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(54) Title: METHODS AND COMPOSITIONS FOR THE DIAGNOSIS AND TREATMENT OF KAWASAKI DISEASE

(57) Abstract: The present invention relates to the determination of levels or expression of particular biomarkers in biological samples which can be utilized to diagnose, prognose, and treat Kawasaki disease in subjects, and further to select subjects who would benefit from a Kawasaki disease therapy other than, or in addition to, IVIG treatment. Accordingly, the present invention encompasses methods and compositions that utilize these biomarkers for the diagnosis, prognosis, and treatment of Kawasaki disease.



METHODS AND COMPOSITIONS FOR THE DIAGNOSIS AND TREATMENT OF KAWASAKI DISEASE

BACKGROUND

5 Kawasaki disease is an acute, systemic vasculitis predominantly affecting young children. Clinical symptoms of Kawasaki disease include persistent fever not managed by antipyretic medications and antibiotics, rash, conjunctival infection, edema and erythema of the extremities, and oropharyngeal erythema.

10 The disease may impact the systemic vasculature, but the coronary arteries and cardiac tissue are particularly susceptible to damage. Inflammation of the coronary arteries and surrounding cardiac tissue may be mild and reversible or may be extensive, leading to cardiac artery aneurysms (ballooning) and stenosis (narrowing) of the arteries. An estimated 25% of Kawasaki disease subjects develop cardiac artery aneurysms or stenosis. While many subjects show recovery of cardiac functions and no angiographic evidence of cardiac artery aneurysms or stenosis following recovery, there can be evidence
15 of continued endothelial and vascular dysfunction even years later. Subjects with larger aneurysms are at higher risk for myocardial infarct (MI) and other cardiovascular events later in life.

There is no specific test available to diagnose Kawasaki disease. Diagnosis largely is a process of ruling out diseases that cause similar signs and symptoms (<http://www.mayoclinic.org/diseases-conditions/kawasaki-disease/basics/tests-diagnosis/con-20024663>, March 3, 2014).

SUMMARY OF THE INVENTION

Applicants have discovered that determination of levels or expression of particular biomarkers (e.g., protein levels, mRNA levels, glycan abundance, and/or the binding properties of IgG) in biological samples can be utilized to diagnose, prognose, and treat Kawasaki disease in subjects, and further to
25 select subjects who would benefit from a Kawasaki disease therapy other than, or in addition to, IVIG treatment. Accordingly, the present invention encompasses methods and compositions that utilize these proteins for the diagnosis, prognosis, and treatment of Kawasaki disease.

In a first aspect, the invention features a method for diagnosing Kawasaki disease in a subject. This method includes the step of determining the level of one or more (e.g., two, three, four, five, six,
30 seven, eight, nine, ten, twelve, fifteen, twenty, twenty-five, thirty, or more) proteins of Table 1 and/or Table 2 e.g., two, three, four, five, six, seven, eight, nine, ten, twelve, fifteen, twenty, twenty-five, thirty, or more and/or determining whether IgG in the sample binds to one or more (e.g., two, three, four, five, six, seven, eight, nine, ten, twelve, fifteen, twenty, twenty-five, thirty, forty, fifty, sixty, or more) peptides of Table 3.

Table 1. Selected Protein Biomarkers

Protein Name	Protein Name
Alpha-1-antichymotrypsin(serpin peptidase inhibitor, clade A, member 3)	apolipoprotein F
lactate dehydrogenase C	zinc finger protein 578
armadillo repeat containing 2	xin actin-binding repeat containing 2
unc-45 homolog A (C. elegans)	lipoprotein, Lp(a)
defensin, alpha 1	armadillo repeat containing 10
S100 calcium binding protein A9	olfactory receptor, family 5, subfamily D, member 14
solute carrier family 26 (anion exchanger), member 3	orosomucoid 1
zeta-chain (TCR) associated protein kinase 70kDa	Alpha-1-antitrypsin (serpin peptidase inhibitor, clade A, member 1)
Zinc finger protein 106 homolog	nitric oxide synthase 2, inducible (Gene)
apolipoprotein B	carcinoembryonic antigen-related cell adhesion molecule 8 (Gene)
Zinc finger protein 161 homolog	interleukin 33 (Gene)
apolipoprotein M	interleukin 6 (interferon, beta 2) (Gene)
apolipoprotein C-II	matrix metalloproteinase 1 (interstitial collagenase) (Gene)

Table 2. Selected Protein Biomarkers

Protein Name	Protein Name
collagen, type VI, alpha 3	Ig lambda-1 chain C regions (Mcg marker)
F-box protein 47	BMP2 inducible kinase
collagen, type VI, alpha 1	antithrombin III (serpin peptidase inhibitor, clade C, member 1)
androgen receptor	pregnancy-zone protein
tumor protein p53 binding protein 1	inter-alpha-trypsin inhibitor heavy chain 1
peptidase inhibitor 16	Ig kappa chain V-II region
cadherin 13	inter-alpha-trypsin inhibitor heavy chain 2
tenascin XB	complement factor H
peroxisome proliferator-activated receptor alpha	Hepatocyte growth factor activator

Protein Name	Protein Name
collagen, type I, alpha 1	attractin
Ig alpha-2 chain C region (A2m marker)	kininogen 1
Ig heavy chain V-III region TIL	coagulation factor XIII, B polypeptide
Ig alpha-1 chain C region	complement factor B
olfactomedin 1	complement component 1, r subcomponent
complement factor H	extracellular matrix protein 1
prostaglandin D2 synthase 21kDa (brain)	complement component 1, s subcomponent
sex hormone-binding globulin	complement component 6
Ig kappa chain V-I region	vitamin D binding protein (group-specific component)
gelsolin	

Table 3 Selected IgG Binding Peptides

SEQ ID NO.	Peptide Sequence	SEQ ID NO.	Peptide Sequence
1	AKFLGQSTYIAGYHQVD	35	AMLGGMWAAYYFPFVPG
2	FWSKMKPSEETYTFYRD	36	YYWATGPEGPFRHPGAR
3	FDRSDYMSFHLDDNITI	37	DMEFTVFDIDMEKHYYK
4	IRIETPYKDTEDGKYF	38	HYWDYQQLGFQGHLDHR
5	LGLLQAITRNSWVDSAF	39	DQHFWWGPTGRAPMNYG
6	KHWEFMQFDIGYIEKF	40	TNGFHIPFYSDFAQSAAA
7	NIPSNQHATEIQVDGYH	41	MKYDVWKFYNGDDMRVS
8	ENFEYHLYDSMIGYEVH	42	WYDNMYKTGFYRMYLLT
9	ERPDPATYFMPGRDDQY	43	EFQDYNHSDVNMSNHPY
10	DNAPYYYREEWHKEFNK	44	YIPEQQPHEADNLYKDA
11	YHWDVQNTFYSLMLPS	45	FYTLPHRPLYYYGYVAS
12	GDFSDYAPTLTQKASYG	46	HRWLEEANTEYMTMNSI
13	QYAFHNLDQNGTVFGNR	47	GPINAYQKQDYSIEPEH
14	YVTNMMINMNYSSLSYS	48	AGDQYVRIDKSTRISNI
15	HWVLSDGYREVYSYNSY	49	SMKTVEADWYTYEPWWH

SEQ ID NO.	Peptide Sequence	SEQ ID NO.	Peptide Sequence
16	AYHSQLYIDYKDTEWIFY	50	NHIYIVQTAYGVTGETS
17	DHPYFVIWDRYKPVHTY	51	MFAYHRAWPVWSSVLHV
18	HEHPPYLGMTAYELAQD	52	WPNPYFYHKKDTYWAHY
19	GQWSGQGYWYDPFDNMK	53	NYDHLLGQYPIRNWWSL
20	TTHFLKDRFESTNHDVY	54	ESMDVWVPYGYKFTQYW
21	VEDPRVGHSFLQDANY	55	THMEHDFHIPLMYKYM
22	DPVQIFNTAEHSGPYIR	56	VTPLEIIEQIREHLDIK
23	HDHFRGGKFILSTQAIW	57	LQKPFDDYMKWQVDNE
24	NYPLPKYYYNWFEPRVW	58	VMQRWPENHFLQTHYDD
25	QYDDPDWQIHYKLEARG	59	WWNWRSAKEDVAYPS
26	QDPYMDLHYDKNQIEQA	60	GWVLDDWSSHINYYIE
27	RGPHNFEIAETDAQMIE	61	FSPHYQYVGLFPYVKYI
28	DAYTNQDISSEEHMHR	62	ANLVYMWGSVHTSDPQ
29	GALEWIYYAGPKPGYWE	63	SPVMYSIANYKYQTMHL
30	PHSTHQIFYKSYETDMA	64	DQEISYLSHTDLFVGR
31	IYWGPMSTGHLPSQAQF	65	YEPTDVYLYRKLATKD
32	YTHFWWLDKYMRYEVAT	66	PVPQERTDLFTGAHRAL
33	FLTEYYEYQNNLFHAFR	67	REVYHEIKSGRAIEIYM
34	SRGDAAAWGILFDANWK	68	HDAIWYDWNPYPSKHES

According to this method, an increased level (e.g., an increase by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more, or an increase by more than 1.2-fold, 1.4-fold, 1.5-fold, 1.8-fold, 2.0-fold, 3.0-fold, 3.5-fold, 4.5-fold, 5.0-fold, 10-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold, 1000-fold, or more) of at least one protein of Table 1, as compared to a reference (e.g., a control, such as a predetermined control value, or a sample from a subject that does not have Kawasaki disease), and/or a decreased level (e.g., a decrease by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more; or a decrease by less than 0.01-fold, 0.02-fold, 0.1-fold, 0.3-fold, 0.5-fold, 0.8-fold, or less) of at least one protein of Table 2, as compared to a reference (e.g., a control, such as a predetermined control value, or a sample from a subject that does not have Kawasaki disease), and/or increased binding (e.g., an increase by 5%, 10%, 15%, 20%, 25%,

30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more, or an increase by more than 1.2-fold, 1.4-fold, 1.5-fold, 1.8-fold, 2.0-fold, 3.0-fold, 3.5-fold, 4.5-fold, 5.0-fold, 10-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold, 1000-fold, or more) of IgG in said sample to a peptide of Table 3, as compared to a reference (e.g., a control, such as a predetermined control value, or a sample from a subject that does not have Kawasaki disease) is indicative of the subject having Kawasaki disease.

In some embodiments, the method further includes the step of determining the level of one or more (e.g., two, three, four, five, six, seven, eight, nine, ten, twelve, fifteen, twenty, twenty-five, thirty, or more) additional biomarkers in the biological sample. In certain embodiments, the one or more additional biomarkers are a protein of Table 4, Table 5, Table 6, Table 7, Table 8, and/or Table 9; an mRNA of Table 10, Table 11, Table 12, and/or Table 13; and/or a glycan of Table 14, Table 15, Table 16, and/or Table 17.

Table 4. Selected Protein Biomarkers

L-lactate dehydrogenase
CD44 antigen
Ankyrin repeat domain-containing protein 26
Lysosome-associated membrane glycoprotein 2
Basal cell adhesion molecule
Multimerin-1
Transforming growth factor-beta-induced protein ig-h3
Methylcytosine dioxygenase TET2
Alpha-1-antichymotrypsin
Cystatin-C
Aftiphilin
Death domain containing 1
Xin actin-binding repeat containing 2
Metastasis associated 1 family, member 3

Table 5. Selected Protein Biomarkers

Plasminogen
Vitamin K-dependent protein C
Coagulation factor XI
Apolipoprotein F
HGF activator
Bone marrow stromal cell antigen 1
Mannan-binding lectin serine peptidase 2
HCG2014417, isoform CRA a
T-lymphoma invasion and metastasis-inducing protein 1

Table 6. Selected Protein Biomarkers

IgA
Hemoglobin subunit gamma-1
Hemoglobin subunit gamma-2
Protein S100-A9
Protein S100-A8
Leucine-rich alpha-2-glycoprotein
orosomucoid 1

orosomuroid 2
Actin, cytoplasmic 1
Haptoglobin-related protein
Cathepsin D
C-reactive protein
Xin actin-binding repeat containing 2
Beta-galactoside alpha-2,6-sialyltransferase 1
Complement component 9
Haptoglobin
Matrix metalloproteinase 9
Nidogen-1

Table 7. Selected Protein Biomarkers

Gelsolin
Kallistatin
Kininogen-1
Lumican
Alpha-2-HS-glycoprotein
butyrylcholinesterase
Coagulation factor IX
Protein C
Ig heavy chain V-III region BRO
Selenoprotein P
Cadherin-13
Myosin-8
Androgen receptor
Afamin
Biotinidase
Neural cell adhesion molecule L1-like protein
Coagulation factor X
Tenascin XB
Titin

Table 8. Selected Protein Biomarkers

Olfactory receptor, family 5, subfamily D, member 14
Orosomuroid 2
Polycystin (PKD) family receptor for egg jelly
Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3
Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1
Complement component 9
C-reactive protein, pentraxin related
Haptoglobin-related protein
Haptoglobin
Orosomuroid 1
Leucine-rich alpha-2-glycoprotein
Protein S100-A9
Lipopolysaccharide binding protein
Beta-galactoside alpha-2,6-sialyltransferase 1
Hemoglobin, alpha 1
Protein S100-A8
Hemoglobin, delta
Fc fragment of IgG, low affinity IIIa receptor (CD16a)
Hemoglobin, gamma G

Hemaglobin, gamma A
Hemaglobin, beta
Kinesin family member 20B
Lysozyme
Actin, alpha, cardiac muscle 1
Inter-alpha-trypsin inhibitor heavy chain 3
Inter-alpha-trypsin inhibitor heavy chain family, member 4
Actin, beta-like 2
Hemaglobin, epsilon 1
IgA
Protein phosphatase 6, regulatory subunit 2
Dedicator of cytokinesis 3
Cathepsin D
Centrosomal protein 290 kDa

Table 9. Selected Protein Biomarkers

Complement component 2
Complement factor H-related 3
Clusterin
Kallikrein B, plasma (Fletcher factor) 1
Macrophage stimulating 1 (hepatocyte growth factor-like)
Biotinidase
Peptidoglycan recognition protein 2
Fibulin 1
Kininogen 1
Gelsolin
Mannan-binding lectin serine peptidase 1 (C4/C2 activating component of Ra-reactive factor)
Alpha-1-microglobulin/bikunin precursor
Apolipoprotein H (beta-2-glycoprotein I)
Sex hormone-binding globulin
Collagen, type I, alpha 1
Inter-alpha-trypsin inhibitor heavy chain 2
Inter-alpha-trypsin inhibitor heavy chain 1
TBC1 domain family, member 8B (with GRAM domain)
Afamin
Heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa)
BMP2 inducible kinase
Collagen, type VI, alpha 3
Kallistatin
Lumican
Cadherin 13
Neural cell adhesion molecule L1-like protein
Androgen receptor
Hepatocyte growth factor activator
Insulin-like growth factor binding protein, acid labile subunit
Tenascin XB
Alpha-2-HS-glycoprotein
Glycosylphosphatidylinositol specific phospholipase D1
Retinol binding protein 4, plasma
Serpin peptidase inhibitor, clade C (antithrombin), member 1
butyrylcholinesterase
Complement factor H
Centrosomal protein 70 kDa

Coagulation factor XIII, B polypeptide
Thrombospondin 4
Fibronectin 1
Insulin-like growth factor 2 (somatomedin A)
Protein C
Selenoprotein P, plasma 1
Neuropilin 1
Neural cell adhesion molecule 1
alpha-1-B glycoprotein
Coagulation factor XIII, A1 polypeptide
Extracellular matrix protein 1
Apolipoprotein C-1
Carboxypeptidase B2 (plasma)
Attractin
Zinc finger protein 217
Sex hormone-binding globulin
Pregnancy zone protein
Collagen, type XI, alpha 2
Selectin L
Ectonucleotide pyrophosphatase/ phosphodiesterase 2
Coagulation factor II (thrombin)
Apolipoprotein D
Plasmoginen
Insulin growth factor binding protein 3
Dehydrogenase/reductase (SDR family) member 11
Quiescin Q6 sulfhydryl oxidase 1

Table 10. Selected mRNA Biomarkers

CD80
Beta-glucuronidase

5

Table 11. Selected mRNA Biomarkers

carcinoembryonic antigen-related cell adhesion molecule 8
S100 calcium binding protein A12
Versican

Table 12 Selected mRNA Biomarkers

Fc fragment of IgG, high affinity Ia, receptor (CD64)
S100 calcium binding protein A12
Matrix metalloproteinase 9
Tumor necrosis factor receptor superfamily, member 1A
Versican
Colony stimulating factor 2 receptor, beta, low affinity (granulocyte-macrophage)
Interleukin 1, beta
Interleukin 1 receptor antagonist
S100 calcium binding protein A8
S100 calcium binding protein A11
Tumor necrosis factor receptor superfamily, member 1B
Tumor necrosis factor (ligand) superfamily, member 13b
Fc fragment of IgG, low affinity Iia receptor (CD32)

Chemokine (C-C motif) receptor 2
Actin, beta
Tumor necrosis factor

Table 13. Selected mRNA Biomarkers

Beta-glucuronidase
CD80
Killer cell lectin-like receptor subfamily G, member 1

Table 14. Selected IgG and IgG Glycan Biomarkers

GP38HA
GP23HA
GP41HA
GP28HA
GP37HA
GP42HA
GP43HA
GP43LA
GP44HA
GP44LA
GP9HA
GP9LA

5

Table 15. Selected IgG and IgG Glycan Biomarkers

GP6HA
GP4HA
GP26HA
GP11HA
GP5HA
GP7HA
GP2HA
GP12HA
IgG2_G0F_BGlcNAc
IgG2_BGlcNAc
GP3HA
IgG2_G1F_BGlcNAc
GP13HA
GP10HA
IgG3_4_A1
GP12LA
GP14HA
GP14LA
GP16LA
GP18HA
GP18LA
GP19LA
GP20HA
GP20LA
GP24LA
GP29LA
GP32HA
GP32LA
GP34HA
GP34LA

GP38LA
GP3LA

Table 16. Selected IgG and IgG Glycan Biomarkers

GP41LA
GP42LA
GP42HA
GP41HA
GP43LA
GP43HA
GP37HA
GP44HA
GP44LA
GP23HA
GP35HA
GP31HA
IgA_G1_S1

Table 17. Selected IgG and IgG Glycan Biomarkers

GP13HA
GP18HA
GP12LA
GP20LA
GP18LA
GP12HA
GP11HA
GP19HA
GP3HA
GP10HA
GP5HA
GP6HA
GP13LA
GP24LA
GP8HA
GP7HA
GP32LA
GP26HA
GP5LA
GP19LA
GP6LA
GP2HA
GP20HA

- 5 By this method, a subject can be further diagnosed for a predisposition to develop a secondary Kawasaki disease symptom, e.g., a cardiac artery aneurysm or stenosis. According to this method, an increased level (e.g., an increase by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more; or an increase by more than 1.2-fold, 1.4-fold, 1.5-fold, 1.8-fold, 2.0-fold, 3.0-fold, 3.5-fold, 4.5-fold, 5.0-fold, 10-fold, 15-fold, 20-
- 10 fold, 30-fold, 40-fold, 50-fold, 100-fold, 1000-fold, or more) of a protein of Table 4, Table 6, and/or Table 8, an mRNA of Table 10 or Table 12, and/or a glycan of Table 14 or Table 16, as compared to a reference (e.g., a control, such as a predetermined control value, or a sample from a subject that does not have Kawasaki disease or a subject that has Kawasaki disease and responded positively to IVIG

treatment) and/or a decreased level (e.g., a decrease by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more; or a decrease by less than 0.01-fold, 0.02-fold, 0.1-fold, 0.3-fold, 0.5-fold, 0.8-fold, or less) of a protein of Table 5, Table 7, and/or Table 9, an mRNA of Table 11 or Table 13, and/or a glycan of Table 15 or Table 17, as compared to a reference (e.g., a control, such as a predetermined control value, or a sample from a subject that does not have Kawasaki disease or a subject that has Kawasaki disease and responded positively to IVIG treatment) is indicative of said subject having a predisposition to develop a secondary Kawasaki disease symptom, e.g., a cardiac artery aneurysm or stenosis.

In a second aspect, the invention features a method for diagnosing whether a subject has a predisposition to develop cardiac artery aneurysms or stenosis (e.g., without an initial biomarker-based Kawasaki disease diagnosis). This method includes the step of determining the level of one or more (e.g., two, three, four, five, six, seven, eight, nine, ten, twelve, fifteen, twenty, twenty-five, thirty, or more) biomarkers of Table 4, Table 5, Table 6, Table 7, Table 8, Table 9, Table 10, Table 11, Table 12, Table 13, Table 14, Table 15, Table 16, and/or Table 17 in a biological sample obtained from the subject. According to this method, an increased level (e.g., an increase by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more, or an increase by more than 1.2-fold, 1.4-fold, 1.5-fold, 1.8-fold, 2.0-fold, 3.0-fold, 3.5-fold, 4.5-fold, 5.0-fold, 10-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold, 1000-fold, or more) of at least one protein of Table 4, Table 6, and/or Table 8, an mRNA of Table 10 or Table 12, and/or a glycan of Table 14 or Table 16, as compared to a reference (e.g., a control, such as a predetermined control value, or a sample from a subject that does not have Kawasaki disease), and/or a decreased level (e.g., a decrease by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more; or a decrease by less than 0.01-fold, 0.02-fold, 0.1-fold, 0.3-fold, 0.5-fold, 0.8-fold, or less) of at least one protein of Table 5, Table 7, and/or Table 9, an mRNA of Table 11 or Table 13, and/or a glycan of Table 15 or Table 17, as compared to a reference (e.g., a control, such as a predetermined control value, or a sample from a subject that does not have Kawasaki disease) is indicative of a subject having a predisposition to develop cardiac artery aneurysms or stenosis.

In a third aspect, the invention features a method for classifying a subject. Such classification includes predicting the response to a Kawasaki disease therapy in a subject, selecting a subject that may benefit from a Kawasaki disease therapy, selecting a subject who may benefit from IVIG therapy, or predicting the responsiveness of a subject to IVIG therapy. This method includes the step of determining the level of one or more (e.g., two, three, four, five, six, seven, eight, nine, ten, twelve, fifteen, twenty, twenty-five, thirty, or more) proteins of Table 1 and/or Table 2 in a biological sample obtained from the subject and/or determining whether IgG in the sample binds to one or more (e.g., two, three, four, five, six, seven, eight, nine, ten, twelve, fifteen, twenty, twenty-five, thirty, forty, fifty, sixty, or more) peptides of Table 3. According to this method, a subject is classified based on at least one or more of the proteins of Table 1 having an increased level (e.g., an increase by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or

more, or an increase by more than 1.2-fold, 1.4-fold, 1.5-fold, 1.8-fold, 2.0-fold, 3.0-fold, 3.5-fold, 4.5-fold, 5.0-fold, 10-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold, 1000-fold, or more), as compared to a reference (e.g., a control, such as a predetermined control value, or a sample from a subject that does not have Kawasaki disease), and/or at least one or more of the proteins of Table 2 having a decreased level (e.g., a decrease by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more; or a decrease by less than 0.01-fold, 0.02-fold, 0.1-fold, 0.3-fold, 0.5-fold, 0.8-fold, or less), as compared to a reference (e.g., a control, such as a predetermined control value, or a sample from a subject that does not have Kawasaki disease), and/or increased binding (e.g., an increase by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more, or an increase by more than 1.2-fold, 1.4-fold, 1.5-fold, 1.8-fold, 2.0-fold, 3.0-fold, 3.5-fold, 4.5-fold, 5.0-fold, 10-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold, 1000-fold, or more) of IgG in said sample to a peptide of Table 3, as compared to a reference (e.g., a control, such as a predetermined control value, or a sample from a subject that does not have Kawasaki disease).

In some embodiments, the method further includes the step of determining the level of one or more (e.g., two, three, four, five, six, seven, eight, nine, ten, twelve, fifteen, twenty, twenty-five, thirty, or more) additional biomarkers in the biological sample. In certain embodiments, the one or more additional proteins are any protein of Table 4, Table 5, Table 6, Table 7, Table 8, and/or Table 9; an mRNA of Table 10, Table 11, Table 12, and/or Table 13; and/or a glycan of Table 14, Table 15, Table 16, and/or Table 17. By this step a subject can be further classified (for example, by determining the likelihood of a subject to develop cardiac artery aneurysms or stenosis, predicting the response to a Kawasaki disease therapy, selecting a subject that may benefit from a Kawasaki disease therapy other than, or in addition to, IVIG therapy, or predicting the responsiveness of a subject to IVIG therapy) based on one or more of the proteins of Table 4, Table 6, and/or Table 8, one or more mRNA of Table 10 or Table 12, and/or one or more glycan of Table 14 or Table 16 having an increased level (e.g., an increase by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more; or an increase by more than 1.2-fold, 1.4-fold, 1.5-fold, 1.8-fold, 2.0-fold, 3.0-fold, 3.5-fold, 4.5-fold, 5.0-fold, 10-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold, 1000-fold, or more), as compared to a reference (e.g., a control, such as a predetermined control value, or a sample from a subject that does not have Kawasaki disease or a subject that has Kawasaki disease and responded positively to IVIG treatment) and/or one or more of the proteins of Table 5, Table 7, and/or Table 9, one or more mRNA of Table 11 or Table 13, and/or one or more glycan of Table 15 or Table 17 having a decreased level (e.g., a decrease by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more; or a decrease by less than 0.01-fold, 0.02-fold, 0.1-fold, 0.3-fold, 0.5-fold, 0.8-fold, or less), as compared to a reference (e.g., a control, such as a predetermined control value, or a sample from a subject that does not have Kawasaki disease or a subject that has Kawasaki disease and responded positively to IVIG treatment).

In a fourth aspect, the invention features a method for classifying a subject. Such classification includes determining the likelihood of a subject to develop cardiac artery aneurysms or stenosis,

predicting the response to a Kawasaki disease therapy, selecting a subject that may benefit from a Kawasaki disease therapy other than, or in addition to, IVIG therapy, or predicting the responsiveness of a subject to IVIG therapy. The method includes: determining the level of one or more (e.g., two, three, four, five, six, seven, eight, nine, ten, twelve, fifteen, twenty, twenty-five, thirty, or more) biomarkers of Table 4, Table 5, Table 6, Table 7, Table 8, Table 9, Table 10, Table 11, Table 12, Table 13, Table 14, Table 15, Table 16, and/or Table 17 in a biological sample. According to this method, a subject is classified based on at least one or more of the Table 4, Table 6, and/or Table 8, one or more mRNA of Table 10 or Table 12, and/or one or more glycan of Table 14 or Table 16 having an increased level (e.g., an increase by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more; or an increase by more than 1.2-fold, 1.4-fold, 1.5-fold, 1.8-fold, 2.0-fold, 3.0-fold, 3.5-fold, 4.5-fold, 5.0-fold, 10-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold, 1000-fold, or more), as compared to a reference (e.g., a control, such as, a predetermined control value, or a sample from a subject that does not have Kawasaki disease or a subject that has Kawasaki disease and responded positively to IVIG treatment) and/or one or more Table 5, Table 7, and/or Table 9, one or more mRNA of Table 11 or Table 13, and/or one or more glycan of Table 15 or Table 17 having a decreased level (e.g., a decrease by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more; or a decrease by less than 0.01-fold, 0.02-fold, 0.1-fold, 0.3-fold, 0.5-fold, 0.8-fold, or less), as compared to a reference (e.g., a control, such as a predetermined control value, or a sample from a subject that does not have Kawasaki disease or a subject that has Kawasaki disease and responded positively to IVIG treatment).

In a fifth aspect, the invention features a method for treating Kawasaki disease. The method includes: (a) determining the level of one or more (e.g., two, three, four, five, six, seven, eight, nine, ten, twelve, fifteen, twenty, twenty-five, thirty, or more) proteins from Table 1 or Table 2 in a biological sample obtained from the subject and/or determining whether IgG in the sample binds to one or more (e.g., two, three, four, five, six, seven, eight, nine, ten, twelve, fifteen, twenty, twenty-five, thirty, forty, fifty, sixty, or more) peptides of Table 3.; and (b) administering a Kawasaki disease therapy to the subject if the level of the one or more proteins is indicative that the subject may benefit from a Kawasaki disease therapy (e.g., administration of IVIG). In this method, an increased level (e.g., an increase by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more; or an increase by more than 1.2-fold, 1.4-fold, 1.5-fold, 1.8-fold, 2.0-fold, 3.0-fold, 3.5-fold, 4.5-fold, 5.0-fold, 10-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold, 1000-fold, or more) of a protein of Table 1, as compared to a reference (e.g., a control, such as a predetermined control value, or a sample from a subject that does not have Kawasaki disease), is indicative that the subject may benefit from a Kawasaki disease therapy, and/or a decreased level (e.g., a decrease by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more; or a decrease by less than 0.01-fold, 0.02-fold, 0.1-fold, 0.3-fold, 0.5-fold, 0.8-fold, or less) of a protein of Table 2, as compared to a reference (e.g., a control, such as a predetermined control value, or a sample from a subject that does not have Kawasaki disease), and/or increased binding (e.g., an increase by 5%, 10%, 15%, 20%, 25%, 30%, 35%,

40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more, or an increase by more than 1.2-fold, 1.4-fold, 1.5-fold, 1.8-fold, 2.0-fold, 3.0-fold, 3.5-fold, 4.5-fold, 5.0-fold, 10-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold, 1000-fold, or more) of IgG in said sample to a peptide of Table 3, as compared to a reference (e.g., a control, such as a predetermined control value, or a sample from a subject that does not have Kawasaki disease) is indicative that the subject may benefit from a Kawasaki disease therapy.

In some embodiments, the method further includes, prior to the determining step, the step of selecting a subject having a fever and one or more of: red eyes; a red swollen tongue; red skin on the palms on the hands and/or soles of the feet; peeling skin on the hands and/or feet; a rash on the main part of the body and/or in the genital area; and swollen lymph nodes and/or the step of obtaining a biological sample from said subject.

In other embodiments, the method further includes, between step (a) and step (b), the step of comparing the level of said one or more proteins to a reference (e.g., a predetermined control value) and/or comparing the binding of IgG in the sample to a reference (e.g., a predetermined control value).

In some embodiments, the method further includes (c) determining the level of one or more (e.g., two, three, four, five, six, seven, eight, nine, ten, twelve, fifteen, twenty, twenty-five, thirty, or more) biomarkers from Table 4, Table 5, Table 6, Table 7, Table 8, Table 9, Table 10, Table 11, Table 12, Table 13, Table 14, Table 15, Table 16, and/or Table 17 in a biological sample obtained from the subject; and (d) administering a Kawasaki disease therapy other than, or in addition to, IVIG therapy (e.g., a Kawasaki disease therapy including one or more anticoagulants such as enoxaparin and/or clopidogrel, an anti-inflammatory such as aspirin, and/or one or more immunosuppressant drugs such as infliximab, cyclosporine, and/or prednisone) to the subject if the level of the one or more biomarkers is indicative that the subject may benefit from a Kawasaki disease therapy other than, or in addition to, IVIG therapy. In this method, an increased level (e.g., an increase by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more; or an increase by more than 1.2-fold, 1.4-fold, 1.5-fold, 1.8-fold, 2.0-fold, 3.0-fold, 3.5-fold, 4.5-fold, 5.0-fold, 10-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold, 1000-fold, or more) of a protein of Table 4, Table 6, and/or Table 8, an mRNA of Table 10 or Table 12, and/or a glycan of Table 14 or Table 16 as compared to a reference (e.g., a control, such as a predetermined control value, or a sample from a subject that does not have Kawasaki disease), is indicative that the subject may benefit from a Kawasaki disease therapy other than, or in addition to, IVIG therapy (e.g., a Kawasaki disease therapy including one or more anticoagulants such as enoxaparin and/or clopidogrel, an anti-inflammatory such as aspirin, and/or one or more immunosuppressant drugs such as infliximab, cyclosporine, and/or prednisone); and/or a decreased level (e.g., a decrease by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more; or a decrease by less than 0.01-fold, 0.02-fold, 0.1-fold, 0.3-fold, 0.5-fold, 0.8-fold, or less) of a protein of Table 5, Table 7, and/or Table 9, an mRNA of Table 11 or Table 13, and/or a glycan of Table 15 or Table 17 as compared to a reference (e.g., a control, such as a predetermined control value, or a sample from a subject that does not have Kawasaki disease), is indicative that the subject may benefit from a Kawasaki disease therapy other than, or in addition to, IVIG therapy.

In a sixth aspect, the invention features a method of treating Kawasaki disease. The method includes: (a) determining the level of one or more (e.g., two, three, four, five, six, seven, eight, nine, ten, twelve, fifteen, twenty, twenty-five, thirty, or more) biomarkers from Table 4, Table 5, Table 6, Table 7, Table 8, Table 9, Table 10, Table 11, Table 12, Table 13, Table 14, Table 15, Table 16, and/or Table 17 in a biological sample obtained from the subject; and (b) administering a Kawasaki disease therapy other than, or in addition to, IVIG therapy (e.g., a Kawasaki disease therapy including one or more anticoagulants such as enoxaparin and/or clopidogrel, an anti-inflammatory such as aspirin, and/or one or more immunosuppressant drugs such as infliximab, cyclosporine, and/or prednisone) to the subject if the level of the one or more biomarkers is indicative that the subject may benefit from a Kawasaki disease therapy other than, or in addition to, IVIG therapy. In this method, an increased level (e.g., an increase by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more; or an increase by more than 1.2-fold, 1.4-fold, 1.5-fold, 1.8-fold, 2.0-fold, 3.0-fold, 3.5-fold, 4.5-fold, 5.0-fold, 10-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold, 1000-fold, or more) of a protein of Table 4, Table 6, and/or Table 8, an mRNA of Table 10 or Table 12, and/or a glycan of Table 14 or Table 16 as compared to a reference (e.g., a control, such as a predetermined control value, or a sample from a subject that does not have Kawasaki disease), is indicative that the subject may benefit from a Kawasaki disease therapy other than, or in addition to, IVIG therapy (e.g., a Kawasaki disease therapy including one or more anticoagulants such as enoxaparin and/or clopidogrel, an anti-inflammatory such as aspirin, and/or one or more immunosuppressant drugs such as infliximab, cyclosporine, and/or prednisone); and/or a decreased level (e.g., a decrease by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more; or a decrease by less than 0.01-fold, 0.02-fold, 0.1-fold, 0.3-fold, 0.5-fold, 0.8-fold, or less) of a protein of Table 5, Table 7, and/or Table 9, an mRNA of Table 11 or Table 13, and/or a glycan of Table 15 or Table 17 as compared to a reference (e.g., a control, such as a predetermined control value, or a sample from a subject that does not have Kawasaki disease), is indicative that the subject may benefit from a Kawasaki disease therapy other than, or in addition to, IVIG therapy.

Any of the methods herein that rely upon protein measurement can also be adapted for use with the measurement of mRNA levels for the protein. Accordingly, in a seventh aspect, the invention features a method for diagnosing Kawasaki disease in a subject, diagnosing whether a subject has a predisposition to develop cardiac artery aneurysms or stenosis, classifying a subject, or treating Kawasaki disease. This method includes the step of determining the level of mRNA encoding one or more (e.g., two, three, four, five, six, seven, eight, nine, ten, twelve, fifteen, twenty, twenty-five, thirty, or more) proteins of Tables 1-9 in a biological sample obtained from the subject. According to this method, increased level of mRNA (e.g., an increase by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more; or an increase by more than 1.2-fold, 1.4-fold, 1.5-fold, 1.8-fold, 2.0-fold, 3.0-fold, 3.5-fold, 4.5-fold, 5.0-fold, 10-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold, 1000-fold, or more) encoding a protein of Table 1, Table 4, Table 6, and/or Table 8 as compared to a reference (e.g., a control, such as a predetermined control value, or a sample from a subject that does not have Kawasaki disease), is a basis for

classification of the subject and/or is indicative of the subject having Kawasaki disease, of the subject having a predisposition to develop cardiac artery aneurysms or stenosis, that the subject may benefit from a Kawasaki disease therapy, or that the subject may benefit from a Kawasaki disease therapy other than, or in addition to, IVIG therapy; and/or decreased mRNA level (e.g., a decrease by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more; or a decrease by less than 0.01-fold, 0.02-fold, 0.1-fold, 0.3-fold, 0.5-fold, 0.8-fold, or less) of a protein of Table 2, Table 5, Table 7, and/or Table 9 as compared to a reference (e.g., a control, such as a predetermined control value, or a sample from a subject that does not have Kawasaki disease), is a basis for classification of the subject and/or is indicative of the subject having Kawasaki disease, of the subject having a predisposition to develop cardiac artery aneurysms or stenosis, that the subject may benefit from a Kawasaki disease therapy, or that the subject may benefit from a Kawasaki disease therapy other than, or in addition to, IVIG therapy.

In some embodiments, the method further includes the step of administering a Kawasaki disease therapy or a Kawasaki disease therapy other than, or in addition to, IVIG therapy if the mRNA level of the one or more proteins is indicative that the subject may benefit from a Kawasaki disease therapy or a Kawasaki disease therapy other than, or in addition to, IVIG therapy.

In other embodiments, the method further includes prior to determining the expression level, extracting mRNA from the biological sample and reverse transcribing the mRNA into cDNA to obtain a treated biological sample.

In certain embodiments, the mRNA level is determined by an amplification-based assay (e.g., PCR, quantitative PCR, or real-time quantitative PCR), amplification-free assay (e.g., Nanostring), microdroplet based assay, nanopore based assay, or bead based assays (e.g., Luminex, nanoparticles, Nanosphere).

Next generation sequencing methods may also be used with the methods of the invention. Next generation sequencing methods are sequencing technologies that parallelize the sequencing process, producing thousands or millions of sequences concurrently (see, for example, Hall, *J. Exp. Biol.* 209(Pt.9):1518-1525 (2007) for a review of next generation methods). Next generation sequencing methods include, but are not limited to, polony sequencing, 454 pyrosequencing, Illumina (Solexa) sequencing, SOLiD sequencing, Ion Torrent semiconductor sequencing, DNA nanoball sequencing, Heliscope single molecule sequencing, single molecule real time sequencing, nanopore DNA sequencing (see, for example, Dela Torre et al. *Nanotechnology*, 23(38):385308, 2012), tunneling currents DNA sequencing (see, for example, Massimiliano, *Nanotechnology*, 24:342501, 2013), sequencing by hybridization (see, for example, Qin et al. *PLoS One*, 7(5):e35819, 2012), sequencing with mass spectrometry (see, for example, Edwards et al. *Mutation Research*, 573(1-2):3-12, 2005), microfluidic Sanger sequencing (see, for example, Kan et al. *Electrophoresis*, 25(21-22):3564-3588, 2004), microscopy-based sequencing (see, for example, Bell et al. *Microscopy and microanalysis : the official journal of Microscopy Society of America, Microbeam Analysis Society, Microscopical Society of Canada*, 18(5):1-5, 2012), and RNA polymerase sequencing (see, for example, Pareek et al. *J. Applied Genetics*, 52(4):413-415, 2011).

In an eighth aspect, the invention features a kit or device for selecting a subject that may benefit from a Kawasaki disease therapy. The kit or device includes a set of two or more (e.g., three, four, five,

six, seven, eight, nine, ten, twelve, fifteen, twenty, twenty-five, thirty, or more) distinct binding agents, each of the binding agents being capable of specifically binding to at least one protein from Table 1 and/or Table 2, wherein each binding agent binds a different protein and/or one or more peptides from Table 3.

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In a ninth aspect, the invention features a kit or device for selecting a subject that may benefit from a Kawasaki disease therapy. The kit or device includes a set of two or more (e.g., three, four, five, six, seven, eight, nine, ten, twelve, fifteen, twenty, twenty-five, thirty, or more) distinct reagents, each or the reagents being capable of detecting at least one mRNA that encodes a protein from Table 1 and/or Table 2, wherein each reagent detects a different mRNA that encodes a protein from Table 1 and/or Table 2.

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Optionally the kit or device also includes instructions for use of the kit or device to determine the level of the proteins in a biological sample and/or instructions for use of the kit or device to determine the binding of IgG in the sample to the one or more peptides of Table 3.

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In some embodiments of any of the foregoing kits or devices, the kit or device further includes a set of one or more (e.g., two, three, four, five, six, seven, eight, nine, ten, twelve, fifteen, twenty, twenty-five, thirty, or more) binding agents, each of the binding agents being capable of specifically binding to at least one protein, or the mRNA which encodes the protein, from Table 4, Table 5, Table 6, Table 7, Table 8, and/or Table 9; an mRNA or protein product of an mRNA of Table 10, Table 11, Table 12, and/or Table 13; and/or a glycan of Table 14, Table 15, Table 16, and/or 17.

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In a tenth aspect, the invention features a kit or device for selecting a subject that may benefit from a Kawasaki disease therapy other than, or in addition to, IVIG therapy. The kit or device includes a set of two or more (e.g., two, three, four, five, six, seven, eight, nine, ten, twelve, fifteen, twenty, twenty-five, thirty, or more) binding agents, each of the binding agents being capable of specifically binding to at least one protein from Table 4, Table 5, Table 6, Table 7, Table 8, and/or Table 9; an mRNA or protein product of an mRNA of Table 10, Table 11, Table 12, and/or Table 13; and/or a glycan of Table 14, Table 15, Table 16, and/or 17 wherein each binding agent binds a different biomarker.

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In an eleventh aspect, the invention features a kit or device for selecting a subject that may benefit from a Kawasaki disease therapy. The kit or device includes a set of two or more (e.g., three, four, five, six, seven, eight, nine, ten, twelve, fifteen, twenty, twenty-five, thirty, or more) distinct reagents, each or the reagents being capable of detecting at least one mRNA that encodes a protein from Table 4, Table 5, Table 6, Table 7, Table 8, and/or Table 9 wherein each reagent detects a different mRNA that encodes a protein from Table 4, Table 5, Table 6, Table 7, Table 8, and/or Table 9.

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In a twelfth aspect, the invention features a method for diagnosing Kawasaki disease in a subject. The method includes determining the level of one or more proteins in a biological sample obtained from the subject with any of the foregoing kits or devices, wherein an increased level (e.g., an increase by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more; or an increase by more than 1.2-fold, 1.4-fold, 1.5-

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fold, 1.8-fold, 2.0-fold, 3.0-fold, 3.5-fold, 4.5-fold, 5.0-fold, 10-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold, 1000-fold, or more) of a protein of Table 1, as compared to a reference (e.g., a control, such as a predetermined control value, or a sample from a subject that does not have Kawasaki disease), and/or a decreased level (e.g., a decrease by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more; or a decrease by less than 0.01-fold, 0.02-fold, 0.1-fold, 0.3-fold, 0.5-fold, 0.8-fold, or less) of a protein of Table 2, as compared to a reference (e.g., a control, such as a predetermined control value, or a sample from a subject that does not have Kawasaki disease), and/or increased binding (e.g., an increase by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more, or an increase by more than 1.2-fold, 1.4-fold, 1.5-fold, 1.8-fold, 2.0-fold, 3.0-fold, 3.5-fold, 4.5-fold, 5.0-fold, 10-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold, 1000-fold, or more) of IgG in said sample to a peptide of Table 3, as compared to a reference (e.g., a control, such as a predetermined control value, or a sample from a subject that does not have Kawasaki disease) is indicative of the subject having Kawasaki disease.

In some embodiments, the method further includes the step of determining the level of one or more (e.g., two, three, four, five, six, seven, eight, nine, ten, twelve, fifteen, twenty, twenty-five, thirty, or more) additional biomarkers in the biological sample. In certain embodiments, the one or more additional biomarkers are any biomarker of Table 4, Table 5, Table 6, Table 7, Table 8, Table 9, Table 10, Table 11, Table 12, Table 13, Table 14, Table 15, Table 16, and/or Table 17. A subject can be further diagnosed with a predisposition to develop cardiac artery aneurysms or stenosis based on one or more of the proteins of Table 4, Table 6, and/or Table 8, an mRNA of Table 10 or Table 12, and/or a glycan of Table 14 or Table 16 having an increased level (e.g., an increase by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more; or an increase by more than 1.2-fold, 1.4-fold, 1.5-fold, 1.8-fold, 2.0-fold, 3.0-fold, 3.5-fold, 4.5-fold, 5.0-fold, 10-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold, 1000-fold, or more), as compared to a reference (e.g., a control, such as a predetermined control value, or a sample from a subject that does not have Kawasaki disease or a subject that has Kawasaki disease and responded positively to IVIG treatment) and/or one or more of the proteins of Table 5, Table 7, and/or Table 9, an mRNA of Table 11 or Table 13, and/or a glycan of Table 15 or Table 17 having a decreased level (e.g., a decrease by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more; or a decrease by less than 0.01-fold, 0.02-fold, 0.1-fold, 0.3-fold, 0.5-fold, 0.8-fold, or less), as compared to a reference (e.g., a control, such as a predetermined control value, or a sample from a subject that does not have Kawasaki disease or a subject that has Kawasaki disease and responded positively to IVIG treatment) is indicative of the subject having a predisposition to develop cardiac artery aneurysms or stenosis.

In a thirteenth aspect, the invention features a method for diagnosing whether a subject has a predisposition to develop cardiac artery aneurysms or stenosis. This method includes the step of determining the level of one or more (e.g., two, three, four, five, six, seven, eight, nine, ten, twelve, fifteen, twenty, twenty-five, thirty, or more) biomarkers of Table 4, Table 5, Table 6, Table 7, Table 8, Table 9, Table 10, Table 11, Table 12, Table 13, Table 14, Table 15, Table 16, and/or Table 17 in a biological sample obtained from the subject with any of the foregoing kits or devices. According to this method, an

increased level (e.g., an increase by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more, or an increase by more than 1.2-fold, 1.4-fold, 1.5-fold, 1.8-fold, 2.0-fold, 3.0-fold, 3.5-fold, 4.5-fold, 5.0-fold, 10-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold, 1000-fold, or more) of at least one protein of Table 4, Table 6 and/or Table 8, and/or at least one glycan of Table 11, as compared to a reference (e.g., a control, such as a predetermined control value, or a sample from a subject that does not have Kawasaki disease), and/or a decreased level (e.g., a decrease by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more; or a decrease by less than 0.01-fold, 0.02-fold, 0.1-fold, 0.3-fold, 0.5-fold, 0.8-fold, or less) of at least one protein of Table 5, Table 7, and/or Table 9, at least one mRNA of Table 11 or Table 13, and/or at least one glycan of Table 15 or Table 17, as compared to a reference (e.g., a control, such as a predetermined control value, or a sample from a subject that does not have Kawasaki disease) is indicative of a predisposition to develop cardiac artery aneurysms or stenosis in said subject.

In a fourteenth aspect, the invention features a method for treating Kawasaki disease in a subject. The method includes the steps of (a) determining the level of one or more proteins in a biological sample obtained from the subject and/or binding of IgG in the sample to one or more peptides with any of the foregoing kits or devices; and (b) administering a Kawasaki disease therapy to the subject if the level of the one or more proteins is indicative that the subject may benefit from a Kawasaki disease therapy (e.g., administration of IVIG). In this method, an increased level (e.g., an increase by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more; or an increase by more than 1.2-fold, 1.4-fold, 1.5-fold, 1.8-fold, 2.0-fold, 3.0-fold, 3.5-fold, 4.5-fold, 5.0-fold, 10-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold, 1000-fold, or more) of a protein of Table 1, as compared to a reference (e.g., a control, such as a predetermined control value, or a sample from a subject that does not have Kawasaki disease), is indicative that the subject may benefit from a Kawasaki disease therapy, and/or a decreased level (e.g., a decrease by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more; or a decrease by less than 0.01-fold, 0.02-fold, 0.1-fold, 0.3-fold, 0.5-fold, 0.8-fold, or less) of a protein of Table 2, as compared to a reference (e.g., a control, such as a predetermined control value, or a sample from a subject that does not have Kawasaki disease), and/or increased binding (e.g., an increase by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more, or an increase by more than 1.2-fold, 1.4-fold, 1.5-fold, 1.8-fold, 2.0-fold, 3.0-fold, 3.5-fold, 4.5-fold, 5.0-fold, 10-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold, 1000-fold, or more) of IgG in said sample to a peptide of Table 3, as compared to a reference (e.g., a control, such as a predetermined control value, or a sample from a subject that does not have Kawasaki disease) is indicative that the subject may benefit from a Kawasaki disease therapy.

In a fifteenth aspect, the invention features a further method for treating Kawasaki disease in a subject. This method includes the steps of (a) determining the level of one or more biomarkers in a biological sample obtained from the subject with any of the foregoing kits or devices; and (b)

administering a therapy other than, or in addition to, IVIG therapy to the subject if the level of the one or more proteins is indicative that the subject may benefit from a Kawasaki disease therapy other than, or in addition to, IVIG therapy. In this method, an increased level (e.g., an increase by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more; or an increase by more than 1.2-fold, 1.4-fold, 1.5-fold, 1.8-fold, 2.0-fold, 3.0-fold, 3.5-fold, 4.5-fold, 5.0-fold, 10-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold, 1000-fold, or more) of a protein of Table 4, Table 6, and/or Table 8, an mRNA of Table 10 or Table 12, and/or a glycan of Table 14 or Table 16 as compared to a reference (e.g., a control, such as a predetermined control value, or a sample from a subject that does not have Kawasaki disease), is indicative that the subject may benefit from a Kawasaki disease therapy other than, or in addition to, IVIG therapy (e.g., a Kawasaki disease therapy including one or more anticoagulants such as enoxaparin and/or clopidogrel, an anti-inflammatory such as aspirin, and/or one or more immunosuppressant drugs such as infliximab, cyclosporine, and/or prednisone); and/or a decreased level (e.g., a decrease by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more; or a decrease by less than 0.01-fold, 0.02-fold, 0.1-fold, 0.3-fold, 0.5-fold, 0.8-fold, or less) of a protein of Table 5, Table 7, and/or Table 9, an mRNA of Table 11 or Table 13, and/or a glycan of Table 15 or Table 17 as compared to a reference (e.g., a control, such as a predetermined control value, or a sample from a subject that does not have Kawasaki disease), is indicative that the subject may benefit from a Kawasaki disease therapy other than, or in addition to, IVIG therapy.

In other embodiments of any of the foregoing methods, the Kawasaki disease therapy includes administration of IVIG to the subject (e.g., in high doses such as greater than 400 mg/kg, 500 mg/kg, 600 mg/kg, 700 mg/kg, 800 mg/kg, 900 mg/kg, 1 g/kg, 1.1 g/kg, 1.2 g/kg, 1.3 g/kg, 1.4 g/kg, 1.5 g/kg, 1.6 g/kg, 1.7 g/kg, 1.8 g/kg, 1.9 g/kg, 2.0 g/kg, 2.10 g/kg, 2.20 g/kg, 2.3 g/kg, 2.4 g/kg, 2.5 g/kg or more). In some embodiments, IVIG is administered to the subject between the fifth and ninth day after the appearance of symptoms. In other embodiments of any of the foregoing methods, the Kawasaki disease therapy includes administration of one or more anticoagulants (e.g., enoxaparin and/or clopidogrel or a pharmaceutically acceptable salt thereof) to the subject. In some embodiments of any of the foregoing methods, the Kawasaki disease therapy includes administration of an anti-inflammatory agent (e.g., aspirin). In other embodiments of any of the foregoing methods, the Kawasaki disease therapy includes administration of one or more immunosuppressant drugs (e.g., infliximab, cyclosporine, and/or prednisone).

In certain embodiments of any of the foregoing methods, the subject has one or more of: a fever; red eyes; a rash on the main part of the body and/or in the genital area; red, dry, cracked lips; a red, swollen tongue; swollen, red skin on the palms of the hands and/or soles of the feet; swollen lymph nodes; irritability; peeling of the skin on the hands and/or feet; joint pain; diarrhea; vomiting; and abdominal pain. For example, the subject may have a fever (e.g., a fever lasting more than four days) and one or more of: red eyes; a red swollen tongue; red skin on the palms of the hands and/or soles of the feet; peeling of the skin on the hands and/or feet; a rash on the main part of the body and/or in the genital area; and swollen lymph nodes.

In other embodiments of any of the foregoing methods, the subject exhibits the clinical symptoms of cardiac artery aneurysms and/or stenosis of the arteries. In yet other embodiments of any of the foregoing methods, the subject has not been diagnosed with cardiac artery aneurysms and/or stenosis of the arteries prior to determining the level of the one or more proteins. In certain embodiments of any of the foregoing methods, the subject has a white blood cell count and/or a C-reactive protein measurement that is not indicative of inflammation.

In some embodiments of any of the foregoing methods, the biological sample is obtained from the subject prior to the commencement of IVIG therapy. In other embodiments of any of the foregoing methods, the biological sample is obtained from the subject after commencement of IVIG therapy. In certain embodiments of any of the foregoing methods, the biological sample is obtained from the subject with 24 hours after commencement of IVIG therapy. In some embodiments of any of the foregoing methods, the biological sample is a tissue sample, whole blood, plasma, urine, saliva, pancreatic juice, bile, or serum sample. In certain embodiments of any of the foregoing methods, the biological sample is a plasma sample.

In some embodiments of any of the foregoing methods, the biological sample is processed prior to determining the level of the one or more the proteins, e.g., the biological sample is centrifuged, the biological sample is filtered, the biological sample is diluted, the biological sample is treated with reagents (e.g., digesting enzymes or reducing reagents), the biological sample is fractionated to remove more abundant proteins (e.g., proteins present at concentrations greater than 0.01 g/dL, greater than 0.02 g/dL, greater than 0.05 g/dL, greater than 0.1 g/dL, greater than 0.2 g/dL, greater than 0.5 g/dL, greater than 1.0 g/dL, greater than 2.0 g/dL, greater than 3.0 g/dL), such as, albumins, globulins (e.g., haptoglobin, alpha2-macroglobulin, IgG, IgA, and IgM), alpha1-acid glycoprotein, apolipoprotein AI, apolipoprotein AII, complement C3, transthyretin, antitrypsin, transferrin, and fibrinogen and/or enrich for less abundant proteins, such as, any protein from Tables 1, 2, 4, or 5. In some embodiments, the biological sample is subjected to centrifugation to remove red blood cells. In certain embodiments, the biological sample is filtered (e.g., spin filtered). In some embodiments, the biological sample is diluted. In other embodiments, the biological sample is subjected to cold alcohol fractionation. In certain embodiments, the biological sample is subjected to chromatographic separation (e.g., using an immunoaffinity-based column). In some embodiments, the biological sample is concentrated. In other embodiments, the biological sample is buffer exchanged. In certain embodiments, the biological sample is treated with a digesting enzyme (e.g., trypsin).

In other embodiments any of the foregoing methods further include contacting the biological sample with one or more binding agents capable of specifically binding to the one or more proteins, one or more peptides of Table 3, one or more mRNAs of Table 10, Table 11, Table 12, and/or Table 13, and/or one or more glycans of Table 14, Table 15, Table 16, and/or Table 17.

In any of the aspects and embodiments described herein, the protein level and/or binding of IgG in the sample is determined by one or more of a hybridization assay, an immunoassay, liquid chromatography, mass spectrometry, and/or fluorescence *in situ* hybridization assay (e.g., Northern analysis, ELISA, immunohistochemical analysis, microarray, chip, microfluidic chip, sequencing, or Western blotting).

In certain embodiments of any of the foregoing methods, the subject is less than 18 years old (e.g., less than 17 years old, less than 16 years old, less than 15 years old, less than 14 years old, less than 13 years old, less than 12 years old, less than 11 years old, less than 10 years old, less than 9 years old, less than 8 years old, less than 7 years old, less than 6 years old, less than 5 years old, less than 4 years old, less than 3 years old, less than 2 years old, less than 1 year old, less than 6 months old). In some embodiments of any of the foregoing methods, the subject is Asian (e.g., Japanese or Korean) or Afro-Caribbean.

In other embodiments of any of the foregoing methods, the level of the one or more proteins and/or binding of IgG in the sample is determined at least twice within 365 days (e.g., twice within 180 days, within 90 days, within 60 days, within 30 days, within 14 days, within 7 days). In certain embodiments, the level of the one or more proteins and/or binding of IgG in the sample is determined at least once prior to the commencement of IVIG therapy and at least once after commencement of IVIG therapy.

In certain embodiments any of the foregoing kits or devices also include instructions for use of the kit or device to determine the level of the proteins in a biological sample, the binding of IgG to a peptide of Table 3, the expression level of an mRNA of Table 10, Table 11, Table 12, and/or Table 13, and/or the abundance of a glycan of Table 14, Table 15, Table 16, and/or Table 17.

In other embodiments of any of the foregoing methods, the method further includes the step of recording the result in a print or computer readable media. In other embodiments, the method further includes the step of informing (e.g., providing the results of the determining step on printable media) the subject that he or she has Kawasaki disease, may benefit from a Kawasaki disease therapy, may benefit from IVIG therapy, may have an increased likelihood to develop cardiac artery aneurysms and/or stenosis, may have a predisposition to develop cardiac artery aneurysms and/or stenosis, may benefit from a therapy other than, or in addition to, IVIG therapy, or may benefit from therapy that includes one or more anticoagulants, an anti-inflammatory agent, and/or one or more immunosuppressant drugs.

In some embodiments of any of the aspects described herein, the binding agent is an antibody. In other embodiments of any of the aspects described herein, one or more of the binding agents and/or peptides of Table 3 are provided on a solid support (e.g., as a microarray). In any of the aspects and embodiments described herein, the one or more proteins, one or more peptides of Table 3, one or more mRNAs of Table 10, Table 11, Table 12, and/or Table 13, one or more glycans of Table 14, Table 15, Table 16 and/or Table 17, and/or set of binding agents and/or peptides include or consist of any combination described herein. In any of the aspects and embodiments described herein, the one or more peptides of Table 3 may be attached to a solid support by a linker (e.g., an N-terminal or C-terminal cysteine, or an N-terminal or C-terminal cysteine-serine-glycine group).

Also provided herein are methods of monitoring a subject with Kawasaki disease. The diagnostic kits and methods disclosed herein can be used to determine an optimal treatment plan for a subject or to determine the efficacy of a treatment plan for a subject. For example, the subject can be treated for Kawasaki disease and the prognosis of the disease can be determined by the diagnostic kits and methods disclosed herein. In particular embodiments, a diagnostic kit or method is used to determine if a subject has Kawasaki disease. A diagnostic kit or method can include a screen for protein level and/or IgG binding profiles by any useful detection method (e.g., unlabeled, fluorescence, radiation, or

chemiluminescence). A diagnostic test can further include one or more binding agents (e.g., one or more of probes, primers, peptides, small molecules, aptamers, or antibodies) to detect the level of these proteins or mRNAs encoding these proteins. In certain embodiments, the diagnostic kit includes the use of one or more proteins associated with Kawasaki disease and/or one or more peptides of Table 3 in a diagnostic platform, which can be optionally automated.

Also provided herein are general strategies to develop diagnostic tests which can be used to diagnose Kawasaki disease based on the level of proteins, binding of IgG to one or more peptides of Table 3, mRNAs of Table 10, Table 11, Table 12, and/or Table 13, one or more glycans of Table 14, Table 15, Table 16 and/or Table 17 disclosed herein. These strategies can be used to develop tests that use one or more of these proteins, peptides, mRNAs, and/or glycans, any combination of one or more of these proteins, peptides, mRNAs and/or glycans, one or more of these proteins, peptides, mRNAs, and/or glycans in combination with any other biomarkers found to be associated with Kawasaki disease, and/or one or more of these proteins, peptides, mRNAs, and/or glycans in combination with one or more reference biomarkers not associated with Kawasaki disease.

Also provided herein are methods of determining the likelihood of a subject to develop cardiac artery aneurysms or stenosis. Accordingly, the invention also includes methods of diagnosing a subject that would benefit from a therapy other than or in addition to, IVIG therapy by performing any of the methods or using any of the compositions or kits described herein.

Other features and advantages of the invention will be apparent from the following description and the claims.

Definitions

As used herein, the term "about" means $\pm 10\%$ of the recited value.

The term "array" or "microarray," as used herein refers to an ordered arrangement of hybridizable array elements, preferably protein probes (e.g., antibodies), on a substrate. The substrate can be a solid substrate, such as a glass slide, beads, or microfluidic chip, or a semi-solid substrate, such as nitrocellulose membrane.

The term "Afro-Caribbean" refers to a person of Caribbean descent (i.e., is from or has an ancestor from the Caribbean Region, as classified by the United Nations Department of Economic and Social Affairs) and has an ancestor that emigrated from Africa to the Caribbean Region in the period since 1492.

The term "anticoagulant" refers to a drug that works to prevent the coagulation of blood, such as coumarins, thienopyridines (e.g., clopidogrel), heparin, low molecular weight heparin (e.g., enoxaparin), inhibitors of factor Xa, or thrombin inhibitors.

The term "anti-inflammatory agent" refers to a drug that reduces inflammation in a subject, e.g., non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, ibuprofen, and naproxen.

The term "Asian" refers to a person of Asian descent (i.e., is from or has an ancestor from the Eastern Asia or Southeastern Asia Regions, as classified by the United Nations Department of Economic and Social Affairs). For example, a person from, or having an ancestor from, Japan (i.e., someone who is Japanese); or a person from, or having an ancestor from, Korea (i.e., someone who is Korean) are Asian.

By a “binding agent” is meant any compound (e.g., a probe, primer, protein, small molecule, aptamer, or antibody) capable of specifically binding a target. By “specifically binds” is meant binding that is measurably different from a non-specific interaction. Specific binding can be measured, for example, by determining binding of a molecule compared to binding of a control molecule. For example, specific binding can be determined by competition with a control molecule that is similar to the target, for example, an excess of non-labeled target. In this case, specific binding is indicated if the binding of the labeled target to a binding agent is competitively inhibited by excess unlabeled target. The term “specific binding,” “specifically binding,” or “specifically binds to” a particular protein as used herein can be exhibited, for example, by a molecule having a K_D for the target of 10^{-4} M or lower, alternatively 10^{-5} M or lower, alternatively 10^{-6} M or lower, alternatively 10^{-7} M or lower, alternatively 10^{-8} M or lower, alternatively 10^{-9} M or lower, alternatively 10^{-10} M or lower, alternatively 10^{-11} M or lower, alternatively 10^{-12} M or lower, or a K_D in the range of 10^{-4} M to 10^{-12} M or 10^{-6} M to 10^{-10} M or 10^{-7} M to 10^{-9} M. As will be appreciated by the skilled artisan, affinity and K_D values are inversely related. A high affinity for a target is measured by a low K_D value. In one embodiment, the term “specific binding” refers to binding where a binding agent binds to a particular protein, mRNA, or glycan without substantially binding to any other protein, mRNA, or glycan.

By “biological sample” or “sample” is meant a fluid or solid sample from a subject. Biological samples may include cells; nucleic acid, protein, or membrane extracts of cells; or blood or biological fluids including (e.g., plasma, serum, saliva, urine, bile). Solid biological samples include samples taken from feces, the rectum, central nervous system, bone, breast tissue, renal tissue, the uterine cervix, the endometrium, the head or neck, the gallbladder, parotid tissue, the prostate, the brain, the pituitary gland, kidney tissue, muscle, the esophagus, the stomach, the small intestine, the colon, the liver, the spleen, the pancreas, thyroid tissue, heart tissue, lung tissue, the bladder, adipose tissue, lymph node tissue, the uterus, ovarian tissue, adrenal tissue, testis tissue, the tonsils, and the thymus. Fluid biological samples include samples taken from the blood, serum, plasma, pancreatic fluid, CSF, semen, prostate fluid, seminal fluid, urine, saliva, sputum, mucus, bone marrow, lymph, and tears. Samples may be obtained by standard methods including, e.g., venous puncture and surgical biopsy. In certain embodiments, the biological sample is a blood, plasma, or serum sample.

By “classifying a subject” is meant predicting a response to a Kawasaki disease therapy by a subject; selecting a subject that may benefit from a Kawasaki disease therapy; selecting a subject who may benefit from IVIG therapy; predicting the responsiveness of a subject to IVIG therapy; determining the likelihood of a subject to develop cardiac artery aneurysms or stenosis; or selecting a subject that may benefit from a Kawasaki disease therapy other than, or in addition to, IVIG therapy.

By “diagnosing” is meant identifying a molecular or pathological state, disease or condition, such as the identification of Kawasaki disease or cardiac artery aneurysm and/or stenosis, or to refer to identification of a subject having Kawasaki disease who may benefit from a particular treatment regimen.

By “determining the level of a protein, mRNA, or glycan” is meant the detection of a protein, mRNA, or glycan by methods known in the art either directly or indirectly. “Directly determining” means performing a process (e.g., performing an assay or test on a sample or “analyzing a sample” as that term is defined herein) to obtain the physical entity or value. “Indirectly determining” refers to receiving the physical entity or value from another party or source (e.g., a third party laboratory that directly acquired

the physical entity or value). Methods to measure protein level generally include, but are not limited to, western blotting, immunoblotting, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), immunoprecipitation, immunofluorescence, surface plasmon resonance, chemiluminescence, fluorescent polarization, phosphorescence, immunohistochemical analysis, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, liquid chromatography (LC)-mass spectrometry, microcytometry, microscopy, fluorescence activated cell sorting (FACS), and flow cytometry, as well as assays based on a property of a protein including, but not limited to, enzymatic activity or interaction with other protein partners. Methods to measure mRNA and glycan levels are known in the art. Exemplary methods are provided herein.

By “determining the binding of IgG” is meant the detection of binding of IgG in a sample (e.g., a plasma sample) to a binding agent (e.g., a peptide of Table 3) by methods known in the art. “Directly determining” means performing a process (e.g., performing an assay or test on a sample or “analyzing a sample” as that term is defined herein) to obtain the physical entity or value. “Indirectly determining” refers to receiving the physical entity or value from another party or source (e.g., a third party laboratory that directly acquired the physical entity or value). Methods to measure binding generally include, but are not limited to, western blotting, immunoblotting, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), immunoprecipitation, immunofluorescence, surface plasmon resonance, chemiluminescence, fluorescent polarization, phosphorescence, immunohistochemical analysis, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, liquid chromatography (LC)-mass spectrometry, microcytometry, microscopy, fluorescence activated cell sorting (FACS), flow cytometry, peptide arrays, protein arrays and microarrays.

The term “immunosuppressant drug,” as used herein refers to a drug that inhibits or prevents activity of the immune system including glucocorticoids such as prednisone, cytostatics such as methotrexate, antibodies such as infliximab, drugs acting on immunophilins such as cyclosporine.

By “informing a subject” is meant providing the subject or the parent or legal guardian of the subject the results of the determining step and/or analysis of the results verbally and/or on printable media.

The term “IVIG” as used herein refers to intravenous immunoglobulin, a blood product containing pooled, polyvalent IgG extracted from the plasma of over one thousand blood donors. The term “IVIG therapy” refers to a treatment including the administration of IVIG to a subject, e.g., in high doses, such as, 2 g/kg.

By “Kawasaki disease therapy” is meant any therapy in the art for the treatment of Kawasaki disease, such as, therapeutic agents or modalities for Kawasaki disease. Common treatments for Kawasaki disease include administration of IVIG (i.e., IVIG therapy); salicylates (e.g., aspirin); corticosteroids (e.g., prednisone); IL-1 receptor antagonists; anticoagulants (e.g., enoxaparin and/or clopidogrel); anti-TNF agents (e.g., infliximab); or any combination thereof.

The terms “kit or device,” as used herein, refer to a set of articles and/or equipment, such as reagents, instruments, and systems, intended for use in diagnosis or prognosis of disease or other conditions, including determination of the state of health, in order to cure, mitigate, treat, or prevent disease or its sequelae. The kits and devices of the invention are intended for use in the collection, preparation, and/or examination of biological samples taken from the subject. For example, the kits and

devices of the invention may be used for biochemical estimation or the qualitative detection of a protein.

The kits and devices of the invention may include general purpose reagents and analyte specific reagents. A "general purpose reagent" refers to a chemical reagent that has general laboratory

application, used to collect, prepare, and/or examine specimens from the human body for diagnostic

purposes, and is not labeled or otherwise intended for a specific diagnostic application. An "analyte

specific reagent" refers to antibodies, both polyclonal and monoclonal, specific receptor proteins, ligands, nucleic acids, and other binding agents which, through specific binding or chemical reaction with

substances in a biological sample, are intended for use in a diagnostic application for identification and

quantification of an individual chemical substance or ligand in biological samples. The kits and devices of

the invention may include a label which states the name of the kit or device, the intended use or uses of

the device (e.g., the diagnosis of Kawasaki disease), a statement of warnings or precautions for users of

any hazardous substances contained in the kit or device and any other warnings appropriate to user

hazards, the established name of the reagents, quantity, proportion, or concentration of all active

ingredients and for reagents derived from biological activity, the source and measure of its activity,

storage instructions, and/or instructions for manipulation of products requiring mixing or reconstitution.

The kit may also include instructions for detection read out and interpretation.

By "level" is meant a level of a protein, glycan, or mRNA, as compared to a reference. The

reference can be any useful reference, as defined herein. By a "decreased level" or an "increased level"

of a protein is meant a decrease or increase in protein level, as compared to a reference (e.g., a

decrease or an increase by about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about

35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%,

about 80%, about 85%, about 90%, about 95%, about 100%, about 150%, about 200%, about 300%,

about 400%, about 500%, or more; a decrease or an increase of more than about 10%, about 15%, about

20%, about 50%, about 75%, about 100%, or about 200%, as compared to a reference; a decrease or an

increase by less than about 0.01-fold, about 0.02-fold, about 0.1-fold, about 0.3-fold, about 0.5-fold, about

0.8-fold, or less; or an increase by more than about 1.2-fold, about 1.4-fold, about 1.5-fold, about 1.8-fold,

about 2.0-fold, about 3.0-fold, about 3.5-fold, about 4.5-fold, about 5.0-fold, about 10-fold, about 15-fold,

about 20-fold, about 30-fold, about 40-fold, about 50-fold, about 100-fold, about 1000-fold, or more). A

level of a protein may be expressed in mass/vol (e.g., g/dL, mg/mL, µg/mL, ng/mL) or percentage relative

to total protein, glycan, or mRNA in a sample. By "protein level profile" is meant one or more protein level

values determined for a sample.

By "processing a sample" is meant any process carried out on the sample prior to the

determination of the level or expression of the protein. Exemplary processing steps include, but are not

limited to, centrifugation of the sample, fractionation of the sample, treatment with reagents (e.g.,

digesting enzymes or reducing reagents), and/or dilution of the sample. By "fractionation of a sample" is

meant the general processes of separating the various components of a sample. For example, the

components of the sample may be separated by chromatography (e.g., ion exchange chromatography).

In some cases, the most abundant proteins, such as, proteins present at greater than 0.01, greater than

0.02, greater than 0.05, greater than 0.1 g/dL (e.g., greater than 0.2 g/dL, greater than 0.5 g/dL, greater

than 1.0 g/dL, greater than 2.0 g/dL, greater than 3.0 g/dL) are depleted from the sample by

chromatography to enhance the sensitivity for less abundant proteins, such as, proteins present at less

than 0.2 g/dL (e.g., less than 0.1 g/dL, less than 0.05 g/dL, less than 0.01 g/dL). Columns/kits for the depletion of abundant proteins are known in the art, for example, MARS Human-6 and Human-7 from Agilent Technologies deplete the 6 and 7 most abundant proteins from human plasma.

By “reagent” is meant a polynucleotide sequence or polypeptide sequence capable of detecting a target sequence, or a fragment thereof.

By a “reference” is meant any useful reference used to compare protein or mRNA levels related to Kawasaki disease and/or binding of IgG to a peptide of Table 3. The reference can be any sample, standard, standard curve, or level that is used for comparison purposes. The reference can be a normal reference sample or a reference standard or level. A “reference sample” can be, for example, a control, e.g., a predetermined negative control value such as a “normal control” or a prior sample taken from the same subject; a sample from a normal healthy subject, such as a normal cell or normal tissue; a sample (e.g., a cell or tissue) from a subject not having Kawasaki disease; a sample from a subject that is diagnosed with cardiac artery aneurysms or stenosis; a sample from a subject that has been treated for Kawasaki disease; or a sample of a purified protein (e.g., any described herein) at a known normal concentration. By “reference standard or level” is meant a value or number derived from a reference sample. A “normal control value” is a pre-determined value indicative of non-disease state, e.g., a value expected in a healthy control subject. Typically, a normal control value is expressed as a range (“between X and Y”), a high threshold (“no higher than X”), or a low threshold (“no lower than X”). A subject having a measured value within the normal control value for a particular biomarker is typically referred to as “within normal limits” for that biomarker. A normal reference standard or level can be a value or number derived from a normal subject not having Kawasaki disease; a subject that is diagnosed with cardiac artery aneurysms or stenosis; a subject that has been treated for Kawasaki disease. In preferred embodiments, the reference sample, standard, or level is matched to the sample subject sample by at least one of the following criteria: age, weight, sex, disease stage, and overall health. A standard curve of levels of a purified protein, e.g., any described herein, within the normal reference range can also be used as a reference.

“Response” as used herein indicates a subject’s response to a Kawasaki disease therapy, e.g., a response can be a positive response such that symptoms will be alleviated as a result of the Kawasaki disease therapy.

By “selecting a subject” is meant to choose a subject directly or indirectly in preference to others based on an analysis, e.g., analysis of results of the methods of the invention or clinical evaluation. *Directly selecting* means performing a process (e.g., performing an analysis) to choose a subject. *Indirectly selecting* refers to receiving the results of an analysis from another party or source (e.g., a third party laboratory that directly performed the analysis).

By “solid support” is meant a structure capable of storing, binding, or attaching one or more binding agents.

By “subject” is meant a human (e.g., a child less than 18 years old, less than 13 years old, less than 8 years old, less than 5 years old, less than 4 years old, less than 3 years old, less than 2 years old, or less than 1 year old). A subject to be treated with a pharmaceutical composition described herein may be one who has been diagnosed by a medical practitioner as having such a disease or condition (e.g.,

Kawasaki disease) or one at risk for developing a disease or condition (e.g., cardiac artery aneurysm or stenosis).

By "target sequence" is meant a portion of a gene or a gene product, including the mRNA and related cDNA.

By "therapeutic agent" is meant any agent that produces a healing, curative, stabilizing, or ameliorative effect.

A "therapeutically effective amount" of a compound may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the compound to elicit a desired response in the individual. A therapeutically effective amount encompasses an amount in which any toxic or detrimental effects of the compound are outweighed by the therapeutically beneficial effects. A therapeutically effective amount also encompasses an amount sufficient to confer benefit, e.g., clinical benefit.

By "treating" is meant administering a composition (e.g., a pharmaceutical composition) for therapeutic purposes or administering treatment to a subject already having a condition or disorder to improve the subject's condition or to reduce the likelihood of a condition or disorder. By "treating a condition or disorder" is meant that the condition or disorder and/or the symptoms associated with the condition or disorder are, e.g., alleviated, reduced, cured, or placed in a state of remission. By "reduce the likelihood of" is meant reducing the severity, the frequency, and/or the duration of a disorder (e.g., cardiac artery aneurysms and/or stenosis) or symptoms thereof. Reducing the likelihood of cardiac artery aneurysms and/or stenosis is synonymous with prophylaxis or the chronic treatment of cardiac artery aneurysms and/or stenosis.

Other features and advantages of the invention will be apparent from the following Detailed Description and the claims.

Detailed Description of the Invention

There is no specific test available to diagnose Kawasaki disease. Diagnosis largely is a process of ruling out diseases that cause similar signs and symptoms, including: scarlet fever; juvenile rheumatoid arthritis; Stevens-Johnson syndrome; toxic shock syndrome; measles; certain tick-borne illnesses. A doctor may do a physical examination and perform other tests to help in the diagnosis or prognosis. These tests may include urine tests, blood tests, electrocardiogram, and echocardiogram. (<http://www.mayoclinic.org/diseases-conditions/kawasaki-disease/basics/tests-diagnosis/con-20024663>, March 3, 2014).

The present invention relates to the identification of biomarkers (e.g., protein levels, mRNA levels, glycan abundance, or IgG binding) that identify subjects having Kawasaki disease and/or being predisposed to develop Kawasaki disease-related cardiac artery aneurysms or stenosis. Such differential levels of proteins, mRNAs, glycans, and/or differential binding of IgG in samples can be used to diagnose, prognose, and classify subjects with Kawasaki disease and/or a predisposition to develop cardiac artery aneurysms or stenosis from healthy controls. Accordingly, the kits and methods described herein are useful for treating or diagnosing Kawasaki disease and/or related cardiac artery aneurysms or stenosis. Also described herein are diagnostic kits (e.g., on a solid support, such as an array or chip) which can be used to perform such methods.

Proteins

Applicants have discovered that the levels of certain proteins can be utilized to diagnose, prognose, and treat Kawasaki disease, as well as to select subjects who would benefit from either IVIG therapy or a Kawasaki disease therapy other than, or in addition to, IVIG therapy. Proteins, the levels of which are of interest in the methods and compositions of the invention, include those in Table 18.

Table 18. Proteins with levels relevant to Kawasaki disease

Protein Name	Accession No.	Gene Symbol
collagen, type VI, alpha 3	E7ENL6	COL6A3
F-box protein 47	Q5MNV8	FBXO47
collagen, type VI, alpha 1	P12109	COL6A1
androgen receptor	P10275	AR
tumor protein p53 binding protein 1	Q12888	TP53BP1
peptidase inhibitor 16	Q6UXB8	PI16
cadherin 13	P55290	CDH13
tenascin XB	G5E9A9	TNXB
tenascin XB	B0UYX3	TNXB
peroxisome proliferator-activated receptor alpha	Q07869	PPARA
collagen, type I, alpha 1	P02452	COL1A1
Ig alpha-2 chain C region (A2m marker)	P01877	IGHA2
Ig heavy chain V-III region TIL	P01765	HV304
Ig alpha-1 chain C region	P01876	IGHA1
olfactomedin 1	Q6IMJ5	OLFM1
complement factor H	Q5TFM2	CFH
prostaglandin D2 synthase 21kDa (brain)	P41222	PTGDS
sex hormone-binding globulin	I3L145	SHBG
Ig kappa chain V-I region	P01593	KV101
gelsolin	P06396	GSN
Ig lambda-1 chain C regions (Mcg marker)	P0CG04	IGLC1
BMP2 inducible kinase	Q9NSY1	BMP2K
serpin peptidase inhibitor, clade C (antithrombin), member 1	P01008	SERPINC1
pregnancy-zone protein	P20742	PZP
inter-alpha-trypsin inhibitor heavy chain 1	P19827	ITIH1
Ig kappa chain V-II region	P01617	KV204
inter-alpha-trypsin inhibitor heavy chain 2	P19823	ITIH2
complement factor H	P08603	CFH
Hepatocyte growth factor activator	Q04756	HGFAC
attractin	O75882	ATRN
kininogen 1	P01042	KNG1
coagulation factor XIII, B polypeptide	P05160	F13B
complement factor B	B4E1Z4	CFB
complement component 1, r subcomponent	H0YFH3	C1R
complement component 1, r subcomponent	P00736	C1R
extracellular matrix protein 1	Q16610	ECM1
complement component 1, s	P09871	C1S

Protein Name	Accession No.	Gene Symbol
subcomponent		
kininogen 1	C9JEX1	KNG1
complement component 6	P13671	C6
vitamin D binding protein (group-specific component)	P02774	GC
Alpha-1-antichymotrypsin(serpin peptidase inhibitor, clade A, member 3)	P01011	SERPINA3
lactate dehydrogenase C	F5H5G7	LDHC
Alpha-1-antichymotrypsin(serpin peptidase inhibitor, clade A, member 3)	G3V3A0	SERPINA3
armadillo repeat containing 2	Q8NEN0	ARMC2
unc-45 homolog A (C. elegans)	Q9H3U1	UNC45A
defensin, alpha 1	P59665	DEFA1
S100 calcium binding protein A9	P06702	S100A9
solute carrier family 26 (anion exchanger), member 3	P40879	SLC26A3
zeta-chain (TCR) associated protein kinase 70kDa	P43403	ZAP70
Zinc finger protein 106 homolog	Q9H2Y7	ZFP106
apolipoprotein B	P04114	APOB
Zinc finger protein 161 homolog	J3QLI2	ZFP161
apolipoprotein M	O95445	APOM
apolipoprotein C-II	P02655	APOC2
apolipoprotein F	F5GXS5	APOF
zinc finger protein 578	I3L1Y6	ZNF578
xin actin-binding repeat containing 2	A4UGR9	XIRP2
lipoprotein, Lp(a)	P08519	LPA
armadillo repeat containing 10	Q8N2F6	ARMC10
olfactory receptor, family 5, subfamily D, member 14	Q8NGL3	OR5D14
orosomucoid 1	P02763	ORM1
Alpha-1-antitrypsin (serpin peptidase inhibitor, clade A, member 1)	P01009	SERPINA1
nitric oxide synthase 2, inducible (Gene)	4843	NOS2
carcinoembryonic antigen-related cell adhesion molecule 8 (Gene)	1088	CEACAM8
interleukin 33 (Gene)	90865	IL33
interleukin 6 (interferon, beta 2) (Gene)	3569	IL6
Plasminogen	P00747	PLG
Vitamin K-dependent protein C	P04070	PROC
Coagulation factor XI	P03951	F11
Apolipoprotein F	F5GXS5	APOF
CD44 antigen	E7EPC6	CD44
Ankyrin repeat domain-containing protein 26	Q9UPS8	ANKRD26
Lysosome-associated membrane glycoprotein 2	B4E2S7	LAMP2
Basal cell adhesion molecule	P50895	BCAM
Multimerin-1	Q13201	MMRN1
Transforming growth factor-beta-induced protein ig-h3	G8JLA8	TGFB1
Methylcytosine dioxygenase TET2	E7EQS8	TET2
Alpha-1-antichymotrypsin	P01011	SERPINA3
Cystatin-C	P01034	CST3

Protein Name	Accession No.	Gene Symbol
matrix metalloproteinase 1 (interstitial collagenase) (Gene)	4312	MMP1
Aftiphilin	Q6uLP2	AFTPH
Death domain containing 1	Q6ZMT9	DTHD1
Metastasis associated 1 family, member 3	E7EV10	MTA
HGF activator	Q04756	HGFAC
Bone marrow stromal cell antigen 1	Q10588	BST1
Mannan-binding lectin serine peptidase 2	O00187	MASP2
HCG2014417, isoform CRA a	B7Z718	AGAP2
T-lymphoma invasion and metastasis-inducing protein 1	F5GZ53	TIAM1
Hemoglobin subunit gamma-1	P69891	HBG1
Hemoglobin subunit gamma-2	P69892	HBG2
Protein S100-A9	P06702	S100A9
Protein S100-A8	P05109	S100A8
Leucine-rich alpha-2-glycoprotein	P02750	LRG1
Actin, cytoplasmic 1	P60709	ACTB
Haptoglobin-related protein	P00739	HPR
Cathepsin D	P07339	CTSD
C-reactive protein	P02741	CRP
Kallistatin	P29622	SERPINA4
Kininogen-1	C9JEX1	KNG1
Lumican	P51884	LUM
Alpha-2-HS-glycoprotein	P02765	AHSG
Butyrylcholinesterase	P06276	BCHE
Coagulation factor IX	P00740	F9
Ig heavy chain V-III region BRO	P01766	HV305
Selenoprotein P	P49908	SEPP1
Cadherin-13	P55290	CDH13
Myosin-8	P13535	MYH8
Orosomucoid 2	P19652	ORM2
Polycystin (PKD) family receptor for egg jelly	Q9NTG1	PKDREJ
Complement component 9	P02748	C9
Complement component 2	P06681	C2
Complement factor H-related 3	Q6NSD3	CFHR3
Clusterin	P10909	CLU
Kallikrein B, plasma (Fletcher factor) 1	H0YAC1	KLKB1
Macrophage stimulating 1 (hepatocyte growth factor-like)	P26927	MST1
Biotinidase	F8W1Q3	BTD
Peptidoglycan recognition protein 2	Q96PD5	PGLYRP2
Fibulin 1	B1AHL2	FBLN1
fibulin 1	P23142	FBLN1
Mannan-binding lectin serine peptidase 1 (C4/C2 activating component of Ra-reactive factor)	P48740	MASP1
Alpha-1-microglobulin/bikunin precursor	P02760	AMBP

Protein Name	Accession No.	Gene Symbol
Mannan-binding lectin serine peptidase 1 (C4/C2 activating component of Ra-reactive factor)	F8W876	MASP1
Apolipoprotein H (beta-2-glycoprotein I)	P02749	APOH
TBC1 domain family, member 8B (with GRAM domain)	J3KN75	TBC1D8B
Afamin	P43652	AFM
Heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa)	P11021	HSPA5
IgA	NA	IGA
ST6 beta-galactosamide alpha-2,6-sialyltransferase 1	NA	ST6
haptoglobin	P00738	HP
Matrix metalloproteinase 9	P14780	MMP9
Nidogen-1	P14543	NID1
Neural cell adhesion molecule L1-like protein	O00533	CHL1
Coagulation factor X	P00742	F10
Titin	Q8WZ42	TTN
Insulin-like growth factor binding protein, acid labile subunit	P35858	IGFALS
Glycosylphosphatidylinositol specific phospholipase D1	P80108	GPLD1
Retinol binding protein 4, plasma	Q5VY30	RBP4
Centrosomal protein 70 kDa	Q8NHQ1	CEP70
Thrombospondin 4	P35433	THBS4
Fibronectin 1	F8W7G7	FN1
Insulin-like growth factor 2 (somatomedin A)	P01344	IGF2
Neuropilin 1	O14786	NRP1
Neural cell adhesion molecule 1 alpha-1-B glycoprotein	E9PLH7	NCAM1
Coagulation factor XIII, A1 polypeptide	P04217	A1BG
Coagulation factor XIII, A1 polypeptide	P00488	F13A1
Apolipoprotein C-1	P02654	APOC1
Carboxypeptidase	Q961Y4	CPB2
Zinc finger protein 217	O75362	ZNF217
Collagen, type XI, alpha 2	H0YHY3	COL11A2
Selectin L	P14151	SELL
Ectonucleotide pyrophosphatase/phosphodiesterase 2	Q13822	ENPP2
Coagulation factor II (thrombin)	P00734	F2
Apolipoprotein D	P05090	APOD
Insulin growth factor binding protein 3	P17936	IGFBP3
Dehydrogenase/reductase (SDR family) member 11	A8MXC2	DHRS11
Quiescin Q6 sulfhydryl oxidase 1	O00391	QSOX1
Lipopolysaccharide binding protein	P18428	LBP
Hemoglobin, alpha 1	P69905	HBA1
Hemoglobin, delta	P02042	HBD
Fc fragment of IgG, low affinity IIIa receptor (CD16a)	P08637	FCGR3A
Hemoglobin, gamma G	P69892	HBG2
Hemoglobin, gamma A	P69891	HBG1
Hemoglobin, beta	P68871	HBB
Kinesin family member 20B	Q96Q89	KIF20B

Protein Name	Accession No.	Gene Symbol
Lysozyme	P61626	LYZ
Actin, alpha, cardiac muscle 1	P68032	ACTC1
Inter-alpha-trypsin inhibitor heavy chain 3	Q06033	ITIH3
Inter-alpha-trypsin inhibitor heavy chain family, member 4	Q14624	ITIH4
Inter-alpha-trypsin inhibitor heavy chain family, member 4	B7ZKJ8	ITIH4
Inter-alpha-trypsin inhibitor heavy chain family, member 4	E9PGN5	ITIH4
Actin, beta-like 2	Q562R	ACTBL2
Hemaglobin, epsilon 1	P02100	HBE1
Protein phosphatase 6, regulatory subunit 2	B3KMJ7	PPP6R2
Dedicator of cytokinesis 3	Q8IZD9	DOCK3
Centrosomal protein 290 kDa	O15078	CEP290

As indicated above, proteins useful for diagnosing Kawasaki disease, or selecting or classifying a subject that may benefit from a Kawasaki disease therapy include those in Tables 1 and 2. Proteins useful for selecting or classifying a subject that may benefit from a therapy other than, or in addition to, 5 IVIG therapy include those in Tables 4, 5, 6, and 7.

Applicants have discovered that the level of binding of IgG in samples to certain proteins can be utilized to diagnose, prognose, and treat Kawasaki disease, as well as to select subjects who would benefit from a Kawasaki disease therapy. Proteins, the binding of which are of interest in the methods and compositions of the invention, include those comprising an amino acid sequence of any one of SEQ 10 ID NOs:1 to 68.

Genes

Applicants have discovered that the mRNA expression levels of certain genes can be utilized to diagnose, prognose, and treat Kawasaki disease, as well as to select subjects who would benefit from 15 either IVIG therapy or a Kawasaki disease therapy other than, or in addition to, IVIG therapy. Genes, the mRNA levels of which are of interest in the methods and compositions of the invention, include those in Table 19.

Table 19. Genes with mRNA levels relevant to Kawasaki disease

Gene Name	Accession No.	Gene Symbol
CD80 molecule	941	CD80
Matrix metalloproteinase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)	4318	MMP9
Colony stimulating factor 2 receptor, beta, low-affinity	1439	CSF2RB
Tumor necrosis factor receptor superfamily, member 1A	7132	TNFRSF1A
carcinoembryonic antigen-related cell adhesion molecule 8	1088	CEACAM8
Beta-glucuronidase	2990	GUSB
S100 calcium binding protein A12	6283	S100A12
Versican	1462	VCAN
Fc fragment of IgG, high affinity	2209	FCGR1A

Gene Name	Accession No.	Gene Symbol
Ia, receptor (CD64)		
S100 calcium binding protein A11	6282	S100A11
Tumor necrosis factor receptor superfamily, member 1B	7133	TNFRSF1B
Interleukin 1, beta	3553	IL1B
Interleukin 1 receptor antagonist	3557	IL1RN
S100 calcium binding protein A8	6279	S100A8
Tumor necrosis factor (ligand) superfamily, member 13b	10673	TNFSF13B
Killer cell lectin-like receptor subfamily G, member 1	10219	KLRG1
Fc fragment of IgG, high affinity IIa, receptor (CD32)	2212	FCGR2A
chemokine (C-C motif) receptor 2	729230	CCR2
Actin, beta	60	ACTB
Tumor necrosis factor	7124	TNF

As indicated above, genes useful for selecting or classifying a subject that may benefit from a therapy other than, or in addition to, IVIG therapy include those in Table 10, Table 11, Table 12, and/or Table 13.

5 Glycans

Applicants have discovered that the levels of certain glycans on IgG and IgA can be utilized to diagnose, prognose, and treat Kawasaki disease, as well as to select subjects who would benefit from either IVIG therapy or a Kawasaki disease therapy other than, or in addition to, IVIG therapy. Glycans, the levels of which are of interest in the methods and compositions of the invention, include those in

10 Table 20.

Table 20. Glycans with levels relevant to Kawasaki disease

Fraction	Structure
GP6HA	FA2[3]G1
GP4HA	A2[6]BG1
GP26HA	FA2BG2S[3,6]2 FA2BG2S[6,6]2
GP11HA	FA2G2
GP5HA	FA2[6]G1
GP7HA	FA2[6]BG1
GP2HA	FA2
GP12HA	FA2BG2
IgG2_G0F_BGlcNAc	
IgG2_BGlcNAc	
GP3HA	M5 FA2B A2[6]G1
GP38HA	A4G4S[3,3,3,3]4
GP23HA	A2G2S[3,6]2
IgG2_G1F_BGlcNAc	
GP13HA	A2[3]BG1S[3]1 A2[3]BG1S[6]1 M7 D1

Fraction	Structure
	FA2[3]G1S[3]1 FA2[3]G1S[6]1
GP10HA	A2BG2
GP41HA	A4G4S[3,3,3,3]4
GP28HA	A3BG3S[3,6]2
IgG3_4_A1	IgG3 isoform A2F1G1S1
GP37HA	A4F1G3S[3,3,6]3 A4F1G3S[3,6,6]3
GP42HA	A4F1G4S[3,3,3,6]4
GP43HA	A4G4LacS[3,3,3,6]4
GP43LA	A4G4LacS[3,3,3,6]4
GP44HA	A4F3G4S[3,3,3,3]4
GP44LA	A4F3G4S[3,3,3,3]4
GP9HA	A1[3]G1S[3]1 A2G2
GP9LA	A1[3]G1S[3]1 A2G2
GP12LA	FA2BG2 A2[3]BG1S[3]1 A2[3]BG1S[6]1
GP14HA	FA2[3]BG1S[3]1
GP14LA	FA2[3]BG1S[3]1
GP16LA	A2BG2S[6]1
GP18HA	FA2G2S[6]1 FA2BG2S[3]1
GP18LA	FA2G2S[6]1 FA2BG2S[3]1
GP19LA	FA2BG2S[6]1
GP20HA	A2G2S[3,6]2 A3G3S[3]1
GP20LA	A2G2S[3,6]2 A3G3S[3]1
GP24LA	A2BG2S[6,6]2
GP29LA	A4G4S[3]1
GP32HA	A3G3S[3,3,6]3
GP32LA	A3G3S[3,3,6]3
GP34HA	A3G3S[3,3,6]3
GP34LA	A3G3S[3,3,6]3
GP38LA	A4G4S[3,3,3,3]4
GP3LA	M5 FA2B A2[6]G1
GP41LA	A4G4S[3,3,3,3]4
GP42LA	A4F1G4S[3,3,3,6]4
GP42HA	A4F1G4S[3,3,3,6]4
GP41HA	A4G4S[3,3,3,3]4
GP43LA	A4G4LacS[3,3,3,6]4
GP43HA	A4G4LacS[3,3,3,6]4
GP37HA	A4F1G3S[3,3,3]3 A4F1G3S[3,3,6]3 A4F1G3S[3,6,6]3
GP44HA	A4F3G4S[3,3,3,3]4
GP44LA	A4F3G4S[3,3,3,3]4

Fraction	Structure
GP23HA	A2G2S[3,6]2
GP35HA	A3F1G3S[3,3,3]3
GP31HA	A3G3S[3,3,3]3
IgA_G1_S1	A2,G1,S1
GP13HA	A2[3]BG1S[3]1 A2[3]BG1S[6]1 M7 D1
GP18HA	FA2G2S[6]1
GP12LA	FA2BG2
GP20LA	A2G2S[3,6]2
GP18LA	FA2G2S[6]1
GP12HA	FA2BG2
GP11HA	FA2G2
GP19HA	FA2BG2S[3]1; FA2BG2S[6]1
GP3HA	M5; FA2B; A2[6]G1
GP10HA	A2BG2
GP5HA	FA2[6]G1
GP6HA	FA2[3]G1
GP13LA	A2[3]BG1S[3]1; A2[3]BG1S[6]1; M7 D1; FA2[3]G1S[3]1
GP24LA	A2BG2S[6,6]2
GP8HA	FA2[3]BG1; M6 D3
GP7HA	FA2[6]BG1
GP32LA	A3G3S[3,3,6]3
GP26HA	FA2BG2S[3,6]2 FA2BG2S[6,6]2
GP5LA	FA2[6]G1
GP19LA	FA2BG2S[3]1 FA2BG2S[6]1
GP6LA	FA2[3]G1
GP2HA	FA2
GP20HA	A2G2S[3,6]2

G; N-glycans, P; plasma, fraction #, LA; low abundant fraction, HA; high abundant fraction.

Glycan annotations according to Oxford Symbol nomenclature. All N-glycans have two core

GlcNAcs; F at the start of the abbreviation indicates a core a(1-6)fucose linked to inner GlcNAc; Mx,

number (x) of mannose on core GlcNAcs; Ax, number of antenna (GlcNAc) on trimannosyl core; A2,

biantennary with both GlcNAcs as b(1-2) linked; A3, triantennary with a GlcNAc linked b(1-2) to both

mannose and a third GlcNAc linked b(1,4) to the a(1-3) linked mannose; A3'; triantennary with a

GlcNAc linked b(1-2) to both mannose and the third GlcNAc linked b(1-6) mannose; B, bisecting

GlcNAc linked b(1-4) to b(1-3) mannose; Gx, number (x) of b1-4 linked galactose on the antenna; Fx,

number (x) of linked fucose on antenna, (4) or (3) after the F indicates that the Fuc is a(1-4) or a(1-3)

linked to the GlcNAc; Sx, number (x) sialic acids linked to galactose; the number 3 or 6 in parenthesis

after S indicates whether the sialic acid is in an a(2-3) or a(2-6) linkage. See Harvey et al. Proposal

for a standard system for drawing structural diagrams of N- and O-linked carbohydrates and related

compounds. Proteomics 2009, 9:3796-801.

As indicated above, glycans useful for diagnosing Kawasaki disease, selecting or classifying a subject that may benefit from a Kawasaki disease therapy and/or selecting or classifying a subject that may benefit from a therapy other than, or in addition to, IVIG therapy include those in Table 14, Table 15, Table 16, and/or Table 17.

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Diagnostic and classification methods

The present invention features methods and compositions to diagnose Kawasaki disease. The kits and methods of the invention may be used alone or as a companion diagnostics with other diagnostic or therapeutic approaches, as an early molecular screen to distinguish Kawasaki disease from other diseases and disorders with similar symptoms. More specifically, alterations in the level of one or more proteins described herein (e.g., proteins of Table 1 and/or Table 2) and/or binding of IgG to a protein of Table 3 in a test sample as compared to a normal reference can be used to diagnose Kawasaki disease and/or distinguish Kawasaki disease from diseases or disorders with similar symptoms, thereby allowing subject classification.

Further, the present invention features methods and compositions useful in determining the likelihood of a subject to develop cardiac artery aneurysms and/or stenosis. For example, the methods and compositions of the invention may be used to determine if a subject may benefit from a Kawasaki disease therapy other than, or in addition to, IVIG therapy, by determining the levels of one or more biomarkers of Table 4, Table 5, Table 6, Table 7, Table 8, Table 9, Table 10, Table 11, Table 12, Table 13, Table 14, Table 15, Table 16, and/or Table 17.

The methods of the invention can be used to diagnose, prognose, or classify a subject, for example, an increase in the level (e.g., an increase by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more, or an increase by more than 1.2-fold, 1.4-fold, 1.5-fold, 1.8-fold, 2.0-fold, 3.0-fold, 3.5-fold, 4.5-fold, 5.0-fold, 10-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold, 1000-fold, or more, as compared to a reference) of the biomarker(s) (e.g., a protein of Table 1) may indicate a subject has Kawasaki disease and/or may benefit from a Kawasaki disease therapy. Similarly, a decrease in the level (e.g., a decrease by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more; or a decrease by less than 0.01-fold, 0.02-fold, 0.1-fold, 0.3-fold, 0.5-fold, 0.8-fold, or less, as compared to a reference) of the biomarker(s) (e.g., a protein of Table 2) may indicate a subject has Kawasaki disease and/or may benefit from a Kawasaki disease therapy. An increase (e.g., an increase by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more, or an increase by more than 1.2-fold, 1.4-fold, 1.5-fold, 1.8-fold, 2.0-fold, 3.0-fold, 3.5-fold, 4.5-fold, 5.0-fold, 10-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold, 1000-fold, or more, as compared to a reference) in binding of IgG in a sample to a protein comprising an amino acid sequence of Table 3 may indicate a subject has Kawasaki disease and/or may benefit from a Kawasaki disease therapy.

Alternatively, an increase in the level (e.g., an increase by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more, or an increase by more than 1.2-fold, 1.4-fold, 1.5-fold, 1.8-fold, 2.0-fold, 3.0-fold, 3.5-fold, 4.5-fold, 5.0-fold, 10-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold, 1000-fold, or more, as

compared to a reference) of the biomarker(s) (e.g., a protein of Table 4) may indicate a subject is predisposed to develop cardiac artery aneurysms or stenosis and/or may benefit from a Kawasaki disease therapy other than, or in addition to, IVIG therapy. Similarly, a decrease in the level (e.g., a decrease by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more; or a decrease by less than 0.01-fold, 0.02-fold, 0.1-fold, 0.3-fold, 0.5-fold, 0.8-fold, or less, as compared to a reference) of a biomarker(s) (e.g., a protein of Table 5) may indicate a subject is predisposed to develop cardiac artery aneurysms or stenosis and/or may benefit from a Kawasaki disease therapy other than, or in addition to, IVIG therapy.

To carry out the methods of the invention, a sample can be obtained by any method known in the art. For instance, samples from a subject may be obtained by venipuncture, resection, bronchoscopy, fine needle aspiration, bronchial brushings, or from sputum, pleural fluid, urine, or blood, such as serum or plasma. Proteins can be detected in these samples. By screening such biological samples, a simple early diagnosis or differential diagnosis can be achieved for Kawasaki disease. In addition, the progress of therapy can be monitored by testing such biological samples for target proteins and/or binding of IgG to a protein of Table 3. Furthermore, the prediction of outcome or response to therapy can similarly be tested using such biological samples for target proteins and/or binding of IgG to a protein of Table 3.

In certain embodiments, the sample may be contacted with an antibody specific for the target protein under conditions sufficient for an antibody-protein complex to form, and detection of the complex. The presence of the biomarker may be detected in a number of ways, such as by Western blotting or ELISA procedures using any of a wide variety of tissues or samples, including plasma or serum. A wide range of immunoassay techniques using such an assay format are available, see, e.g., U.S. Pat. Nos. 4,016,043, 4,424,279, and 4,018,653. These include both single-site and two-site or "sandwich" assays of the noncompetitive types, as well as traditional competitive binding assays. These assays also include direct binding of a labeled antibody to a target biomarker.

Another method involves immobilizing the target biomarkers (e.g., on a solid support) and then exposing the immobilized target to a specific antibody, which may or may not contain a label. Depending on the amount of target and the strength of the label's signal, a bound target may be detectable by direct labeling with the antibody. Alternatively, a second labeled antibody, specific to the first antibody is exposed to the target-first antibody complex to form a target-first antibody-second antibody tertiary complex. The complex is detected by the signal emitted by a label, e.g., an enzyme, a fluorescent label, a chromogenic label, a radionuclide containing molecule (i.e., a radioisotope), or a chemiluminescent molecule.

Variations on the forward assay include a simultaneous assay, in which both sample and labeled antibody are added simultaneously to a bound antibody. These techniques are well known to those skilled in the art, including any minor variations as will be readily apparent. In a typical forward sandwich assay, a first antibody having specificity for the biomarker is either covalently or passively bound to a solid surface (e.g., a glass or a polymer surface, such as those with solid supports in the form of tubes, beads, discs, or microplates), and a second antibody is linked to a label that is used to indicate the binding of the second antibody to the molecular marker.

In alternative methods, the expression of a protein in a sample may be examined using immunohistochemistry ("IHC") and staining protocols. IHC staining of tissue sections has been shown to

be a reliable method of assessing or detecting presence of proteins in a sample. IHC and immunofluorescence techniques use an antibody to probe and visualize cellular antigens in situ, generally by chromogenic or fluorescent methods. The tissue sample may be fixed (i.e., preserved) by conventional methodology (see, e.g., "Manual of Histological Staining Method of the Armed Forces Institute of Pathology," 3rd edition (1960) Lee G. Luna, HT (ASCP) Editor, The Blakston Division McGraw-Hill Book Company, New York; *The Armed Forces Institute of Pathology Advanced Laboratory Methods in Histology and Pathology* (1994) Ulreka V. Mikel, Editor, Armed Forces Institute of Pathology, American Registry of Pathology, Washington, D.C.). One of skill in the art will appreciate that the choice of a fixative is determined by the purpose for which the sample is to be histologically stained or otherwise analyzed. By way of example, neutral buffered formalin, Bouin's or formaldehyde, may be used to fix a sample. Generally, the sample is first fixed and is then dehydrated through an ascending series of alcohols, infiltrated and embedded with paraffin or other sectioning media so that the tissue sample may be sectioned. Alternatively, one may section the tissue and fix the sections obtained. The primary and/or secondary antibody used for immunohistochemistry typically will be labeled with a detectable moiety, such as a radioisotope, a colloidal gold particle, a fluorescent label, a chromogenic label, or an enzyme-substrate label.

Alternatively, the levels of biomarkers may be detected without the use of binding agents. In some instances, biological samples as described herein are analyzed, for example, by one or more, enzymatic methods, chromatographic methods, mass spectrometry (MS) methods, chromatographic methods followed by MS, electrophoretic methods, electrophoretic methods followed by MS, nuclear magnetic resonance (NMR) methods, and combinations thereof. In some instances, the biological sample is treated with one or more enzymes (e.g., trypsin). Exemplary chromatographic methods include, but are not limited to, Strong Anion Exchange chromatography using Pulsed Amperometric Detection (SAX-PAD), liquid chromatography (LC), high performance liquid chromatography (HPLC), ultra performance liquid chromatography (UPLC), thin layer chromatography (TLC), amide column chromatography, and combinations thereof. Exemplary mass spectrometry (MS) include, but are not limited to, tandem MS, LC-MS, LC-MS/MS, matrix assisted laser desorption ionisation mass spectrometry (MALDI-MS), Fourier transform mass spectrometry (FTMS), ion mobility separation with mass spectrometry (IMS-MS), electron transfer dissociation (ETD-MS), Multiple Reaction Monitoring (MRM), and combinations thereof. Exemplary electrophoretic methods include, but are not limited to, capillary electrophoresis (CE), CE-MS, gel electrophoresis, agarose gel electrophoresis, acrylamide gel electrophoresis, SDS-polyacrylamide gel electrophoresis (SDS-PAGE) followed by Western blotting using antibodies that recognize specific glycan structures, and combinations thereof. Exemplary nuclear magnetic resonance (NMR) include, but are not limited to, one-dimensional NMR (1D-NMR), two-dimensional NMR (2D-NMR), correlation spectroscopy magnetic-angle spinning NMR (COSY-NMR), total correlated spectroscopy NMR (TOCSY-NMR), heteronuclear single-quantum coherence NMR (HSQC-NMR), heteronuclear multiple quantum coherence (HMQC-NMR), rotational nuclear overhauser effect spectroscopy NMR (ROESY-NMR), nuclear overhauser effect spectroscopy (NOESY-NMR), and combinations thereof.

Any of the methods herein that rely upon protein measurement can also be adapted for use with the measurement of mRNA levels for the protein. The level of mRNA can be determined using methods

known in the art. Methods to measure mRNA levels generally include, but are not limited to, sequencing, northern blotting, RT-PCR, gene array technology, and RNase protection assays.

Binding Agents

Any binding agent that specifically binds a target biomarker may be used in the methods of the invention. The binding agent may be, e.g., a protein (e.g., an antibody), small molecule, or aptamer capable of specifically binding a target.

Preferably, each binding agent specifically binds to one biomarker in a sample. For determining the level of a biomarker, the measurement of antibodies specific to a biomarker of the invention in a subject may be used for the diagnosis of Kawasaki disease. The binding agent may optionally contain a label, such as a radioisotope, a colloidal gold particle, a fluorescent label, a chromogenic label, an enzyme-substrate label, or a chemiluminescent label.

Sample Processing

In some embodiments of any of the foregoing methods, the biological sample is processed prior to determining the level of the one or more the biomarkers, e.g., the biological sample is centrifuged, the biological sample is filtered, the biological sample is diluted, the biological sample is treated with reagents (e.g., digesting enzymes or reducing reagents), the biological sample is fractionated to remove more abundant proteins (e.g., proteins present at concentrations greater than 0.01 g/dL, greater than 0.02 g/dL, greater than 0.05 g/dL, greater than 0.1 g/dL, greater than 0.2 g/dL, greater than 0.5 g/dL, greater than 1.0 g/dL, greater than 2.0 g/dL, greater than 3.0 g/dL), such as, albumins, globulins (e.g., haptoglobin, alpha2-macroglobulin, IgG, IgA, and IgM), alpha1-acid glycoprotein, apolipoprotein AI, apolipoprotein AII, complement C3, transthyretin, antitrypsin, transferrin, and fibrinogen and/or enrich for less abundant proteins, such as, any protein from Tables 1, 2, 4, and/or 5.

For example, a blood sample may be obtained, and prior to determining the level of one or more proteins, the sample may be centrifuged to remove red blood cells (i.e., to provide a plasma sample). The plasma sample may be spin filtered and diluted. Subsequently, the sample may be chromatographically separated using an immunoaffinity-based column to remove more abundant proteins (e.g., the 10-20, e.g., 10, 12, 14, 16, 18, 20 most abundant proteins) and enrich for less abundant proteins. The enriched sample may be concentrated and buffer exchanged, followed by treatment with a digesting enzyme, e.g., trypsin. Determination of protein levels may then be carried out on the processed sample.

Methods for predicting and monitoring response to Kawasaki disease therapies

The invention further features methods for predicting response to a Kawasaki disease therapy in a subject before or after administration of one or more Kawasaki disease therapies. These methods may be carried out generally as described above or as known in the art with respect to sample collection and assay format. For example, these methods may be carried out by collecting a sample, e.g., a blood or plasma sample from a subject; measuring the level of one or more biomarkers described herein (e.g., proteins of Table 1, 2, 4, 5, 6, 7, 8, and/or 9, genes of Table 10, Table 11, Table 12, and/or Table 13, and/or glycans of Table 14, Table 15, Table 16, and/or Table 17) in the sample and/or determining the

binding of IgG in the sample to a protein comprising an amino acid sequence of Table 3; comparing to a control sample; and making a prediction about whether the subject will be responsive to a Kawasaki disease therapy. The method also can be used to predict whether a subject, who has been diagnosed with Kawasaki disease, will respond positively to a Kawasaki disease therapy such as a therapeutic (e.g.,
 5 IVIG) or a combination of therapeutics (e.g., IVIG and one or more anticoagulants, an anti-inflammatory agent, and/or one or more immunosuppressant drugs).

A prediction of a positive response refers to a case where the Kawasaki disease symptoms will be alleviated and/or the risk of mortality will be reduced as a result of the Kawasaki disease therapy.

In the methods of predicting response to a Kawasaki disease therapy, the level of the protein(s)
 10 binding of IgG, gene(s), and/or glycan(s) can be determined relative to a control value. A control value can be a range or average value from a normal subject or a population of normal subjects; a value from a sample from a subject or population of subjects who have undergone a Kawasaki disease therapy and have reduced symptoms following therapy; a value from the same subject before the subject was diagnosed or before the subject started treatment.

The methods of the invention can be used to predict whether a subject will be responsive to a
 15 Kawasaki disease therapy, for example, an increase in the level (e.g., an increase by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more, or an increase by more than 1.2-fold, 1.4-fold, 1.5-fold, 1.8-fold, 2.0-fold, 3.0-fold, 3.5-fold, 4.5-fold, 5.0-fold, 10-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-
 20 fold, 1000-fold, or more, as compared to a reference) of the biomarker(s) (e.g., a protein of Table 1) may indicate a positive response to a Kawasaki disease therapy. Similarly, a decrease in the level (e.g., a decrease by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more; or a decrease by less than 0.01-fold, 0.02-fold, 0.1-fold, 0.3-fold, 0.5-fold, 0.8-fold, or less, as compared to a reference) of the biomarker(s)
 25 (e.g., a protein of Table 2) may indicate a positive response to a Kawasaki disease therapy. Also, increased binding (e.g., an increase by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more, or an increase by more than 1.2-fold, 1.4-fold, 1.5-fold, 1.8-fold, 2.0-fold, 3.0-fold, 3.5-fold, 4.5-fold, 5.0-fold, 10-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold, 1000-fold, or more, as compared to a reference)
 30 of IgG in the sample may indicate a positive response to a Kawasaki disease therapy.

Alternatively, an increase in the level (e.g., an increase by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more, or an increase by more than 1.2-fold, 1.4-fold, 1.5-fold, 1.8-fold, 2.0-fold, 3.0-fold, 3.5-fold, 4.5-fold, 5.0-fold, 10-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold, 1000-fold, or more, as
 35 compared to a reference) of the biomarker(s) (e.g., a protein of Table 4) may indicate a poor response to a Kawasaki disease therapy. Similarly, a decrease in the level (e.g., a decrease by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500%, or more; or a decrease by less than 0.01-fold, 0.02-fold, 0.1-fold, 0.3-fold, 0.5-fold, 0.8-fold, or less, as compared to a reference) of the biomarker(s) (e.g., a protein of Table 5) may
 40 indicate a poor response to a Kawasaki disease therapy.

The methods of the invention can be used to predict a subject's response to a Kawasaki disease therapy and classify the subject as a "responder," e.g., a subject with protein levels and/or binding of IgG indicative of a positive response to a Kawasaki disease therapy (e.g., IVIG), or a "non-responder," e.g., a subject with protein levels and/or binding of IgG indicative of a poor response to a Kawasaki disease therapy (e.g., a subject that may benefit from a therapy other than, or in addition to, IVIG therapy).

The prediction can be made prior to administration of a first Kawasaki disease therapy. Alternatively, the prediction can be made after administration of the first Kawasaki disease therapy, or after administration of a first Kawasaki disease therapy but before a second Kawasaki disease therapy. Furthermore, the prediction can be made at any time during the course of a Kawasaki disease therapy.

The methods described herein can also be used to monitor Kawasaki disease status (e.g., progression or regression) during therapy or to optimize dosage of one or more therapeutic agents for a subject. For example, alterations (e.g., an increase or a decrease as compared to either the positive reference sample or the level diagnostic for Kawasaki disease) can be detected to indicate an improvement in Kawasaki disease status. In this embodiment, the levels of the protein(s), binding of IgG, gene(s), and/or glycan(s) may be measured repeatedly as a method of not only diagnosing disease, but also monitoring the treatment, prevention, or management of the disease.

In order to monitor the status of Kawasaki disease in a subject, subject samples may be compared to reference samples taken early in the diagnosis of the disorder. Such monitoring may be useful, for example, in assessing the efficacy of a particular therapeutic agent (e.g., IVIG) in a subject, determining dosages, or in assessing disease progression or status. For example, levels of any of the proteins, genes, and/or glycans described herein, and/or binding of IgG, or any combination thereof can be monitored in a subject, and as the levels or activities increase or decrease, relative to control, the dosage or administration of therapeutic agents may be adjusted.

Methods of treatment

The invention also features a method for treatment of Kawasaki disease in a subject by contacting a biological sample from the subject with one or more binding agents capable of specifically binding one or more biomarkers (e.g., one or more proteins of Table 6); determining if the level in said biological sample is changed relative to a control value; predicting a response to a Kawasaki disease therapy in said subject based on the level of one or more of said biomarkers; and, if the prediction is positive, administering a Kawasaki disease therapy.

The methods can also be used to determine the proper dosage (e.g., the therapeutically effective amount) of a therapeutic agent for the subject, the proper type of therapeutic agent, or whether a therapy should be administered.

Several therapeutic agents have been used in the treatment of Kawasaki disease. These include, without limitation, administration of IVIG (i.e., IVIG therapy); salicylates (e.g., aspirin); corticosteroids (e.g., prednisone); IL-1 receptor antagonists; anticoagulants (e.g., enoxaparin and/or clopidogrel); infliximab; or any combination thereof. Approximately 25% of subjects do not respond to IVIG treatment. These predisposed subjects are at a higher risk for developing cardiac artery aneurysms or stenosis. These subjects, as classified herein, may be administered low molecular weight heparin (LMWH), such as enoxaparin, in addition to IVIG, for an extended period of time, e.g., weeks to months.

Other therapies that may be administered in addition to IVIG, include, but are not limited to, anti-TNF agents (e.g., adalimumab, infliximab, or etanercept); anti-IL treatment (e.g., anti-IL-1a, IL-1b, IL-1RA); statins (e.g., atorvastatin, pravastatin); corticosteroids (e.g., prednisolone, methylprednisolone); immunomodulators (e.g., cyclosporine A, methotrexate), anti-CD20 therapy (e.g., rituximab); plasma exchange, warfarin, and fibrinogen receptor glycoprotein IIb/IIIa, such as Abciximab.

Diagnostic kits

The invention also provides test kits and devices. For example, a diagnostic test kit can include one or more binding agents and, if desired, components for detecting and/or evaluating binding between the binding agent and a biomarker. For detection, one or more of binding agents may be labeled. In further embodiments, one or more of the binding agents may be substrate-bound, such that a biomarker-antibody interaction can be established by determining the amount of label attached to the substrate following binding between the antibody and the biomarker. A conventional ELISA is a common, art-known method for detecting antibody-substrate interaction and can be provided as the kit of the invention.

A kit that determines an alteration in the level of a protein, binding of IgG, to a peptide, gene, and/or glycan relative to a reference, such as the level present in a normal control, is useful as a diagnostic kit in the methods of the invention. Such a kit or device may further include a reference sample or standard curve indicative of a positive reference or a normal control reference.

Desirably, the kit will contain instructions for the use of the kit. In one example, the kit contains instructions for the use of the kit for the diagnosis of Kawasaki disease. In yet another example, the kit contains instructions for the use of the kit to monitor therapeutic treatment or dosage regimens. In yet another example, the kit contains instructions for the use of the kit to predict outcome, response to therapy, or disease recurrence. In a further example, the instructions include one or more metrics (e.g., metrics to be used as references).

Examples

The following examples are intended to illustrate the invention. They are not meant to limit the invention in any way.

Methods

Plasma proteomics

Proteomics on human plasma samples were carried out as follows. In order to enhance sensitivity for less abundant species, the top 14 most abundant proteins were depleted from plasma samples prior to proteomics analysis using the Agilent MARS column. Briefly, 50µl of human plasma was spin filtered and diluted with the sample buffer and applied to a MARS column. Two fractions, eluted from the column, were collected: fraction 1 representing less abundant proteins (LA Frn1) and fraction 2 representing highly abundant serum proteins (HA Frn2). Both fractions were concentrated and buffer exchanged. LA Frn1 was digested by trypsin, followed by proteomics analysis. Lower abundant fraction-1 tryptic digests were subjected to nano-LC-MS/MS analysis. Separations were carried out using an Ultimate3000 RSLCnano system. Chromatography was carried using analytical EASY-Spray PepMap RSLC, 25cm x 75µm id, C18, 2µm and 100Å nano column thermostatically controlled at 50°C and at

300nL/min with a linear gradient from 1% to 38% acetonitrile/water both containing 0.1% (v/v) FA for a total duration of 150 minutes. The separation step was followed by a 30 minute washing step with 99% acetonitrile/water followed by a 20 minute equilibration step with 99% water/acetonitrile both containing 0.1% (v/v) FA. 1.0 µg of each sample was injected into the column. Data dependent MS-MS was performed on the top 25 precursor ions from the full MS scan on the Orbi-Velos MS instrument.

1µg protein from each sample was injected into the nano-LC-MS/MS system. The data quality was reproducible across all samples. Proteome discoverer with Sequest search engine was used for database searching of each sample against Uniprot human protein database. ~450 proteins were identified from each sample. The distribution of peptide spectral matches (PSM) was plotted for each sample, and the overall assessment indicated that most samples contained equivalent numbers of PSM as observed.

Overall, ca. 440 protein signatures were acquired per sample using the set criteria.

Log ratios were calculated from the average normalized spectral counts from pairwise comparisons to get the fold change of proteins across the groups. T-test was conducted to determine the significance of the change. Further, the data were filtered to include changes with less than or equal to 0.05 T-test value.

Soluble plasma proteins

The concentrations of plasma IgG and IgA were determined using the Total Human IgG Immunoenzymetric Assay and the Human Immunoglobulin A Immunoenzymetric Assay from Cygnus Technologies (Southport, NC). The Human FcγR3B ELISA Kit from MyBioSource (San Diego, CA) was used to quantify the concentration of soluble CD16 in the patient samples. The concentration of α2,6-sialyltransferase (ST6GAL1) in the plasma samples was quantified using the α2,6-Sialyltransferase Assay Kit from Immuno-Biological Laboratories (Minneapolis, MN). The plasma samples were diluted in diluents provided with each ELISA. The manufacturer's instructions supplied with the assay kits were followed for the quantitation of each analyte. Absorbance readings for all assays were determined using a SpectraMax M2 spectrophotometer (Molecular Devices, Sunnyvale, CA) and analysis was performed using SoftMax Pro 5.2 software (Molecular Devices).

IgG and IgA glycomics

Methods for glycosylation analysis of Ig proteins is known in the art, for example, as described in Pucic et al, Molecular and Cellular Proteomics, 2011 Oct(10): 1-15.

Tryptic digests of Protein G enriched plasma fraction were subjected to nano-LC-MS/MS analysis. Two IVIg samples were used along with the KD samples as controls throughout the sample prep and data analysis. All separations were carried out using an Ultimate3000 RSLCnano system. Chromatography was carried using analytical EASY-Spray PepMap RSLC, 25cm x 75µm id, C18, 2µm and 100Å nano column thermostatically controlled at 50°C and at 300nL/min with a linear gradient from 1% to 38% acetonitrile/water both containing 0.1% (v/v) FA for a total duration of 70 minutes. The separation step was followed by a 30 minutes washing step with 99% acetonitrile/water followed by a 20 minutes equilibration step with 99% water/acetonitrile both containing 0.1% (v/v) FA. 1.0 µg of each

sample was injected on column. Data dependent MS-MS was performed on top 25 precursor ions from the full MS scan on the Orbi-Velos MS instrument.

Data analysis was performed using Xcalibur software- Qual and Quan browser. Area of each of the glycopeptides (IgG1, IgG2, IgG3/4 and IgA) was extracted from the base peak chromatogram. All the glycopeptide species m/z and retention time were captured in a processing method that was used for quantitation across all the samples. Peak integration was manually checked for accuracy in the quan browser. The raw abundances/area of all the glycopeptides for all the 30 KD samples and two IVIg controls was then exported in excel. The data was normalized based on the total glycopeptide abundance for each specific IgG (1,2, 3/4) and IgA protein within the sample. Based on these data analysis, we reported the relative abundance of each of the IgG1, IgG2, IgG3/4, and IgA glycopeptide species per sample. Up to this point the samples were all blinded. Once we had the measurement values, then for interpretation we used the unblinded sample description wherein we could group the samples as Febrile controls, Acute phase KD, IVIg treated, and LMWH treated patient samples.

Total plasma glycome

To low- and high-abundant protein plasma fractions (25µL) were added: 2µL of sample buffer (0.625 mL of 0.5M Tris, pH 6.6, 1mL of 10% SDS, and 3.375mL of water), 2µL of water, and 1µL of 0.5M dithiothreitol (DTT), followed by incubation at 65°C for 15min. The samples were then alkylated by adding 1µL of 100mM iodoacetamide and incubated for 30min in the dark at room temperature. The samples were then set into gel blocks by adding 22.5 µL of 30% (w/w) acrylamide/0.8% (w/v) bis-acrylamide stock solution (37.5:1.0, Protogel, National Diagnostics, Hesse, Hull, UK), 11.25µL of 1.5M Tris (pH 8.8), 1µL of 10% SDS, 1µL of 10% ammonium peroxodisulfate (APS), and finally 1µL of N,N,N',N'-tetramethyl-ethylenediamine (TEMED), mixed, and then left to set. The gel blocks were transferred to a filter plate and washed with 1mL of acetonitrile with vortexing on a plate mixer (Sarstedt, Leicester, UK) for 10min, followed by removal of the liquid. Washing procedure was repeated twice with 1mL of 20mM NaHCO₃ (pH 7.0) followed by 1mL of acetonitrile. N-glycans were released by adding 50µL of 0.1 U/mL PNGaseF (Prozyme, CA, USA) in 20mM NaHCO₃ (pH 7.0) to each sample and incubating overnight at 37°C. The released glycans were collected by washing the gel pieces with 3 × 200µL of water, 200µL of acetonitrile, 200µL of water, and finally 200µL of acetonitrile. The released glycans were dried, 20µL of 1% formic acid was added, and the mixture was incubated at room temperature for 40 min and then re-dried. Samples were labeled by adding 5µL of 2AB labeling solution, vortexed, incubated for 30min at 65°C, vortexed again, and incubated for a further 90min. Excess 2AB was removed using Whatman 3MM chromatography paper cleanup: 1-cm square pieces of prewashed, dried Whatman 3MM chromatography paper were folded into quarters and placed into a filter plate (Whatman protein precipitation plate prewashed with 200µL of acetonitrile followed by 200µL of water). The 5µL of 2AB-labeled samples were applied to the paper and left to dry/bind for 15min. The excess 2AB was washed off the paper by vortexing with 1.8mL of acetonitrile for 15min and then removing the acetonitrile using a vacuum manifold; this procedure was repeated four times. The labeled glycans were eluted from the paper by vortexing with 900µL of water for 30min and then collected by vacuum into a 2-mL 96-well plate. This was repeated with a further 900µL of water. The eluates were dried and re-constituted in H₂O/ACN (v/v 30/70). Released and

labeled glycans were subsequently fractionated by normal phase chromatography. Ultra Performance Hydrophilic interaction liquid chromatography (UPLC-HILIC) was carried out on a 1.7 μ m Waters BEH Glycan (150mm x 2.1mm) column as detailed in Mittermayr et al. "Multiplexed analytical glycomics: Rapid and confident IgG N-glycan structural elucidation," J. Prot. Res. 2011;10:3820-9 with retention times expressed as glucose units (GU) with the following conditions: Solvent A was 50mM formic acid adjusted to pH 4.4 with ammonia solution. Solvent B was acetonitrile. The column temperature was set to 30°C. The 30min method was used with a linear gradient of 30-47% with solvent A (=70-53% solvent B) at 0.56mL/min for 23min followed by 47-70% solvent A and finally reverting back to 30% solvent A to complete the run method. Samples were injected in 60% acetonitrile. Samples were run once and peaks were identified by their GU values which were compared with the GlycoBase 3.1 structural library for serum N-Glycans (<http://glycobase.nibrt.ei>).

Glycan HILIC data represent the relative percentage areas from HILIC profiles. Therefore, the data are compositional, and convey the relative amounts of glycan structures in a sample rather than the absolute quantities. Compositional data are subject to an awkward constant sum constraint, that is, the values sum to a constant value such as one or one hundred percent. For this reason, the logit transform was used to map the data onto real space.

Gene expression

RNA was purified from whole blood collected in Paxgene tubes. Reverse transcription was performed on 0.5 μ g of RNA with random hexamer priming (Invitrogen) and reverse transcriptase (New England Biolabs). Expression analysis of 48 genes was performed by Real Time-Quantitative Polymerase Chain Reaction (RT-qPCR) performed on the Roche LightCycler 480 II. The $\Delta\Delta C_p$ method (Pfaffl 2001) was used to quantify transcripts using the average C_p value of housekeeping genes ACTB, GUSB and RPS14 to normalize each sample. $-\Delta C_p$ was used as an arbitrary unit measure of transcript quantity.

Study Design

Subject samples selected for this study were between 2 to 33 months of age, Hispanic, males treated for Kawasaki Disease at Kawasaki Disease Center at Rady Children's Hospital.

Thirty subject samples, consisting of blood plasma, DNA, and RNA were used in the study. The Kawasaki Disease group consisted of 3 subjects that received IVIG and LOVENOX® treatment (Note: Subject 1 also received a second dose of IVIG 14 days after the first dose); and 5 subjects that received IVIG. A control group was selected consisting of 5 age and sex matched non-Kawasaki, febrile infants.

Samples were categorized according to the stage of disease or treatment into:

Group A – acute Kawasaki

Group B – sub-acute, post IVIG

Group C – on LOVENOX®;

Group D – convalescent

Febrile controls have only acute samples available.

All samples were randomized and blinded for each analytical platform that was applied.

The subject's clinical information, including treatment information, is provided in Table 21.

Table 21. Subject clinical information

Subject ID#	CA status*	Treatment**	
1	2	IVIG x 2	Second IVIG given 2 weeks after initial treatment for cardiac indications
2	4	IVIG x 2, infliximab	Second IVIG + infliximab for cardiac indications
3	2	IVIG x 2	Second IVIG for cardiac indications
4	2	IVIG x 2	Second IVIG for treatment resistance
5	3	IVIG x 2	Second IVIG for treatment resistance
6	2	IVIG x 2	Second IVIG for cardiac indications
7	1	IVIG x 2	Second IVIG for treatment resistance
8	3	IVIG x 2	Second IVIG for treatment resistance

*Coronary artery status: 1= normal echo, 2=coronary artery aneurysms, 3+ transiently dilated, 4= giant aneurysms

**Subjects treated with second IVIG for "treatment resistance" had fever $\geq$ 36 h post-end of IVIG infusion; subjects treated with second IVIG for cardiac indications had abnormal echo and therefore received 2 infusions of IVIG to maximize the anti-inflammatory benefit

The sample information is provided in Table 22.

Table 22. Sample information

Sample ID#	Stage	Day	Treatment	Age (months)
1A	Acute KD	4	IVIG/LMWH	2.4
1B	Post IVIG	12		
1C	on Lovenox	21		
1D	Convalescent	693		
2A	Acute KD	8	IVIG/LMWH	3.9
2B	Post IVIG	4		
2C	on Lovenox	13		
2D	Convalescent	NA		
3A	Acute KD		IVIG/LMWH	14.6
3B	Post IVIG	NA		
3C	on Lovenox	37		
3D	Convalescent	364		
4A	Acute KD		IVIG	3.4
4B	Post IVIG	6		
4D	Convalescent	26		
5A	Acute KD		IVIG	2.4
5B	Post IVIG	16		
5D	Convalescent	33		
6A	Acute KD		IVIG	15.2
6B	Post IVIG	12		
6D	Convalescent	21		
7A	Acute KD		IVIG	19.6
7B	Post IVIG	21		
7D	Convalescent	490		
8A	Acute KD		IVIG	33.3
8B	Post IVIG	15		
8D	Convalescent	22		
9A	Acute	NA	Control	
10A	Acute		Control	
11A	Acute		Control	
12A	Acute		Control	
13A	Acute		Control	

High risk subjects 1-3 received IVIG/LMWH treatment. Subjects 4-8 received IVIG treatment leading to disease resolution. Subjects 9-13 were febrile control.

Example 1. Identification of proteins indicative of Kawasaki disease

Shot-gun proteomics identified ~450 unique proteins on average per sample. In the targeted comparison of Kawasaki disease subjects at the acute stage (1A-8A) with the febrile control (9A-13A), 39 proteins were differentially expressed with 22 down-regulated and 17 up-regulated with a p-value <0.05 as a filter. These differentially expressed proteins belong to: inflammatory response pathway (S100A9, ORM1 ↑), Statin pathway (Apolipoproteins↑), Complement/Coagulation cascades (CFH, Serpin A1, C1), and Autophagy (GSN ↑), and they were among those altered in Kawasaki compared to the Febrile control.

A list of proteins that were found to be significantly different between the Kawasaki disease group (1A-8A) and the febrile control group (9A-13A) are shown in Table 23.

Table 23. Proteins that are significantly different (p<0.05) in KD group versus febrile control

Accession #	Description	p-value	Febrile Control Value	Acute KD Value	Log2FC Acute
E7ENL6	collagen, type VI, alpha 3	0.0140	5.41	1.27	-2.1
Q5MNV8	F-box protein 47	0.0100	3.71	0.98	-1.9
P12109	collagen, type VI, alpha 1	0.0161	2.70	0.76	-1.8
P10275	androgen receptor	0.0018	4.01		1.30
Q12888	tumor protein p53 binding protein 1	0.0003	2.01	0.76	-1.4
Q6UXB8	peptidase inhibitor 16	0.0333	10.67	4.14	-1.4
P55290	cadherin 13	0.0017	2.13	0.83	-1.4
G5E9A9	tenascin XB	0.0308	8.19	3.50	-1.2
B0UYX3	tenascin XB	0.0365	7.42	3.20	-1.2
Q07869	peroxisome proliferator-activated receptor alpha	0.0377	1.55	0.69	-1.2
P02452	collagen, type I, alpha 1	0.0168	3.57	1.75	-1.0
P01877	Ig alpha-2 chain C region (A2m marker)	0.0485	9.80	4.96	-1.0
P01765	Ig heavy chain V-III region TIL	0.0111	1.34	0.69	-1.0
P01876	Ig alpha-1 chain C region	0.0420	17.95	9.19	-1.0
Q6IMJ5	olfactomedin 1	0.0311	3.36	1.74	-1.0
Q5TFM2	complement factor H	0.0026	92.22	48.12	-0.9
P41222	prostaglandin D2 synthase 21kDa (brain)	0.0211	2.45	1.30	-0.9
I3L145	sex hormone-binding globulin	0.0007	12.83	6.97	-0.9
P01593	Ig kappa chain V-I region	0.0131	3.02	1.66	-0.9
P06396	gelsolin	0.0003	70.33	39.42	-0.8
P0CG04	Ig lambda-1 chain C regions (Mcg marker)	0.0102	12.65	7.15	-0.8
Q9NSY1	BMP2 inducible kinase	0.0130	1.34	0.76	-0.8
P01008	serpin peptidase inhibitor, clade C (antithrombin), member 1	0.0250	215.36	124.42	-0.8
P20742	pregnancy-zone protein	0.0430	10.65	6.20	-0.8
P19827	inter-alpha-trypsin inhibitor heavy chain 1	0.0447	193.28	115.09	-0.7

Accession #	Description	p-value	Febrile Control Value	Acute KD Value	Log2FC Acute
P01617	Ig kappa chain V-II region	0.0202	1.90	1.14	-0.7
P19823	inter-alpha-trypsin inhibitor heavy chain 2	0.0437	186.02	114.81	-0.7
P08603	complement factor H	0.0046	226.25	141.04	-0.7
Q04756	Hepatocyte growth factor activator	0.0203	6.81	4.42	-0.6
O75882	attractin	0.0433	13.74	8.92	-0.6
P01042	kininogen 1	0.0134	97.64	63.38	-0.6
P05160	coagulation factor XIII, B polypeptide	0.0401	15.43	10.20	-0.6
B4E1Z4	complement factor B	0.0052	213.26	149.14	-0.5
H0YFH3	complement component 1, r subcomponent	0.0079	33.02	23.48	-0.5
P00736	complement component 1, r subcomponent	0.0109	36.72	27.34	-0.4
Q16610	extracellular matrix protein 1	0.0263	20.87	15.70	-0.4
P09871	complement component 1, s subcomponent	0.0085	40.93	31.31	-0.4
C9JEX1	kininogen 1	0.0195	65.08	50.06	-0.4
P13671	complement component 6	0.0163	59.56	46.05	-0.4
P02774	vitamin D binding protein (group-specific component)	0.0259	396.66	320.83	-0.3
P01011	Alpha-1-antichymotrypsin(serpin peptidase inhibitor, clade A, member 3)	0.0029	387.52	536.07	0.5
F5H5G7	lactate dehydrogenase C	0.0106	0.90	1.36	0.6
G3V3A0	Alpha-1-antichymotrypsin(serpin peptidase inhibitor, clade A, member 3)	0.0006	152.86	246.65	0.7
Q8NEN0	armadillo repeat containing 2	0.0193	1.00	1.69	0.8
Q9H3U1	unc-45 homolog A (C. elegans)	0.0145	0.88	1.52	0.8
P59665	defensin, alpha 1	0.0048	1.01	1.75	0.8
P06702	S100 calcium binding protein A9	0.0358	6.13	12.50	1.0
P40879	solute carrier family 26 (anion exchanger), member 3	0.0467	1.13	2.38	1.1
P43403	zeta-chain (TCR) associated protein kinase 70kDa	0.0094	1.01	2.15	1.1
Q9H2Y7	Zinc finger protein 106 homolog	0.0093	0.78	1.76	1.2
P04114	apolipoprotein B	0.0021	159.94	363.53	1.2
J3QLI2	Zinc finger protein 161 homolog	0.0013	1.55	3.59	1.2
O95445	apolipoprotein M	0.0095	0.79	1.84	1.2
P02655	apolipoprotein C-II	0.0446	1.66	4.20	1.3
F5GXS5	apolipoprotein F	0.0145	0.67	1.82	1.4
I3L1Y6	zinc finger protein 578	0.0402	0.67	2.14	1.7
A4UGR9	xin actin-binding repeat containing 2	0.0001	1.00	3.49	1.8
P08519	lipoprotein, Lp(a)	0.0475	0.90	3.54	2.0
Q8N2F6	armadillo repeat containing 10	0.0069	0.56	2.54	2.2
Q8NGL3	olfactory receptor, family 5,	0.0201	1.57	7.17	2.2

Accession #	Description	p-value	Febrile Control Value	Acute KD Value	Log2FC Acute
	subfamily D, member 14				
P02763	orosomucoid 1	0.0251	17.47	162.97	3.2
P01009	Alpha-1-antitrypsin (serpin peptidase inhibitor, clade A, member 1)	0.0374	27.65	314.28	3.5
4843	nitric oxide synthase 2, inducible (Gene)	0.0491	0.049135	0.00028	1.8
1088	carcinoembryonic antigen-related cell adhesion molecule 8 (Gene)	0.0274	0.027424	0.00684	2.2
90865	interleukin 33 (Gene)	0.0432	0.043233	0.000154	3.8
3569	interleukin 6 (interferon, beta 2) (Gene)	0.0100	0.00164	0.091973	5.8
4312	matrix metalloproteinase 1 (interstitial collagenase) (Gene)	0.0115	7.84E-05	0.124912	10.6

SERPINA3 protein is an example of a measurement that separates Febrile Controls and Acute Kawasaki Disease. SERPINA3 is considered to be an acute phase response protein observed to be increased during certain types of inflammatory response. CRP is another protein that belongs to the acute phase response group. However, CRP does not appear to consistently separate Febrile Controls from Acute Kawasaki Disease subjects. IVIG treatment appears to reduce levels of both of these proteins as evidenced by reduced levels after IVIG treatment.

Example 2. Identification of proteins indicative of development of cardiac artery aneurysm or stenosis

Current standard of care for Kawasaki disease is treatment with IVIG/ASA at the time of diagnosis at the acute stage. As there is no test or diagnostic tool to identify subjects "at risk" for developing cardiac aneurysm at the acute stage, the aneurysm treatment (LOVENOX®) is generally delayed until echocardiogram imaging is provided later in the course of disease, often when a coronary artery aneurysm has already occurred. A test or diagnostic tool that identifies subjects "at risk" earlier could prevent the development of coronary artery aneurysms and the resulting long term effects (e.g., increased risk of heart attack and other cardiovascular events later in life).

To identify proteins indicative of development of cardiac artery aneurysms or stenosis, a group of Kawasaki disease subjects (1A, 2A, 3A) who were subjected to IVIG/LOVENOX® treatment (based on the clinical outcome) was compared with a group of subjects that were treated with IVIG only (4A, 5A, 6A, 7A, 8A). All subjects in the IVIG/LOVENOX® group developed coronary aneurysm. These subjects may be genetically predisposed to developing coronary artery aneurysm even with the appropriate standard of care treatment.

Ten proteins were significantly altered in the high risk group: three were up-regulated (PROC, F11, APOF) and seven down-regulated (CD44, ANKRD26, LAMP2, BCAM, MMRN1, TGFBI, TET2). The differential levels of these proteins in subjects that developed coronary artery aneurysms or stenosis indicates these proteins are useful for selecting or classifying subjects predisposed to coronary artery aneurysms or stenosis. This early selection may allow for treatment with anticoagulants (e.g., enoxaparin

and/or clopidogrel) or other therapies (e.g., infliximab, cyclosporine, and/or prednisone) to begin before the development of coronary artery aneurysms or stenosis, rather than in response to their development as is the current practice.

These protein identifiers were mapped to the following functions:

- a. Proteins related to autophagy - CD44 and LAMP2
- b. Proteins from complement/coagulation cascades - PROC and F11 are lower in IVIG/LOVENOX® group versus IVIG groups, suggesting that these protein markers for wound healing are present in higher expression in the IVIG responders.
- c. Cell adhesion related proteins such as BCAM, TGFB1, and MMRN1 show higher expression in the high risk group as compared with the IVIG responders.

A list of proteins that were found to be significantly different between the IVIG/Lovenox® group (1A-3A) and the IVIG only group (4A-8A) are shown in Table 19.

Table 24. Proteins that are significantly different ($p < 0.05$) in IVIG/LOVENOX® group versus IVIG only group

Accession No	Description	IVIG/LOVENOX® Value	IVIG value	Log fold change	p-value
P00747	Plasminogen	43.78	60.98	-0.5	0.0408
P04070	Vitamin K-dependent protein C	0.99	2.81	-1.5	0.0014
P03951	Coagulation factor XI	2.17	5.25	-1.3	0.0235
F5GXS5	Apolipoprotein F	0.99	2.32	-1.2	0.0326
F5H5G7	L-lactate dehydrogenase	1.60	1.22	0.4	0.0064
E7EPC6	CD44 antigen	3.61	2.08	0.8	0.0148
Q9UPS8	Ankyrin repeat domain-containing protein 26	5.23	2.56	1.0	0.0199
B4E2S7	Lysosome-associated membrane glycoprotein 2	2.01	0.98	1.0	0.0234
P50895	Basal cell adhesion molecule	2.39	1.10	1.1	0.0018
Q13201	Multimerin-1	2.84	1.23	1.2	0.0035
G8JLA8	Transforming growth factor-beta-induced protein ig-h3	7.12	2.94	1.3	0.0206
E7EQS8	Methylcytosine dioxygenase TET2	2.88	0.98	1.6	0.0067
P01011	Alpha-1-antichymotrypsin	610.74	491.26	0.3	0.0110
P01034	Cystatin-C	2.62	1.96	0.4	0.0197

In an effort to identify further proteins indicative of development of cardiac artery aneurysms or stenosis, a group of Kawasaki disease subjects (1, 2, 3, 4, and 6) who developed aneurysms based on electrocardiogram imaging was compared with a group of subjects that either did not develop aneurysms or were only dilated and not considered to have developed full aneurysms (5, 7, and 8). The subjects that developed aneurysms may be genetically predisposed to developing coronary artery aneurysm even with the appropriate standard of care treatment.

Student's t-test were used to determine if any significant differences were observed between aneurysm and non-aneurysm groups. Of the 647 measurements, 10 and proteins had a p-value less than 0.05 (see Table 25).

Table 25. Proteins that are significantly different ($p < 0.05$) in aneurysm group versus non-aneurysm group

Gene Symbol	Analytic	Accession Number	P-value	Log2 FC no aneurysm/aneurysm
HGFAC	PRO	Q04756	1.15E-02	-0.6
BST1	PRO	Q10588	4.23E-02	-1
AFTPH	PRO	Q6ULP2	4.86E-02	2
DTHD1	PRO	Q6ZMT9	4.35E-02	0.9
MASP2	PRO	O00187	4.27E-02	-0.6
XIRP2	PRO	A4UGR9	4.35E-02	0.6
MTA3	PRO	E7EV10	6.65E-05	1.7
hCG_2014417	PRO	B7Z718	3.45E-02	-1.4
TET2	PRO	E7EQS8	3.21E-02	1.4
TIAM1	PRO	F5GZ53	3.95E-02	-1.6

Example 3. Identification of proteins with different binding to plasma IgG between samples of acute Kawasaki disease and febrile control

A 10K random peptide array was tested for IgG reactivities in plasma samples of KD.

A total of 68 out of 10,000 peptides were found to have significantly different binding to plasma IgG between acute KD and febrile control ($p < 0.05$).

Using the 68 peptides, an agglomerative hierarchical clustering technique (via Ward's minimum variance method) was used to naturally break the data into a hierarchy of "similar" clusters. Here, the natural break created two clusters. The 68 peptides identified have the sequences of SEQ ID NOs: 1-68, provided in Table 3, supra. The 68 peptides were attached to the microarray by cysteine-serine-glycine linkers. The peptides (including the linker sequence) are listed in Table 26.

Table 26. Peptides identified with different IgG binding between febrile control and Kawasaki disease acute stage samples

SEQ ID NO:	Peptide Sequence	p-value
69	CSGAKFLGQSTYIAGYHQVD	7.67E-07
70	CSGFWSKMKPSEEYTTFYRD	0.001
71	CSGFDRSDYMSFHLDDNITI	0.001
72	CSGIRIETPYKDTEDGKYF	0.001
73	CSGLGLLQAITRNSWVDSAF	0.001
74	CSGKHWEFMQFDIGYIYEF	0.001
75	CSGNIPSNQHATEIQVDGYH	0.002
76	CSGENFEYHLYDSMIGYEVH	0.002
77	CSGERPDPATYFMPGRDDQY	0.002
78	CSGDNAPYYYREEWHKEFNK	0.003
79	CSGYHWDVQNTFYSLMLPS	0.003
80	CSGGDFSDYAPTLTQKASYG	0.003
81	CSGQYAFHNLDQNGTVFGNR	0.003
82	CSGYVTNMMINMNYSSLSYS	0.003

SEQ ID NO:	Peptide Sequence	p-value
83	CSGHWVLSDGYREVYSYNSY	0.003
84	CSGAYHSQLYIDYKDTEWFY	0.004
85	CSGDHPYFVIWDYKPVHTY	0.004
86	CSGHEHPYLGMTAYELAQD	0.004
87	CSGGQWSGQGYWYDPFDNMK	0.004
88	CSGTTHFLKDRFESTNHDVY	0.004
89	CSGVEDPRVGHSFLQDANY	0.004
90	CSGDPVQIFNTAEHSGPYIR	0.004
91	CSGHDHFRGGKFILSTQAIW	0.005
92	CSGNYPLPKYYYNWFEPVW	0.005
93	CSGQYDDPDWQIHYKLEARG	0.005
94	CSGQDPYMDLHYDKNQIEQA	0.006
95	CSGRGPHNFEIAETDAQMIE	0.006
96	CSGDAYTNQDISEEEHMHRY	0.006
97	CSGGALEWIIYAGPKPGYWE	0.006
98	CSGPHSTHQIFYKSYETDMA	0.007
99	CSGIYWGPMSTGHLPSQAQF	0.007
100	CSGYTHFWWLDKYMRYEVAT	0.007
101	CSGFLTEYYEQNNLFHAFR	0.007
102	CSGSRGDAAAWGILFDANWK	0.008
103	CSGAMLGGMWAAYYPPVPG	0.008
104	CSGYYWATGPEGPFRRHPGAR	0.008
105	CSGDMEFTVFDIDMEKHYYK	0.008
106	CSGHYWDYQQLGFGHLDHR	0.009
107	CSGDQHFVWGPTGRAPMNYG	0.009
108	CSGTNGFHIPFYSDFAQSAAA	0.009
109	CSGMKYDVWKFYNGDDMRVS	0.009
110	CSGWYDNMYKTGFYRMYLLT	0.009
111	CSGEFQDYNHSDVNMSNHPY	0.009
112	CSGYIPEQQPHEADNLYKDA	0.010
113	CSGFYTLPHRPLYYYGYVAS	0.010
114	CSGHRWLEEANTEYMTMNSI	0.010
115	CSGGPINAYQKQDYSIEPEH	0.010
116	CSGAGDQYVRIDKSTRISNI	0.011
117	CSGSMKTVEADWYTYEPWWH	0.011
118	CSGNHIYIVQTAYGVTGETS	0.011
119	CSGMFAYHRAWPVWSSVLHV	0.012
120	CSGWPNPYFYHKKDTYWAHY	0.012
121	CSGNYDHLLGQYPIRNWWSL	0.012
122	CSGESMDVWVPYGYKFTQYW	0.012
123	CSGTHMEHDFHIPLMYKYM	0.012
124	CSGVTPLEIIEQIREHLDIK	0.013
125	CSGLQKPFDYMKEWQVDNE	0.013
126	CSGVMQRWPENHFLQTHYDD	0.013

SEQ ID NO:	Peptide Sequence	p-value
127	CSGWWNWR SAYKEGDVAYPS	0.013
128	CSGGWVLDDWSSHHINYIE	0.013
129	CSGFSPHYQYVGLFPYVKYI	0.013
130	CSGANLVYMWGSAVHTSDPQ	0.013
131	CSGSPVMYSIANYKYQTMHL	0.013
132	CSGDQEISYLNSTDLFVGR	0.014
133	CSGYEPTDVYLTYRKLATKD	0.014
134	CSGPVPQERTDLFTGAHRAL	0.014
135	CSGREVYHEIKSGRAIEIYM	0.014
136	CSGHDAIWYDWNYPYPSKHES	0.014

Example 4. Identification of biomarkers indicative of development of cardiac artery aneurysm or stenosis

The predisposition for development of aneurysms can also be examined by looking at the difference in gene expression between the aneurysm group (Subjects 1 and 2) and the non-aneurysm group (Subjects 3-8). Measurements that show persistently different values in the Sub-acute samples in Subjects 1 and 2 were identified by selecting those measurements where the absolute value of the standard normal deviate was greater than 2.58 for both subjects. ($Z=2.58$ is the critical value for the two-tailed 99% confidence interval of the normal distribution). Sixty three measurements were observed to be persistently perturbed, either up or down, in subjects 1 and 2 at the sub-acute phase as shown in Table 27.

Table 27. Biomarkers that are differentially expressed in aneurysm group versus non-aneurysm group after treatment with IVIG

Measurement	Anal.	Acute Subj. 1	Acute Subj. 2	Acute Subj. 3-8	Sub-acute Subj. 1	Sub-acute Subj. 2	Sub-Acute Subj. 3-8	Conv. Subj. 1	Conv. Subj. 2	Conv. Subj. 3-8
ACTB	PRO	3.46	5.74	2.67	2.67	3.84	0.82	5.74	2.80	0.0
AFM	PRO	-3.35	-3.47	4.03	-3.41	-3.22	0.70	-2.42	-0.46	0.0
AHSG	PRO	-2.86	-2.63	2.63	-3.27	-4.57	1.42	-4.96	-1.66	0.0
AR	PRO	-10.28	-3.67	-1.38	-9.48	-5.43	-2.42	0.30	-2.31	0.0
BCHE	PRO	-0.30	-4.10	-1.58	-4.66	-4.19	-0.36	-3.38	-1.89	0.0
BTD	PRO	-3.87	-1.67	1.99	-3.10	-3.19	2.02	-2.04	0.62	0.0
C9	PRO	4.49	3.84	4.15	4.18	3.04	1.31	3.30	1.97	0.0
CD80	GE	-5.03	-5.08	-1.53	-4.33	-5.78	-0.98	-0.38	NA	0.0
CDH13	PRO	-2.02	-4.53	9.51	-4.41	-4.89	-0.98	-1.82	0.78	0.0
CEACAM8	GE	4.98	8.87	-1.69	3.80	3.60	-0.45	4.51	NA	0.0
CHL1	PRO	-1.14	-1.43	-0.24	-2.82	-2.78	0.36	-2.05	-2.11	0.0
CRP	PRO	4.65	4.17	2.89	3.30	3.08	-0.62	2.24	1.73	0.0
CTSD	PRO	12.48	12.56	-0.22	11.18	10.87	2.05	-0.18	11.43	0.0
F10	PRO	1.09	1.23	4.05	-3.20	-3.35	0.72	-1.31	0.51	0.0
F9	PRO	2.74	0.54	3.42	-4.92	-6.45	1.32	-0.99	2.45	0.0
GP12LA	GLY	-4.68	-5.39	-5.13	-3.96	-8.08	0.52	-3.08	NA	0.0

Measurement	Anal.	Acute Subj. 1	Acute Subj. 2	Acute Subj. 3-8	Sub-acute Subj. 1	Sub-acute Subj. 2	Sub-acute Subj. 3-8	Conv. Subj. 1	Conv. Subj. 2	Conv. Subj. 3-8
GP13HA	GLY	- 11.39	- 11.40	-6.67	-7.83	-6.26	2.76	-5.28	NA	0.0
GP14HA	GLY	-0.93	-1.81	-2.63	-2.86	-3.30	1.99	-1.10	NA	0.0
GP14LA	GLY	-4.56	-5.34	-2.08	-2.78	-3.90	0.23	-1.44	NA	0.0
GP16LA	GLY	-4.83	-5.61	-3.35	-9.05	-5.29	0.14	-3.37	NA	0.0
GP18HA	GLY	-9.30	- 11.24	-6.92	-5.11	-5.57	-5.29	-4.19	NA	0.0
GP18LA	GLY	-1.99	-9.33	-3.58	-5.33	-9.96	-0.07	-2.99	NA	0.0
GP19LA	GLY	-1.38	-4.51	-2.25	-2.92	-5.86	-0.21	-0.62	NA	0.0
GP20HA	GLY	-6.37	-3.41	-7.20	- 16.34	- 10.72	-2.93	-7.11	NA	0.0
GP20LA	GLY	-9.24	-7.52	-0.89	- 16.51	- 11.69	-1.55	-5.65	NA	0.0
GP24LA	GLY	-2.96	-2.91	-5.57	-5.51	-3.13	-0.81	-0.75	NA	0.0
GP29LA	GLY	-2.36	1.15	-5.36	-6.00	3.84	-1.62	-0.95	NA	0.0
GP32HA	GLY	-4.04	-3.19	-1.63	-6.21	-5.19	-0.38	-0.34	NA	0.0
GP32LA	GLY	-3.04	-4.29	-1.70	-4.11	-4.48	0.29	-1.29	NA	0.0
GP34HA	GLY	-2.44	-2.13	2.25	-4.35	-3.66	-2.22	0.46	NA	0.0
GP34LA	GLY	-2.58	-3.32	-3.65	-3.32	-3.58	-0.18	-0.45	NA	0.0
GP37HA	GLY	2.57	3.33	-1.24	2.72	3.23	-0.59	2.47	NA	0.0
GP38LA	GLY	-1.61	-2.65	-5.11	-4.93	3.33	-0.91	0.78	NA	0.0
GP3LA	GLY	-5.97	-3.02	-4.26	-3.01	-4.92	0.74	-4.69	NA	0.0
GP42HA	GLY	3.26	4.01	-1.60	2.69	3.69	-0.15	2.67	NA	0.0
GP43HA	GLY	5.61	6.14	0.12	5.85	6.18	-0.34	3.13	NA	0.0
GP43LA	GLY	3.74	4.22	-5.21	3.90	4.48	-0.99	2.11	NA	0.0
GP44HA	GLY	7.89	9.42	-0.60	8.49	9.84	-0.21	3.32	NA	0.0
GP44LA	GLY	3.62	5.22	-7.90	3.83	5.58	5.31	1.38	NA	0.0
GP9HA	GLY	4.83	5.35	-4.02	3.99	5.20	0.85	-2.26	NA	0.0
GP9LA	GLY	2.69	6.69	-9.94	4.12	6.16	-0.37	-2.38	NA	0.0
GSN	PRO	-4.66	-6.50	4.79	-4.15	-4.87	2.93	-2.77	-0.87	0.0
GUSB	GE	-4.91	-4.67	-4.48	-3.96	-5.12	-0.39	-2.03	NA	0.0
HP	PRO	1.45	2.93	2.93	2.78	2.79	1.40	2.57	1.21	0.0
HPR	PRO	1.91	3.90	2.36	3.79	3.72	1.23	3.46	0.97	0.0
IgA	ELISA	0.26	10.99	-2.98	3.88	11.39	-1.47	7.17	NA	0.0
LRG1	PRO	6.05	6.39	3.40	3.37	3.93	0.93	2.43	3.62	0.0
LUM	PRO	-1.79	-4.21	4.43	-6.10	-6.03	1.65	-5.21	-0.78	0.0
MMP9	PRO	8.18	5.57	-7.51	2.96	3.82	-0.42	3.29	NA	0.0
NID1	PRO	0.01	4.30	4.08	2.66	4.15	-0.53	0.16	-1.98	0.0
ORM1	PRO	13.42	16.46	-0.61	9.34	13.34	0.21	1.68	-1.78	0.0
ORM2	PRO	5.79	13.52	2.75	6.30	6.85	2.31	4.60	0.23	0.0
PROC	PRO	-3.03	-4.13	2.73	-4.21	-2.81	-0.36	-1.64	-1.00	0.0
S100A12	GE	9.08	6.74	-1.11	3.91	5.55	-1.57	4.74	NA	0.0
S100A8	PRO	3.78	4.69	3.36	3.21	3.88	0.85	3.93	2.67	0.0
SERPINA4	PRO	-6.97	-4.28	1.85	-5.80	-3.28	0.48	-2.53	-0.15	0.0
ST6GAL1	ELISA	2.25	5.80	-3.01	3.70	6.25	-1.47	4.86	NA	0.0
TNXB	PRO	0.65	-4.38	3.15	-3.77	-3.47	0.07	-6.52	-0.27	0.0
TNXB_1	PRO	0.37	-5.16	0.45	-3.54	-3.90	0.83	-7.82	-0.62	0.0
TTN	PRO	-0.04	0.53	0.51	- 12.01	-2.65	1.11	0.26	-1.27	0.0
VCAN	GE	2.75	3.57	-1.37	4.53	3.68	-0.32	4.50	NA	0.0

PRO, protein from shot-gun proteomics; ELISA, soluble plasma protein; GE, gene expression; GLY, serum protein glycans; GP, fraction number; HA, high-abundant fraction; LA, low abundant fraction.

Example 5. Identification of biomarkers indicative of response to IVIG treatment

Measurements made on samples from KD subjects were analyzed to examine the effect of IVIG treatment. Acute phase samples were compared to samples collected after IVIG treatment.

A total of 690 analytes per sample from 21 biological samples with the complete data sets was used for the analysis. Samples were divided into an "Acute Disease" group and a "Sub-acute" group. Differences between the two groups were examined by Student's t-tests after variance stabilizing transformation. Transformations were performed by analytic: plasma proteomics, $\log_2$; RT-qPCR $-\Delta C_p$, none; plasma protein ELISA, none; plasma glycans, $\log_2$; Ig glycopeptides, logit. A total of 157 measurements with $P \leq 0.01$ were considered to be significantly different between the two groups (Acute Value vs. Sub-acute Value) and are listed in table 28.

Table 28. Biomarkers that are differentially expressed in acute stage versus after treatment with IVIG

Analyte	Analytic	Accession No/ glycan structure	Gene name	p-value	Acute Stage Value	Post-treatment Value
CRP	PRO	P02741	C-reactive protein, pentraxin-related	1.07E-09	15.7	0.9
HPR	PRO	P00739	haptoglobin-related protein	6.21E-09	107.2	9.02
ITIH2	PRO	P19823	inter-alpha-trypsin inhibitor heavy chain 2	3.22E-08	122.08	259.05
HP	PRO	P00738	haptoglobin	3.85E-08	249.76	29.02
GSN	PRO	P06396	gelsolin	5.31E-08	41.19	73.46
AFM	PRO	P43652	afamin	5.46E-08	30.22	74.24
AMBP	PRO	P02760	alpha-1-microglobulin/bikunin precursor	2.27E-07	31.07	59.83
FCGR1A	GE	2209	Fc fragment of IgG, high affinity Ia, receptor (CD64)	2.90E-07	-0.74	-3.72
BTD	PRO	F8W1Q3	biotinidase	4.26E-07	4.82	8.22
SERPINA 4	PRO	P29622	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 4	6.12E-07	5.84	14.94
C9	PRO	P02748	complement component 9	6.66E-07	50.32	32.85
ORM2	PRO	P19652	orosomucoid 2	6.85E-07	31.78	4.93
S100A12	GE	6283	S100 calcium binding protein A12	1.42E-06	0.76	-2.41
LUM	PRO	P51884	lumican	1.51E-06	15.28	26.94
CDH13	PRO	P55290	cadherin 13	2.31E-06	0.8	1.96

Analyte	Analytic	Accession No/ glycan structure	Gene name	p-value	Acute Stage Value	Post-treatment Value
ORM1	PRO	P02763	orosomucoid 1	3.15E-06	159.54	8.7
MMP9	GE	4318	matrix metalloproteinase 9	3.36E-06	4.31	2.17
GP13HA	GLY	A2[3]BG1S [3]1 A2[3]BG1S [6]1 M7 D1 FA2[3]G1S [3]1		3.77E-06	1.29	1.95
CHL1	PRO	O00533	cell adhesion molecule L1-like	4.59E-06	0.93	4.93
AR	PRO	P10275	androgen receptor	6.17E-06	1.14	3.14
KNG1	PRO	P01042	kininogen 1	6.94E-06	67.17	115.34
LRG1	PRO	P02750	leucine-rich alpha-2-glycoprotein 1	7.32E-06	49.06	21.97
HGFAC	PRO	Q04756	HGF activator	1.62E-05	4.17	9.41
S100A9	PRO	P06702	S100 calcium binding protein A9	2.52E-05	13.5	2.98
ITIH1	PRO	P19827	inter-alpha-trypsin inhibitor heavy chain 1	2.94E-05	127.79	248.86
MST1	PRO	P26927	macrophage stimulating 1 (hepatocyte growth factor-like)	3.10E-05	11.58	18.42
IGFALS	PRO	P35858	insulin-like growth factor binding protein, acid labile subunit	3.49E-05	9.52	20.4
SERPINA 3	PRO	G3V3A0	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3	3.53E-05	227.39	124.78
PGLYRP2	PRO	Q96PD5	peptidoglycan recognition protein 2	3.69E-05	28.53	47.95
GP18HA	GLY	FA2G2S[6] 1		3.86E-05	4.04	6.66
GP12LA	GLY	FA2BG2		4.25E-05	0.06	0.09
TNFRSF1 A	GE	7132	tumor necrosis factor receptor superfamily, member 1A	4.69E-05	-3.89	-5.19
TNXB	PRO	G5E9A9	tenascin XB	5.35E-05	3.02	9.09
CLU	PRO	P10909	clusterin	5.84E-05	22.8	32.62
AHSG	PRO	P02765	alpha-2-HS-glycoprotein	6.16E-05	195.41	284.02
SERPINA 3	PRO	P01011	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3	6.35E-05	486.42	298.31
LBP	PRO	P18428	lipopolysaccharide binding protein	6.53E-05	10.3	4.44
GPLD1	PRO	P80108	glycosylphosphatidylinositol specific phospholipase D1	6.79E-05	2.89	8.13

Analyte	Analytic	Accession No/ glycan structure	Gene name	p-value	Acute Stage Value	Post-treatment Value
TNXB	PRO	B0UYX3	tenascin XB	6.79E-05	2.69	8.64
RBP4	PRO	Q5VY30	retinol binding protein 4, plasma	7.66E-05	20.88	70.17
FBLN1	PRO	B1AHL2	fibulin 1	7.67E-05	10.65	16.87
GP20LA	GLY	A2G2S[3,6] 2		7.96E-05	4.45	6.27
ST6GAL1	ELISA	NA	ST6 beta-galactosamide alpha-2,6-sialyltransferase 1	8.04E-05	33.66	11.47
GP18LA	GLY	FA2G2S[6] 1		8.97E-05	1.25	1.58
VCAN	GE	1462	versican	1.02E-04	-0.85	-2.62
SERPINC 1	PRO	P01008	serpin peptidase inhibitor, clade C (antithrombin), member 1	1.15E-04	118.4	195.9
GP12HA	GLY	FA2BG2		1.32E-04	0.29	0.54
HBA1	PRO	P69905	hemoglobin, alpha 1	1.35E-04	88.8	4.91
BCHE	PRO	P06276	butyrylcholinesterase	1.42E-04	4.48	9.23
S100A8	PRO	P05109	S100 calcium binding protein A8	1.52E-04	4.57	2.22
HSPA5	PRO	P11021	heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa)	1.75E-04	1	2.05
KLKB1	PRO	H0YAC1	kallikrein B, plasma (Fletcher factor) 1	2.0E-04	13.66	21.26
GP41LA	GLY	A4G4S[3,3, 3,3]4		2.11E-04	0.63	0.35
CSF2RB	GE	1439	colony stimulating factor 2 receptor, beta, low-affinity (granulocyte-macrophage)	2.15E-04	-2.41	-4.16
HBD	PRO	P02042	hemoglobin, delta	2.45E-04	40.53	3.68
CFH	PRO	P08603	complement factor H	2.55E-04	143.84	215.03
GP11HA	GLY	FA2G2		2.61E-04	2.84	4.8
GP19HA	GLY	FA2BG2S[3]1; FA2BG2S[6]1		3.24E-04	0.76	1.44
GUSB	GE	2990	glucuronidase, beta	3.46E-04	-5.18	-4.9
SERPINA 1	PRO	P01009	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1	3.55E-04	264.17	21.64
FCGR3A	ELISA	NA	Fc fragment of IgG, low affinity IIIa, receptor (CD16a)	3.74E-04	8.95	5.12
CFH	PRO	Q5TFM2	complement factor H	4.03E-04	50.22	93.38
GP42LA	GLY	A4F1G4S[3,3,3,6]4		4.1E-04	1.2	0.5

Analyte	Analytic	Accession No/ glycan structure	Gene name	p-value	Acute Stage Value	Post-treatment Value
GP42HA	GLY	A4F1G4S[3,3,3,6]4		4.16E-04	1.78	0.63
GP3HA	GLY	M5; FA2B; A2[6]G1		4.22E-04	1.26	1.99
CEP70	PRO	Q8NHQ1	centrosomal protein 70kDa	4.45E-04	1.93	3.55
GP10HA	GLY	A2BG2		4.49E-04	0.03	0.06
HBG2	PRO	P69892	hemoglobin, gamma G	4.61E-04	18.05	1.45
HBG1	PRO	P69891	hemoglobin, gamma A	4.63E-04	18.05	1.45
GP41HA	GLY	A4G4S[3,3,3,3]4		4.75E-04	0.9	0.47
HBB	PRO	P68871	hemoglobin, beta	5.42E-04	81.49	7.7
IL1B	GE	3553	interleukin 1, beta	5.71E-04	-3.25	-5.13
GP43LA	GLY	A4G4LacS[3,3,3,6]4		5.71E-04	1.27	0.35
IL1RN	GE	3557	interleukin 1 receptor antagonist	6.60E-04	-1.59	-3.34
F13B	PRO	P05160	coagulation factor XIII, B polypeptide	6.85E-04	10.83	20.03
GP5HA	GLY	FA2[6]G1		7.79E-04	2.68	4.93
S100A8	GE	6279	S100 calcium binding protein A8	7.99E-04	1.03	-1.14
APOH	PRO	P02749	apolipoprotein H (beta-2-glycoprotein I)	8.13E-04	87.26	130.48
FBLN1	PRO	P23142	fibulin 1	8.21E-04	10.7	16.65
GP43HA	GLY	A4G4LacS[3,3,3,6]4		8.23E-04	1.28	0.32
THBS4	PRO	P35443	thrombospondin 4	8.79E-04	3.84	8.92
GP37HA	GLY	A4F1G3S[3,3,3]3 A4F1G3S[3,3,6]3 A4F1G3S[3,6,6]3		9.41E-04	0.48	0.25
FN1	PRO	F8W7G7	fibronectin 1	9.44E-04	42.92	111.05
GP44HA	GLY	A4F3G4S[3,3,3,3]4		9.51E-04	0.9	0.24
IGF2	PRO	P01344	insulin-like growth factor 2 (somatomedin A)	1.01E-03	0.87	1.77
S100A11	GE	6282	S100 calcium binding protein A11	1.03E-03	2.62	1.19
FN1	PRO	P02751	fibronectin 1	1.04E-03	47.41	120.85
PROC	PRO	P04070	protein C (inactivator of coagulation factors Va and VIIIa)	1.06 E-03	2.07	3.68
GP44LA	GLY	A4F3G4S[3,3,3,3]4		1.15E-03	0.47	0.11
GP6HA	GLY	FA2[3]G1		1.32E-03	0.95	1.94
GP13LA	GLY	A2[3]BG1S		1.41E-03	0.43	0.66

Analyte	Analytic	Accession No/ glycan structure	Gene name	p-value	Acute Stage Value	Post-treatment Value
		[3]1; A2[3]BG1S [6]1; M7 D1; FA2[3]G1S [3]1				
KNG1	PRO	C9JEX1	kininogen 1	1.56E-03	51.86	65.54
GP24LA	GLY	A2BG2S[6, 6]2		1.63E-03	1.66	2.03
SEPP1	PRO	P49908	selenoprotein P, plasma, 1	1.76E-03	2.97	4.76
GP8HA	GLY	FA2[3]BG1 ; M6 D3;		1.85E-03	0.81	1.12
NRP1	PRO	O14786	neuropilin 1	1.91E-03	1.26	2.36
NCAM1	PRO	E9PLH7	neural cell adhesion molecule 1	1.94E-03	2.16	3.79
PKDREJ	PRO	Q9NTG1	polycystin (PKD) family receptor for egg jelly	1.97E-03	2.37	0.9
TNFRSF1 B	GE	7133	tumor necrosis factor receptor superfamily, member 1B	1.99E-03	-1.94	-2.88
KIF20B	PRO	Q96Q89	kinesin family member 20B	2.04E-03	4.47	2.22
LYZ	PRO	P61626	lysozyme	2.05E-03	1.66	0.78
COL1A1	PRO	P02452	collagen, type I, alpha 1	2.05E-03	1.8	3.88
GP7HA	GLY	FA2[6]BG1		2.12E-03	0.69	1.22
GP23HA	GLY	A2G2S[3,6] 2		2.4E-03	38.79	32.23
A1BG	PRO	P04217	alpha-1-B glycoprotein	2.47E-03	100.11	131.28
F13A1	PRO	P00488	coagulation factor XIII, A1 polypeptide	2.52E-03	6.73	14.07
ACTC1	PRO	P68032	actin, alpha, cardiac muscle 1	2.6 E-03	5.8	3.25
ECM1	PRO	Q16610	extracellular matrix protein 1	2.62E-03	14.69	19.83
GP32LA	GLY	A3G3S[3,3, 6]3		2.63E-03	0.65	1.14
TNFSF13 B	GE	10673	tumor necrosis factor (ligand) superfamily, member 13b	2.64E-03	-1.73	-2.71
APOC1	PRO	P02654	apolipoprotein C-I	2.74E-03	1.65	3.27
GP35HA	GLY	A3F1G3S[3,3,3]3		2.79E-03	2.87	1.96
CPB2	PRO	Q96IY4	carboxypeptidase B2 (plasma)	2.79E-03	4.11	7.78
ATRNL	PRO	O75882	attractin	2.85E-03	7.93	12.91
GP26HA	GLY	FA2BG2S[3,6]2 FA2BG2S[6,6]2		2.86E-03	0.65	0.99
ZNF217	PRO	O75362	zinc finger protein 217	2.89E-03	3.95	7.72
SHBG	PRO	I3L145	sex hormone-binding	2.93E-03	8	13.57

Analyte	Analytic	Accession No/ glycan structure	Gene name	p-value	Acute Stage Value	Post-treatment Value
			globulin			
PZP	PRO	P20742	pregnancy-zone protein	3.05E-03	7.04	16.74
ITIH3	PRO	Q06033	inter-alpha-trypsin inhibitor heavy chain 3	3.20E-03	98.25	59.4
COL11A2	PRO	H0YHY3	collagen, type XI, alpha 2	3.37E-03	0.93	2.24
SELL	PRO	P14151	selectin L	3.41E-03	4.98	7.56
ENPP2	PRO	Q13822	ectonucleotide pyrophosphatase/phosphodiesterase 2	3.59E-03	1.5	3.99
GP5LA	GLY	FA2[6]G1		3.62E-03	0.06	0.08
GP19LA	GLY	FA2BG2S[3]1 FA2BG2S[6]1		3.73E-03	0.17	0.21
CD80	GE	941	CD80 (costimulatory molecule)	3.78E-03	-9.17	-8.24
F2	PRO	P00734	coagulation factor II (thrombin)	3.80E-03	88.77	118.4
ITIH4	PRO	Q14624	inter-alpha-trypsin inhibitor heavy chain family, member 4	3.99E-03	396.21	362.86
ACTBL2	PRO	Q562R1	actin, beta-like 2	4.08E-03	3.78	2.58
ITIH4	PRO	B7ZKJ8	inter-alpha-trypsin inhibitor heavy chain family, member 4	4.08E-03	396.42	362.46
KLRG1	GE	10219	killer cell lectin-like receptor subfamily G, member 1	4.11E-03	-3.67	-2.9
ITIH4	PRO	E9PGN5	inter-alpha-trypsin inhibitor heavy chain family, member 4	4.17E-03	395.42	362.13
HBE1	PRO	P02100	hemoglobin, epsilon 1	4.51E-03	11.98	1.25
IgA	ELISA	NA	IgA	4.58E-03	1.11	0.41
GP6LA	GLY	FA2[3]G1		4.79E-03	0.01	0.03
GP2HA	GLY	FA2		4.85E-03	2.98	4.99
APOD	PRO	P05090	apolipoprotein D	5.18E-03	0.94	1.89
CRY2	PRO	Q49AN0	cryptochrome 2	5.32E-03	2.37	1.18
PLG	PRO	P00747	plasminogen	5.42E-03	57.53	72.3
PPP6R2	PRO	B3KMJ7	protein phosphatase 6, regulatory subunit 2	5.45E-03	4.28	0.65
DOCK3	PRO	Q8IZD9	dedicator of cytokinesis 3	5.47E-03	2.6	0.84
FCGR2A	GE	2212	Fc fragment of IgG, low affinity IIa, receptor (CD32)	5.73E-03	2.06	0.48
CCR2	GE	729230	chemokine (C-C motif) receptor 2	6.66E-03	-2.4	-3.4
MASP1	PRO	P48740	mannan-binding lectin serine peptidase 1 (C4/C2 activating	6.69E-03	7.73	12.25

Analyte	Analytic	Accession No/ glycan structure	Gene name	p-value	Acute Stage Value	Post-treatment Value
			component of Ra-reactive factor)			
GP31HA	GLY	A3G3S[3,3,3]3		6.74E-03	0.42	0.26
ACTB	GE	60	actin, beta	7.45E-03	4.74	4.15
CTSD	PRO	P07339	cathepsin D	7.61E-03	4.15	0.83
TNF	GE	7124	tumor necrosis factor	7.65E-03	-4.82	-5.53
CEP290	PRO	O15078	centrosomal protein 290kDa	7.71E-03	3.24	1.43
IGFBP3	PRO	P17936	insulin-like growth factor binding protein 3	8.01E-03	2.06	3.55
DHRS11	PRO	A8MXC2	dehydrogenase/reductase (SDR family) member 11	8.04E-03	0.67	2.07
QSOX1	PRO	O00391	quiescin Q6 sulfhydryl oxidase 1	8.16E-03	3.04	4.86
MASP1	PRO	F8W876	mannan-binding lectin serine peptidase 1 (C4/C2 activating component of Ra-reactive factor)	8.22E-03	5.31	8.56
IgA_G1_S1	IgGLY	A2,G1,S1		8.36E-03	8.79	2.12
GP20HA	GLY	A2G2S[3,6]2		8.60E-03	3.53	4.55

Analytic annotations: PRO; shot-gun proteomics, GE; gene expression, GLY; plasma glycomics, IgGLY; site-specific Ig glycosylation. Glycan annotations according to Oxford Symbol nomenclature. All N-glycans have two core GlcNAcs; F at the start of the abbreviation indicates a core $\alpha(1-6)$ fucose linked to inner GlcNAc; Mx, number (x) of mannose on core GlcNAcs; Ax, number of antenna (GlcNAc) on trimannosyl core; A2, biantennary with both GlcNAcs as $\beta(1-2)$ linked; A3, triantennary with a GlcNAc linked $\beta(1-2)$ to both mannose and a third GlcNAc linked $\beta(1,4)$ to the $\alpha(1-3)$ linked mannose; A3'; triantennary with a GlcNAc linked $\beta(1-2)$ to both mannose and the third GlcNAc linked $\beta(1-6)$ mannose; B, bisecting GlcNAc linked $\beta(1-4)$ to $\beta(1-3)$ mannose; Gx, number (x) of $\beta(1-4)$ linked galactose on the antenna; Fx, number (x) of linked fucose on antenna, (4) or (3) after the F indicates that the Fuc is $\alpha(1-4)$ or $\alpha(1-3)$ linked to the GlcNAc; Sx, number (x) sialic acids linked to galactose; the number 3 or 6 in parenthesis after S indicates whether the sialic acid is in an $\alpha(2-3)$ or $\alpha(2-6)$ linkage.

Other Embodiments

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure that come within known or customary practice within the art to which the invention pertains and may be applied to the essential features herein before set forth.

All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

What is claimed is:

CLAIMS

1. A method for treating Kawasaki disease in a subject, said method comprising:

- (a) selecting a subject having a fever and one or more of: red eyes; a red swollen tongue; red skin on the palms of the hands and/or soles of the feet; peeling of the skin on the hands and/or feet; a rash on the main part of the body and/or in the genital area; and swollen lymph nodes;
- (b) obtaining a biological sample from said subject;
- (c) determining the level of one or more proteins from Table 1 and/or Table 2 in said biological sample;
- (d) comparing the level of said one or more proteins to a predetermined control value; and
- (e) administering a Kawasaki disease therapy to said subject if the level of said one or more proteins is indicative that said subject may benefit from a Kawasaki disease therapy;

wherein an increased level of at least one protein of Table 1, as compared to a reference, and/or a decreased level of at least one protein of Table 2, as compared to a reference, is indicative that the subject may benefit from a Kawasaki disease therapy.

2. A method for treating Kawasaki disease in a subject, said method comprising:

- (a) selecting a subject having a fever and one or more of: red eyes; a red swollen tongue; red skin on the palms of the hands and/or soles of the feet; peeling of the skin on the hands and/or feet; a rash on the main part of the body and/or in the genital area; and swollen lymph nodes;
- (b) administering a Kawasaki disease therapy to said subject;
- (c) obtaining a biological sample from said subject;
- (d) determining the level of one or more proteins from Table 6, Table 7, Table 8, and/or Table 9, one or more mRNAs from Table 10, Table 11, Table 12, and/or Table 13, and/or one or more glycans of Table 14, Table 15, Table 16, and/or Table 17, in said biological sample;
- (e) comparing the level of said one or more proteins to a predetermined control value; and
- (f) administering a Kawasaki disease therapy, other than, or in addition to IVIG, to said subject if the level of said one or more proteins is indicative that said subject may benefit from a Kawasaki disease therapy other than, or in addition to, IVIG;

wherein an increased level of at least one protein of Table 6 and/or Table 8, at least one mRNA of Table 10 and/or 12, and/or at least one glycan of Table 14 and/or 16, as compared to a reference, and/or a decreased level of at least one protein of Table 7 and/or Table 9, at least one mRNA of Table 11 and/or Table 13, and/or at least one glycan of Table 15 and/or Table 17, as compared to a reference, is indicative that the subject may benefit from a Kawasaki disease therapy other than, or in addition to, IVIG.

3. A method for treating Kawasaki disease in a subject, said method comprising:

- (a) selecting a subject having a fever and one or more of: red eyes; a red swollen tongue; red skin on the palms of the hands and/or soles of the feet; peeling of the skin on the hands and/or feet; a rash on the main part of the body and/or in the genital area; and swollen lymph nodes;
- (b) obtaining a biological sample from said subject;

(c) determining the binding of IgG in said biological sample to one or more proteins comprising an amino acid sequence of any one of SEQ ID NOs: 1 to 68;

(d) comparing the binding of IgG to said one or more proteins to a predetermined control value; and

(e) administering a Kawasaki disease therapy to said subject if the binding of IgG to said one or more proteins is indicative that said subject may benefit from a Kawasaki disease therapy;

wherein increased binding of IgG to at least one protein comprising an amino acid sequence of any one of SEQ ID NOs: 1 to 68, as compared to a reference, is indicative that the subject may benefit from a Kawasaki disease therapy.

4. A method for treating Kawasaki disease in a subject, said method comprising:

(a) determining the level of one or more proteins from Table 1 and/or Table 2 in a biological sample obtained from said subject; and

(b) administering a Kawasaki disease therapy to said subject if the level of said one or more proteins is indicative that said subject may benefit from a Kawasaki disease therapy;

wherein an increased level of at least one protein of Table 1, as compared to a reference, and/or a decreased level of at least one protein of Table 2, as compared to a reference, is indicative that the subject may benefit from a Kawasaki disease therapy.

5. A method for treating Kawasaki disease in a subject, said method comprising:

(a) administering a Kawasaki disease therapy to said subject;

(b) determining the level of one or more proteins from Table 6, Table 7, Table 8, and/or Table 9, one or more mRNAs from Table 10, Table 11, Table 12, and/or Table 13, and/or one or more glycans from Table 14, Table 15, Table 16, and/or Table 17, in a biological sample obtained from said subject; and

(c) administering a Kawasaki disease therapy, other than, or in addition to, IVIG to said subject if the level of said one or more proteins is indicative that said subject may benefit from a Kawasaki disease therapy other than, or in addition to, IVIG;

wherein an increased level of at least one protein of Table 6 and/or Table 8, at least one mRNA of Table 10 and/or 12, and/or at least one glycan of Table 14 and/or Table 16, as compared to a reference, and/or a decreased level of at least one protein of Table 7 and/or Table 9, at least one mRNA of Table 11 and/or Table 13, and/or at least one glycan of Table 15 and/or Table 17, as compared to a reference, is indicative that the subject may benefit from a Kawasaki disease therapy.

6. A method for treating Kawasaki disease in a subject, said method comprising:

(a) the binding of IgG in said biological sample to one or more proteins comprising an amino acid sequence of any one of SEQ ID NOs: 1 to 68; and

(b) administering a Kawasaki disease therapy to said subject if the binding of IgG to said one or more proteins is indicative that said subject may benefit from a Kawasaki disease therapy;

wherein increased binding of IgG to at least one protein comprising an amino acid sequence of any one of SEQ ID NOs:1 to 68, as compared to a reference, is indicative that the subject may benefit from a Kawasaki disease therapy.

7. The method of any one of claims 1 to 6, further comprising:

determining the level of one or more proteins from Table 4 and/or Table 5 in a biological sample obtained from said subject; and

administering a therapy other than, or in addition to, IVIG therapy if the level of said one or more proteins is indicative that said subject may benefit from a Kawasaki disease therapy other than, or in addition to, IVIG therapy;

wherein an increased level of at least one protein of Table 4, as compared to a reference and/or a decreased level of at least one protein of Table 5, as compared to a reference, is indicative that the subject may benefit from a Kawasaki disease therapy, other than or in addition to, IVIG therapy.

8. The method of any one of claims 1 to 7, wherein said one or more proteins comprises complement factor H, collagen type 1 alpha 1, gelsolin, alpha-1-antichymotrypsin, and sex hormone binding globulin.

9. A method of treating Kawasaki disease in a subject, said method comprising:

(a) determining the level of one or more proteins from Table 4, Table 5, Table 6, Table 7, Table 8, and/or Table 9, one or more mRNAs of Table 10, Table 11, Table 12, and/or Table 13, and/or one or more glycans of Table 14, Table 15, Table 16, and/or Table 17 in a biological sample obtained from said subject; and

(b) administering a therapy other than, or in addition to, IVIG therapy if the level of said one or more proteins is indicative that said subject may benefit from a Kawasaki disease therapy other than, or in addition to, IVIG therapy;

wherein an increased level of at least one protein of Table 4, Table 6, and/or Table 8, at least one mRNA of Table 10 or Table 12, and/or at least one glycan of Table 14 or Table 16, as compared to a reference, and/or a decreased level of at least one protein of Table 5, Table 7, and/or Table 9, at least one mRNA of Table 11 or Table 13, and/or at least one glycan of Table 15 or Table 17, as compared to a reference, is indicative that the subject may benefit from a Kawasaki disease therapy other than, or in addition to, IVIG therapy.

10. The method of claims 1 or 4, wherein said one or more proteins from Table 1 and/or Table 2 comprises two or more proteins from Table 1 and/or Table 2.

11. The method of claim 1 or 4, wherein said one or more proteins from Table 1 and/or Table 2 comprises three or more proteins from Table 1 and/or Table 2.

12. The method of any one of claims 7 to 9, wherein said one or more proteins from Table 4 and/or Table 5 comprises two or more proteins from Table 4 and/or Table 5.

13. The method of any one of claims 7 to 9, wherein said one or more proteins from Table 4 and/or Table 5 comprises three or more proteins from Table 4 and/or Table 5.

14. The method of any one of claims 1 to 13, further comprising contacting the biological sample with one or more binding agents capable of specifically binding to the one or more proteins.

15. The method of any one of claims 1 to 14, wherein said Kawasaki disease therapy comprises administration of IVIG to said subject.

16. The method of any one of claims 1 to 15, wherein said Kawasaki disease therapy comprises administration of an anticoagulant to said subject.

17. The method of claim 16, wherein said anticoagulant is enoxaparin or a pharmaceutically acceptable salt thereof.

18. The method of any one of claims 4 to 17, wherein said subject has one or more of: a fever; red eyes; a rash on the main part of the body and/or in the genital area; red, dry, cracked lips; a red, swollen tongue; swollen, red skin on the palms of the hands and/or soles of the feet; swollen lymph nodes; irritability; peeling of the skin on the hands and/or feet; joint pain; diarrhea; vomiting; and abdominal pain.

19. The method of claim 18, wherein said subject has a fever and one or more of: red eyes; a red swollen tongue; red skin on the palms of the hands and/or soles of the feet; peeling of the skin on the hands and/or feet; a rash on the main part of the body and/or in the genital area; and swollen lymph nodes.

20. The method of claim 19, wherein said fever lasts for more than four days.

21. The method of any one of claims 1 to 20, wherein said subject exhibits the clinical symptoms of cardiac artery aneurysms and/or stenosis of the arteries.

22. The method of any one of claims 1 to 21, wherein said subject has not been diagnosed with cardiac artery aneurysms and/or stenosis of the arteries prior to determining the level of said one or more proteins.

23. The method of any one of claims 1 to 22, wherein a biological sample is obtained from said subject prior to the commencement of IVIG therapy.

24. The method of any one of claims 1 to 22, wherein a biological sample is obtained from said subject after commencement of IVIG therapy.

25. The method of claim 24, wherein said biological sample is obtained from said subject within 24 hours after commencement of IVIG therapy.

26. The method of any one of claims 1 to 25, wherein said biological sample is a tissue sample, whole blood, plasma, urine, saliva, pancreatic juice, bile, or serum sample.

27. The method of claim 26, wherein said biological sample is a plasma sample.

28. The method of any one of claims 1 to 27, further comprising processing said biological sample prior to determining the level of said one or more said proteins.

29. The method of claim 28, wherein processing said biological sample comprises fractionation of said biological sample.

30. The method of claim 29, wherein said fractionation comprises the removal of proteins present at concentrations greater than 0.01 g/dL.

31. The method of any one of claims 1 to 30, wherein said level of said one or more proteins is determined by a hybridization assay, an immunoassay, a mass spectrometric assay, a single molecule detection assay, and/or a fluorescence *in situ* hybridization assay.

32. The method of claim 31, wherein said level of said one or more proteins is determined by an immunoassay.

33. The method of claim 32, wherein said immunoassay is an ELISA assay.

34. The method of any one of claims 1 to 33, wherein said subject is less than 13 years old.

35. The method of claim 34, wherein said subject is less than 5 years old.

36. The method of any one of claims 1 to 35, wherein said subject is Asian or Afro-Caribbean.

37. The method of claim 36, wherein said subject is Asian.

38. The method of claim 37, wherein said subject is Japanese or Korean.

39. The method of any one of claims 1 to 38, wherein said level of said one or more proteins and/or binding of IgG in said sample is determined at least twice within 365 days.

40. A kit for selecting a subject that may benefit from a Kawasaki disease therapy, said kit comprising a set of two or more binding agents, each of said binding agents being capable of specifically binding to at least one protein from Table 1 and/or Table 2, and/or one or more proteins comprising an amino acid sequence of any one of SEQ ID NOs:1 to 68.

41. The kit of claim 40, wherein said set comprises binding agents capable of specifically binding to complement factor H, collagen type 1 alpha 1, gelsolin, alpha-1-antichymotrypsin, and sex hormone binding globulin.

42. The kit of claim 40 or 41, further comprising a set of one or more binding agents, each of which is capable of specifically binding to at least one protein from Table 4, Table 5, Table 6, Table 7, Table 8, and/or Table 9, at least one mRNA from Table 10, Table 11, Table 12, and/or Table 13, and/or at least one glycan from Table 14, Table 15, Table 16, and/or Table 17.

43. A kit for selecting a subject that may benefit from a Kawasaki disease therapy other than, or in addition to, IVIG therapy, said kit comprising a set of two or more binding agents, each of said binding agents being capable of specifically binding to at least one protein from Table 4, Table 5, Table 6, Table 7, Table 8, and/or Table 9, at least one mRNA from Table 10, Table 11, Table 12, and/or Table 13, and/or at least one glycan from Table 14, Table 15, Table 16, and/or Table 17.

44. The kit of any one of claims 40 to 43 further comprising instructions for use of said kit to determine the level of proteins in a biological sample.

45. The kit of any one of claims 40 to 44, wherein said binding agents are provided on a solid support.

46. The kit of any one of claims 40 to 45, wherein said binding agents are antibodies.

47. A method for diagnosing Kawasaki disease in a subject, said method comprising determining the level of one or more proteins in a biological sample obtained from said subject and/or the binding of IgG in said sample to one or more proteins comprising an amino acid sequence of any one of SEQ ID NOs:1 to 68, with a kit of any one of claims 40 to 42, wherein an increased level of at least one protein of Table 1, as compared to a reference, and/or a decreased level of at least one protein of Table 2, as compared to a reference, and/or increased binding of IgG to at least one protein comprising an amino acid sequence of any one of SEQ ID NOs:1 to 68 is indicative of said subject having Kawasaki disease.

48. A method for diagnosing a predisposition to develop cardiac artery aneurysms or stenosis in a subject, said method comprising determining the level of one or more proteins in a biological sample obtained from said subject with a kit of claims 42 or 43, wherein an increased level of at least one protein of Table 4, Table 6, and/or Table 8, at least one mRNA of Table 10 or Table 12, and/or at least one glycan of Table 14 or Table 16, as compared to a reference, and/or a decreased level of at least one

protein of Table 5, Table 7, and/or Table 9, at least one mRNA of Table 11 or Table 13, and/or at least one glycan of Table 15 or Table 17, as compared to a reference, is indicative of said subject having a predisposition to develop cardiac artery aneurysms or stenosis.

49. A method for classifying a subject as one who may benefit from a Kawasaki disease therapy, said method comprising determining the level of one or more proteins in a biological sample obtained from said subject and/or the binding of IgG in said sample to one or more proteins comprising an amino acid sequence of any one of SEQ ID NOs:1 to 68 with a kit of any one of claims 40 to 42, wherein said subject is classified based on an increased level of at least one protein of Table 1, as compared to a reference, and/or a decreased level of at least one protein of Table 2, as compared to a reference, and/or increased binding of IgG in said sample to at least one protein comprising an amino acid sequence of any one of SEQ ID NOs:1 to 68.

50. A method of classifying a subject as one who may benefit from a Kawasaki disease therapy other than, or in addition to, IVIG therapy, said method comprising determining the level of one or more proteins in a biological sample obtained from said subject with a kit of claims 42 or 43, wherein said subject is classified based on an increased level of at least one protein of Table 4, Table 6, and/or Table 8, at least one mRNA of Table 10 or Table 12, and/or at least one glycan of Table 14 or Table 16, as compared to a reference, and/or a decreased level of at least one protein of Table 5, Table 7, and/or Table 9, at least one mRNA of Table 11 or Table 13, and/or at least one glycan of Table 15 or Table 17, as compared to a reference.

51. A method for treating Kawasaki disease in a subject, said method comprising:

(a) determining the level of one or more proteins in a biological sample obtained from said subject and/or binding of IgG in said sample to one or more proteins comprising an amino acid sequence of any one of SEQ ID NOs:1 to 68 with a kit of any one of claims 40 to 42;

(b) administering a Kawasaki disease therapy if the level of said one or more proteins and/or binding of IgG in said sample is indicative that said subject may benefit from a Kawasaki disease therapy; wherein an increased level of at least one protein of Table 1, as compared to a reference, and/or a decreased level of at least one protein of Table 2, as compared to a reference, and/or increased binding of IgG in said sample to at least one protein comprising an amino acid sequence of any one of SEQ ID NOs:1 to 68, is indicative that said subject may benefit from a Kawasaki disease therapy.

52. A method for treating Kawasaki disease in a subject, said method comprising:

(a) determining the level of one or more proteins in a biological sample obtained from said subject with a kit of claims 42 or 43;

(b) administering a therapy other than, or in addition to, IVIG therapy if the level of said one or more proteins is indicative that said subject may benefit from a Kawasaki disease therapy other than, or in addition to, IVIG therapy;

wherein an increased level of at least one protein of Table 4, Table 6, and/or Table 8, at least one mRNA of Table 10 or Table 12, and/or at least one glycan of Table 14 or Table 16, as compared to a

reference, and/or a decreased level of at least one protein of Table 5, Table 7, and/or Table 9, at least one mRNA of Table 11 or Table 13, and/or at least one glycan of Table 15 or Table 17, as compared to a reference, is indicative that the subject may benefit from a Kawasaki disease therapy other than, or in addition to, IVIG therapy.