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Analysis of the etiology of intravenous immunoglobulin-resistant Kawasaki disease using iPSCs technology

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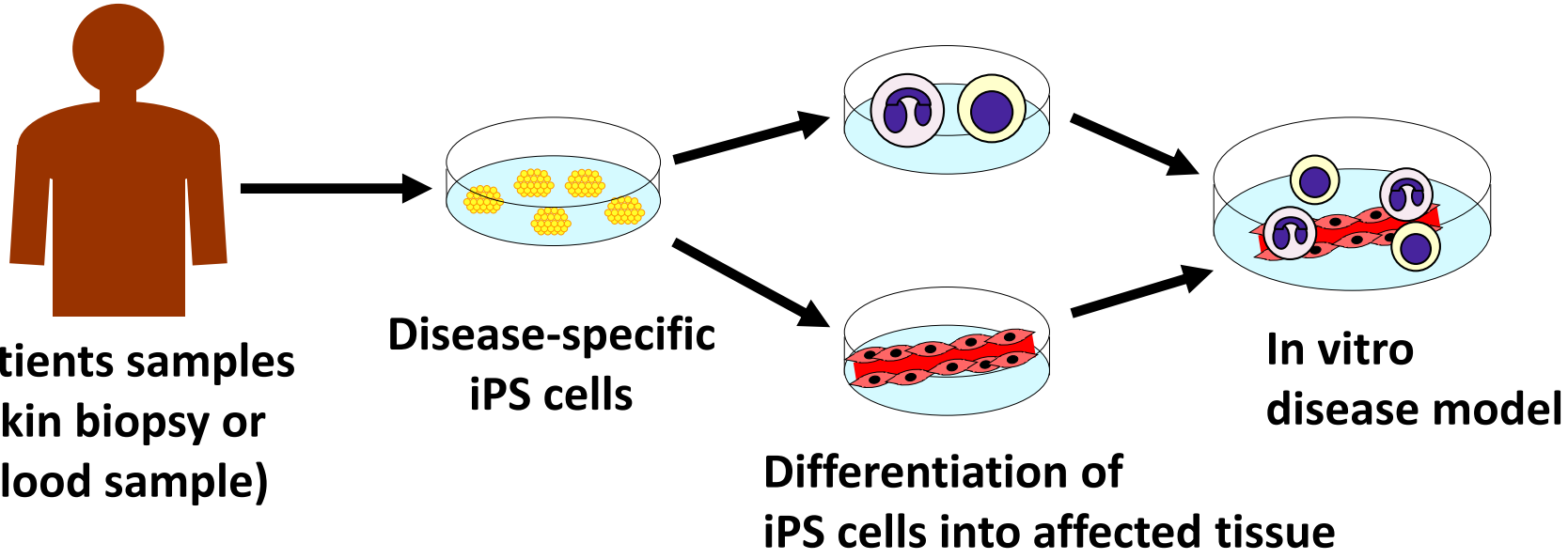
Background

The mechanism of IVIG-resistant KD has been analyzed using the leukocyte mRNA levels, however, vascular endothelial cells (ECs), closely related to the vasculitis of KD, were not analyzed in the previous report.

I propose a hypothesis that ECs are mainly involved in the etiology of IVIG-resistant KD.

Disease modeling and drug screening using patient-specific iPS cells



Human coronary artery endothelial cells did not contain disease-related genetic information.

We selected vascular endothelial cells derived from human iPS cells which carry on corresponding genetic information of disease.

Objective

The purpose of this study is to establish new in vitro disease models of vasculitis using induced pluripotent stem cell (iPSC) technology, and clarify the mechanisms of IVIG-resistance in KD.

Method

- Dermal fibroblasts or T cells from 2 IVIG-resistant and 2 IVIG-responsive KD patients were reprogrammed by episomal vectors encoding Oct3/4, Sox2, Klf4, L-Myc, LIN28, and p53 shRNA.

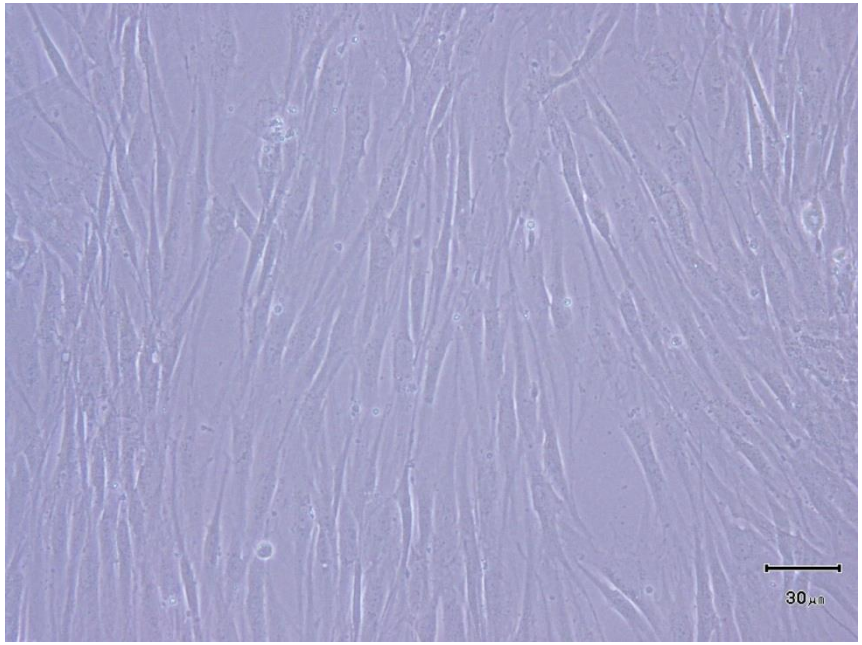
- The iPSC lines were then differentiated into vascular endothelial cells (ECs), by using a previously-reported differentiation method, and the ECs samples were subjected to the microarray analyses.

Patient	Age	Sex	Tissue	Responsiveness for IVIG
Pt. 1	12 y.o.	M	dermal tissue	IVIG non-responder, CAL +
Pt. 2	14 y.o.	M	dermal tissue	IVIG non-responder, CAL +
Pt. 3	16 y.o.	F	Peripheral blood	IVIG responder, CAL -
Pt. 4	16 y.o.	F	dermal tissue	IVIG responder, CAL -

CAL; coronary artery lesion

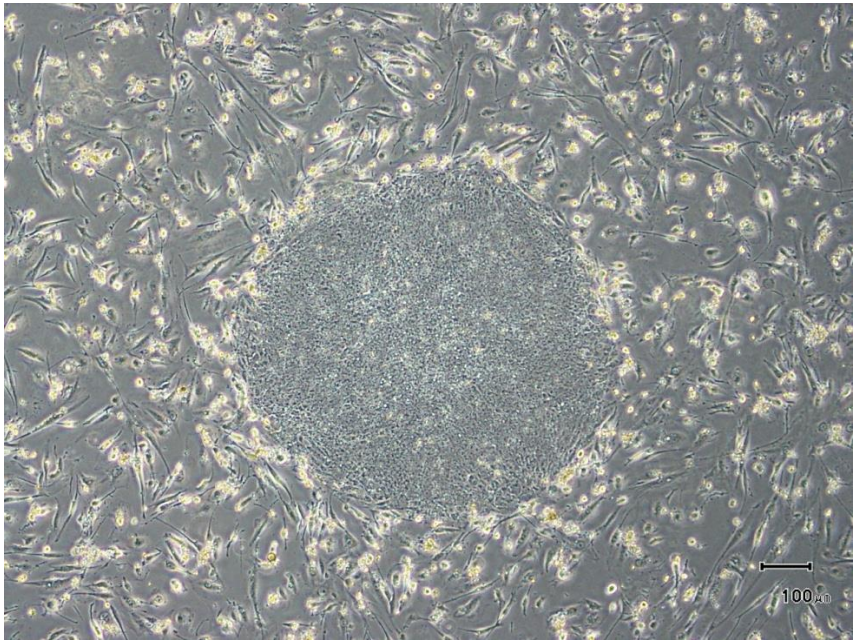
Human iPS cells could be induced from KD patients' fibroblasts

Pt. 2
Fibroblast

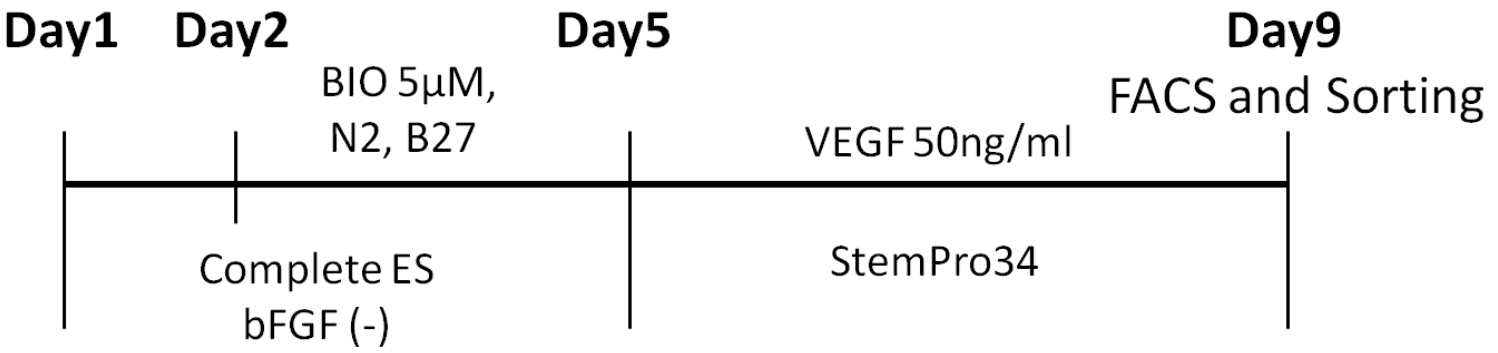


OCT3/4
SOX2
KLF4
L-MYC
LIN28
p53shRNA

Pt. 2
iPS Cells

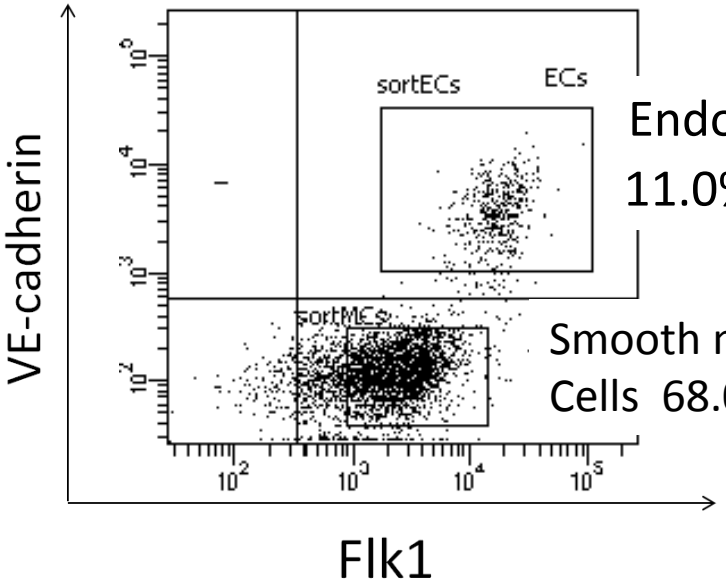


The KD patient-derived iPSCs could be differentiated into vascular endothelial cells



Taura D, et al. 2009

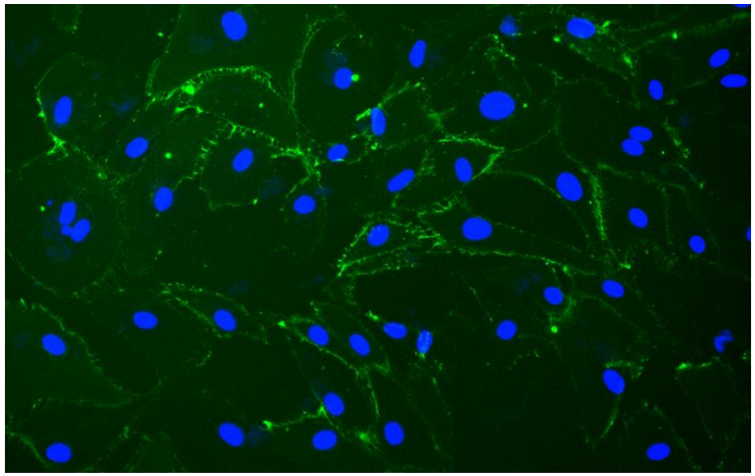
Pt. 2 clone 3



Endothelial cells
11.0%

Smooth muscle
Cells 68.0%

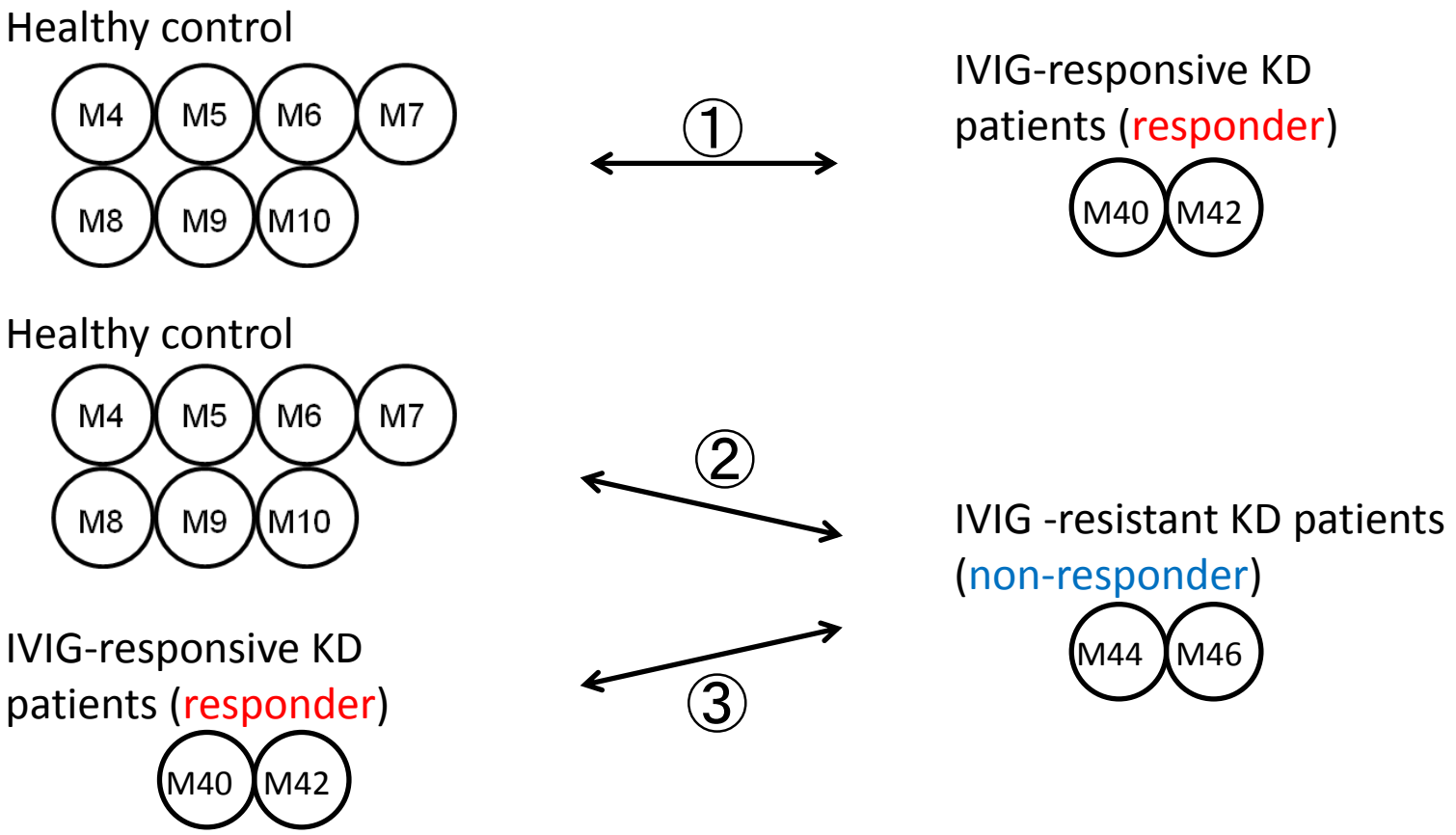
Pt. 1 clone 1



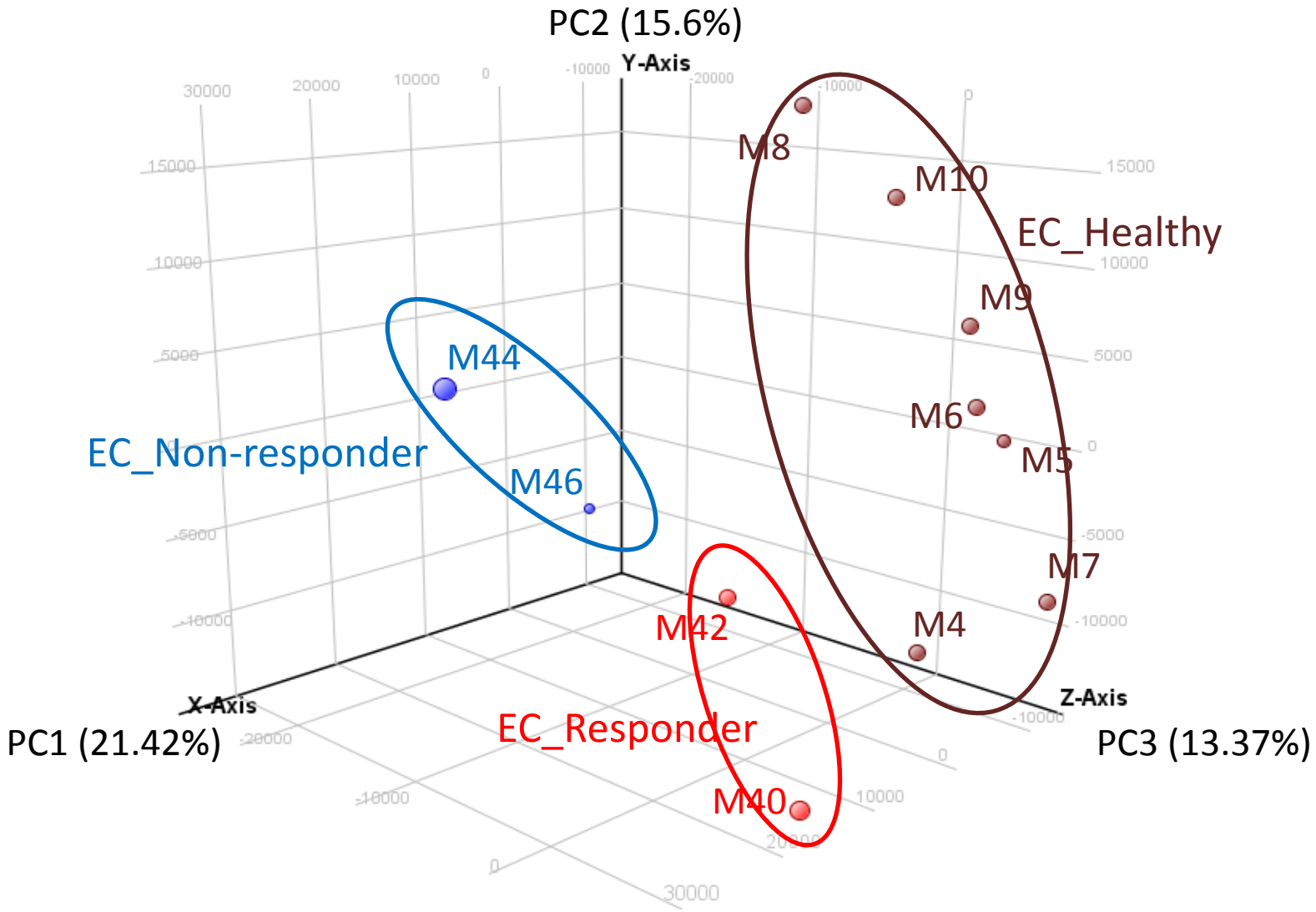
CD31/nuclei

Microarray Protocol for iPS-ECs

iPS-derived ECs (iPS-ECs)



Principle Component Analysis (PCA): iPS - ECs

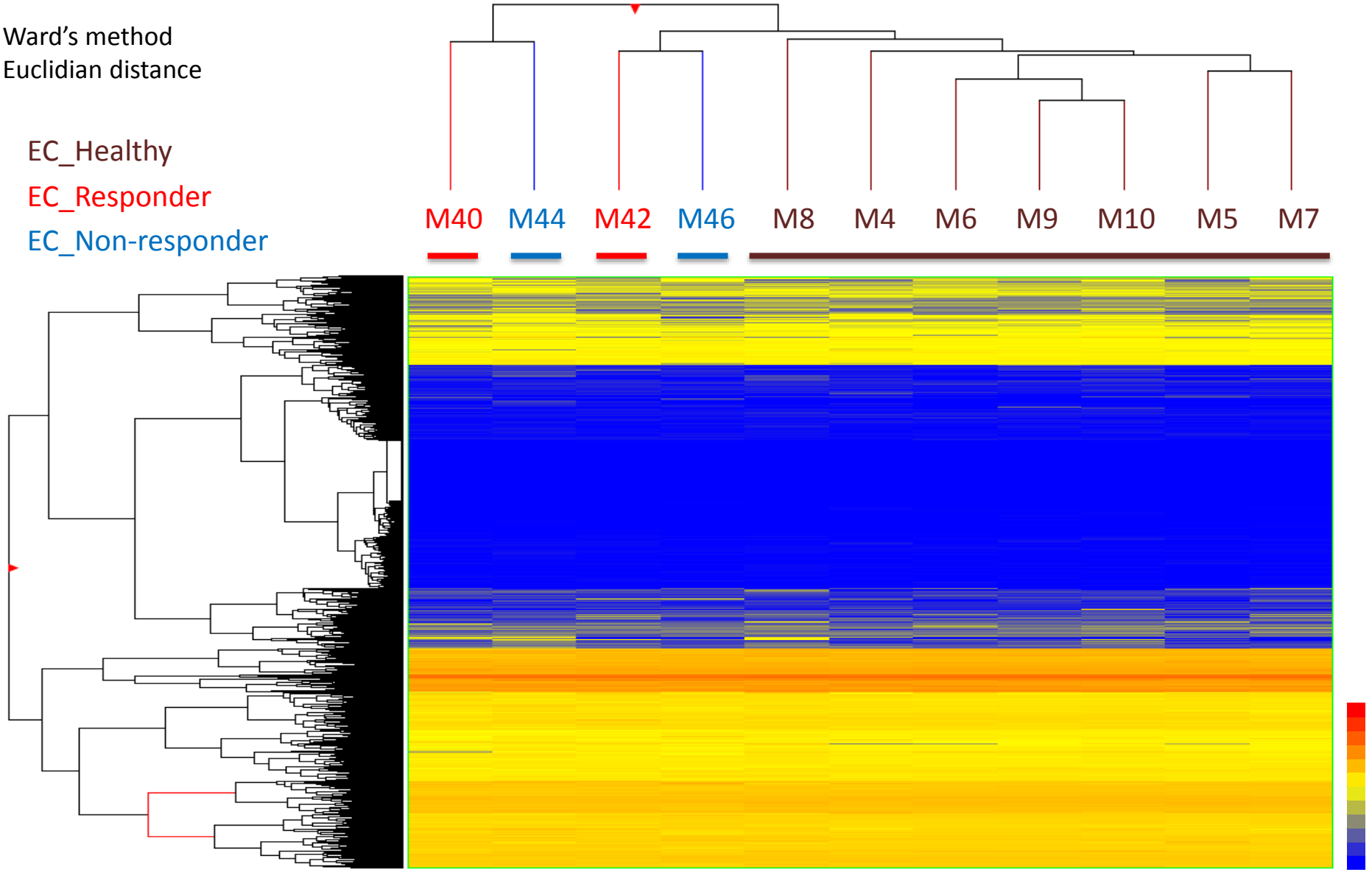


PCA involves a mathematical procedure that transforms a number of correlated variables into a smaller number of uncorrelated variables, and accentuation the characteristics of data.

Clustering Analysis: iPS - ECs

Ward's method
Euclidian distance

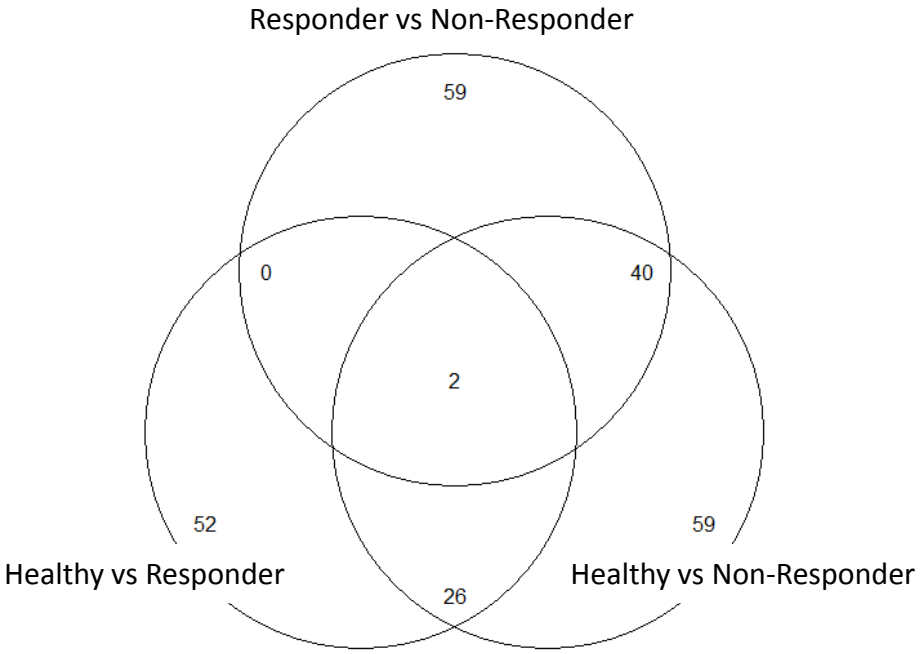
EC_Healthy
EC_Responder
EC_Non-responder



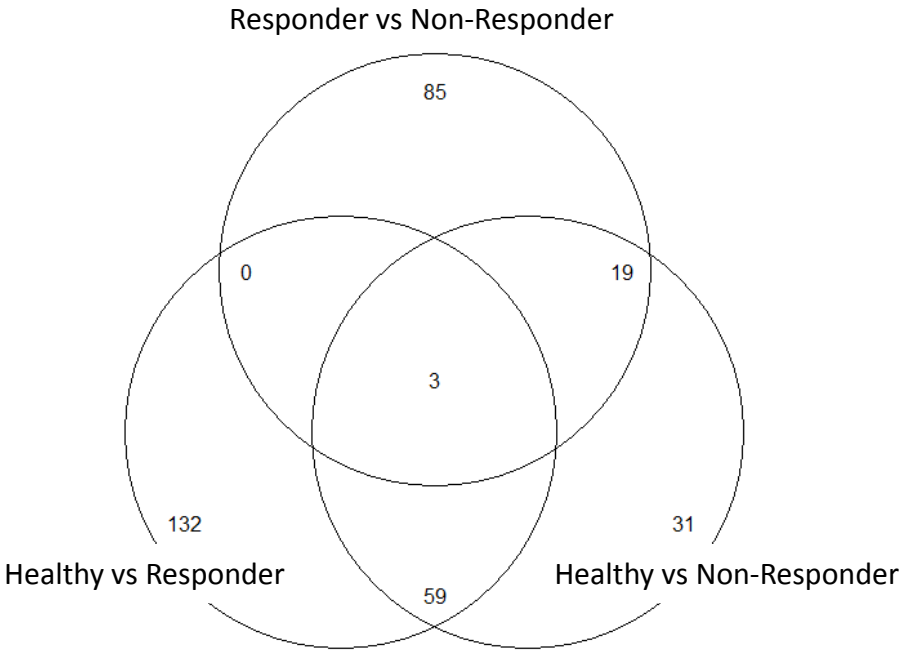
Selection of genes that were two fold up-regulated and two fold down-regulated: iPS - ECs

			up-regulate	down-regulate
1	EC	Healthy vs Patient	**58**	139
1-1	EC	Healthy vs Responder	**80**	194
1-2	EC	Healthy vs Non-responder	**127**	112
1-3	EC	Responder vs Non-responder	**101**	107

up-regulate



down-regulate



Gene Ontology (GO) analysis (iPS - ECs : Healthy < 4 KD Patient)

Term	Count	%	Genes	PValue
blood vessel development	6	20.68966	BGN, COL1A2, COL15A1, COL1A1, GJA4, THY1	4.84E-06
vasculature development	6	20.68966	BGN, COL1A2, COL15A1, COL1A1, GJA4, THY1	5.45E-06
skin morphogenesis	2	6.896552	COL1A2, COL1A1	0.004428
tissue morphogenesis	3	10.34483	ACTC1, COL1A2, COL1A1	0.016499
blood vessel morphogenesis	3	10.34483	BGN, COL15A1, THY1	0.022245
epidermis morphogenesis	2	6.896552	COL1A2, COL1A1	0.027379
skin development	2	6.896552	COL1A2, COL1A1	0.031694
collagen fibril organization	2	6.896552	COL1A2, COL1A1	0.031694
cell adhesion	4	13.7931	LAMC3, COL15A1, CPXM2, THY1	0.039403
biological adhesion	4	13.7931	LAMC3, COL15A1, CPXM2, THY1	0.039545
cell junction assembly	2	6.896552	GJA4, THY1	0.044532
skeletal system development	3	10.34483	INHBA, COL1A2, COL1A1	0.047546

GO analysis (iPS - ECs: Healthy < Non-responder)

Term	Count	%	Genes	PValue
blood vessel development	10	10.20408	SEMA5A, BMP4, BGN, COL1A2, COL15A1, COL1A1, GJA5, **Gene X**, **MMP2**, THY1	**1.20E-06**
vasculature development	10	10.20408	SEMA5A, BMP4, BGN, COL1A2, COL15A1, COL1A1, GJA5, **Gene X**, **MMP2**, THY1	**1.46E-06**
blood circulation	8	8.163265	ACTC1, PTGS1, COL1A2, CARTPT, NPPB, SERPING1, NPR3, **Gene X**	**1.73E-05**
circulatory system process	8	8.163265	ACTC1, PTGS1, COL1A2, CARTPT, NPPB, SERPING1, NPR3, **Gene X**	**1.73E-05**
skeletal system development	9	9.183673	BMP4, INHBA, COL1A2, PBX1, NPR3, COL1A1, GJA5, COL11A1, **MMP2**	**7.71E-05**
regulation of blood pressure	6	6.122449	ACTC1, PTGS1, COL1A2, CARTPT, NPPB, NPR3	7.90E-05
tissue morphogenesis	7	7.142857	BMP4, BMP10, ACTC1, COL1A2, PBX1, COL1A1, COL11A1	1.41E-04
trabecula formation	3	3.061224	BMP10, COL1A1, **MMP2**	**5.32E-04**
neuron differentiation	9	9.183673	SEMA5A, BMP4, SLITRK4, UNC5B, STMN2, ROBO2, **Gene X**, SLIT3, THY1	**6.62E-04**
cell adhesion	11	11.22449	SEMA5A, SORBS3, NLGN4Y, TTYH1, COL15A1, BCAM, ROBO2, COL11A1, **Gene X**, SPON1, THY1	**9.28E-04**
biological adhesion	11	11.22449	SEMA5A, SORBS3, NLGN4Y, TTYH1, COL15A1, BCAM, ROBO2, COL11A1, **Gene X**, SPON1, THY1	**9.38E-04**
axon guidance	5	5.102041	SEMA5A, UNC5B, ROBO2, **Gene X**, SLIT3	**0.001284**

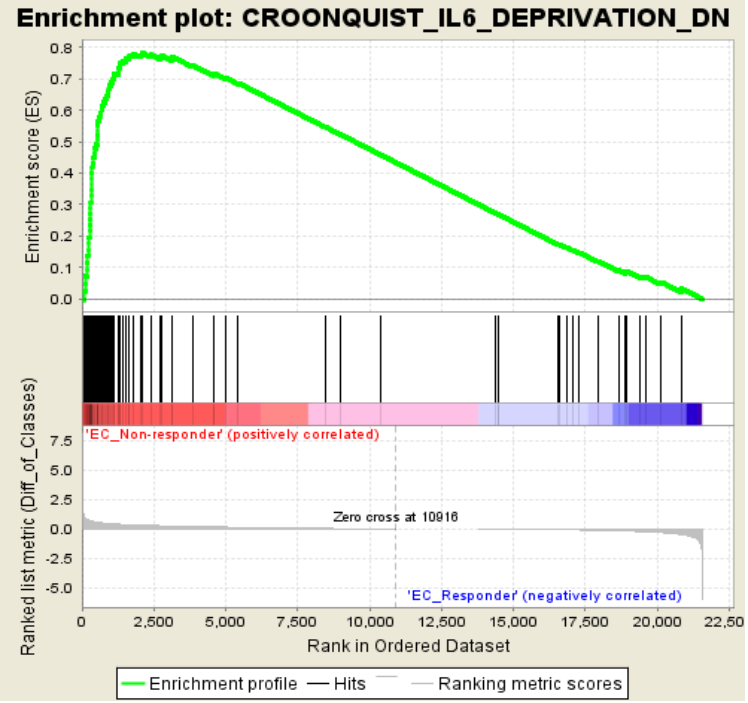
Gene x; Chemoattractant active on leukocyte. MMP-2; Expressed on coronary artery walls of KD patient with CAL.

GO analysis (iPS - ECs: Responder < Non-responder)

Term	Count	%	Genes	Pvalue
cell adhesion	10	12.5	LAMA2, VWF, EMCN, NLGN4Y, CD34, COL15A1, ROBO2, **Gene X**, CYR61, SPON1	**0.001255**
biological adhesion	10	12.5	LAMA2, VWF, EMCN, NLGN4Y, CD34, COL15A1, ROBO2, **Gene X**, CYR61, SPON1	**0.001268**
angiogenesis	5	6.25	EMCN, MEOX2, COL15A1, **Gene X**, CYR61	**0.002478**
blood vessel morphogenesis	5	6.25	EMCN, MEOX2, COL15A1, **Gene X**, CYR61	**0.008681**
regulation of organelle organization	5	6.25	HOXA13, DLGAP5, TMSB4Y, **Gene X**, SYNPO	**0.009557**
chromosome organization	7	8.75	CDCA8, UTY, DLGAP5, HMGA2, TSPYL5, TOP2A, KDM5D	0.010374
blood vessel development	5	6.25	EMCN, MEOX2, COL15A1, **Gene X**, CYR61	**0.014405**
vasculature development	5	6.25	EMCN, MEOX2, COL15A1, **Gene X**, CYR61	**0.015614**
branching morphogenesis of a tube	3	3.75	PBX1, **Gene X**, CYR61	**0.025836**
positive regulation of cellular component organization	4	5	HOXA13, DLGAP5, ROBO2, SYNPO	0.032264
morphogenesis of a branching structure	3	3.75	PBX1, **Gene X**, CYR61	**0.032826**
positive regulation of organelle organization	3	3.75	HOXA13, DLGAP5, SYNPO	0.040467

Gene Set Enrichment Analysis (GSEA)
iPS - ECs: responder < Non-Responder

EC,
Non-responder

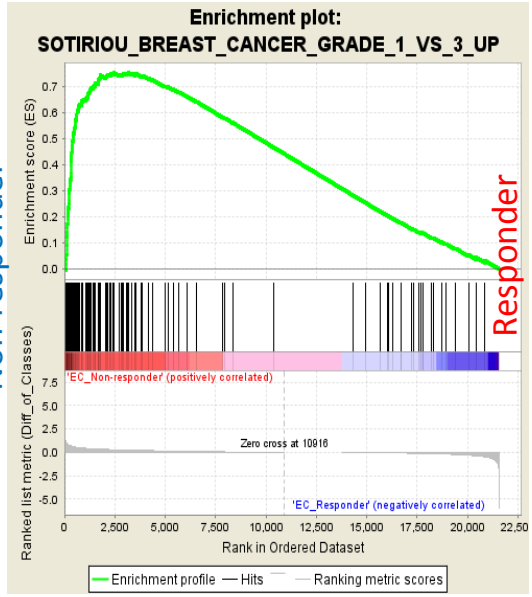


EC,
responder

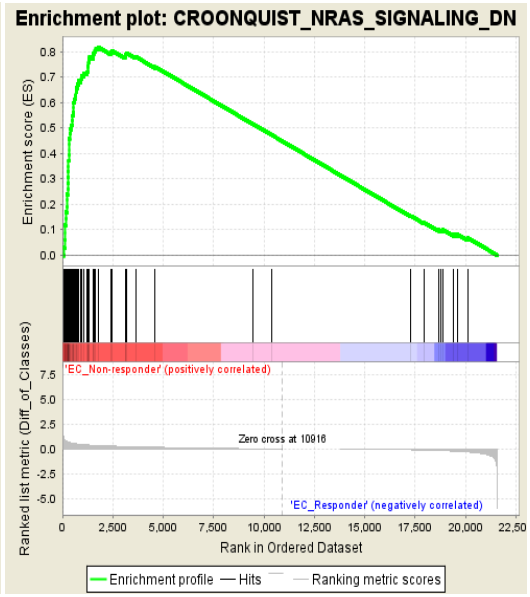
- Gene Set Enrichment Analysis (GSEA) is a computational method that determines whether a pre-defined set of genes from database shows statistically significant differences between two biological states.
- GSEA revealed that the gene sets related to IL-6, were up-regulated in iPS-ECs from IVIG-resistant KD patients.

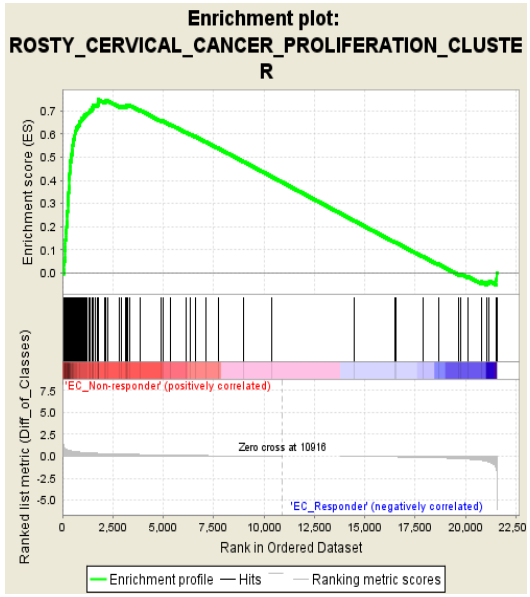
Gene Set Enrichment Analysis (GSEA)
iPS - ECs: responder < Non-Responder

Non-responder



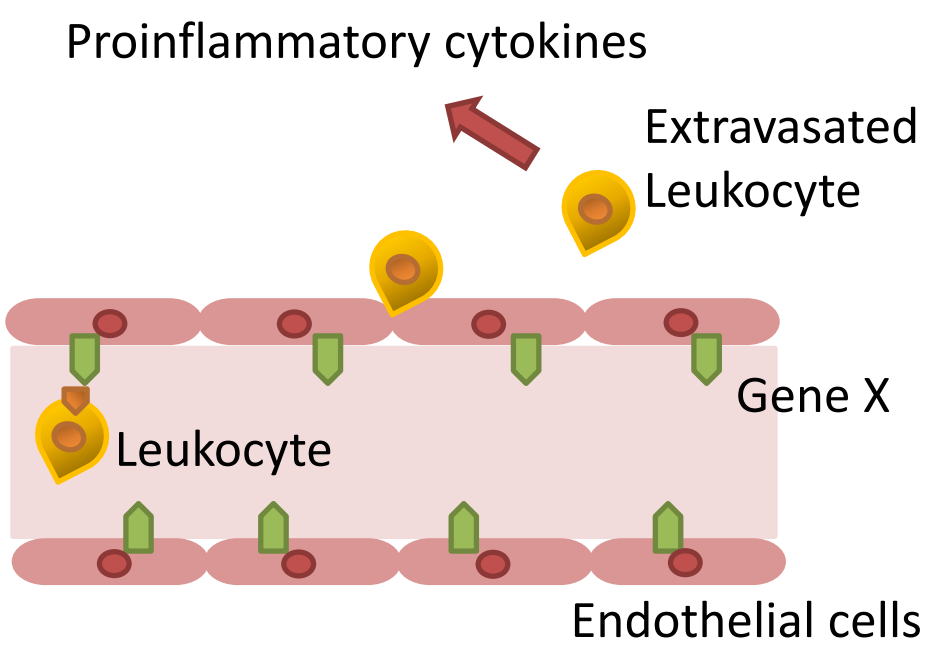
Responder





- GSEA revealed that the gene sets related to NRAS (a member of the RAS oncogene family), breast cancer and cervical carcinoma were up-regulated in iPS-ECs from IVIG-resistant KD patients.

Discussion



< hypothesis>

Gene X is expressed on leukocytes and endothelial cells, and acts as a positive regulator of leukocyte migration.

In KD patients with IVIG unresponsiveness, numerous leukocytes infiltrate the vascular wall, and leukocytes produce proinflammatory cytokines.

It might be suggested that gene X would be the key molecule of IVIG unresponsiveness.

Conclusions

- Taking into account that the concentration of IL-6 has been reported to be elevated in acute phase of IVIG-resistant KD, our results suggest that the up-regulation of IL-6 related genes in ECs might be involved in the pathogenesis of IVIG-resistant KD.

- It was speculated that Gene X, related to transmigration of leukocyte, might be closely related to both the responsiveness for IVIG treatment and the severity of Kawasaki disease.