心障害の発症、非発症の差を検出するには、患者の 10%が心障害発症群となると仮定すると、300 例の解析可能な患者がエントリーできた場合、270 例が心障害の発生無し、30 例が発生ありとなる。この状態での、心障害発生無し群における、疫学要因の暴露割合(頻度)と心障害発生群の割合の差がどの程度あれば、両側検定、有意水準 5%で、検出率 80%となるかを計算した(表 3)。この結果から本研究の想定の 300 例の患者では、暴露頻度の差が約 20%を超える要因についての検出が期待される。これはオッズ比は 3 以上または 0.33 未満に相当している。

D 考察

我々は、川崎病への MMP 遺伝子群の関与を中心に、遺伝疫学的な解析を行ってきた。しかしながら、本年度の大部分は、患者のリクルートの方法、検体の処理、輸送体制の構築、シクロスポリン投与のプロトコール作成に多くの時間がかかり、遺伝子解析に必要な十分なサンプル数が集まらなかった。そのため、複数 MMP 遺伝子の一般集団における遺伝子多型の頻度調査と連鎖不平衡地図の作成を主に行なった。また、今後、本研究期間中にリクルート可能な症例数から見込める疾患発症、冠動脈瘤などの発症に関係する疫学指標の検出力を検討した。遺伝子多型からの川崎病患者の IVIG 不応例、心障害発症予測に関しては、ITPKC の SNP itpkc_3 (rs28493229)について冠動脈瘤有り群と無し群間での OR は 1.22 であり、川崎病患者内での心障害の発症の有無と遺伝子多型の相関を見いだすことは、本研究で想定している患者数では不可能であると思われる。IVIG 不応例に関しても川崎病患者のたかだか十数パーセントであることから状況は同じである。遺伝子多型を川崎病の診療に生かすことができる可能性としては、シクロスポリン投与例でのシクロスポリンの代謝、血中濃度に影響を与える可能性のある候補遺伝子の検討が考えられる。この薬物代謝に関わる候補遺伝子としては、CYP3A4、CYP3A5、MDR1 などが考えられる。しかしながら、IVIG の 2 回投与でも無効でシクロスポリン投与となる症例数も数十例以下とみられ、血中濃度では数倍、効果の有無の判断でも 5−6 以上の OR を示す要因でないと検出は難しいことが予想される。

E 結論

川崎病の発症リスクに影響を与える遺伝子、遺伝子多型の発見に向けた基本調査を行なった。

F 健康危険情報

該当無し。

G 研究発表

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2. 学会発表

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(3) 保育所通園歴の有無による血清 IgE 値の変化はCD14遺伝子-550C/Tの遺伝型に影響される 服部 聡、真下陽一、船水真紀子、下条直樹、岡本美孝、河野陽一、羽田 明、鈴木洋一 日本アレルギー学会平成 20 年 10 月 27-29 日 東京

(4) アレルギー感作における Gene-Environmental Interaction について（シンポジウム） 日本小児アレルギー学会平成20年12月13-14日 横浜

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H 知的財産権の出願・登録状況（予定を含む。）

該当無し。

図1 MMP遺伝子クラスター領域

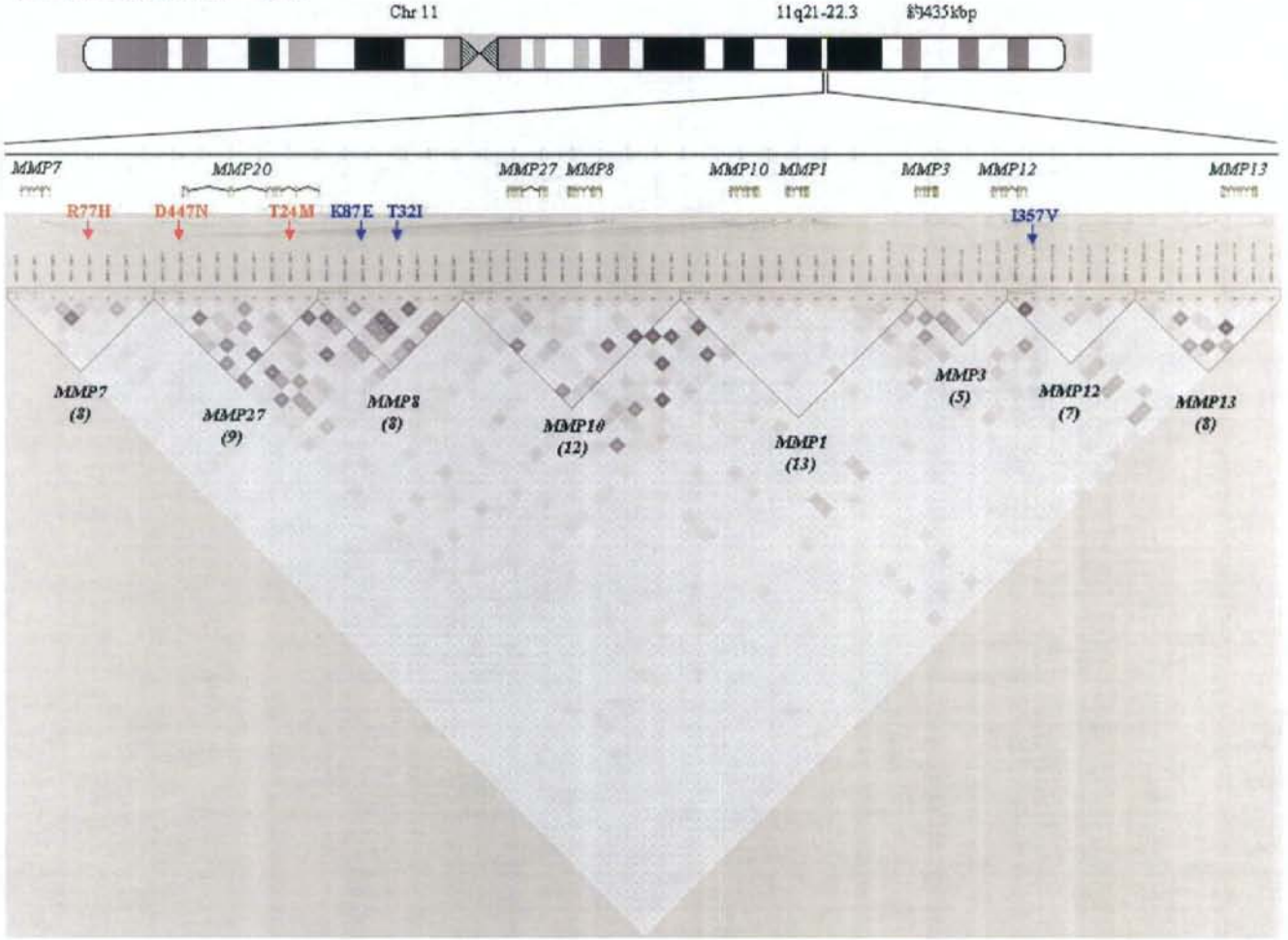
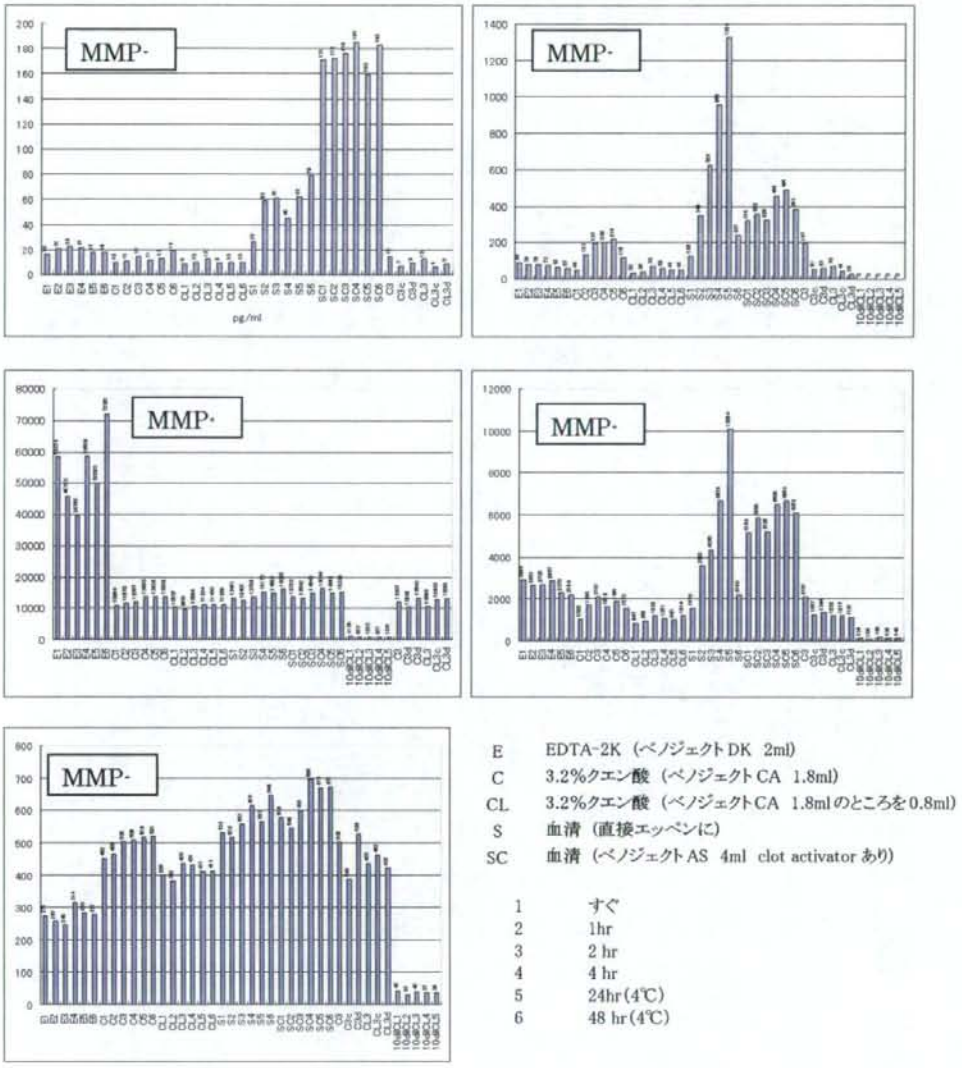


図1

図 2 各種マトリックスメタロプロテアーゼの血清、血漿中蛋白レベル



E　EDTA-2K（ベノジェクトDK　2ml）
C　3.2%クエン酸（ベノジェクトCA　1.8ml）
CL　3.2%クエン酸（ベノジェクトCA　1.8mlのところを0.8ml）
S　血清（直接エッペンに）
SC　血清（ベノジェクトAS　4ml　clot activator あり）

1　すぐ
2　1hr
3　2 hr
4　4 hr
5　24hr(4℃)
6　48 hr(4℃)

濃度の単位は pg/ml。上図に示していない MMP12、MMP13 は測定感度以下であった。

表1 MMP 遺伝子群の一塩基多型(SNP)　一般集団における遺伝子型とアレル頻度

gene	genotype/allele	number (%)
MMP1		
SNP1	Genotyp G/G	233 (0.693)
	G/A	92 (0.274)
	A/A	11 (0.033)
	Allele G	558 (0.830)
	A	114 (0.170)
SNP3	Genotyp T/T	200 (0.610)
	T/C	115 (0.351)
	C/C	13 (0.040)
	Allele T	515 (0.785)
	C	141 (0.215)
SNP4	Genotyp G/G	81 (0.253)
	G/T	158 (0.494)
	T/T	81 (0.253)
	Allele G	320 (0.500)
	T	320 (0.500)
SNP5	Genotyp A/A	258 (0.772)
	A/G	72 (0.216)
	G/G	4 (0.012)
	Allele A	588 (0.880)
	G	80 (0.120)
SNP6	Genotyp A/A	98 (0.293)
	A/T	177 (0.530)
	T/T	59 (0.177)
	Allele A	373 (0.558)
	T	295 (0.442)
SNP7	Genotyp T/T	236 (0.704)
	T/C	90 (0.269)
	C/C	9 (0.027)
	Allele T	562 (0.839)
	C	108 (0.161)
SNP8	Genotyp G/G	143 (0.427)
	G/A	147 (0.439)
	A/A	45 (0.134)
	Allele G	433 (0.646)
	A	237 (0.354)
SNP9	Genotyp G/G	249 (0.741)
	G/A	82 (0.244)
	A/A	5 (0.015)
	Allele G	580 (0.863)
	A	92 (0.137)
SNP10	Genotyp A/A	236 (0.704)
	A/G	90 (0.269)
	G/G	9 (0.027)
	Allele A	562 (0.839)
	G	108 (0.161)
SNP11	Genotyp C/C	128 (0.376)
	C/T	168 (0.501)
	T/T	41 (0.122)
	Allele C	420 (0.627)
	T	250 (0.373)
SNP12	Genotyp T/T	202 (0.603)
	T/C	113 (0.337)
	C/C	20 (0.060)
	Allele T	517 (0.772)
	C	153 (0.228)
SNP13	Genotyp G/G	312 (0.929)
	G/A	24 (0.071)
	A/A	0 (0.000)
	Allele G	648 (0.964)
	A	24 (0.036)
MMP7		
rs181	Genotyp A/A	297 (0.884)
	A/G	39 (0.116)
	G/G	0 (0.000)
	Allele A	633 (0.942)
	G	39 (0.058)
SNP2	Genotyp G/G	177 (0.527)
	G/A	139 (0.414)
	A/A	20 (0.060)
	Allele G	493 (0.734)
	A	179 (0.266)
SNP3	Genotyp T/T	119 (0.355)
	T/C	159 (0.475)
	C/C	57 (0.170)
	Allele T	397 (0.593)
	C	273 (0.407)
SNP4	Genotyp G/G	233 (0.693)
	G/A	92 (0.274)
	A/A	11 (0.033)
	Allele G	558 (0.830)
	A	114 (0.170)
SNP5	Genotyp T/T	280 (0.833)
	T/G	55 (0.164)
	G/G	1 (0.003)
	Allele T	615 (0.915)
	G	57 (0.085)
SNP6	Genotyp C/C	229 (0.682)
	C/T	97 (0.289)
	T/T	10 (0.030)
	Allele C	555 (0.826)
	T	117 (0.174)
SNP7	Genotyp A/A	99 (0.295)
	A/G	186 (0.554)
	G/G	51 (0.152)
	Allele A	384 (0.571)
	G	288 (0.429)
SNP8	Genotyp T/T	128 (0.388)
	T/C	152 (0.461)
	C/C	50 (0.152)
	Allele T	408 (0.618)
	C	252 (0.382)

gene	genotype/allele	number (%)
MMP3		
rs1612	Genotype 6T/6T	243 (0.723)
	6T/5T	85 (0.253)
	5T/5T	8 (0.024)
	Allele 6T	571 (0.850)
	5T	101 (0.150)
SNP1	Genotype A/A	118 (0.351)
	A/G	171 (0.509)
	G/G	47 (0.140)
	Allele A	407 (0.606)
	G	265 (0.394)
SNP2	Genotype G/G	150 (0.446)
	G/A	149 (0.443)
	A/A	37 (0.110)
	Allele G	449 (0.668)
	A	223 (0.332)
SNP3	Genotype A/A	256 (0.762)
	A/T	77 (0.229)
	T/T	3 (0.009)
	Allele A	589 (0.876)
	T	83 (0.124)
SNP4	Genotype T/T	91 (0.271)
	T/C	184 (0.548)
	C/C	61 (0.182)
	Allele T	366 (0.545)
	C	306 (0.455)
MMP8		
SNP1	Genotype T/T	148 (0.442)
	T/C	158 (0.472)
	C/C	29 (0.087)
	Allele T	454 (0.678)
	C	216 (0.322)
SNP2	Genotype T/T	283 (0.845)
	T/C	50 (0.149)
	C/C	2 (0.006)
	Allele T	616 (0.919)
	C	54 (0.081)
SNP3	Genotype T/T	180 (0.537)
	T/G	139 (0.415)
	G/G	16 (0.048)
	Allele T	499 (0.745)
	G	171 (0.255)
SNP4	Genotype T/T	182 (0.543)
	T/C	138 (0.412)
	C/C	15 (0.045)
	Allele T	502 (0.749)
	C	168 (0.251)
SNP5	Genotype G/G	294 (0.675)
	G/A	41 (0.122)
	A/A	1 (0.003)
	Allele G	629 (0.936)
	A	43 (0.064)
SNP6	Genotype G/G	184 (0.548)
	G/A	137 (0.408)
	A/A	15 (0.045)
	Allele G	505 (0.751)
	A	167 (0.249)
SNP7	Genotype C/C	224 (0.667)
	C/T	105 (0.313)
	T/T	7 (0.021)
	Allele C	553 (0.823)
	T	119 (0.177)
SNP8	Genotype G/G	168 (0.500)
	G/T	136 (0.405)
	T/T	32 (0.095)
	Allele G	472 (0.702)
	T	200 (0.298)
MMP12		
SNP1	Genotype A/A	323 (0.961)
	A/G	13 (0.039)
	G/G	0 (0.000)
	Allele A	659 (0.981)
	G	13 (0.019)
SNP2	Genotype C/G	264 (0.793)
	C/G	63 (0.189)
	G/G	6 (0.018)
	Allele C	591 (0.887)
	G	75 (0.113)
SNP3	Genotype T/T	180 (0.541)
	T/C	125 (0.375)
	C/C	28 (0.084)
	Allele T	485 (0.728)
	C	181 (0.272)
SNP4	Genotype G/G	142 (0.426)
	G/C	144 (0.432)
	C/C	47 (0.141)
	Allele G	428 (0.643)
	C	238 (0.357)
SNP5	Genotype G/G	253 (0.757)
	G/A	76 (0.228)
	A/A	5 (0.015)
	Allele G	582 (0.871)
	A	86 (0.129)
SNP6	Genotype A/A	307 (0.922)
	A/G	26 (0.078)
	G/G	0 (0.000)
	Allele A	640 (0.961)
	G	26 (0.039)
SNP7	Genotype A/A	307 (0.922)
	A/G	26 (0.078)
	G/G	0 (0.000)
	Allele A	640 (0.961)
	G	26 (0.039)

gene	genotype/allele	number (%)
MMP10		
SNP1	Genotype C/C	130 (0.387)
	C/T	165 (0.491)
	T/T	41 (0.122)
	Allele C	425 (0.632)
	T	247 (0.368)
SNP2	Genotype G/G	252 (0.750)
	G/A	81 (0.241)
	A/A	3 (0.009)
	Allele G	585 (0.871)
	A	87 (0.129)
SNP3	Genotype T/T	202 (0.601)
	T/C	114 (0.339)
	C/C	20 (0.060)
	Allele T	518 (0.771)
	C	154 (0.229)
SNP4	Genotype G/G	312 (0.929)
	G/C	24 (0.071)
	C/C	0 (0.000)
	Allele G	648 (0.964)
	C	24 (0.036)
SNP5	Genotype CA/CA	133 (0.397)
	CA/-	162 (0.484)
	-/-	40 (0.119)
	Allele CA	428 (0.639)
	-	242 (0.361)
SNP6	Genotype C/C	297 (0.887)
	C/G	36 (0.107)
	G/G	2 (0.006)
	Allele C	630 (0.940)
	G	40 (0.060)
SNP7	Genotype T/T	250 (0.744)
	T/A	80 (0.238)
	A/A	6 (0.018)
	Allele T	580 (0.863)
	A	92 (0.137)
SNP8	Genotype C/C	95 (0.283)
	C/T	172 (0.512)
	T/T	69 (0.205)
	Allele C	362 (0.539)
	T	310 (0.461)
SNP9	Genotype C/C	234 (0.696)
	C/T	93 (0.277)
	T/T	9 (0.027)
	Allele C	561 (0.835)
	T	111 (0.165)
SNP10	Genotype G/G	123 (0.366)
	G/T	160 (0.476)
	T/T	53 (0.158)
	Allele G	406 (0.604)
	T	266 (0.396)
SNP11	Genotype G/G	270 (0.804)
	G/A	61 (0.182)
	A/A	5 (0.015)
	Allele G	601 (0.894)
	A	71 (0.106)
SNP12	Genotype T/T	258 (0.768)
	T/A	71 (0.211)
	A/A	7 (0.021)
	Allele T	587 (0.874)
	A	85 (0.126)
MMP17		
SNP1	Genotype A/A	100 (0.444)
	A/C	96 (0.427)
	C/C	29 (0.129)
	Allele A	296 (0.658)
	C/C	154 (0.342)
SNP5	Genotype C/C	88 (0.395)
	C/T	90 (0.404)
	T/T	45 (0.202)
	Allele C	266 (0.596)
	T/T	180 (0.404)
SNP9	Genotype G/G	124 (0.301)
	G/A	207 (0.502)
	A/A	81 (0.197)
	Allele G	455 (0.552)
	A	369 (0.448)
SNP10	Genotype G/G	302 (0.731)
	G/A	102 (0.247)
	A/A	9 (0.022)
	Allele G	706 (0.855)
	A	120 (0.145)
SNP13	Genotype A/A	119 (0.290)
	A/C	180 (0.439)
	C/C	111 (0.271)
	Allele A	418 (0.510)
	C/C	402 (0.490)
SNP17	Genotype C/C	116 (0.520)
	C/G	84 (0.377)
	G/G	23 (0.103)
	Allele C	316 (0.709)
	G/G	130 (0.291)
SNP19	Genotype T/T	105 (0.477)
	T/G	92 (0.418)
	G/G	23 (0.105)
	Allele T	302 (0.686)
	G	138 (0.314)
SNP21	Genotype G/G	207 (0.932)
	G/A	15 (0.068)
	A/A	0 (0.000)
	Allele G	429 (0.966)
	A	15 (0.034)

gene	genotype/allele	number (%)
MMP13		
SNP1	Genotyp C/C	161 (0.483)
	C/T	142 (0.426)
	T/T	30 (0.090)
	Allele C	464 (0.697)
	T	202 (0.303)
SNP2	Genotyp G/G	308 (0.925)
	G/A	23 (0.069)
	A/A	2 (0.006)
	Allele G	639 (0.959)
	A	27 (0.041)
SNP3	Genotyp T/T	222 (0.667)
	T/A	102 (0.306)
	A/A	9 (0.027)
	Allele T	546 (0.820)
	A	120 (0.180)
SNP4	Genotyp G/G	87 (0.261)
	G/A	170 (0.511)
	A/A	76 (0.228)
	Allele G	344 (0.517)
	A	322 (0.483)
SNP5	Genotyp C/C	298 (0.895)
	C/A	33 (0.099)
	A/A	2 (0.006)
	Allele C	629 (0.944)
	A	37 (0.056)
SNP6	Genotyp A/A	193 (0.580)
	A/G	123 (0.369)
	G/G	17 (0.051)
	Allele A	509 (0.764)
	G	157 (0.236)
SNP7	Genotyp A/A	198 (0.593)
	A/G	118 (0.353)
	G/G	18 (0.054)
	Allele A	514 (0.769)
	G	154 (0.231)
SNP8	Genotyp T/T	308 (0.925)
	T/A	23 (0.069)
	A/A	2 (0.006)
	Allele T	639 (0.959)
	A	27 (0.041)
MMP25		
SNP2	Genotyp C/C	264 (0.786)
	C/A	70 (0.208)
	A/A	2 (0.006)
	Allele C	598 (0.890)
	A	74 (0.110)
SNP3	Genotyp T/T	150 (0.446)
	T/A	152 (0.452)
	A/A	34 (0.101)
	Allele T	452 (0.673)
	A	220 (0.327)
SNP4	Genotyp T/T	125 (0.372)
	T/C	157 (0.467)
	C/C	54 (0.161)
	Allele T	407 (0.606)
	C	265 (0.394)
SNP5	Genotyp G/G	251 (0.747)
	G/A	81 (0.241)
	A/A	4 (0.012)
	Allele G	583 (0.868)
	A	89 (0.132)
SNP6	Genotyp A/A	159 (0.473)
	A/C	147 (0.438)
	C/C	30 (0.089)
	Allele A	465 (0.692)
	C	207 (0.308)
SNP7	Genotyp G/G	207 (0.616)
	G/A	115 (0.342)
	A/A	14 (0.042)
	Allele G	529 (0.787)
	A	143 (0.213)
SNP8	Genotyp T/T	281 (0.836)
	T/A	53 (0.158)
	A/A	2 (0.006)
	Allele T	615 (0.915)
	A	57 (0.085)
SNP9	Genotyp T/T	221 (0.660)
	T/C	105 (0.313)
	C/C	9 (0.027)
	Allele T	547 (0.816)
	C	123 (0.184)

gene	genotype/allele	number (%)
MMP24		
SNP1	Genotyp G/G	78 (0.232)
	G/A	177 (0.527)
	A/A	81 (0.241)
	Allele G	333 (0.496)
	A	339 (0.504)
SNP2	Genotyp C/C	175 (0.521)
	C/G	142 (0.423)
	G/G	19 (0.057)
	Allele C	492 (0.732)
	G	180 (0.268)
SNP3	Genotyp G/G	275 (0.823)
	G/A	57 (0.171)
	A/A	2 (0.006)
	Allele G	607 (0.909)
	A	61 (0.091)
SNP4	Genotyp T/T	286 (0.851)
	T/C	48 (0.143)
	C/C	2 (0.006)
	Allele T	620 (0.923)
	C	52 (0.077)
SNP5	Genotyp A/A	272 (0.810)
	A/G	59 (0.176)
	G/G	5 (0.015)
	Allele A	603 (0.897)
	G	69 (0.103)
SNP6	Genotyp C/C	273 (0.813)
	C/T	56 (0.167)
	T/T	7 (0.021)
	Allele C	602 (0.896)
	T	70 (0.104)
SNP7	Genotyp C/C	264 (0.786)
	C/T	67 (0.199)
	T/T	5 (0.015)
	Allele C	595 (0.885)
	T	77 (0.115)
SNP8	Genotyp A/A	208 (0.621)
	A/C	111 (0.331)
	C/C	16 (0.048)
	Allele A	527 (0.787)
	C	143 (0.213)
SNP9	Genotyp A/A	226 (0.673)
	A/G	97 (0.289)
	G/G	13 (0.039)
	Allele A	549 (0.817)
	G	123 (0.183)
SNP10	Genotyp A/A	186 (0.555)
	A/G	130 (0.388)
	G/G	19 (0.057)
	Allele A	502 (0.749)
	G	168 (0.251)
MMP27		
SNP1	Genotyp C/C	209 (0.633)
	C/T	112 (0.339)
	T/T	9 (0.027)
	Allele C	530 (0.803)
	T	130 (0.197)
SNP2	Genotyp T/T	172 (0.518)
	T/C	130 (0.392)
	C/C	30 (0.090)
	Allele T	474 (0.714)
	C	190 (0.286)
SNP3	Genotyp G/G	289 (0.876)
	G/A	41 (0.124)
	A/A	0 (0.000)
	Allele G	619 (0.938)
	A	41 (0.062)
SNP4	Genotyp G/G	82 (0.245)
	G/T	164 (0.490)
	T/T	89 (0.266)
	Allele G	328 (0.490)
	T	342 (0.510)
SNP5	Genotyp T/T	94 (0.281)
	T/A	170 (0.507)
	A/A	71 (0.212)
	Allele T	358 (0.534)
	A	312 (0.466)
SNP6	Genotyp A/A	282 (0.842)
	A/T	51 (0.152)
	T/T	2 (0.006)
	Allele A	615 (0.918)
	T	55 (0.082)
SNP7	Genotyp C/C	210 (0.627)
	C/T	117 (0.349)
	T/T	8 (0.024)
	Allele C	537 (0.801)
	T	133 (0.199)
SNP8	Genotyp G/G	287 (0.857)
	G/A	47 (0.140)
	A/A	1 (0.003)
	Allele G	621 (0.927)
	A	49 (0.073)
SNP9	Genotyp C/C	187 (0.558)
	C/T	126 (0.376)
	T/T	22 (0.066)
	Allele C	500 (0.746)
	T	170 (0.254)

表 2　300 例の川崎病(MCLS)患者と 350 例の対象集団の症例対照研究において、遺伝子多型と疾患との相関を検出できるアレル頻度差とオッズ比(OR)
検定は両側検定、有意水準＝0.05、検出力=80%の場合

MCLS 群でアレル頻度が上昇する場合

対照	患者	頻度差	OR	Lower 95%CI	Upper 95%CI
0.050	0.090	0.04	1.88	2.16	16.1
0.075	0.127	0.05	1.67	1.16	2.41
0.100	0.152	0.05	1.61	1.16	2.25
0.200	0.256	0.06	1.45	1.12	1.68
0.300	0.374	0.07	1.39	1.11	1.74
0.400	0.477	0.08	1.37	1.10	1.71
0.500	0.578	0.08	1.37	1.10	1.71
0.600	0.675	0.08	1.38	1.10	1.71
0.700	0.769	0.07	1.43	1.11	1.83
0.800	0.859	0.06	1.52	1.13	2.05
0.900	0.942	0.04	1.80	1.18	2.75

MCLS 群でアレル頻度が低下する場合

対照	患者	頻度差	OR	Lower 95%CI	Upper 95%CI
0.050	0.021	−0.029	0.41	0.22	0.79
0.075	0.039	−0.036	0.50	0.30	0.83
0.100	0.058	−0.04	0.55	0.36	0.85
0.200	0.142	−0.06	0.56	0.49	0.89
0.300	0.231	−0.07	0.70	0.55	0.90
0.400	0.325	−0.08	0.72	0.57	0.91
0.500	0.422	−0.08	0.73	0.59	0.91
0.600	0.532	−0.07	0.73	0.59	0.91
0.700	0.626	−0.07	0.72	0.57	0.90
0.800	0.734	−0.07	0.69	0.53	0.89
0.900	0.848	−0.05	0.62	0.44	0.86

表 3　300 例の川崎病患者において、冠動脈瘤の出現との相関を検出できる要因の割合差とオッズ比(OR)

川崎病患者の 10%に冠動脈瘤が発症する(CAL+)と仮定、
検定は両側検定、有意水準＝0.05、検出力=80%の場合

CAL+群で暴露割合が上昇する場合

CAL−	CAL+	割合差	OR	Lower 95%CI	Upper 95%CI
0.05	0.24	0.19	5.90	2.16	16.10
0.08	0.28	0.20	4.75	1.90	11.89
0.10	0.22	0.12	4.16	1.75	9.90
0.20	0.45	0.25	3.26	1.50	7.10
0.30	0.56	0.26	3.02	1.40	6.50
0.40	0.67	0.27	2.99	1.35	6.64
0.50	0.76	0.26	3.12	1.31	7.42
0.60	0.84	0.24	3.47	1.27	9.48
0.70	0.91	0.21	4.39	1.22	15.86
0.80	0.97	0.17	8.08	0.97	67.26

CAL＋群で暴露割合が低下する場合

CAL−	CAL+	割合差	OR	Lower 95%CI	Upper 95%CI
0.20	0.03	−0.17	0.12	0.02	1.03
0.30	0.09	−0.21	0.23	0.06	0.82
0.40	0.16	−0.24	0.29	0.11	0.79
0.50	0.24	−0.26	0.32	0.13	0.76
0.60	0.33	−0.27	0.33	0.52	0.74
0.70	0.44	−0.26	0.33	0.15	0.71
0.80	0.55	−0.25	0.31	0.14	0.67
0.90	0.68	−0.22	0.24	0.10	0.57

厚生労働科学研究費補助金（創薬基盤推進研究事業）
分担研究報告書

統一プロトコールによる川崎病の治療と遺伝子解析－１

研究分担者　寺井　勝　東京女子医科大学八千代医療センター小児科　教授

研究要旨:

研究代表者を中心に協議を重ねて川崎病の統一治療プロトコールを作成した。本研究では、東京女子医科大学八千代医療センターならびに関連施設における川崎病患者に対してシクロスポリン A の治療効果ならびに安全性を検討、さらに川崎病感受性遺伝子である ITPKC のタイプとの関連を解析することを目的とした。東京女子医科大学八千代医療センターにおける 2008 年 1 月～09 年 3 月の新規川崎病入院患者のうち IVIG+アスピリン療法の初期投与をおこなった 73 例を対象とした。初回 IVIG に反応せず解熱が得られなかった 16 例（21.9%）に追加 IVIG （2g/kg）を施行した。このうち、9 例は追加 IVIG に反応し、解熱が得られた。一方、追加 IVIG 投与にも解熱しなかった 7 例の患者のうち、月齢 4 ヵ月以上の 6 例の患者に対してシクロスポリン 4mg/kg を経口で 3 週間投与した。その結果、6 例中 4 例ではすみやかな解熱効果が得られたが、2 例では解熱効果が得られずシクロスポリンを増量した。このうち 1 例(1.3%)は、シクロスポリン A 用量を 8mg/kg に上げても解熱せず、冠状動脈に中等度の動脈瘤（4-5mm）が出現した。残りの 72 例（98.7%）には冠動脈病変は認めなかった。シクロスポリンによる重篤な有害事象は認められなかった。関連施設においては、現在、協力を得られた施設において倫理委員会に申請中である。

A　研究目的:

本研究の目的は、川崎病においていわゆる免疫グロブリン（IVIG）不応症例に対して、新たにシクロスポリン A を用いたプロトコールを当該研究班で作成し、治療投与の有効性と安全性を確かめるものである。IVIG 不応症例では冠動脈瘤の発生頻度が高く、また有効な治療法が確立していないため新たな治療法の開発が急務である。免疫抑制剤であるシクロスポリン A は、尾内、羽田が発見した川崎病感受性遺伝子である ITPKC 遺伝子が関与する炎症活性化の経路を抑制することが知られていることから、川崎病の治療にシクロスポリンが有効であることが期待される。

B　研究方法

治療プロトコールを主任研究者の統括のもとに作成した。本治療プロトコールでは、IVIG 治療の反応性、シクロスポリンの有効性と ITPKC 遺伝子型の相関を 3 年間かけて解析する。また、シクロスポリン治療そのものの問題点、安全性を 3 年間かけて検証する。

C　研究結果

08年1月～09年3月に新規川崎病として入院した患者のうち、IVIG＋アスピリン療法の初期投与をおこなった73例を対象とした。このうち、初回IVIGに反応せず、解熱が得られなかった16例（21.9%）に追加IVIG（2g/kg）を施行した。このうち、9例は追加IVIGに反応し、解熱が得られた。一方、追加IVIG投与にも解熱しなかった7例の患者のうち、プロトコールに準じて、月齢4ヵ月以上の6例の患者に対してシクロスポリン4mg/kgを経口（分2、食前）で3週間投与した。その結果、6例中4例ではすみやかな解熱効果が得られ、検査所見も改善した。一方、2例では解熱効果が得られずシクロスポリンを増量した。このうち1例(1.3%)は、シクロスポリンAの用量を8mg/kgに上げても解熱せず、冠状動脈に中等度の動脈瘤（4-5mm)が出現した。残りの72例（98.7%）には冠動脈病変は認めなかった。シクロスポリンによる重篤な有害事象は認められなかった。冠動脈瘤を発生した1例に一過性の多毛がみられた。また、臨床症状のない高K血症を一過性に認めた。

D　考察：

シクロスポリンに反応する症例は劇的に効果があることが示された。ただし、シクロスポリンに反応しない症例も少なからず存在し、そのITPKC遺伝子型を分担研究者の尾内が解析している。

E　結論

今後も症例を積み重ねて解析し、場合によってはシクロスポリンのプロトコールの改訂を行っていく必要がある。また、関連施設の協力を得て、多施設研究として行う場合の診療上の問題点などを明らかにしていく必要がある。また、IVIG不応症例のサイトカインプロファイルを解析することを今後予定している。

F　健康危険情報：　該当せず

G　研究発表：

1．論文発表

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H　知的財産権の出願・登録状況（予定を含む。）：　該当せず

厚生労働科学研究費補助金（創薬基盤推進研究事業）
分担研究報告書

統一プロトコールによる川崎病の治療と遺伝子解析

研究分担者　鈴木啓之　（歌山県立医科大学小児科　准教授

研究要旨

研究代表者を中心に協議を重ねて川崎病の統一治療プロトコールを作成した。本研究では、川崎病初期治療において初回 IVIG および追加 IVIG に対する反応性、さらに追加 IVIG に不応な症例に対するシクロスポリン A(CyA)の治療効果ならびに安全性を検討し、プロトコールに同意を得て参加した全症例の川崎病感受性遺伝子である ITPKC のタイプとの関連を解析することを目的とした。和歌山県立医科大学小児科および関連 6 施設において平成 20 年 5 月から統一プロトコールでの治療を開始した。

平成 20 年 5 月から平成 21 年 3 月 17 日までに和歌山医大小児科に入院した新規川崎病入院患児の内、統一プロトコール治療を行った 19 例を対象とした。この内当科で初期治療を行ったのは 14 例、他の 5 例は関連施設での初回・追加 IVIG 不応の転院症例であった。当科で初期治療を行った 14 例の内訳は、初回 IVIG 反応例 11 例、追加 IVIG 反応例 2 例、初回・追加 IVIG 不応例は 1 例であった。この 1 例と関連施設からの転院 5 例の計 6 例に CyA を経口で 2-3 週間投与した。6 例中 5 例では速やかな解熱効果が得られたが、1 例では充分な解熱効果が得られず CyA を 5mg/kg に増量し、解熱した。冠動脈病変は CyA 投与 6 例中 1 例に生じたが、本症例は追加 IVIG 中にすでに冠動脈拡張を生じ、CyA を開始した 8 病日に右側冠動脈に 5-6mm の瘤を認めた。CyA 投与後に速やかに解熱したが、瘤は 10-11mm 大の巨大瘤に進展した。CyA による重篤な有害事象は認められなかった。

関連 6 施設の倫理申請が平成 21 年 1 月までに承認され、2 月から症例登録を開始し、統一治療プロトコール＋DNA 採取を開始した。現在登録症例は 7 例である。

A. 研究目的

本研究の目的は、川崎病治療において統一プロトコールで治療を行い、初回 IVIG および追加 IVIG に対する反応性、さらに追加 IVIG に不応な症例に対するシクロスポリン A(CyA)の治療効果ならびに安全性を確かめ、これらの治療反応性と川崎病感受性遺伝子である ITPKC 遺伝子型との関連を解析するものである。IVIG 不応症例では冠動脈瘤の発生頻度が高いが、まだ有効な治療法が確立していないため新たな治療法の開発が急務である。免疫抑制剤である CyA は、尾内、羽田が発見した川崎病感受性遺伝子の ITPKC 遺伝子が関与する炎症活性化の経路

を抑制することが知られていることから、川崎病の治療にCyAが有効であることが期待される。

B 研究方法

治療プロトコールを主任研究者の統括のもとに作成した。本治療プロトコールでは、IVIG治療の反応性、CyAの有効性とITPKC遺伝子型の相関を3年間かけて解析する。また、CyA治療そのものの問題点、安全性を3年間かけて検証する。

C 研究結果

08年5月～09年3月に新規川崎病として入院した患者のうち、IVIG+アスピリン療法の初期投与をおこなった19例を対象とした。このうち、初期治療を当科で行ったのは14例で、初回IVIGに反応せず、解熱が得られなかった3例（21.4%）に追加IVIG（2g/kg）を施行した。このうち、2例は追加IVIGに反応し、解熱が得られた。一方、追加IVIG投与にも解熱しなかった1例と関連施設から初回IVIG・追加IVIG不応で当科に紹介された5例の計6例に対して、プロトコールに準じて、CyA 4mg/kgを経口（分2、食前）で2-3週間投与した。その結果、6例中5例ではすみやかな解熱効果が得られ、検査所見も改善した。一方、1例では解熱効果が得られずCyAを増量し、解熱した。また別の1例はCyAを開始した8病日に右側冠動脈に5-6mmの瘤を認め、CyA投与後に速やかに解熱したが、瘤は10-11mm大の巨大瘤に進展した。残りの18例（94.7%）には冠動脈病変は認めなかった。CyAによる重篤な有害事象は認められなかった。臨床症状のない高K血症を一過性に認めた。

D 考察

初回・追加IVIGに不応な症例にCyAは劇的に効果があることが示された。ただし、CyAに反応が充分でない症例も少なからず存在し、そのITPKC遺伝子型を分担研究者の尾内が解析している。また、CyAは冠動脈障害が生じた後に投与してもその進展を阻止できないことが示唆され、初回IVIG不応の時点など、CyAのより早期投与を考慮する必要がある。

E 結論

今後も症例を積み重ねて解析し、場合によってはCyAのプロトコールの改訂を行っていく必要がある。また、関連施設の協力を得て、多施設研究として行う場合の診療上の問題点などを明らかにしていく必要がある。また、IVIG不応症例のサイトカインプロファイルを解析することを今後予定している。

F 健康危険情報： 該当せず

G 研究発表：

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REVIEW

Susceptibility genes for Kawasaki disease: toward implementation of personalized medicine

Akira Hata[1] and Yoshihiro Onouchi[2]

Kawasaki disease (KD) is an acute systemic vasculitis syndrome, which primarily affects in children under the age of 5 years. In 20–25% of cases, if untreated, coronary artery lesions develop, making KD the leading cause of acquired heart disease in children in both Japan and the United States. Since 1970, 19 nationwide surveys of KD in Japan have been conducted every 2 years and the data are stored in a database. Even though the etiology of KD remains unknown, despite enthusiastic research spanning more than 40 years, we have learnt a great deal about KD from this enormous database. These 19 epidemiologic studies indicate a strong genetic influence on the disease susceptibility, prompting us and other researchers to identify the responsible genes for KD by applying either the candidate gene approach or the genome-wide approach. We have employed a genome-wide linkage study using affected sibling pair data of KD in Japan and have identified several susceptibility loci. Further analysis focusing on a region of chromosome 19, where one of the linked loci was detected, identified a predisposing gene, which codes inositol 1,4,5-trisphosphate 3-kinase C (ITPKC). In this review, we summarize the cumulative knowledge regarding KD, and then outline our hypothesis of the role ITPKC plays in KD susceptibility and our trial that aims toward the implementation of personalized medicine for KD.
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Keywords: coronary artery legions; inositol 1,4,5-trisphosphate 3-kinase C; Kawasaki's disease; personalized medicine

Kawasaki disease (KD; OMIM611775), alternatively called mucocutaneous lymph node syndrome (MCLS), was initially described by the Japanese pediatrician Tomisaku Kawasaki in 1967.[1–3] In the early 1970s, KD was independently recognized at the University of Hawaii and the disease is now known to occur in children of all races.[4]

KD is basically a self-limited vasculitis syndrome affecting systemic small- and medium-sized blood vessels. The symptoms include prolonged fever unresponsive to antibiotics, nonsuppurative cervical lymphadenopathy, and changes in the skin and mucous membranes such as rash, edema, conjunctival injection, erythema of the oral cavity, lips, and palms, and desquamation of the fingertips.

EPIDEMIOLOGY

Started in 1970, The Japan Kawasaki Disease Research Committee has conducted nationwide surveillance of KD every 2 years. In the most recent survey for 2005–2006—the 19th survey—a total of 1543 out of 2183 hospitals responded.[5] A significant number of findings were obtained as follows.

1. After three major outbreaks of KD in 1979, 1982, and 1986, the average annual incidence in Japan has rapidly increased to more than 10 000 patients since 2005, which is about 1.5 times higher than the number of patients of the 1979 outbreak. Owing to the decreasing birth rate in Japan, the incidence rate has been increasing more rapidly than the number of patients, reaching 188.1 (male 212.0; female 163.0) per 100 000 children aged 0–4 years in 2006, which is 2.4 times higher than that of the 1979 outbreak (Figure 1).
2. In the 19th survey, 65.9% of the patients were under 3 years of age and peak incidence was at 6- to 8-month olds in both sexes, which is less than the peak of 9- to 11-month olds found in the 18th survey for 2003–2004.
3. There is a consistent predominance of males with regard to incidence. The highest male/female ratio was 1.49 in the age group of 9 to 11 month olds.
4. The monthly distribution showed a high peak in January and a gradual increase in summer.
5. Geographically, the high incidence areas were limited to certain prefectures, which varied from year to year.

KD is markedly most prevalent in Japan, followed by China, Hong Kong, Korea, and Taiwan.[6–9] In Hawaii, the annual incidence for Japanese Americans is similar to that for Japanese living in Japan.[10] In the United States race-specific incidence rates, estimated from hospital discharge records, indicate that KD is most common among Americans of Asian and Pacific island descent (32.5/100 000 children

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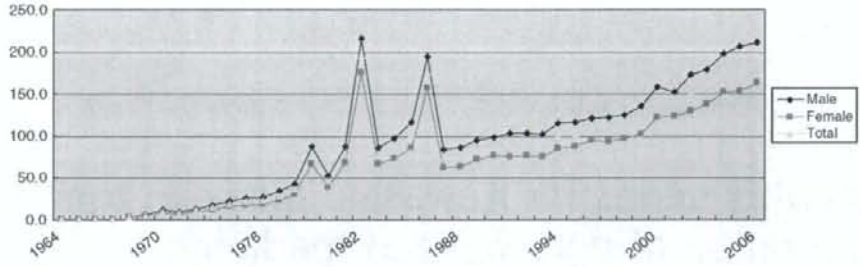

Figure 1 Trends in incidence rate (per 100 000 children aged 0–4 years) of Kawasaki's disease from 1964 to 2006 in Japan.[5]

under 5 years old), of intermediate incidence in African Americans and Hispanics (16.9 and 11.1/100 000 children under 5 years old), and lowest in Caucasians (9.1/100 000 children under 5 years old).[11]

ETIOLOGY AND PATHOGENESIS

The etiology of KD remains unknown. The seasonal variation, geographic distribution, and age-specific distribution strongly suggest an infectious etiology or trigger. To date, however, experiments seeking to identify the infectious agent responsible for KD, using virtually all kinds of techniques, have failed. One plausible hypothesis would be that KD is caused by some unknown, but ubiquitous infectious agent that produces the clinically apparent disease only in individuals with a genetic susceptibility. If this hypothesis is true, the frequency of the susceptible allele should be higher in Asian populations, including Japanese, than in Caucasians. Increased cytokine cascade stimulation and endothelial cell activation in acute KD are well-established findings. Taking into account the hypothesis and laboratory findings, a plausible mechanism of KD onset would be a genetically determined exaggerated immune response triggered by several different unknown microbial agents. Thus, the identification of genetic factors must surely facilitate our understanding of the disease etiology and pathophysiology.

CARDIAC COMPLICATIONS

In the 1960s in Japan, there was controversy as to whether KD was connected to subsequent cardiac complications in a significant number of cases. The first Japanese nationwide survey of KD was conducted in 1970; and from the findings of the first and second surveys, a total of 13 autopsied cases of sudden cardiac death with coronary thrombosis and aneurysm were documented,[3] establishing the link between KD and coronary artery vasculitis.[12,13] It is now known that coronary artery aneurysms develop in 20–25% of untreated patients and may lead to myocardial infarction, sudden death, or ischemic heart disease.[3] Until now, prevention of coronary artery lesions (CAL) is a major clinical issue to be overcome.

TREATMENT

The efficacy of intravenous immunoglobulin (IVIG) administered in the acute phase of KD for reducing the prevalence of CAL is well established.[14–16] Patients should be treated with IVIG ($2\,g\,kg^{-1}$, single infusion) over 8–12 h, together with oral aspirin ($30–50\,mg\,kg^{-1}\,day^{-1}$). Administration of IVIG within the first 10, and preferably 7, days after the onset of fever in combination with aspirin reduces the risk of CAL to 3–5%. Approximately 15–20% of patients with KD fail to respond to initial IVIG therapy, and CAL occurs in 10–15% of patients despite this therapy. Failure to respond is typically defined as persistent fever >36 h after the completion of initial IVIG ($2\,g\,kg^{-1}$) therapy. Kobayashi *et al.*[17] have developed a new risk score that identifies, with high sensitivity and specificity, resistance to IVIG in advance of treatment. They used seven laboratory and demographic variables available before the initiation of primary therapy.

In the case of persistent or recrudescent fever after IVIG therapy, the current treatment trial is to administer one or more repeat doses of IVIG, pulse methylprednisolone ($30\,mg\,kg^{-1}$) for 1–3 days, ulinastatin ($5000\,U\,kg^{-1}$) 3–6 times a day, together with other immunosuppressives such as cyclophosphamide, methotrexate, cyclosporin A (CsA), infliximab, or plasmapheresis. However, full evaluations of these additional therapies have not yet been completed.

EPIDEMIOLOGICAL FINDINGS INDICATING GENETIC PREDISPOSITION FOR KD

A genetic influence on the disease susceptibility is suspected based on the following. First, as mentioned earlier, KD is over-represented among Asian and Asian-American populations.[6–11,18] The fact that Japanese ancestries in Hawaii have higher incidence of KD than other ethnic groups and the same level of incidence as Japanese living in Japan confirms the ethnic genetic difference in susceptibility to KD. Second, KD shows familial aggregation.[19–22] The frequency is 10 times higher than in the general population among siblings of an index case in Japan (relative risk for siblings: $\lambda s=10$). In addition, the frequency of KD was two times higher than predicted in parents of children with KD, indicating the existence of heritable factor(s).

Thus, the identification of susceptible genes could elucidate the fundamental mechanism of KD development and possibly lead to the establishment of a pharmacological therapy or preventive measure.

CANDIDATE GENE APPROACH

One commonly used technique to identify genetic risk factors for multifactorial diseases such as KD is the candidate gene approach, which directly examines the effects of genetic variants of a possibly contributing gene by association study. To date, quite a number of genes have been examined, which are usually selected based on information regarding their function in inflammation, immune response, and other biological mechanisms. Onouchi,[23] one of the authors in this article, has classified elsewhere the majority of the results of the candidate gene approach for KD and, therefore, here we briefly summarize the findings obtained from this approach.

Initial genetic studies of KD were focused on human leukocyte antigen (HLA) class I antigens. The HLA gene cluster is located at chromosome 6p21.3. To date, a number of disorders including rheumatoid arthritis, insulin-dependent diabetes mellitus, and Crohn's disease are known to be associated with the HLA subtype. There is a large ethnic difference in the distribution of HLA alleles. In Japanese

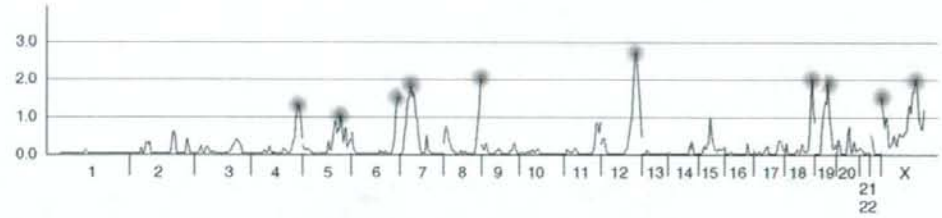

Figure 2 Results of sibling pair linkage analysis. Ten chromosomal regions have positive signals of linkage (MLS > 1.0). Chromosome numbers 1–22 and X are shown on the *x* axis.

KD patients, HLA-Bw22 (now referred to as Bw54) is reported to be predominant.[24,25] On the other hand, HLA-Bw51 was found to be associated with Caucasian[26,27] and Jewish KD patients,[28] but no replication was obtained in Chinese[29] and Korean KD patients.[30] Although several other groups have studied other combinations of HLA subtypes, no consistent association has been detected so far. Considering that no significant linkage was detected near the 6p region in our genome-wide linkage analysis,[31] the involvement of HLA in KD pathogenesis might be unlikely.

Among other candidate genes studied, cytokines and their receptors are in the majority. Those are chemokines and their receptors (CCL3L1,[32] CCR2,[34] CCR3,[34] CCR5,[32–34] CX3CR1,[34] CXCR1,[34] CXCR2,[34] and MCP-1[35]), hematopoietins (interleukin-4 (IL-4)[36–38] and IL-6[39]), IL-1 family (IL-1β,[37] IL-18,[40] and IL-1Ra[37]), IL-10 family (IL-10[41]), platelet-derived growth factor family (vascular endothelial growth factor (VEGF)[42–45] and VEGFR2[42]), and tumor necrosis factor (TNF) family (TNF-α,[46–50] lipoteichoic acid,[47] and CD40L[51,52]). Other candidates include plasma proteins (C-reactive protein[53–55] and MBL2[53,55,56]), matrix metalloproteinase (MMP) and their inhibitors (MMP2,[58] MMP3,[57,58] MMP-9,[58] MMP-12,[58] MMP-13,[58] and tissue inhibitors of metalloproteinase-2[59]), enzymes related to atherosclerosis (methylenetetrahydrofolate reductase (MTHFR),[60] endothelial nitric oxide synthase,[61] and inducible nitric oxide synthase[61]), components of the renin–angiotensin system (angiotensin-converting enzyme[62–65] and AGTR1[64]), and an unclassified group (CD14,[66] FCGR2A,[67,68] SLC11A1,[69] PLA2G7,[70] UGT1A1,[71] MICA,[72] and HMOX1[71]).

KD is recognized as one of the systemic inflammatory response syndromes, in which high levels of cytokines are observed.[73] TNF-α is considered to play an important role in vascular injury in KD, which develops to CAL.[74,75] In accordance with this hypothesis, the usage of infliximab, the monoclonal antibody against TNF-α, has recently been found to be effective as a novel therapy for IVIG-resistant KD.[76–78] Thus, genes for cytokines including TNF-α could be candidates for KD susceptibility. However, consistent results have not been obtained. This is also true for the other candidates mentioned above. Further systematic replication studies would be necessary to draw firm conclusions.

GENOME-WIDE LINKAGE ANALYSIS

Unlike the candidate gene approach, the genome-wide approach can be performed without any *a priori* assumptions about the genes. This approach therefore affords the chance to identify novel gene(s) whose function is unknown. In other words, genome-wide approaches open up the possibility of identifying multiple genetic factors that contribute to KD susceptibility with largely unknown pathogenesis. When we decided to start identifying susceptibility genes for KD about 10 years ago, the available method for the genome-wide approach was linkage analysis with affected sibling pairs. Genetic linkage is based on the phenomenon whereby alleles at loci close together on the same chromosome region will tend to be inherited together, because it is rare for a crossover to occur between the loci at meiosis. We can expect to observe co-segregation of a marker associated with a disease among affected members in the same family, and thus we can test for increased allele sharing between pairs of affected relatives, particularly affected sibling pairs. Linkage is a very long-range phenomenon; only several hundred markers may be sufficient to identify the responsible gene loci from the entire genome. Below we describe our identification of one of the susceptible genes for KD based on the results derived from genome-wide linkage analysis.

In our linkage studies we used the affected sibling pair method, a kind of non-parametric linkage study, with genome-wide distributed microsatellite markers. The total number of markers was 399 and the average interval 9.9 cM. We have recruited more than 80 families including sibling cases all over Japan. The logarithm of the odds (LOD) scores were calculated by estimating number of shared alleles identical by descent for each marker loci. The results of the genome-wide linkage analysis are shown in Figure 2. Ten chromosome regions—4q35, 5q34, 6q27, 7p15, 8q24, 12q24, 18q23, 19q13.2, Xp22, and Xq27—showed maximum LOD scores (MLS) > 1.0 (Figure 2). Among them, the most significant region was 12q24 (MLS 2.69).[31] These peaks, if true, implicate the location of the responsible genes for KD susceptibility. We then performed linkage disequilibrium (LD) mapping to narrow down the candidate loci and identify the genes and their functional variants.

LD MAPPING

LD mapping is usually performed by association study using single nucleotide polymorphisms (SNPs) near the candidate region. We selected SNPs in the vicinity of the 10–25 Mb area to the several linkage peaks from the database. A systematic case–control association study that genotyped these variants identified three significant closely located SNPs in a 150-kb LD block on the 19q13.2 region (Figure 3). A subsequent validation study using different case–control sample sets confirmed the association. We then sequenced the LD block region to find other SNPs. Identifying six additional significant SNPs through association study resulted in total nine significant SNPs. These candidate SNPs were further screened by the transmission disequilibrium test conducted on American multi-ethnic KD patient–parent trios. Of the nine significant SNPs, only four centromeric SNPs showed the same trend of association, indicating one of the four to be a functional SNP (Figure 3).[79]

IDENTIFICATION OF ITPKC AS A SUSCEPTIBILITY GENE FOR KD

As shown in Figure 3, a total of four genes—NUMBL, ADCK4, inositol 1,4,5-trisphosphate 3-kinase C (ITPKC), and FLJ41131—are

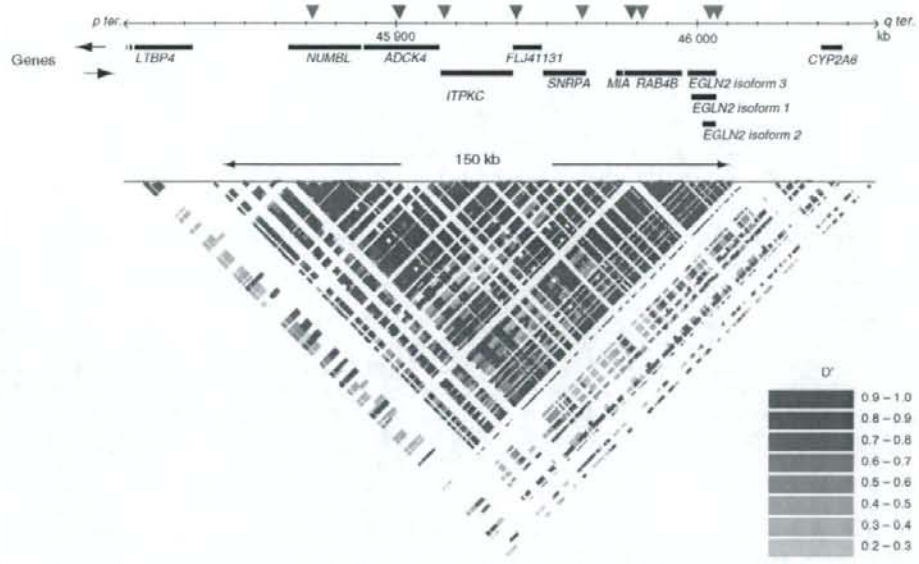

Figure 3 Linkage disequilibrium mapping. Red arrowhead indicates the three SNPs initially identified. Blue arrowhead indicates SNPs identified through detailed analysis of this LD block region. Adapted with permission from Macmillan Publishers Ltd (*Nat. Genet.*),[79] copyright (2008).

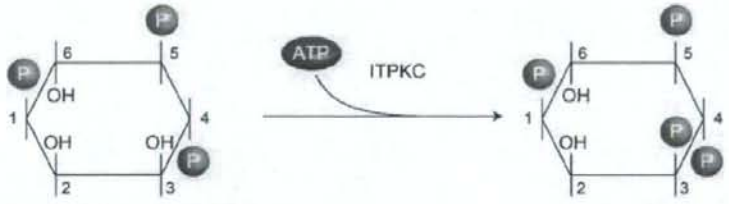

Figure 4 ITPKC phosphorylates IP3 (left) to produce IP4 (right).

located in the region of the four centromeric SNPs. Of the four genes, ITPKC was the most attractive candidate. ITPKC is a kinase of inositol 1,4,5-trsiphosphate (IP3), which is a second messenger molecule in various types of cells including T cells, macrophages, and neutrophils known to be involved in the pathogenesis of KD. IP3 is phosphorylated to become IP4 by ITPKC (Figure 4). Signals from cell surface receptors activated phospholipase C (PLC), which catalyzes the hydrolysis of plasma membrane inositol phospholipids. IP3 is one by-product of PLC-mediated phospholipid breakdown and stimulates the release of Ca^{2+} ions from intracellular stores and also influx of extracellular Ca^{2+} into the cells. In T cells, increased cytoplasmic Ca^{2+} binds calmodulin, and the Ca^{2+}–calmodulin complexes activate calcineurin. Calcineurin then removes phosphates from an inactive nuclear factor of activated T cells (NFAT). Activated NFAT migrates into the nucleus, where it binds to and activates the promoters of several genes, including IL-2 (Ca^{2+}–NFAT pathway) (Figure 5).[80–82] Although a total of three isoenzymes of ITPK proteins (ITPKA, ITPKB, and ITPKC) are known to date, the importance of ITPKC in the immune system has not been identified. We investigated mRNA expression of *ITPKC* in a variety of tissues and revealed that *ITPKC* was strongly induced in stimulated peripheral blood mononuclear cells (PBMCs). Compared with the other isoenzymes, *ITPKC* was most abundantly expressed in PBMCs and leukemic cell lines, and most significantly induced in response to cell stimulation.[79] Therefore, we speculated that ITPKC plays a significant role in the immune system and is probably related to inflammation. The finding of the predicted nuclear factor-κB binding sequence in the promoter of *ITPKC* in an *in silico* study[83] is in accordance with our speculation.

ROLE OF ITPKC IN T-CELL RECEPTOR SIGNAL TRANSDUCTION

We hypothesized that ITPKC negatively regulates T-cell receptor signal transduction by decreasing the amount of IP3 in the cytoplasm. If this is true, increased ITPKC expression should lead to decreased IL-2 expression, and decreased ITPKC expression should lead to increased IL-2 expression. In accordance with our hypothesis, overexpression in Jurkat cells resulted in reduced NFAT activation and *IL-2* expression. On the contrary, knock-down of ITPKC by short hairpin RNA enhances NFAT activity and *IL-2* expression.[79] These data strongly support our hypothesis (Figures 5a and b). Through functional analyses of significant SNPs, we identified that itpkc_3 G/C, an SNP located in intron 1 of *ITPKC*, reduces mRNA expression of *ITPKC* in

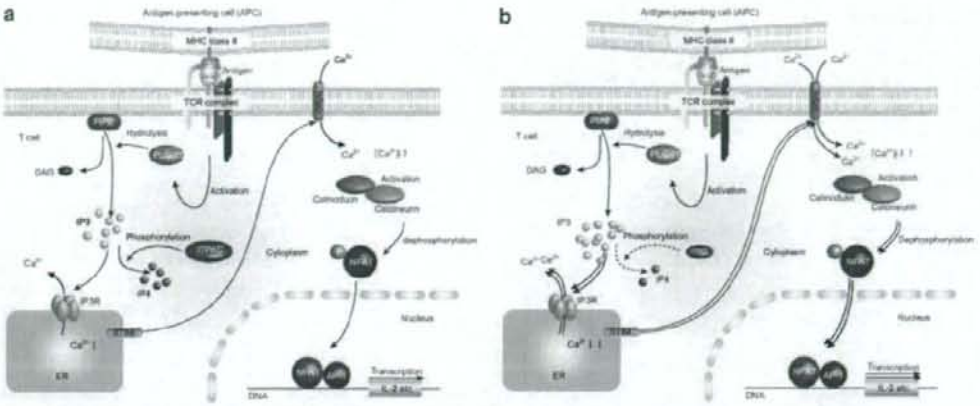

Figure 5 The possible role of ITPKC as a negative regulator in the Ca^{2+}/NFAT pathway. IP3 binds to its receptor expressed on the endoplasmic reticulum (ER) membrane and causes the release of Ca^{2+} into the cytoplasm. Then the depletion of Ca^{2+} store in ER evokes a process termed as store-operated Ca^{2+} entry in which extracellular Ca^{2+} enters through calcium release-activated Ca^{2+} channels on the plasma membrane. Cytoplasmic Ca^{2+} binds calmodulin, which in turn activates calcineurin, a calmodulin-dependent phosphatase. Activated calcineurin dephosphorylates to NFAT in the cytoplasm and leads to nuclear translocation of NFAT. NFAT in the nucleus drives the transcription of genes important in T-cell activation. $[Ca^{2+}]i$, intracellular free Ca^{2+} concentration. Panel **a** denotes in case of higher ITPKC activity, and panel **b** denotes of lower ITPKC activity.

Table 1 Association between itpkc_3 and KD[a]

Japanese

Samples	*itpkc_3 genotype*			*Total*	*Carrier ratio of C allele (%)*	*OR[b]*	*95% CI*	χ^2	*P-value*
	GG	*GC*	*CC*						
KD	376	234	27	637	41	1.89	1.53–2.33	35.8	2.2×10^{-9}
KD with CAL[c]	61	44	2	107	43	2.05	1.37–3.08	12.4	0.00044
KD without CAL[c]	172	94	12	278	38	1.68	1.27–2.21	13.4	0.00025
Control	756	249	29	1034	27				

United States[d]

Samples	*Transmitted C allele*	*Untransmitted C allele*	*OR*	*95% CI*	χ^2	*P-value*
KD	64	30	2.13	1.38–3.29	12.3	0.00045
KD with CAL	37	11	3.36	1.72–6.59	14.1	0.00018
KD without CAL	27	18	1.50	0.63–2.72	1.8	0.18
KD IVIG non-responder	14	3	4.67	1.34–16.24	7.1	0.0076
KD IVIG responder	39	22	1.77	1.05–2.99	4.7	0.030

Abbreviations: CAL, coronary artery lesion; CI, confidence interval; IVIG, intravenous immunoglobulin; KD, Kawasaki's disease; OR, odds ratio.
[a]Adapted with permission from Macmillan Publishers Ltd (*Nat. Genet.*),[79] copyright (2008).
[b]Association study of genotype frequencies in dominant model of inheritance (GG vs GC+CC).
[c]Samples without clinical information were excluded from the analysis.
[d]Transmission disequilibrium test of 209 triads of multiethnic KD patients and their parents.

PBMCs down to 70% by altering splicing efficiency.[79] Relatively reduced ITPKC activity associated with the C allele of itpkc_3 may enhance T-cell activation, leading to the development of KD triggered by unknown infection. Further investigation of the role of human ITPKC and biological significance of itpkc_3 in other immune cells (e.g., macrophages, B cells, and neutrophils) or non-immune cells (e.g., endothelial cells and cardiac myocytes) may elucidate a part of the pathogenesis of KD.

PREDICTION OF CAL FORMATION AND IVIG RESPONSIVENESS

As mentioned above, 15–20% of KD patients respond poorly to IVIG and these patients are at higher risk for developing CAL. The development of clinical measures to prevent CAL is thus the most important issue for pediatricians responsible for KD patients. We can assume that there are several genes related to IVIG responsiveness and CAL formation in KD, and if this is so, prediction by genotyping such

genes would be beneficial in the clinical setting. We stratified our samples based on CAL formation and IVIG responsiveness. The C allele of itpkc_3 was found to be significantly more predominant in patients with CAL and those refractory to IVIG (Table 1),[79] indicating that ITPKC itself is at least one such gene.

In Japan, several cases of successful CsA treatment for IVIG-resistant KD have been reported at meetings. CsA is known to bind to and inhibit the activity of calcineurin, thereby inhibiting the production of cytokines by T cells. The fact that CsA is effective indicates the importance of Ca^{2+}/NFAT pathway activation in the pathogenesis of KD.

We are now conducting a KD prospective cohort study to determine whether or not genotyping can predict IVIG responsiveness and CAL formation, and also whether or not an association between CsA effectiveness and genotype exists. This kind of clinical trial will contribute to the establishment of evidence-based and personalized medicine in the near future.

FURTHER RESEARCH TOWARD PERSONALIZED MEDICINE FOR KD

The International HapMap Consortium genotyped cell lines derived from trios of European and Yoruban descent, providing an extremely rich data set for identifying responsible genes for multifactorial diseases. On the basis of data obtained by the HapMap project completed in 2005—data that are freely available on the project's website—analysis kits for a genome-wide association study were provided by several suppliers. The fact that the genome-wide association study enables 500 000 to 1 million SNPs to be genotyped at a time has made it a mainstream technology. During the next few years, multiple susceptibility genes may be identified from such large-scale studies.

Pathways and molecular networks in which susceptibility genes identified in the genome-wide approach will provide new candidate genes. Based on our findings of the significance of the Ca^{2+}/NFAT pathway, components of this pathway could be candidates. Through rapid progress in molecular biological technology and expanding knowledge of the human genome, we can expect the entire genetic background of KD to be clarified in the near future, bringing us closer to the availability of personalized medicine for KD.

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