UNIVERSITY OF CALIFORNIA, SAN DIEGO

Urotensin 2 in Kawasaki disease pathogenesis

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in

Biology

by

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The Thesis of Cassidy YunJing Huang is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

Co-chair

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LIST OF ABBREVIATIONS

KD- Kawasaki Disease

CAA- Coronary Artery Aneurysm(s)

UTS2- Urotensin 2

UTS2R- Urotensin 2 receptor

SLC8A1- Solute carrier family 8 member 1

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I would like to acknowledge Dr. Jane C. Burns and Dr. Chisato Shimizu for their patience and guidance. Without them, this project would not have been possible. They have inspired me to work hard and produce good work. I would also like to acknowledge the Kawasaki Disease Research Center for their work, which continued to better the lives of countless children affected by Kawasaki disease.

Results and discussion sections, in part, are currently being prepared for submission for publication of the material. Huang, Cassidy Y.; Shimizu, Chisato; Burns, Jane C. The thesis author was the primary investigator and author of this material.

ABSTRACT OF THE THESIS

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by

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Master of Science in Biology

University of California, San Diego, 2016

Professor Jane C. Burns, Chair
Professor Li-Fan Lu, Co-Chair

Background

Genetic variation in calcium signaling pathways is associated with Kawasaki disease (KD) susceptibility and coronary artery aneurysms (CAA). Expression quantitative trait locus analysis for KD-associated variants in calcium/sodium channel gene solute carrier family 8 member 1 (SLC8A1) revealed an effect on expression of urotensin 2 (UTS2). We investigated the role of UTS2 in KD pathogenesis by measuring levels of UTS2 and its receptor in blood and tissues.

Results

UTS2 transcript levels determined by reverse transcription polymerase chain reaction were higher in whole blood of KD subjects homozygous for three risk alleles in SLC8A1 ($p=0.002$-0.006). Increased levels of plasma UTS2 varied as a function of

SLC8A1 genotype (p=0.008-0.04). UTS2 transcript levels in a microarray dataset for 131 subjects remained higher in the convalescent phase of KD in subjects with aneurysms. Immunohistochemical staining identified UTS2 and UTS2R expression in mononuclear inflammatory cells and spindle-shaped cells in the coronary arterial wall of a KD patient with CAA and in a femoral endarterectomy specimen from an adult patient with peripheral aneurysms following KD in childhood.

Discussion

Host genetics influences UTS2 levels, which may contribute to inflammation and cardiovascular damage in KD.

INTRODUCTION

Kawasaki disease

Kawasaki disease (KD) is an acute inflammation of the systemic, medium-sized arteries [1]. KD is currently the most common cause of acquired heart disease in children in developed countries [2]. 85% of KD patients are under five years old [1]. A child is diagnosed with KD according to the following criteria: fever of more than four days and at least four of the following clinical signs: bilateral conjunctival injection (red eyes), mucous membrane changes (cracked lips, strawberry tongue), rash, changes of the extremities (red palms/soles, swollen hands/feet), and unilateral cervical lymphadenopathy (swollen lymph nodes) (Table 1 and Figure 1) [3]. KD is self-limited, which means the systemic symptoms spontaneously resolve even without treatment [1]. However, coronary artery aneurysms (CAA), or the enlargement in diameter of the coronary artery, can be a life-long complication for a subset of KD patients. Intravenous immunoglobulin (IVIG) is now the standard treatment for KD patients [1]. Compared to no treatment, treatment with IVIG within the first 10 days of illness shows faster resolution of inflammation and reduces the frequency of coronary artery abnormalities [4]. The etiology of KD remains unknown after 40 years of research, but one hypothesis is that KD in Japan is triggered by inhalation of a windborne antigen that originates from northeastern China [5].

Pathology of coronary artery aneurysms in KD

CAA can be developed in 20-25% of untreated patients and 5% of patients treated with IVIG, and may progress to myocardial infarction (heart attack) or sudden death [6]. During coronary arteritis, the unknown inflammatory stimulus causes an infiltration of

inflammatory cells such as neutrophils, T cells, monocytes, and macrophages in the coronary arterial wall from both luminal and adventitial sides to all layers of the coronary artery, followed by coronary artery dilation, and CAA formation in a subset of KD subjects[7]. CAA formed during the acute phase of the disease can either remain the same, remodel, or thrombose (clot)[8]. CAA formation can lead to vascular fibrosis (excess collagen deposition in the vascular wall). A separate inflammatory complication of KD is myocarditis (inflammation of the heart muscle), and late myocardial fibrosis (excess collagen deposition in the myocardium), which can cause impaired heart function[9-11].

Evidence of genetic influence in KD

Although the etiology of KD is unknown, previous epidemiologic studies suggest that host genetics influences KD susceptibility[12,13]. The annual incidence of KD is 10-25 times higher in Japan (~300 per 100,000 children < 5 years) than in the US (20.8 per 100,000 children < 5 years) and UK (8.39 per 100,000 children < 5 years)[14-16]. In Hawaii in 1996-2006, the annual incidence of KD among Japanese children < 5 years was 201.5 per 100,000 children, while the annual incidence among white children was 13.7 per 100,000 children[17]. In Rady Children's Hospital in 2004-2014, the incidence of KD for every year is higher among Asian children than among African-American, Caucasian, and Hispanic children (Figure 2). Furthermore, siblings of KD children are 10-40 times more likely to develop KD compared to children with no family history of KD [18]. Parents of KD children are twice as likely to have had KD compared to parents of children with no history of KD [19]. Children whose parents had KD are more likely to have recurrent KD [20]. These data suggest that KD is a genetic disease.

Genetic studies

To understand the pathogenesis and genetic background of KD, several genetic studies were performed. Linkage analysis, which analyzes the chromosomal locations of genes involved in a disease, identified a single nucleotide polymorphism (SNP, a variation of one single nucleotide in a gene) within the inositol 1,4,5-trisphosphate kinase-C (ITPKC) gene associated with KD susceptibility and coronary artery aneurysm formation, and a SNP within the caspase-3 (CASP3) gene associated with KD susceptibility [21,22]. Genome-wide association studies (GWAS), which scan genomes to find genetic variations associated with a trait or disease, identified SNPs within the Fc fragment of IgG, low affinity IIa, receptor (FCGR2A), B lymphoid kinase (BLK), CD40, and human leukocyte antigen (HLA) genes to be associated with KD susceptibility [23-25]. Since KD is a complex genetic disease, and GWAS identify only SNPs with large effects, we performed pathway analysis and gene stability selection to identify genetic variants with smaller effects[26]. Pathway analysis maps associated genes to known pathways. Gene stability analysis determines the genes that drive the pathway associations by knocking out certain genes in a pathway and checking if the pathway association is still present. Pathway analysis was performed using a European GWAS dataset, and identified the 100 pathways significantly associated with KD susceptibility with $p<5x10^{-4}$ [26]. Gene stability analysis identified 26 genes with 116 SNPs. There were seven significant genes in the top three pathways, among which five were involved in calcium signaling pathways. Three SNPs in the gene solute carrier family 8 member 1 (SLC8A1) were validated in a Japanese GWAS data: rs10490051, rs13017968, and rs12989852. KD patients homozygous for the risk alleles had an increased risk of CAA formation. Expression

quantitative trait loci (eQTL) analysis determined that one of the three SLC8A1 variants (rs13017968) influenced the expression of urotensin 2 (UTS2).

Urotensin 2

UTS2 is an 11 amino acid cyclic peptide expressed in monocytes, endothelial cells, vascular smooth muscle cells, and fibroblasts has diverse effects upon binding to the urotensin 2 receptor (UTS2R) including potent vasoconstriction mediated by vascular smooth muscle cells (VSMCs), proliferation of VSMCs and fibroblasts, and chemotaxis of inflammatory cells [27,28]. UTS2 increases expression of monocyte-chemoattractant protein-1 (MCP-1), a chemoattractant for monocytes that was previously reported to be elevated in KD patients [29-32]. UTS2 itself is a chemoattractant for monocytes that express the UTS2R [33]. UTS2 has also been associated with myocardial and vascular fibrosis [34,35]. However, the role of UTS2 in KD has not been previously investigated. In the present study, we tested the hypothesis that KD subjects homozygous for SLC8A1 risk alleles have increased UTS2 expression by studying UTS2 transcript levels in blood, and UTS2 and UTS2R protein concentrations in blood and tissues.

MATERIALS AND METHODS

Subjects

The demographic and clinical characteristics of the 30 KD subjects for whom DNA, RNA, and plasma samples were available, are presented in Table 2. Subjects were grouped by SLC8A1 genotype for the three risk alleles and the data are presented in Supplemental Table 1 (rs10490051), Table 2 (rs13017968), and Table 3 (rs12989852). Allele frequencies for the three SLC8A1 risk loci SNPs (rs10490051, rs13017968, and rs12989852) are presented in Supplemental Table 4 for both the independent cohort of KD subjects and for control subjects from the 1000 Genomes database. All subjects met the American Heart Association (AHA) criteria for KD [3]. The variable z-worst is the maximal standard deviation units from the mean (z-score) measurement of the internal diameters of the proximal right coronary arteries and left anterior descending coronary arteries normalized for body surface area. Dilatation of the coronary arteries were defined as a z-score of ≥ 2.5, CAA were defined as a z-score of ≥ 4.0, and giant CAA were defined as as a z-score of ≥ 10.0. Laboratory data were obtained during the acute phase prior to intravenous-immunoglobulin (IVIG) administration. Parental informed consent and patient assent were obtained from all subjects. The study protocol was reviewed and approved by the University of California, San Diego Institutional Review Board.

Sample collection and RNA extraction

EDTA plasma was collected during the acute phase prior to IVIG administration, and processed within 48 hour of sample collection. Plasma samples were stored at -80°C until use. Whole blood was collected in PAXgene tubes (PreAnalytiX, Homebrechtikon, Switzerland) during the acute phase prior to IVIG administration. PAXgene tubes were

frozen at -20°C until RNA extraction. RNA extraction was performed according to manufacturer's instructions (Qiagen PAXgene blood mRNA kit).

Tissue samples and histology

Formalin fixed, paraffin-embedded tissues were obtained from two subjects with a history of KD, and one control patient. Tissues for Case 1 were obtained at the time of autopsy and tissues for Case 2 were obtained at the time of surgery. The demographic and clinical characteristics of Case 1, Case 2, and the control are presented in Table 3. The complete clinical course of Cases 1 and Case 2 was previously published[10,11,36]. Informed consent was obtained from the subjects or their parents. Histochemical staining with Masson trichrome stains was performed using standard techniques.

Microarray analysis

UTS2 transcript levels in whole blood of 131 paired acute and convalescent KD subjects were analyzed using a published microarray dataset [37]. Microarray probe location was indicated in Supplemental Figure 1. The demographic and acute clinical characteristics of the subjects used for the microarray analysis are presented in Table 4. Supplemental Table 1 (rs10490051), Table 2 (rs13017968), and Table 3 (rs12989852) show the characteristics grouped by SLC8A1 risk loci.

Genotyping

Genomic DNA was extracted from whole blood using Wizard genomic DNA purification kit (Promega, Madison, WI, A1125). KD subjects were genotyped by PCR for SLC8A1 rs10490051, rs13017968, and rs12989852 (Life Technologies, 26251262_10, 2669599_10, and 30967076_10, respectively).

Quantitative reverse transcription-polymerase chain reaction validation of microarray results

To validate published microarray results showing differential UTS2 expression by SLC8A1 genotype, UTS2 transcript levels for an independent cohort of 30 KD subjects were measured by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). cDNA was made from whole blood RNA collected in PAXgene tubes (SuperScript III reverse transcriptase, ThermoFischer, 18080-051). qRT-PCR was performed according to the manufacturer's instructions using commercially available UTS2 Taqman primers (ThermoFischer, Hs00922170_m1). The Taqman PCR product location is shown in Supplemental Fig. 1. The relative abundance of UTS2 transcripts was normalized to the expression levels of TATA box binding protein-associated factor, RNA polymerase I, B (TAF1B) (Life Technologies, Hs00374547).

Plasma UTS2 and MCP-1 measurement using enzyme linked immunosorbent assay

Plasma UTS2 and MCP-1 levels were measured by enzyme linked immunosorbent assay (ELISA) according to manufacturer's instructions (UTS2: Phoenix Pharmaceuticals, Burlingame, CA, EK-071-05, and MCP-1: R&D Systems, Minneapolis, MN, DCP00).

IHC staining of tissues

Tissues were fixed in formalin and embedded in paraffin. Tissue sections were deparaffinized and rehydrated using standard methods. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide (Abcam, Cambridge, MA, Ab93705). Antigen retrieval was performed using citrate buffer in a microwave for 10 minutes. Nonspecific binding was blocked using milk in PBS for 10 minutes (Abcam, Cambridge, MA,

Ab93705). Slides were incubated with primary antibodies, anti-human UTS2 rabbit polyclonal antibody and anti-human UTS2R rabbit polyclonal antibody (Novus Biologicals, Littleton, CO, NBP1-87223, 1:100 dilution and NLS374, 1:100 dilution), at 4°C overnight. Rabbit IgG was used as the primary antibody for the negative staining controls (Dako, Carpinteria, CA, X0936). Bound primary antibodies were detected using a biotinylated secondary antibody, enzyme-labeled streptavidin, and visualized by substrate-chromogen (Abcam, Cambridge, MA, Ab93705).

Statistical analysis

Data were analyzed using GraphPad Prism (GraphPad Software, Inc., La Jolla, CA). Mann-Whitney U test and the Kruskal-Wallis test were used to compare continuous variables. Correlation coefficients were calculated using the Pearson correlation coefficient test. Chi-square test was used to compare equality of proportions. Two-tailed p-value less than 0.05 was considered statistically significant.

RESULTS

UTS2 transcript and protein levels

Using a KD microarray dataset from acute, pre-treatment whole blood, we determined that KD subjects homozygous for the three risk alleles in SLC8A1 (rs10490051, rs10317968 and rs12989852 have higher UTS2 transcript levels (Supplemental Figure 2). This was validated using quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) in whole blood collected pre-treatment from an independent cohort of 30 KD subjects with higher UTS2 levels in risk allele carriers (Figure 3). However, no relationship was found between the risk alleles and expression of the receptor, UTS2R (data not shown). We next determined whether UTS2 plasma protein levels varied as a function of SLC8A1 genotype. Plasma UTS2 levels were higher in KD subjects homozygous for the three risk alleles (Figure 4).

To determine whether UTS2 and UTS2R are differentially expressed as a function of patient characteristics and disease outcome, we analyzed whole blood UTS2 transcript levels in 131 paired acute and convalescent KD subjects [37]. UTS2 transcript levels were significantly lower in the convalescent phase in KD subjects with normal and dilated coronary arteries ($p=0.0001$ and $p=0.004$, respectively) (Figure 5a and b). Levels remained persistently elevated in the convalescent phase for subjects who developed CAA (Figure 5c). Demographic and clinical factors except for ethnicity were similar among KD subjects with different SLC8A1 genotypes (Supplemental Table 5, Table 6, and Table 7). However, subjects with self-declared Asian ancestry were more likely to be homozygous for all three risk alleles. This is consistent with data from the 1000 Genomes database that individuals of Asian descent have a higher allele frequency for the SLC8A1

risk alleles (Supplemental Table 4). No correlation was observed between UTS2 transcript levels and sex, illness day, age, coronary artery z-score, the percentage of lymphocytes in whole blood, and the percentage of monocytes in whole blood of the KD subjects (Supplemental Figure 3). UTS2R was not differentially expressed as a function of KD disease status or coronary artery status (Supplemental Figure 4). Since UTS2R expression in blood was reported to be highest in monocytes[33], we analyzed the correlation between the absolute number of monocytes and UTS2R transcript levels in whole blood, but no correlation was found (Supplemental Figure 5).

Plasma MCP-1 levels as a function of SLC8A1 genotype

Since UTS2 induces MCP-1 secretion in rat adventitial fibroblasts[30], and in the present study, we showed that UTS2 transcript and protein levels are influenced by SLC8A1 genotype, we next determined whether SLC8A1 genotype influences MCP-1 secretion. Plasma MCP-1 levels (pg/ml) did not differ as a function of SLC8A1 genotype for any of the three SLC8A1 risk alleles (Supplemental Figure 6). Similarly, there was no correlation between UTS2 and MCP-1 levels in acute plasma samples (data not shown).

UTS2 and UTS2R expression in tissues

To understand the relationship between the UTS2/UTS2R axis and inflammation of the coronary arteries, we performed IHC staining for UTS2 and UTS2R in the coronary artery of Case 1, a KD patient with CAA who died 10 months after disease onset. The artery had a giant aneurysm with thrombotic occlusion of the lumen, with destruction of the internal elastic lamina and thickening of the intima (Figure 6a). In the thickened intima, UTS2-, UTS2R-, and CD14- positive mononuclear inflammatory cells were present (Figure 6b, c, and d, arrowheads), as well as spindle-shaped cells (arrows).

The normal control coronary artery showed positive staining for UTS2- and UTS2R- in endothelial cells and vascular smooth muscle cells (Figure 6g and h). No spindle-shaped cells or inflammatory cells were observed in the normal coronary artery.

To understand the possible relationship between the UTS2/UTS2R axis and vascular fibrosis in KD, we performed IHC staining for UTS2 and UTS2R in an endarterectomy specimen surgically resected from the femoral artery of Case 2, an adult KD patient with vascular fibrosis [10]. At age three months, Case 2 developed giant CAA and bilateral common femoral artery aneurysms. At age 30 years, he developed claudication from stenosis of the common femoral artery bilaterally and an endarterectomy was performed. Masson's trichrome stain showed collagen deposition in the intima of the iliac artery (Figure 7a). Abundant UTS2- and UTS2R- positive spindle-shaped cells were noted on the luminal side of the thickened intima, where collagen deposition was also observed (Figure 7b and c, arrows).

The results section, in part, is currently being prepared for submission for publication of the material. Huang, Cassidy Y.; Shimizu, Chisato; Burns, Jane C. The thesis author was the primary investigator and author of this material.

DISCUSSION

Here, we show that polymorphisms associated with KD susceptibility in SLC8A1, located on Chromosome 2, influence in *trans* the transcript abundance and protein expression of UTS2 on Chromosome 1. We have recently established the influence of SLC8A1 polymorphisms on susceptibility to KD and risk of CAA [26]. Using our KD microarray database, we showed that SLC8A1 genotype can influence expression of UTS2 transcripts in whole blood, with the highest UTS2 transcript abundance in subjects homozygous for the SLC8A1 risk alleles. In an independent cohort, we confirmed that KD subjects homozygous for all three risk alleles have increased UTS2 transcript levels although only those homozygous for rs10490051 had increased plasma protein levels. UTS2 and UTS2R were expressed in inflammatory cells and spindle-shaped cells with a myofibroblast-like morphology in the coronary artery. UTS2 and UTS2R were also expressed in spindle-shaped cells in an endarterectomy specimen from a remodeled aneurysm in the femoral artery following KD. These data suggest a possible role for UTS2 and UTS2R in vascular inflammation, aneurysm formation, and arterial remodeling.

The recruitment of monocytes to the arterial wall during acute KD could contribute to vessel wall inflammation and aneurysm formation. The exact mechanism of CAA formation in KD has yet to be clarified, but evidence suggests that monocytes and MCP-1, a chemoattractant for monocytes, are involved in the process[38,39]. Monocytes were demonstrated to be more abundant during acute KD, particularly the CD14+CD16+ subpopulation [40]. Studies conducted by Zhang et al determined that UTS2, by binding UTS2R, increases expression and secretion of MCP-1 in rat aorta adventitial

fibroblasts[30]. Segain et al. reported high UTS2R expression in CD14+CD16+ monocytes, and that UTS2 is a chemoattractant for monocytes that express UTS2R[33]. UTS2 mRNA is most abundantly expressed in classical monocytes that are CD14+CD16- [41]. Although increased plasma UTS2 levels did not correlate with increased plasma MCP-1 levels, UTS2 may have a role in monocyte chemotaxis in the arterial wall. The coronary arterial wall of a KD subject who died with giant CAA showed positive staining for UTS2, UTS2R, and CD14 in infiltrating mononuclear inflammatory cells (Figure 6). This suggests a possible role for UTS2 and UTS2R involvement in the recruitment of CD14+ monocytes to the arterial wall, thus contributing to vessel wall inflammation. UTS2 and UTS2R were also expressed in spindle-shaped, myofibroblast-like cells in the coronary artery. It is possible that in KD, the CD14+ monocytes expressing UTS2 and UTS2R can also differentiate into myofibroblasts, as has been reported in systemic sclerosis patients[42]. Although plasma UTS2 levels did not correlate with coronary artery damage, it is likely that expression of UTS2 and recruitment of cells occur in the tissues and not in the periphery. Our results raise the possibility that UTS2 and UTS2R could contribute to inflammation and CAA formation in KD through the induction of myofibroblast transformation in the vascular wall.

Myofibroblasts are spindle-shaped cells that appear after cardiac injury and contribute to cardiac remodeling [43]. Myofibroblasts are formed through the process of epithelial-to-mesenchymal transition (EMT), in which cells of mesenchymal origin, such as fibroblasts, can differentiate into myofibroblasts. The process is triggered by multiple mediators including transforming growth factor (TGF)-β[44]. Markers of myofibroblast phenotypic differentiation include expression of alpha-smooth muscle actin (αSMA),

increased collagen synthesis, and enhanced cell migration [43]. We have previously reported that myofibroblast-like cells and TGFβ play a role in aneurysm formation in KD [9]. Myofibroblast-like cells were found in the coronary arterial wall of KD patients, and were shown to express IL-17, which recruits pro-inflammatory cells to the arterial wall to contribute to aneurysm formation and arterial damage [9]. Studies by Zhang et al. demonstrated that UTS2 can induce increased TGFβ expression and secretion, αSMA expression, collagen synthesis, and cell migration in a time- and concentration-dependent manner in rat adventitial fibroblasts, suggesting that in rats, UTS2 and UTS2R can induce phenotypic differentiation of adventitial fibroblasts to myofibroblasts [29,45]. In the present study, we showed that UTS2 and UTS2R were expressed in myofibroblast-like cells an endarterectomy specimen with dense fibrotic regions with collagen deposition (Figure 7). After formation of CAA, the aneurysmal arterial walls of KD patients may remodel with activated myofibroblasts causing luminal narrowing and occlusion[28].

UTS2R is a G-coupled protein receptor that, upon binding to UTS2, leads to downstream signaling that potentiates L-type calcium channels in cardiomyocytes[46]. Genetic variation in calcium signaling pathways influences KD susceptibility and aneurysm formation [13]. Investigating the role of UTS2 in calcium signaling through UTS2R may provide further insight into the role of UTS2 and UTS2R in KD pathogenesis. Polymorphisms in three calcium-signaling pathway genes, calcium release-activated calcium channel protein 1 (ORAI1), inositol-triphosphate 3-kinase C (ITPKC), and SLC8A1 have been identified and validated to influence KD susceptibility and aneurysm formation [21,26,47]. ORAI1 is a membrane-bound calcium channel activated by stromal interaction molecule 1 (STIM1), a sensor of calcium store depletion in

endoplasmic reticulum[47]. ORAI1 and STIM1 have been shown to participate in the UTS2/UTS2R signaling cascade in the rat coronary artery, in which ORAI1 activation was necessary to induce an enhanced intracellular calcium mobilization during UTS2-induced vasoconstriction[48]. SLC8A1 encodes for the sodium-calcium exchanger member 1 (NCX1), a membrane-bound calcium channel that transports sodium and calcium bi-directionally. NCX1 was shown to be expressed in spindle-shaped, myofibroblast-like cells in the coronary arteries of acute KD patients 7 and 12 days post KD onset. SLC8A1 polymorphisms influence KD susceptibility and CAA formation. KD patients homozygous for the risk allele were shown to have increased disease susceptibility, increased likelihood of CAA formation, and increased resting and stimulated intracellular calcium mobilization [26].

We recognize both strengths and limitations of the present study. This study draws a link between genetic risk factors for KD that serve as an eQTL for UTS2, and increased levels of this protein that may recruit inflammatory cells to the arterial wall and contribute to EMT, a process associated with aneurysm formation. We recognize the descriptive nature of this study that should be viewed as hypothesis generating. Limitations include the small number of KD autopsy tissues available for IHC studies and the inability to genotype the tissue donors due to the poor quality of the DNA.

In summary, we demonstrated that polymorphisms in SLC8A1 influence the transcription of UTS2, with higher UTS2 transcript abundance and plasma protein levels observed in KD subjects homozygous for SLC8A1 risk alleles. Both UTS2 and UTS2R were expressed in mononuclear inflammatory cells and spindle-shaped, myofibroblast-like cells in the coronary arterial wall. These data suggest that UTS2 and UTS2R may

have a pro-inflammatory role associated with monocyte recruitment and myofibroblast generation that contributes to formation of CAA.

The discussion section, in part, is currently being prepared for submission for publication of the material. Huang, Cassidy Y.; Shimizu, Chisato; Burns, Jane C. The thesis author was the primary investigator and author of this material.

FIGURES

Figure 1. Clinical features of KD. Signs are listed clockwise from the top left: bilateral conjunctival injection (bloodshot eyes), changes of the extremities (swollen hands), cervical lymphadenopathy (swollen lymph node in neck), rash, mucous membrane changes (strawberry tongue and red, cracked lips)

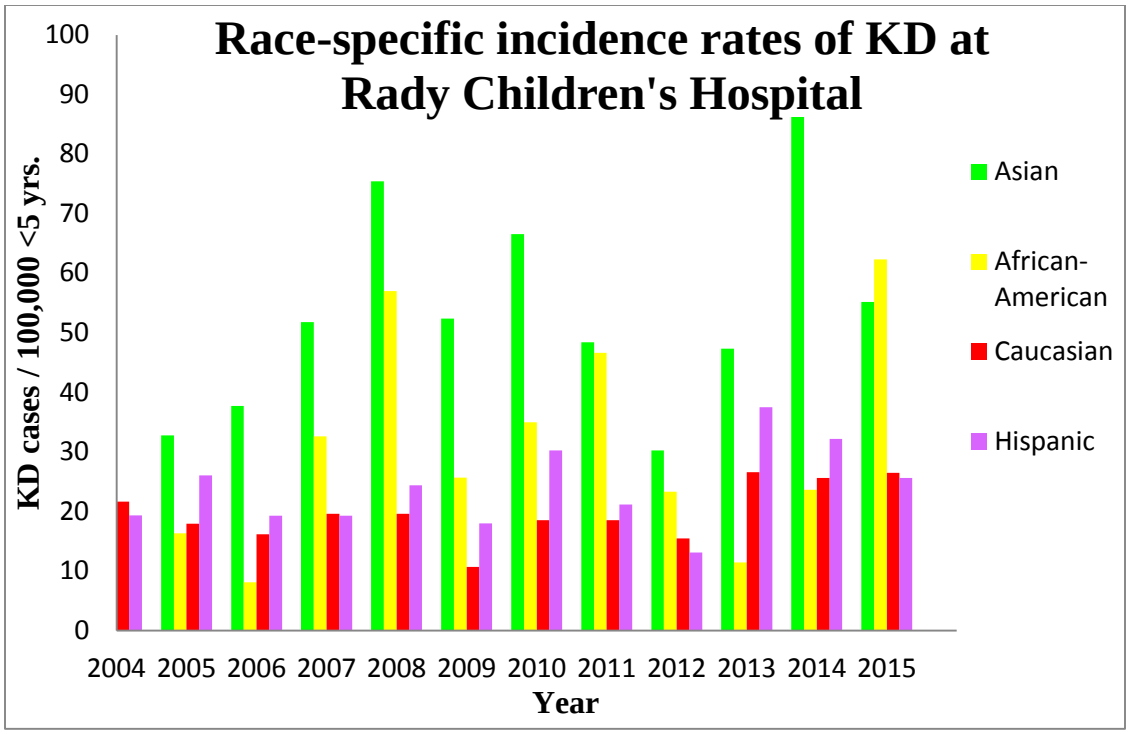


Figure 2. Race-specific incidence rates of KD. Incidence rates were recorded annually from 2004 to 2015. Incidence of Asian KD patients at Rady Children's Hospital are shown in green bars, African-American KD patients in yellow bars, Caucasian patients in red bars, and Hispanic patients in pink bars. KD incidence rates are calculated as number of cases per 100,000 children under five years old.

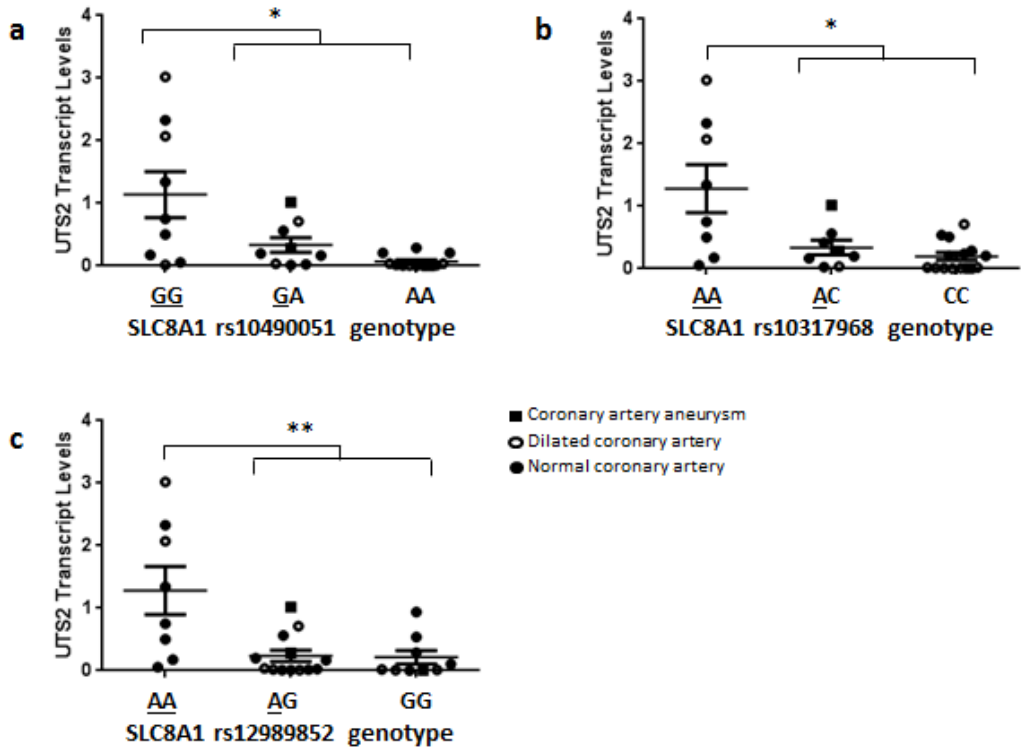


Figure 3. Differential expression of UTS2. Acute UTS2 transcript levels in whole blood of KD patients were stratified by SLC8A1 (**a**) rs10490051 genotypes GG (n=9), GA (n=9), and AA (n=12), (**b**) rs13017968 genotype AA (n=8), AC (n=8), and CC (n=14), and (**c**) rs12989852 genotypes AA (n=8), AG (n=13), and GG (n=9). Risk alleles are underlined. **P*<0.05; ***P*<0.005 for homozygous risk allele vs. all others.

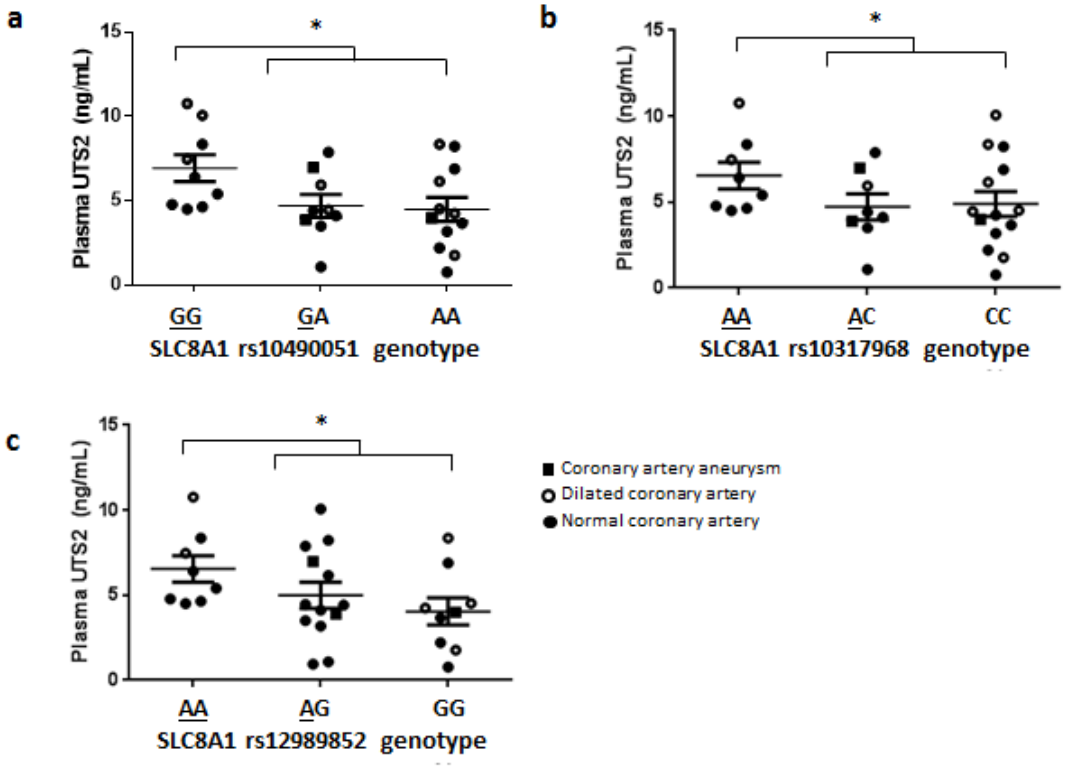


Figure 4. Plasma UTS2 levels. Acute UTS2 levels in plasma of KD patients were stratified by SLC8A1 (**a**) rs10490051 genotypes GG (n=9), GA (n=9), and AA (n=12), (**b**) rs13017968 genotype AA (n=8), AC (n=8), and CC (n=14), and (**c**) rs12989852 genotypes AA (n=8), AG (n=13), and GG (n=9). Risk alleles are underlined. *P<0.05 for homozygous risk allele vs. all others.

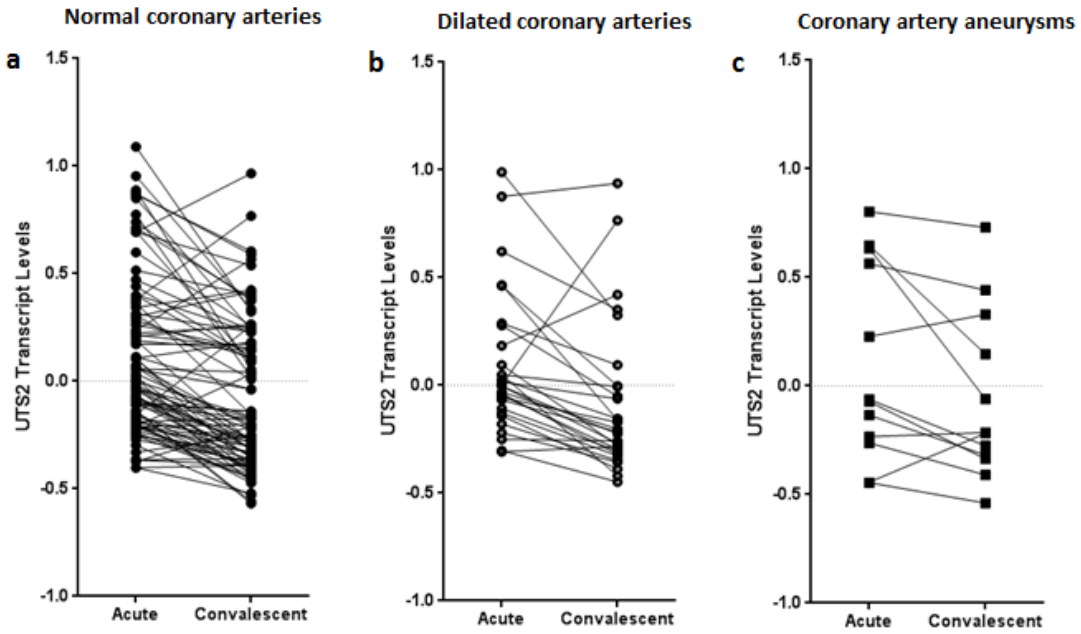


Figure 5. UTS2 transcript levels as a function of coronary artery status. UTS2 transcript levels in whole blood were analyzed for paired acute and convalescent KD subjects: (**a**) Normal coronary arteries (n=90) (*P*=0.0001), (**b**) Dilated coronary arteries (n=29) (*P*=0.004), and (**c**) Coronary artery aneurysms (n=12) (not significant).

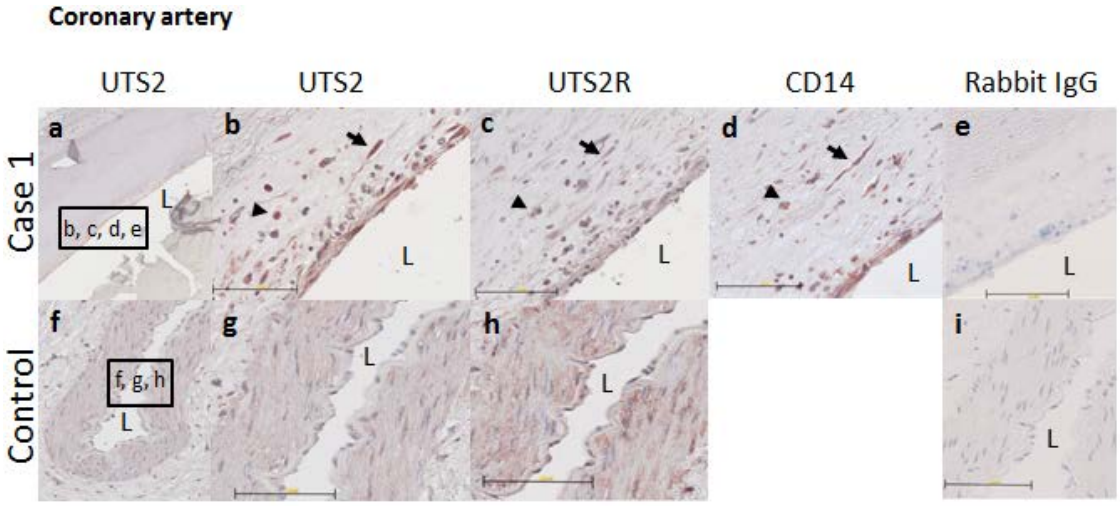


Figure 6. UTS2 and UTS2R expression in coronary artery. The coronary artery of Case 1 was stained for (**a**) UTS2 at 40x, with boxed area indicating location of (**b-e**), (**b**) UTS2, 400x, (**c**) UTS2R, 400x, (**d**) CD14+, 400x, and (**e**) rabbit IgG as a negative staining control at 400x. The control coronary artery was stained for (**f**) UTS2, 40x, with boxed area indicating location of (**g-i**), (**g**) UTS2, 400x, (**h**) UTS2R, 400x, (**i**) rabbit IgG, 400x. Arrows indicate spindle-shaped cells, and arrowheads indicate mononuclear inflammatory cells in thickened intima. L: lumen. Scale bar: 100 μm.

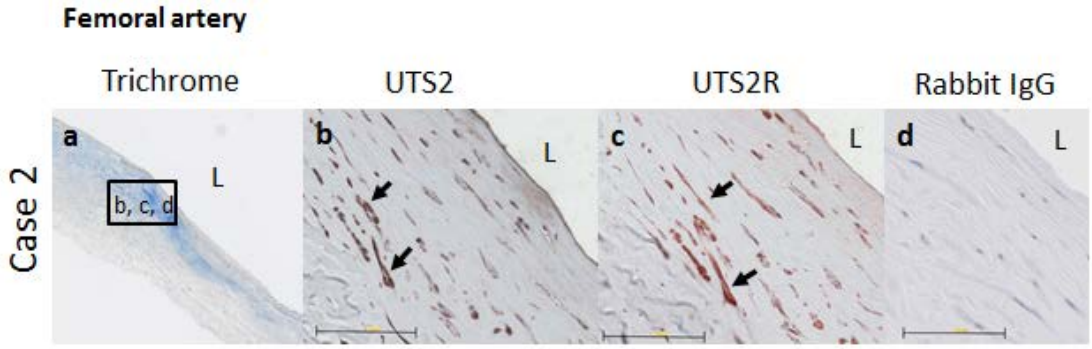


Figure 7. UTS2 and UTS2R expression in femoral artery. The iliac artery of Case 2 was stained for (**a**) trichrome staining shown in 40x magnification, with boxed area indicating location of (**b-d**), (**b**) UTS2 at 400x magnification, (**c**) UTS2R at 400x magnification, and (**d**) rabbit IgG as a negative staining control at 400x magnification. Arrows indicate spindle-shaped cells. L: lumen. Scale bar: 100 μm.

TABLES

Table 1. Clinical features of KD

Epidemiological case definition (classic clinical criteria)
Fever persisting at least 5 days*
Presence of at least 4 principal features:
Changes in extremities
Acute: Erythema of palms, soles; edema of hands, feet
Subacute: Periungual peeling of fingers, toes in weeks 2 and 3
Polymorphous exanthem
Bilateral bulbar conjunctival injection without exudate
Changes in lips and oral cavity: Erythema, lips cracking, strawberry tongue, diffuse injection of oral and pharyngeal mucosae
Cervical lymphadenopathy (>1.5-cm diameter), usually unilateral

Table is modified from previously published AHA clinical features of KD [3]. *In the presence of ≥4 principal criteria, Kawasaki disease diagnosis can be made on day 4 of illness. Experienced clinicians who have treated many Kawasaki disease patients may establish diagnosis before day 4.

Table 2. Demographic and clinical characteristics of KD subjects used for qRT-PCR and ELISA

	KD patients (n=30)
Age	2.1 (1.3-3.7)
Males, n (%)	19 (63)
Ethnicity	
Asian, n (%)	8 (27)
Caucasian, n (%)	5 (17)
Native American, n (%)	1 (3)
Hispanic, n (%)	11 (37)
Mixed, n (%)	5 (16)
Illness day, median (range)*	5 (2-11)
Coronary artery status	
Aneurysm, n (%)	3 (10)
Dilated, n (%)	11 (37)
Normal, n (%)	16 (53)
Z Worst, median (range)*	2.1 (0.5-5.2)
Lab data	
WBC (10^3/uL)	13.5 (11.1-15.5)
Polys (%)	54 (47-62)
Lymphocytes (%)	25 (20-34)
Monocytes (%)	5 (2-9)
Eosinophils (%)	2 (1-4)
Absolute Band Count	1024 (504-1883)
Absolute Neutrophil Count	8917 (6308-10359)
Hemoglobin (mg/dl)	11 (10.3-11.5)
Z-Hemoglobin**	-1.5 (-2.4 - -0.7)
Hematocrit (%)	31.7 (31-34)
Platelets (10^3/mm^3)	378 (289-458)
ESR (mm/h)	57.5 (43-70)
CRP (mg/dL)	6.2 (4.1-11.8)
ALT (iu/L)	47.5 (30.2-86.5)
GGT (iu/L)	42 (20-89)

*All data are represented as a median (interquartile range) unless otherwise specified. **Z-hemoglobin is the hemoglobin concentration normalized for age and expressed as standard deviation units. WBC: white blood count. Polys: polymorphonuclear cells. ESR: erythrocyte sedimentation rate. CRP: C-reactive protein. ALT: alanine aminotransferase. GGT: gamma-glutamyl transferase.

Table 3. Demographic and clinical characteristics of KD and control subjects for IHC staining

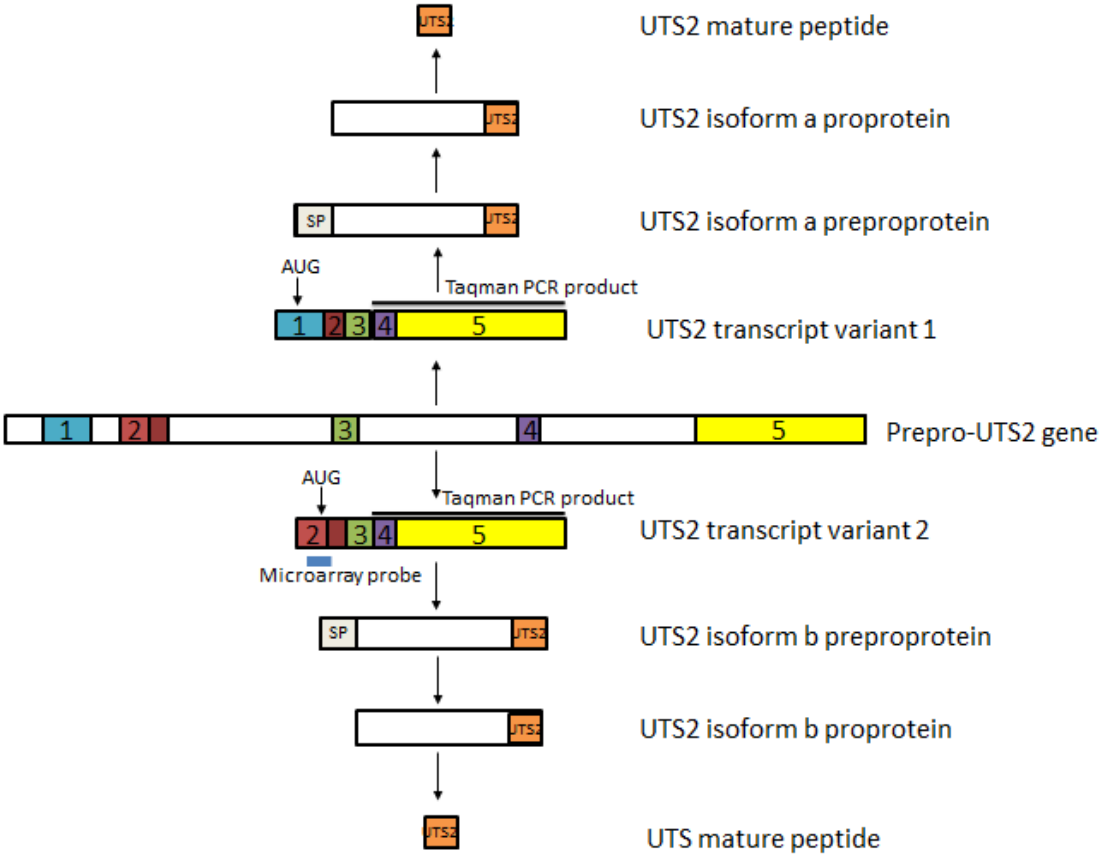
Case	Age/Sex	Ethnicity	Age of KD onset	Procedure	Clinical course	Tissues obtained
1	15m/M	Caucasian	5m	Autopsy	Giant aneurysm at KD onset at 5 months. Death due to cardiac arrest from thrombotic occlusion of giant aneurysm	Coronary artery
2	31y/M	Caucasian	7w	Left iliac endarterectomy	CAA and bilateral axillary aneurysms at KD onset. Developed right lower extremity claudication at age 22 and age 25. At age 30, both femoral arteries were diseased with stenosis. Left iliac artery had thickened intima with diffuse fibrosis and calcification.	Intima of femoral artery
Control	4y/F	N/A	N/A	Autopsy	Congenital diaphragmatic hernia	Coronary artery/ myocardium

Table 4. Demographic and clinical characteristics of KD subjects in microarray dataset

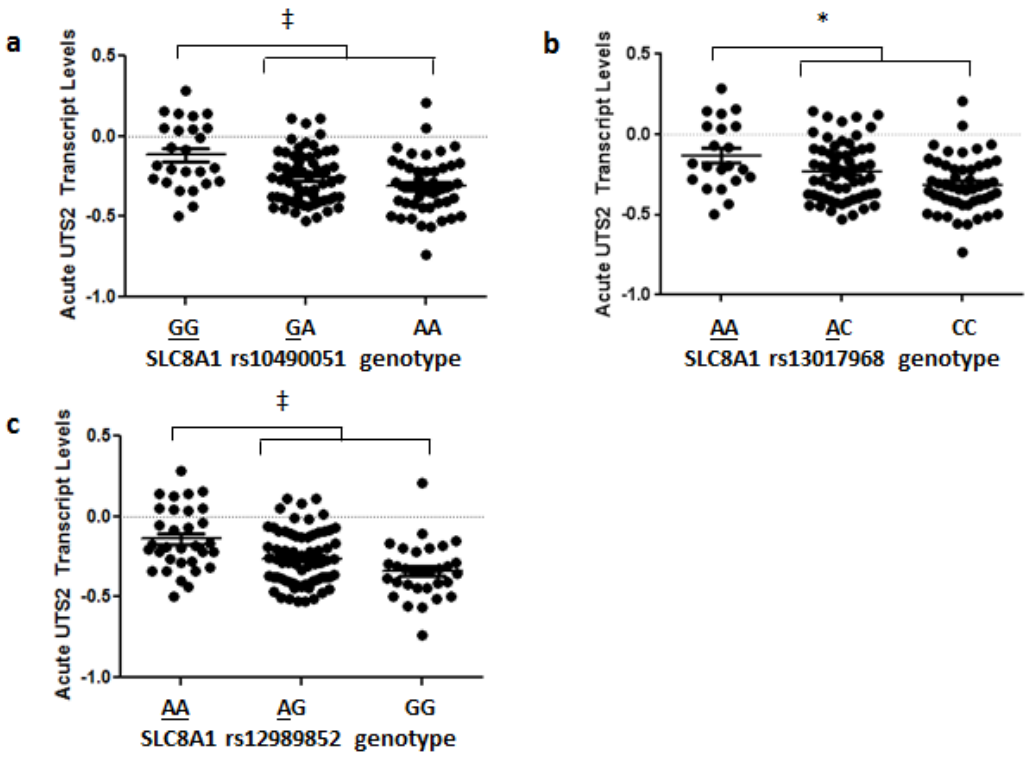
	KD patients (n=131)
Age	2.7 (1.4-4.1)
Males, n (%)	80 (61)
Ethnicity	
Asian, n (%)	22 (17)
African American, n (%)	5 (4)
Caucasian, n (%)	35 (27)
Hispanic, n (%)	32 (24)
Mixed, n (%)	37 (28)
Illness day, median (range)*	6 (2-11)
Coronary artery status	
Aneurysm, n (%)	12 (9)
Dilated, n (%)	29 (22)
Normal, n (%)	90 (69)
Z Worst, median (range)*	1.8 (0.2-18.3)
Lab data	
WBC (10^3/uL)	13.6 (11-19)
Polys (%)	54 (42-63)
Lymphocytes (%)	21 (13-31)
Monocytes (%)	6 (4-8)
Eosinophils (%)	2 (0-3)
Absolute Band Count	1771 (456-2820)
Absolute Neutrophil Count	9027 (6780-12390)
Hemoglobin (mg/dl)	11.3 (10.5-11.8)
Z-Hemoglobin**	-1.5 (-1.9 - -0.4)
Hematocrit (%)	32.8 (31-34)
Platelets (10^3/mm^3)	412 (321-475)
ESR (mm/h)	61 (44-82)
CRP (mg/dL)	8 (4.6-16.4)
ALT (iu/L)	33 (17-109)
GGT (iu/L)	30 (17-93)

*All data are represented as a median (interquartile range) unless otherwise specified. **Z-hemoglobin is the hemoglobin concentration normalized for age and expressed as standard deviation units. WBC: white blood count. Polys: polymorphonuclear cells. ESR: erythrocyte sedimentation rate. CRP: C-reactive protein. ALT: alanine aminotransferase. GGT: gamma-glutamyl transferase.

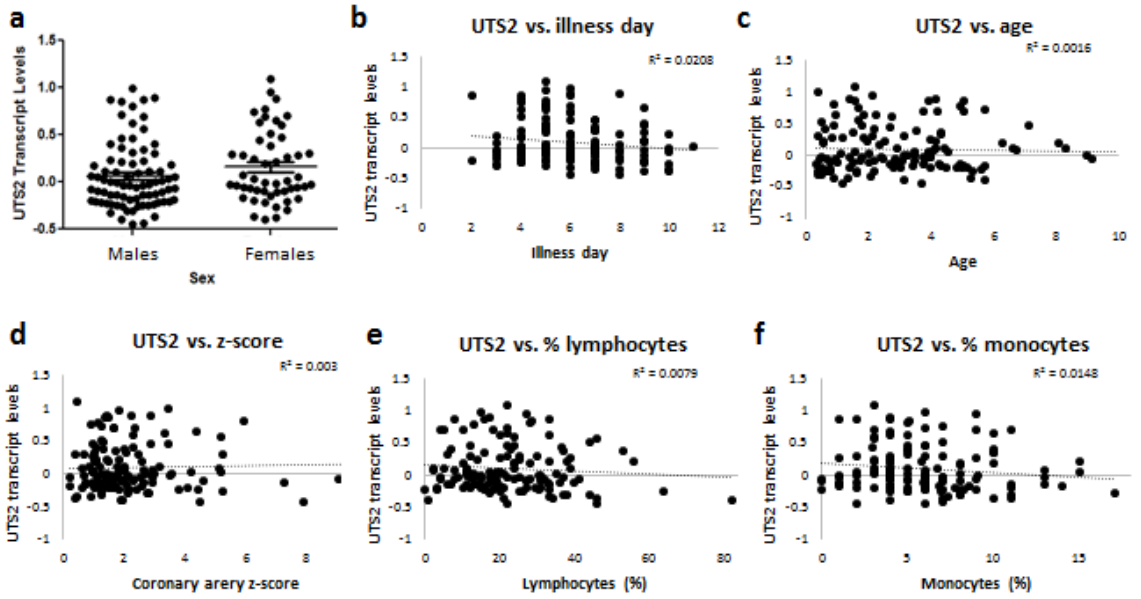
SUPPLEMENTAL FIGURES



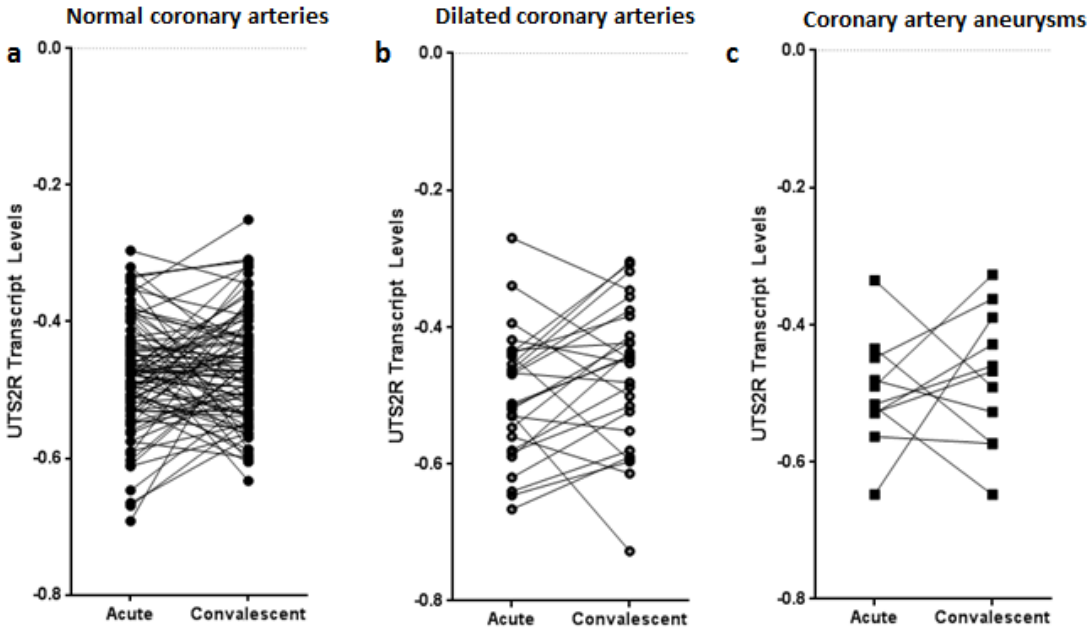
Supplemental Figure 1. Structure of human UTS2. The prepro-UTS2 gene consists of five exons labeled as colored blocks, and is transcribed into two transcript variants, transcript variant 1 and transcript variant 2. Transcript variant 1 and transcript variant 2 are translated into isoform a preproprotein and isoform b preproprotein, respectively. Through proteolytic cleavage, isoform a and isoform b preproprotein produce the same mature peptide. The microarray probe and the Taqman PCR product used in the paper are shown in the diagram.



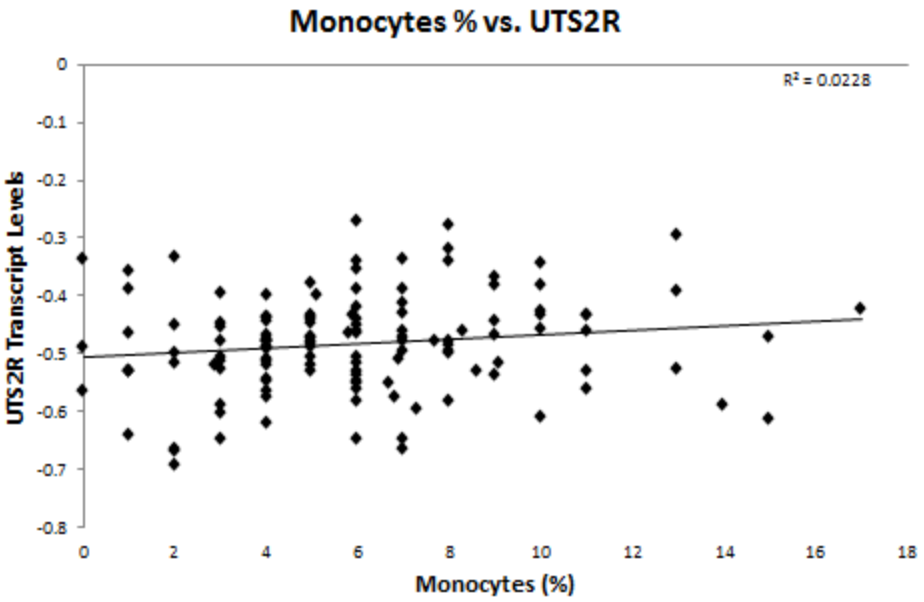
Supplemental Figure 2. UTS2 transcript levels as a function of genotype. Acute UTS2 transcript levels in whole blood were analyzed using a microarray dataset and stratified by (**a**) rs10490051 genotypes GG (n=25), GA (n=58), and AA (n=47), (**b**) rs13017968 genotypes AA (n=21), AC (n=60), and CC (n=50), and (**c**) rs12989852 genotypes AA (n=32), AG (n=67), and GG (n=71). Risk alleles are underlined. ‡P<0.0005, *P<0.05.



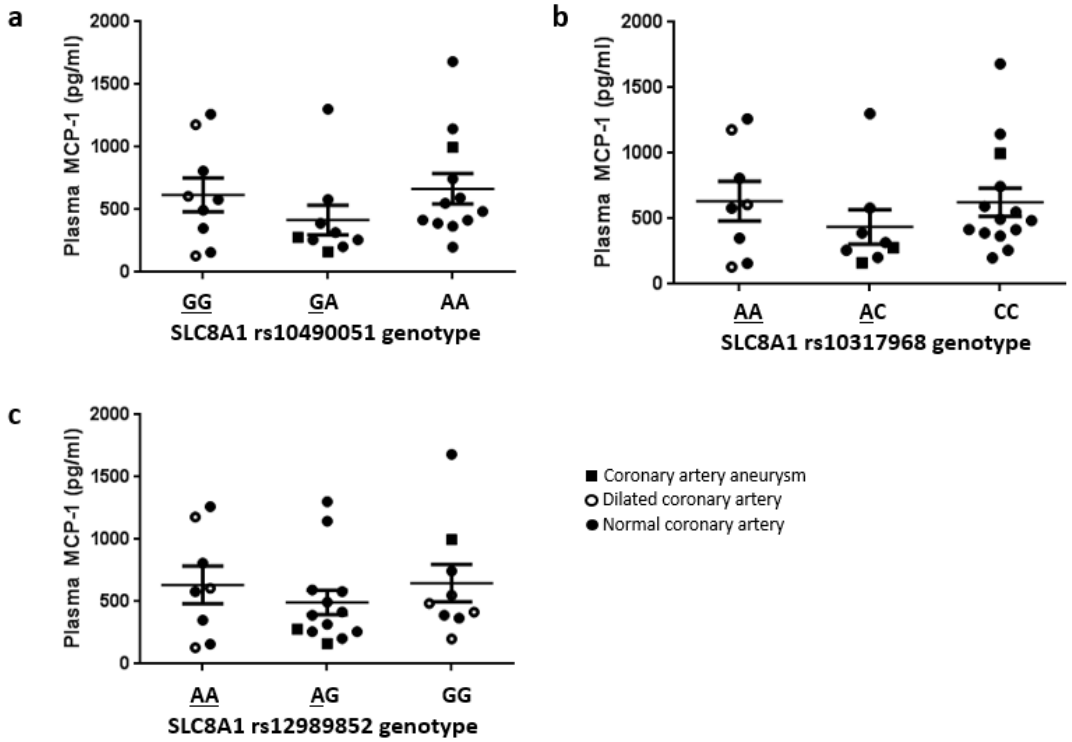
Supplemental Figure 3. UTS2 transcript levels as a function of patient demographic, clinical, and laboratory measurements. Acute UTS2 transcript levels in whole blood were analyzed as a function of (**a**) sex (males n=80, females n=51), (**b**) illness day (first day of fever=Illness Day 1) (R=0.14), (**c**) age (years) (R=0.04), (**d**) coronary artery z-score (R=0.05), (**e**) percent lymphocytes (R=0.09), and (**f**) percent monocytes (R=0.12). None of the correlations were significant.



Supplemental Figure 4. UTS2R transcript levels as a function of KD disease status. UTS2R transcript levels in whole blood were analyzed for (**a**) paired acute and convalescent KD subjects (n=90) with normal coronary arteries, (**b**) paired acute and convalescent KD subjects (n=29) with dilated coronary arteries, and (**c**) paired acute and convalescent KD subjects (n=12) with coronary artery aneurysms (CAA). CAA were defined as a z-score of ≥ 4.0, and dilatation of the coronary arteries were defined as a z-score of ≥ 2.5.



Supplemental Figure 5. Correlation of monocyte percentage and UTS2R transcript levels. Data are measured in whole blood (R=0.15), not significant.



Supplemental Figure 6. Plasma MCP-1 levels. Acute MCP-1 levels were stratified by SLC8A1 (**a**) rs10490051 genotypes GG (n=9), GA (n=9), and AA (n=12), (**b**) rs13017968 genotype AA (n=8), AC (n=8), and CC (n=14), and (**c**) rs12989852 genotypes AA (n=8), AG (n=13), and GG (n=9). Risk alleles are underlined. There was no difference in plasma MCP-1 levels as a function of any of the SLC8A1 genotypes.

SUPPLEMENTAL TABLES

Supplemental Table 1. Demographic and clinical characteristics of KD subjects used for qRT-PCR and ELISA for SLC8A1 rs10490051

rs10490051	GG (n=9)	GA (n=9)	AA (n=12)	p-value
Age	2.1 (2.1-4.5)	2.5 (1.7-4.3)	1.6 (1.1-2.5)	ns
Males, n (%)	6 (67)	5 (55)	8 (67)	ns
Ethnicity				
Asian, n (%)	4 (44)	2 (22)	2 (17)	ns
African American, n (%)	0	0	0	ns
Caucasian, n (%)	1 (11)	3 (33)	2(17)	ns
Hispanic, n (%)	4 (44)	3 (33)	4 (33)	ns
Mixed, n (%)	0	1 (11)	4 (33)	ns
Illness day, median (range)*	5 (4.8-5.3)	6 (4.8-7.3)	5.5 (3.8-7)	ns
Coronary artery status				
Aneurysm, n (%)	0	2 (22)	1 (8)	ns
Dilated, n (%)	3 (33)	2 (22)	5 (42)	ns
Normal, n (%)	6 (66)	5 (55)	6 (50)	ns
Z Worst, median (range)*	2.1 (1.2-2.3)	2.1 (1.3-4.1)	2.1 (1.3-3.0)	ns
Lab data				
WBC (10^3/uL)	14.8 (12.7-15.5)	11.8 (10-13.5)	14 (12.4-15.8)	ns
Polys (%)	55 (50-63)	56 (49-68)	50 (44.5-58)	ns
Lymphocytes (%)	25 (20-25)	22 (16-25)	33 (24.5-36.3)	ns
Monocytes (%)	6 (3-8)	10 (5-13)	4.5 (2-7)	ns
Eosinophils (%)	1 (1-3)	2 (1-7)	3 (1.5-3.5)	ns
Absolute Band Count	1496 (755-1550)	756 (506-1602.5)	1024 (336-2132)	ns
Absolute Neutrophil Count	9765 (8305-11352)	6844 (6132-9638)	8632 (6773.3-10100.3)	ns
Hemoglobin (mg/dl)	11.1 (10.7-11.8)	11.1 (10.3-11.8)	10.9 (10.2-11.3)	ns
Z-Hemoglobin**	-1.5 (-2 - -0.7)	-1.3 (-2.5 - -0.9)	-1.8 (-2.3 - -0.9)	ns
Hematocrit (%)	32 (31.5-35.1)	31.6 (30.4-34.2)	31.6 (31.2-32.5)	ns
Platelets (10^3/mm^3)	361 (242-406)	395 (327-456)	372.5 (329.3-465)	ns
ESR (mm/h)	66 (62-80)	37 (31-47)	57.5 (48.3-69.5)	ns
CRP (mg/dL)	6.3 (4.1-11.8)	6.3 (4.1-11.8)	6.3 (4.1-11.8)	ns
ALT (iu/L)	47.5 (30.3-86.5)	47.5 (30.3-86.5)	47.5 (30.3-86.5)	ns
GGT (iu/L)	42 (20-89)	42 (20-89)	42 (20-89)	ns

*All data are represented as a median (interquartile range) unless otherwise specified. **Z-hemoglobin is the hemoglobin concentration normalized for age and expressed as standard deviation units. WBC: white blood count. Polys: polymorphonuclear cells. ESR: erythrocyte sedimentation rate. CRP: C-reactive protein. ALT: alanine aminotransferase. GGT: gamma-glutamyl transferase. Ns: not significant. P-values are calculated by Kruskal-Wallis test or Chi-square test.

Supplemental Table 2. Demographic and clinical characteristics of KD subjects used for qRT-PCR and ELISA for SLC8A1 rs13017968

rs13017968	AA (n=8)	AC (n=8)	CC (n=14)	p-value
Age	3.2 (2.1-4.9)	3.1 (1.7-5.5)	1.6 (1-2.3)	ns
Males, n (%)	80 (63)	4 (50)	10 (71)	ns
Ethnicity				
Asian, n (%)	4 (50)	2 (25)	2 (14)	ns
African American, n (%)	0	0	0	ns
Caucasian, n (%)	0	3 (37)	3 (21)	ns
Hispanic, n (%)	4 (50)	2 (25)	5 (37)	ns
Mixed, n (%)	0	1 (13)	4 (28)	ns
Illness day, median (range)*	5 (2-8)	6 (3-11)	5 (2-10)	ns
Coronary artery status				
Aneurysm, n (%)	0	2 (25)	1 (7)	ns
Dilated, n (%)	2 (25)	1 (13)	7 (50)	ns
Normal, n (%)	6 (75)	5 (62)	6 (43)	ns
Z Worst, median (range)*	1.8 (1-3.2)	1.8 (1.2-3.1)	2.6 (0.6-4.1)	ns
Lab data				
WBC (10^3/uL)	14.2 (11-15.6)	11.8 (9.6-14.1)	14 (12-15)	ns
Polys (%)	59 (49.2-64.5)	60 (41.7-68)	52.5 (46-57.5)	ns
Lymphocytes (%)	23.5 (19-26)	19.5 (15.5-29.5)	32 (25-35)	ns
Monocytes (%)	5.5 (2.8-7.3)	8 (4.5-12.3)	5 (2-8.5)	ns
Eosinophils (%)	1.5 (0.7-3.3)	2.5 (1.8-8)	2 (1-3)	ns
Absolute Band Count	1129 (700-1620)	1049 (594-1733)	1024 (384-2088)	ns
Absolute Neutrophil Count	9635 (7418-11438)	7777 (5469-9658)	8632 (6869-9919)	ns
Hemoglobin (mg/dl)	11.1 (10.6-11.8)	11.1 (10.3-11.9)	10.8 (10.2-11.2)	ns
Z-Hemoglobin**	-1.3 (-2.2 - -0.6)	-1.25 (-2.5 - -0.8)	-1.8 (-2.1 - -1.1)	ns
Hematocrit (%)	33 (31-35)	32 (30.4-34.4)	31.5 (31-32)	ns
Platelets (10^3/mm^3)	305 (240-404)	366.5 (297-438)	429.5 (333-477)	ns
ESR (mm/h)	66 (59-81)	36 (29-49)	57.5 (47.5-68.3)	ns
CRP (mg/dL)	6.3 (4.1-11.8)	6.2 (4.1-11.8)	6.2 (4.1-11.8)	ns
ALT (iu/L)	47 (30-86)	47 (30-86.5)	47.5 (30.2-86.5)	ns
GGT (iu/L)	42 (20-89)	42 (20-89)	42 (20-89)	ns

*All data are represented as a median (interquartile range) unless otherwise specified. **Z-hemoglobin is the hemoglobin concentration normalized for age and expressed as standard deviation units. WBC: white blood count. Polys: polymorphonuclear cells. ESR: erythrocyte sedimentation rate. CRP: C-reactive protein. ALT: alanine aminotransferase. GGT: gamma-glutamyl transferase. Ns: not significant. P-values are calculated by Kruskal-Wallis test or Chi-square test.

Supplemental Table 3. Demographic and clinical characteristics of KD subjects used for qRT-PCR and ELISA for SLC8A1 rs12989852

rs12989852	AA (n=8)	AG (n=13)	GG (n=9)	p-value
Age	3.2 (2.1-4.9)	2.4 (1.6-3.7)	1.3 (0.9-2)	ns
Males, n (%)	5 (62)	8 (62)	6 (67)	ns
Ethnicity				
Asian, n (%)	4 (50)	3 (23)	1 (11)	ns
African American, n (%)	0	0	0	ns
Caucasian, n (%)	0	5 (39)	1 (11)	ns
Hispanic, n (%)	4 (50)	3 (23)	4 (44)	ns
Mixed, n (%)	0	2 (15)	3 (33)	ns
Illness day, median (range)*	5 (2-8)	6 (3-11)	6 (2-8)	ns
Coronary artery status				
Aneurysm, n (%)	0	2 (15)	1 (11)	ns
Dilated, n (%)	2 (25)	4 (31)	4 (44)	ns
Normal, n (%)	6 (75)	7 (54)	4 (44)	ns
Z Worst, median (range)*	1.8 (1-3.2)	2.1 (0.5-4.8)	2.6 (1-4.1)	ns
Lab data				
WBC (10^3/uL)	14.2 (11.2-15.6)	12.2 (11-15.5)	14.4 (12.8-16.8)	ns
Polys (%)	59 (49-64.5)	53 (47-64)	56 (40-58)	ns
Lymphocytes (%)	23.5 (19-26)	24 (16-25)	34 (32-35)	ns
Monocytes (%)	5.5 (3-7.3)	9 (5-12)	4 (2-5)	ns
Eosinophils (%)	1.5 (0.7-3.3)	2 (1-4)	2.5 (0.8-3.5)	ns
Absolute Band Count	1129 (700-1620)	1049 (489-1785)	1024 (384-2088)	ns
Absolute Neutrophil Count	9635 (7418-11438)	6943 (6132-9720)	9072 (7616-9971)	ns
Hemoglobin (mg/dl)	11.1 (10.6-11.8)	11 (10.3-11.3)	10.9 (10.2-11.3)	ns
Z-Hemoglobin**	-1.3 (-2.2 - -0.6)	-1.5 (-2.5 - -1)	-1.8 (-2.1 - -0.5)	ns
Hematocrit (%)	33 (31-35.2)	31.6 (30.5-32.3)	31.5 (31.2-33.5)	ns
Platelets (10^3/mm^3)	305 (240-404)	395 (327-485)	406 (339-459)	ns
ESR (mm/h)	66.5 (58.7-80.5)	43 (35-49)	66 (55-71)	ns
CRP (mg/dL)	6.3 (4.1-11.8)	6.3 (4.1-11.8)	6.3 (4.1-11.8)	ns
ALT (iu/L)	47 (30-86.5)	47 (30-86.5)	47.5 (30-86.5)	ns
GGT (iu/L)	42 (20-89)	42 (20-89)	42 (20-89)	ns

*All data are represented as a median (interquartile range) unless otherwise specified. **Z-hemoglobin is the hemoglobin concentration normalized for age and expressed as standard deviation units. WBC: white blood count. Polys: polymorphonuclear cells. ESR: erythrocyte sedimentation rate. CRP: C-reactive protein. ALT: alanine aminotransferase. GGT: gamma-glutamyl transferase. Ns: not significant. P-values are calculated by Kruskal-Wallis test or Chi-square test.

Supplemental Table 4. Allele frequencies of selected SLC8A1 SNPs of KD subjects and subjects from 1000 Genomes database

	European		Asian		Hispanic	
SLC8A1 Genotype	1000 Genomes	KD (n=5)	1000 Genomes	KD (n=8)	1000 Genomes	KD (n=11)
rs10490051						
G allele frequency, %	29	50	63	63	35	50
A allele frequency, %	71	50	37	37	65	50
rs13017968						
A allele frequency, %	28	30	62	63	32	50
C allele frequency, %	72	70	38	37	68	50
rs12989852						
A allele frequency, %	40	40	68	69	45	50
G allele frequency, %	60	60	32	31	55	50

Letters in bold indicate risk alleles. Population genetics data for European, East Asian, and American populations are extracted from 1000 Genomes database. Europeans include Utah residents with Northern and Western European ancestry, Finnish in Finland, British in England and Scotland, Iberian populations in Spain, and Toscani in Italy. Asians include Chinese Dai in Xishuangbanna, China, Han Chinese in Beijing, China, Southern Han Chinese, Japanese in Tokyo, Japan, and Kinh in Ho Chi Minh City, Vietnam. Hispanics include Colombian in Medellin, Colombia, Mexican Ancestry in Los Angeles, California, Peruvian in Lima, Peru, and Puerto Rican in Puerto Rico.

Supplemental Table 5. Demographic and clinical characteristics of KD subjects in microarray dataset for SLC8A1 rs10490051

rs10490051	AA (n=25)	AC (n=58)	CC (n=47)	p-value
Age	2.7 (1.4-4.1)	2.7 (1.6-4)	3.1 (1.3-4.3)	ns
Males, n (%)	80 (61)	35 (60)	29 (62)	ns
Ethnicity				
Asian, n (%)	10 (40)	10 (17)	1 (2)	0.0001
African American, n (%)	0	2 (4)	3 (6)	ns
Caucasian, n (%)	4 (16)	17 (29)	15 (32)	ns
Hispanic, n (%)	4 (16)	13 (22)	15 (32)	ns
Mixed, n (%)	7 (28)	16 (28)	13 (28)	ns
Illness day, median (range)*	6 (2-11)	6.5 (2-10)	6 (3-10)	ns
Coronary artery status				
Aneurysm, n (%)	12 (9)	5 (9)	2 (4)	ns
Dilated, n (%)	29 (22)	11 (19)	11 (24)	ns
Normal, n (%)	90 (69)	42 (72)	34 (72)	ns
Z Worst, median (range)*	1.8 (0.2-18.3)	1.8 (0.2-18.3)	1.5 (0.4-9.3)	ns
Lab data				
WBC (10^3/uL)	13.6 (11-19)	13.1 (11.7-18.8)	13.6 (10.6-18.8)	ns
Polys (%)	54 (42-63)	54.5 (47-62)	55.2 (40.5-63.3)	ns
Lymphocytes (%)	21 (13-31)	22 (16-30.3)	19 (11.5-27)	ns
Monocytes (%)	6 (4-8)	6 (4-8.2)	5 (4-7)	ns
Eosinophils (%)	2 (0-3)	2 (1-4)	1 (0-3)	ns
Absolute Band Count	1771 (456-2820)	1355 (377-2661)	1820 (1050-2912)	ns
Absolute Neutrophil Count	9027 (6780-12390)	8307 (7251-11275)	9416 (6780-12676)	ns
Hemoglobin (mg/dl)	11 (10.4-12)	11.3 (10.8-11.6)	11.3 (10.5-12)	ns
Z-Hemoglobin**	-1.1 (-2.3 - -0.4)	-1.1 (-1.8 - -0.5)	-1.2 (-2 - -0.2)	ns
Hematocrit (%)	32.2 (29.6-34.3)	32.9 (31.5-34.1)	32.7 (31.1-35.2)	ns
Platelets (10^3/mm^3)	408 (352-527)	426 (343.8-486.5)	335 (247.5-465.5)	ns
ESR (mm/h)	79 (53-100)	59 (39-80.5)	58 (42-78)	ns
CRP (mg/dL)	12.2 (4.6-19.2)	6.8 (4.9-12.1)	8.3 (5.1-18.6)	ns
ALT (iu/L)	31 (16-55)	27 (17.3-72.5)	44 (20.5-140.3)	ns
GGT (iu/L)	28 (23-130)	28.5 (17-78)	38 (16-98)	ns

*All data are represented as a median (interquartile range) unless otherwise specified. **Z-hemoglobin is the hemoglobin concentration normalized for age and expressed as standard deviation units. WBC: white blood count. Polys: polymorphonuclear cells. ESR: erythrocyte sedimentation rate. CRP: C-reactive protein. ALT: alanine aminotransferase. GGT: gamma-glutamyl transferase. Ns: not significant. P-values are calculated by Kruskal-Wallis test or Chi-square test.

Supplemental Table 6. Demographic and clinical characteristics of KD subjects in microarray dataset for SLC8A1 rs13017968

rs13017968	AA (n=21)	AC (n=60)	CC (n=50)	p-value
Age	1.6 (0.4-15)	2.6 (1.6-3.8)	3.4 (1.4-4.8)	ns
Males, n (%)	12 (57)	38 (63)	30 (60)	ns
Ethnicity				
Asian, n (%)	9 (43)	12 (20)	1 (2)	<0.0001
African American, n (%)	0	0	5 (10)	ns
Caucasian, n (%)	3 (14)	17 (28)	16 (32)	ns
Hispanic, n (%)	4 (19)	13 (22)	15 (30)	ns
Mixed, n (%)	5 (24)	18 (30)	13 (26)	ns
Illness day, median (range)*	5 (3-11)	6 (2-10)	6 (3-10)	ns
Coronary artery status				
Aneurysm, n (%)	4 (19)	5 (8)	3 (6)	ns
Dilated, n (%)	7 (33)	10 (17)	12 (24)	ns
Normal, n (%)	10 (48)	45 (75)	35 (70)	ns
Z Worst, median (range)*	2.6 (0.4-7.9)	1.8 (0.2-18.3)	1.6 (0.2-9.3)	ns
Lab data				
WBC (10^3/uL)	14 (12.5-18.7)	13.3 (11.7-18.7)	13.6 (10.6-19.2)	ns
Polys (%)	54 (44-67)	53 (44-62)	56 (41-63.8)	ns
Lymphocytes (%)	22 (13-33)	24 (16-31)	18.5 (11-26.3)	ns
Monocytes (%)	5.8 (4-8)	6 (4-8.1)	5 (4-7)	ns
Eosinophils (%)	1 (0-3)	2 (1-4)	1 (0-3)	ns
Absolute Band Count	2250 (1024-3168)	1123 (264-2540)	1836 (1045-3139)	ns
Absolute Neutrophil Count	11248 (7544-12272)	8307 (6646-11272)	9420 (6918-12992)	ns
Hemoglobin (mg/dl)	11.1 (10.4-12.2)	11.3 (10.7-11.6)	11.3 (10.5-12)	ns
Z-Hemoglobin**	-0.9 (-1.5 - -0.1)	-1.2 (-1.9 - -0.5)	-1.3 (-2 – -0.3)	ns
Hematocrit (%)	32.8 (31.1-35)	32.8 (31.2-34)	32.9 (31-35)	ns
Platelets (10^3/mm^3)	448 (306-578)	425 (347.3-483.3)	357.5 (254-466.5)	ns
ESR (mm/h)	79 (53-90)	58 (37-86.3)	59.5 (44-77.8)	ns
CRP (mg/dL)	12.2 (4.7-19.2)	6.6 (4.1-12.3)	8.3 (5.2-18.9)	ns
ALT (iu/L)	29 (16-55)	30.5 (18-74)	46 (20.3-139.5)	ns
GGT (iu/L)	26 (18-140)	29 (17-75)	41.5 (17-104)	ns

*All data are represented as a median (interquartile range) unless otherwise specified. **Z-hemoglobin is the hemoglobin concentration normalized for age and expressed as standard deviation units. WBC: white blood count. Polys: polymorphonuclear cells. ESR: erythrocyte sedimentation rate. CRP: C-reactive protein. ALT: alanine aminotransferase. GGT: gamma-glutamyl transferase. Ns: not significant. P-values are calculated by Kruskal-Wallis test or Chi-square test.

Supplemental Table 7. Demographic and clinical characteristics of KD subjects in microarray dataset for SLC8A1 rs12989852

rs12989852	AA (n=32)	AG (n=67)	GG (n=31)	p-value
Age	1.6 (0.7-4)	2.8 (1.6-4)	3.9 (1.9-5)	ns
Males, n (%)	17 (53)	42 (63)	20 (65)	ns
Ethnicity				
Asian, n (%)	10 (31)	10 (15)	1 (3)	0.01
African American, n (%)	2 (6)	3 (5)	0	ns
Caucasian, n (%)	5 (16)	18 (27)	13 (42)	ns
Hispanic, n (%)	7 (22)	17 (25)	8 (26)	ns
Mixed, n (%)	8 (25)	19 (28)	9 (29)	ns
Illness day, median (range)*	6 (2-11)	6 (2-10)	6 (3-10)	ns
Coronary artery status				
Aneurysm, n (%)	4 (13)	5 (7)	2 (7)	ns
Dilated, n (%)	9 (28)	14 (21)	6 (19)	ns
Normal, n (%)	19 (59)	48 (72)	23 (74)	ns
Z Worst, median (range)*	2 (0.4-7.9)	1.7 (0.2-18.3)	1.8 (0.4-9.3)	ns
Lab data				
WBC (10^3/uL)	15.3 (12.7-21.1)	12.7 (10.6-18.4)	13.9 (10.9-19)	ns
Polys (%)	54 (43.5-66.3)	53 (45.5-61.5)	56 (38-65.5)	ns
Lymphocytes (%)	24.5 (14.8-33)	21.2 (13.5-30.7)	17 (10.5-23.5)	ns
Monocytes (%)	5.9 (4-8)	6 (4-8)	5 (4-7)	ns
Eosinophils (%)	1 (0-2)	3 (1-4)	1 (0-2.5)	ns
Absolute Band Count	1980 (801-3003)	1638 (414-2554)	1853 (1190-3688)	ns
Absolute Neutrophil Count	11006 (7323-15679)	8349 (6469-11344)	9424 (7514-12954)	ns
Hemoglobin (mg/dl)	11.3 (10.5-11.9)	11.1 (10.4-11.8)	11.3 (10.9-11.8)	ns
Z-Hemoglobin**	-1 (-1.7 - -0.2)	-1.5 (-1.9 - -0.4)	-1.3 (-1.9 - -0.6)	ns
Hematocrit (%)	33 (31.2-34.2)	32.7 (30.6-35)	32.7 (31.6-34.4)	ns
Platelets (10^3/mm^3)	416 (362.5-539.8)	420 (325.5-471.5)	369 (255-466)	ns
ESR (mm/h)	69.5 (48-90)	60 (38.5-83)	58 (44-78)	ns
CRP (mg/dL)	10.7 (4.6-16.2)	6.8 (4.7-13.8)	11.8 (5.3-19.7)	ns
ALT (iu/L)	39.5 (17.8-137.5)	25 (17-75)	48 (17.4-149.5)	ns
GGT (iu/L)	38 (21.8-125.5)	27 (17-79)	38 (16-134)	ns

*All data are represented as a median (interquartile range) unless otherwise specified. **Z-hemoglobin is the hemoglobin concentration normalized for age and expressed as standard deviation units. WBC: white blood count. Polys: polymorphonuclear cells. ESR: erythrocyte sedimentation rate. CRP: C-reactive protein. ALT: alanine aminotransferase. GGT: gamma-glutamyl transferase. Ns: not significant. P-values are calculated by Kruskal-Wallis test or Chi-square test.

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