB.; Liu, XQ.; Xi, L.; Liang, XC.; Huang, MR.; Chen, S.; Huang, LS.; Liu, XQ.; Chan, KW. & Lau, YL. (2008). Inflammatory gene polymorphisms and susceptibility to kawasaki disease and its arterial sequelae. *Pediatrics*, Vol. 122, No.3, pp. e608-614, (September 2008). ISSN 0031-4005
- Chi, H.; Huang, FY.; Chen, MR.; Chiu, NC.; Lee, HC.; Lin, SP.; Chen, WF.; Lin, CL.; Chan, HW.; Liu, HF.; Huang, LM. & Lee, YJ. (2010). ITPKC gene SNP rs28493229 and Kawasaki disease in Taiwanese children. *Human Molecular Genetics*, Vol.15, No.6, pp. 1147-1151, (March 2010). ISSN 1434-5161
- Chien, YH.; Chang, KW.; Yang, YH.; Lu, MY.; Lin, YT. & Chiang BL. (2003). Association between levels of TNF-alpha and TNF-alpha promoter -308 A/A polymorphism in children with Kawasaki disease. *Journal of the Formosan Medical Association*, Vol.102, No. 3, pp. 147-150, (March 2003). ISSN 0929-6646
- Chun, JK.; Kang, DW.; Yoo, BW.; Shin, JS. & Kim, DS. (2010). Programmed death-1 (PD-1) gene polymorphisms lodged in the genetic predispositions of Kawasaki Disease. *European Journal of Pediatrics*, Vol.169, No.2, pp. 181-185, (February 2010). ISSN 0340-6199
- Fujita, Y.; Nakamura, Y.; Sakata, K.; Hara, N.; Kobayashi, M.; Nagai, M.; Yanagawa, H. & Kawasaki, T. (1989). Kawasaki disease in families. *Pediatrics*, Vol.84, No.4, pp. 666-669, (October 1989). ISSN 1098-4275
- Fukazawa, R.; Sonobe, T.; Hamamoto, K.; Hamaoka, K.; Sakata, K.; Asano, T.; Imai, T.; Kamisago, M.; Ohkubo, T.; Uchikoba, Y.; Ikegami, E.; Watanabe, M. & Ogawa, S. (2004). Possible synergic effect of angiotensin-I converting enzyme gene insertion/deletion polymorphism and angiotensin-II type-1 receptor 1166A/C gene polymorphism on ischemic heart disease in patients with Kawasaki disease. *Pediatric Research*, Vol.56, No.4, pp. 597-601, (October 2004). ISSN 0031-3998
- Furuno, K.; Takada, H.; Yamamoto, K.; Ikeda, K.; Ohno, T.; Khajoev, V.; Mizuno, Y. & Hara T. (2007). Tissue inhibitor of metalloproteinase 2 and coronary artery lesions in Kawasaki disease. *The Journal of Pediatrics*, Vol.151, No.2, pp. 155-160, (August 2007). ISSN 1085-8695
- Holman, RC.; Christensen, KY.; Belay, ED.; Steiner, CA.; Effler, PV.; Miyamura, J.; Forbes, S.; Schonberger, LB. & Melish, M. (2010). Racial/ethnic differences in the incidence of Kawasaki syndrome among children in Hawaii. *Hawaii Medical Journal*, Vol.69, No.8, pp. 194-197, (August 2010). ISSN 0017-8594
- Hong, YM.; Jin, HS.; Park, IS. & Hong, SJ. Association of the matrix metalloproteinase-3 (-439C/G) promoter polymorphism with Kawasaki disease in Korean children. *Heart and Vessels*, Vol.23, No.5, pp. 341-347, (September 2008). ISSN 0910-8327
- Hsieh, YY.; Lin, YJ.; Chang, CC.; Chen, DY.; Hsu, CM.; Wang, YK.; Hsu, KH. & Tsai FJ. (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. *Journal of Clinical Laboratory Analysis*, Vol.24, No.4, pp. 262-268, (n.d. 2010). ISSN 0887-8013
- Hsueh, KC.; Lin, YJ.; Chang, JS.; Wan, L.; Tsai, YH.; Tsai, CH.; Chen, CP. & Tsai FJ. (2008).