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# How specific is the Kawasaki disease blood transcriptome? A four- comparator reanalysis of 437 candidate genes

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## Abstract

**Background.** Blood transcriptional signatures for Kawasaki disease (KD) are usually derived and evaluated against healthy children, although the clinical task is to separate KD from viral and bacterial febrile illnesses. How much of a KD-associated transcriptome remains disease-specific against these mimics is unknown.

**Methods.** We reanalysed the whole-blood discovery cohort GSE73461 (KD 77, viral 94, bacterial 52, healthy 55, undetermined fever 96). A previously locked nine-gene score and a 437-gene candidate pool were carried forward unchanged. Four contrasts were computed (KD versus healthy, viral, bacterial and adenovirus), and each gene was classified by an attenuation index, 1 − log2FC(KD versus mimic)/log2FC(KD versus healthy).

**Results.** The score separated KD from healthy children almost perfectly (AUC 0.989, 95% CI 0.975–0.998), less well from viral illness (0.827, 0.765–0.883) and not from bacterial illness (0.616, 0.514–0.719). Of 353 genes up-regulated versus healthy children, 219 (62.0%) were equally up-regulated in both mimics and 8 (2.3%) retained KD-preference across all comparators; among 51 up-regulated secreted ligands, 26 (51.0%) were shared and 3 (5.9%) KD-preferential. Gene sets selected against different comparators overlapped by 1–24%. Interferon silence separated KD from viral illness (AUC 0.13); no module separated KD from bacterial illness.

**Conclusions.** In this cohort the KD blood transcriptome is largely a generic febrile response, and apparent specificity is a property of the comparator, not the disease. KD-specific biomarker claims, and endothelial ligand axes built on them, should report attenuation against viral and bacterial mimics.


## What is known

- Whole-blood transcriptional signatures distinguish children with Kawasaki disease from healthy children with high accuracy, and several have been proposed as diagnostic tests.
- Kawasaki disease is clinically confounded mainly by viral and bacterial febrile illnesses, not by health.
- Published signatures differ widely in their reported accuracy, and the reasons for this heterogeneity are incompletely understood.

#### What is new

- The same nine-gene score reached an AUC of 0.99 against healthy children, 0.83 against viral illness and 0.62 against bacterial illness; the confidence interval against bacterial illness included 0.5.
- Half of the KD-associated transcripts, and half of the secreted ligand genes, were equally induced in viral and bacterial mimics; only 8 of 437 genes (1.8%) retained KD-preference against all four comparators.
- Gene sets selected against different comparators shared as little as 1% of their members, so “KD-specific” describes a comparison rather than the disease.

## Introduction

Kawasaki disease (KD) is an acute self-limited vasculitis of childhood and the leading cause of acquired heart disease in children in high-income countries [1, 2]. Its diagnosis remains clinical, rests on a constellation of non-specific features, and is easily confused with common viral and bacterial febrile illnesses [3, 7]. Because timely intravenous immunoglobulin reduces the risk of coronary artery abnormalities, a laboratory test that shortened the diagnostic pathway would be valuable, and whole-blood gene expression has been pursued for this purpose for more than fifteen years [3–5].

Two features of that literature are relevant here. First, most studies derive candidate genes from a comparison with healthy children. This contrast is statistically efficient and produces large fold changes, but it answers a question that is not the clinical one: a febrile child without KD is not a healthy child [9]. Second, when the same signature is later applied to a

different febrile population, reported accuracy falls, and the fall is usually attributed to platform, age or ethnicity rather than to the choice of comparator [5, 10].

The distinction matters because “specificity” has two meanings that are easily conflated. A transcript can be specific to KD in the sense that it is not induced by other febrile illnesses, or it can be non-specific in that sense while still being useful for separating KD from health. Only the first kind can support a mechanistic claim, such as the existence of a KD-specific ligand that activates the endothelium. The second kind supports a claim about inflammation in general.

We therefore asked a deliberately narrow question of an existing, well-characterised cohort that contains, on one platform and in one study, children with KD, definite viral illness, definite bacterial illness and health [3]. If a KD-associated transcriptome is compared with each of these groups in turn, how much of it survives, and does the answer depend on which group is used as the comparator? We carried forward a previously locked nine-gene score and a 437-gene candidate pool without re-derivation, computed the four contrasts, and classified every gene by a continuous attenuation index.

## Methods

# Data source and participants

We used the discovery dataset of GSE73461 (Illumina HumanHT-12 v4; whole blood; children under 17 years) [3]. Of 459 deposited samples, one array (GSM1894671) contained no values and was excluded, leaving 458: KD 77, definite viral illness 94, definite bacterial illness 52, healthy controls 55, febrile illness of uncertain viral or bacterial origin 96, juvenile idiopathic arthritis 66 and Henoch–Schönlein purpura 18. The four groups used for the primary contrasts were KD, viral, bacterial and healthy (n = 278); the uncertain group was retained as a clinically realistic “fever of unknown source” comparator, and the two inflammatory disease groups were used only to describe the score’s behaviour outside febrile infection.

Group labels were taken from the deposited sample characteristics. The viral and bacterial groups are aetiologically heterogeneous and are described by the depositors as “definite viral” and “definite bacterial”; the dataset does not resolve individual pathogens. Sex was available for all samples; age was not deposited at sample level and is not analysed.

## Candidate genes and score, carried forward unchanged

The 437-gene candidate pool and the nine-gene score (ADM, ACSL1, PLBD1, UPP1, KCNJ15, LIMK2, TRIM25, LTB4R, SLC2A14) were defined in an earlier analysis of this cohort by weighted gene co-expression network analysis, followed by penalised regression and random forest selection, and were locked before the present analyses [21]. No gene was added or removed, and the score coefficients and scaling parameters were used as locked. The purpose of carrying them forward unchanged is that every result below is a property of a fixed object evaluated against different comparators, not of a model re-fitted to flatter each comparison.

## Contrasts and the attenuation index

We used the moderated t-statistics already computed for this cohort (limma) for three contrasts: KD versus healthy, KD versus viral and KD versus bacterial [12]. For the fourth contrast, KD versus adenovirus, we used a published whole-blood cohort containing children with KD and adenovirus infection (GSE68004; 19 samples in the affected groups), analysed with Welch’s t-test on log2 expression with batch adjustment, as described previously [4].

## For every gene we defined the attenuation index against a mimic M as

# A(M) = 1 − log2FC(KD versus M) / log2FC(KD versus healthy)

A(M) = 0 means the gene is induced in the mimic exactly as in KD (fully shared); A(M) = 1 means the gene is not induced at all in the mimic (KD-preferential); values above 1 indicate that the gene is induced more strongly in the mimic than in KD. The index is undefined for genes not induced versus healthy children and was not computed for them.

# Classification of specificity

Genes were classified with a rule fixed before the analysis. A gene was “mimic-shared” if it was up-regulated versus healthy children ($q < 0.05$) and remained significantly up-regulated versus viral or bacterial illness with less than 50% attenuation. It was “KD-preferential” if it was up-regulated versus healthy children, not significantly different from either mimic ($q \geq 0.05$), attenuated by at least 50% against both, and — where the gene was represented on the adenovirus platform — also not significantly different from adenovirus. Genes meeting the attenuation criteria against the two in-cohort mimics but differing from adenovirus were labelled “KD versus mimics inconsistent”; genes that did not reach significance versus healthy children were recorded separately. The class assigned to every gene, with its direction and every contrast value, is listed gene by gene in Table S1. A threshold sensitivity analysis (attenuation 0.3–0.7; q 0.05 and 0.10) was prespecified.

## Secreted ligands and modules

Secreted ligands were annotated manually from UniProt subcellular localisation and standard nomenclature: cytokines, chemokines, growth factors, alarmins, antimicrobial peptides, complement components and secreted proteases. Surface receptors were annotated separately and were not counted as ligands. This annotation is a curated list, not an unbiased secretome, and is provided in full in the supplement.

Five modules were scored as the mean z-score of their member genes across samples: neutrophil granule (16 genes including DEFA1, DEFA3, DEFA4, CAMP, BPI, ELANE, MPO, PRTN3, CTSG, LCN2, MMP8, MMP9), type I interferon (12 genes including IFI27, IFI44L, RSAD2, MX1, OAS1, ISG15, IFIT1, IFIT3, SIGLEC1, USP18), monocyte/myeloid (8 genes including CD14, FCN1, VCAN, FCGR1A, C1QB, CD163), platelet (6 genes including PPBP, PF4, ITGA2B, GP9, TUBB1, SELP) and adaptive/T cell (7 genes including CD3D, CD3E, CD2, CD8A, IL7R, CCR7, LTB). The definition and complete membership of each module are given in Table S2.

## Comparator-dependent selection experiment

To test whether the choice of comparator affects gene discovery and not only gene evaluation, we selected the top 100 up-regulated genes ($q < 0.05$, positive log2FC) against each of the three in-cohort comparators, then measured the attenuation of each selected set against the other two comparators and against adenovirus. Overlap between the three sets was counted directly.

# Statistics

Group comparisons used Mann–Whitney U tests and Kruskal–Wallis tests. Discrimination was summarised by the area under the receiver operating characteristic curve (AUC) with 95% confidence intervals from 2,000 bootstrap resamples stratified by group. Confidence intervals for fold changes were derived from the moderated t-statistic (SE = log2FC/t; 95% CI = log2FC ± 1.96 SE). Multiple testing used the Benjamini–Hochberg procedure [13]. Analyses were performed in Python 3.13 (NumPy, pandas, scikit-learn, SciPy) and R 4.4 (limma) [12]. Reporting follows TRIPOD + AI where applicable [11]; the study is a secondary analysis of public data and no prediction model was developed.

# Ethics

This study used only de-identified, publicly available data deposited by the original investigators, who obtained ethics approval and consent in their own jurisdictions. No human participants were recruited and no identifiable information was accessed. Under the national regulation governing ethics review of life-science and medical research involving humans,

secondary analysis of legally obtained, de-identified public data is exempt from ethics review; a formal exemption was obtained from our institutional committee.

## Results

# The cohort covers the clinical differential diagnosis

The 458 children comprised 77 with KD, 94 with definite viral illness, 52 with definite bacterial illness, 55 healthy controls and 96 with febrile illness of uncertain origin (Table 1). Male proportion was 54.5% in KD, 70.2% in viral illness, 42.3% in bacterial illness and 52.7% in controls. The distribution of the nine-gene score differed across groups (Kruskal–Wallis $P < 0.001$) and was highest in KD (median 0.95, IQR 0.50–1.26), followed by bacterial illness (0.83, − 0.04 to 1.13).

Table 1
Characteristics of the discovery cohort and discrimination of the nine-gene score by comparator (GSE73461, n = 458).

| Group | n | Male, n (%) | Score, median (IQR) | AUC vs KD (95% CI) |
|---|---|---|---|---|
| Kawasaki disease | 77 | 42 (54.5) | 0.95 (0.50 to 1.26) | — |
| Definite viral illness | 94 | 66 (70.2) | 0.04 (− 0.74 to 0.57) | 0.827 (0.765–0.883) |
| Definite bacterial illness | 52 | 22 (42.3) | 0.83 (− 0.04 to 1.13) | 0.616 (0.514–0.719) |
| Healthy controls | 55 | 29 (52.7) | −1.04 (− 1.35 to − 0.67) | 0.989 (0.975–0.998) |
| Febrile illness, uncertain origin | 96 | 62 (64.6) | 0.23 (− 0.39 to 0.66) | 0.784 (0.715–0.848) |
| Juvenile idiopathic arthritis | 66 | 25 (37.9) | −0.62 (− 0.83 to − 0.33) | 0.975 (0.953–0.992) |
| Henoch–Schönlein purpura | 18 | 9 (50.0) | −0.01 (− 0.28 to 0.29) | 0.850 (0.752–0.934) |

AUC, area under the receiver operating characteristic curve; CI, confidence interval; IQR, interquartile range. Confidence intervals from 2,000 bootstrap resamples.

# Apparent accuracy is a function of the comparator

Against healthy children the score performed almost perfectly: AUC 0.989 (95% CI 0.975–0.998). Against viral illness it fell to 0.827 (0.765–0.883), against febrile illness of uncertain origin to 0.784 (0.715–0.848), and against bacterial illness to 0.616 (0.514–0.719), a confidence interval that includes 0.5 (Fig. 1, Table 1). Against the two non-infectious inflammatory groups the score behaved like a general inflammation meter: AUC 0.975 (0.953–0.992) versus juvenile idiopathic arthritis and 0.850 (0.752–0.934) versus Henoch–Schönlein purpura.

The same pattern appeared at module level (Table 2, Fig. 3). The neutrophil granule module separated KD from healthy children (AUC 0.922) but not from bacterial illness (0.276) — that is, the module was higher in bacterial illness than in KD. The monocyte/myeloid module behaved similarly (0.955 versus healthy, 0.480 versus bacterial). The only module that separated KD from viral illness was type I interferon, and it did so in the opposite direction from what a “KD activation” reading would suggest (AUC 0.127; median module score − 0.62 in KD versus 0.97 in viral illness). No module separated KD from bacterial illness; the best was platelet (AUC 0.696).

Table 2
Module-level discrimination of Kawasaki disease by comparator (AUC).

| Module | vs healthy | vs viral | vs bacterial | vs uncertain origin |
|---|---|---|---|---|
| Neutrophil granule | 0.922 | 0.606 | 0.276 | 0.399 |
| Type I interferon | 0.398 | 0.127 | 0.652 | 0.295 |
| Monocyte / myeloid | 0.955 | 0.598 | 0.480 | 0.552 |
| Platelet | 0.858 | 0.723 | 0.696 | 0.540 |
| Adaptive / T cell | 0.056 | 0.290 | 0.535 | 0.399 |
| AUC values below 0.5 indicate that the module score is higher in the comparator group than in Kawasaki disease. | | | | |

# Half of the candidate pool is shared with mimics

Of the 437 candidate genes, 353 were up-regulated versus healthy children with $q < 0.05$ and a positive fold change. Of these, 219 (62.0%) were also up-regulated against both viral and bacterial illness with less than 50% attenuation and were classified as mimic-shared; 123 (34.8%) were intermediate; and 8 (2.3%) met the KD-preferential criteria against both in-cohort mimics and, where testable, against adenovirus (Table 3, Fig. 2a). Three further genes met the attenuation criteria against viral and bacterial illness but differed significantly from adenovirus. The remaining 84 genes either did not reach significance against healthy children or were not represented on the platform. Counts for every combination of attenuation and q value threshold are given in Table S3.

Table 3
Classification of the 437 candidate genes by specificity against viral and bacterial mimics.

| Class | Definition | n | % of 437 | % of 353 up-regulated |
|---|---|---|---|---|
| Mimic-shared | Up vs healthy ($q < 0.05$) and up vs viral or bacterial with < 50% attenuation | 219 | 50.1 | 62.0 |
| Intermediate | Up vs healthy, attenuated but not meeting either extreme | 123 | 28.1 | 34.8 |
| KD-preferential | Up vs healthy; not different from viral and bacterial ($q \geq 0.05$); ≥ 50% attenuation against both; and, where testable, not different from adenovirus | 8 | 1.8 | 2.3 |
| KD versus mimics inconsistent | Met attenuation criteria against viral and bacterial illness but differed from adenovirus | 3 | 0.7 | 0.8 |
| Not up-regulated or not represented | Did not reach significance versus healthy children, or not on the platform | 84 | 19.2 | — |

The nine-gene score itself illustrates the point: seven of its nine members (ACSL1, PLBD1, UPP1, KCNJ15, LIMK2, TRIM25, SLC2A14) were mimic-shared, and the remaining two (ADM, LTB4R) were intermediate (attenuation 0.52 and 0.59 against viral illness). No member of the score was KD-preferential.

# Secreted ligands are shared almost without exception

Fifty-five of the 437 candidates encode secreted proteins; 51 of these were up-regulated versus healthy children. Twenty-six (51.0%) were mimic-shared, 20 (39.2%) intermediate and 3 (5.9%) KD-preferential (Fig. 2b). The shared group includes the ligands most often proposed as mediators of KD vasculopathy: interleukin-1β (IL1B), the alarmins S100A8, S100A9 and S100A12, resistin (RETN), nicotinamide phosphoribosyltransferase (NAMPT), matrix metalloproteinase-9 (MMP9), oncostatin M (OSM), CXCL16 and TNFSF13B. For these genes the KD-versus-healthy difference was reproduced, in whole or in large part, by viral and bacterial illness.

The three KD-preferential ligands were the α-defensin DEFA1, the cathelicidin precursor CAMP and complement C1q C chain (C1QC); their fold changes and attenuation values are given in Table 4 and Fig. 4. Two of the three (DEFA1, CAMP) are neutrophil granule peptides. DEFA1 was in fact higher in bacterial illness than in KD (log2FC − 0.84 versus bacterial), so its KD-preference is relative to viral illness rather than absolute.

Table 4
The eleven genes retaining KD-preference against both in-cohort mimics.

| Gene | log2FC vs healthy | q | log2FC vs viral | q | log2FC vs bacterial | q | log2FC vs adenovirus | q | A(viral) | A(bacterial) |
|---|---|---|---|---|---|---|---|---|---|---|
| *TNFAIP6* | 1.86 | < 0.001 | 0.29 | 0.12 | 0.24 | 0.46 | 0.60 | 0.015 | 0.84 | 0.87 |
| *DEFA1* | 1.67 | < 0.001 | 0.04 | 0.94 | −0.84 | 0.062 | 0.64 | 0.15 | 0.98 | 1.50 |
| *CAMP* | 1.66 | < 0.001 | 0.27 | 0.30 | 0.15 | 0.78 | 0.60 | 0.083 | 0.84 | 0.91 |
| *RAP1GAP* | 1.47 | < 0.001 | 0.57 | 0.096 | 0.40 | 0.43 | NA | NA | 0.61 | 0.72 |
| *IL1RN* | 1.47 | < 0.001 | 0.27 | 0.14 | 0.27 | 0.33 | 0.87 | < 0.001 | 0.82 | 0.82 |
| *ASGR2* | 1.17 | < 0.001 | 0.20 | 0.16 | 0.06 | 0.88 | 0.09 | 0.69 | 0.83 | 0.95 |
| *IGF2BP3* | 1.14 | < 0.001 | −0.06 | 0.72 | 0.11 | 0.74 | 0.40 | 0.025 | 1.05 | 0.91 |
| *GPER1* | 1.13 | < 0.001 | 0.28 | 0.065 | 0.004 | 0.99 | 0.17 | 0.14 | 0.75 | 1.00 |
| *HIST1H4H* | 1.07 | < 0.001 | −0.02 | 0.93 | 0.24 | 0.15 | 0.14 | 0.61 | 1.02 | 0.77 |
| *ABCA1* | 1.04 | < 0.001 | 0.26 | 0.095 | −0.14 | 0.62 | 0.25 | 0.22 | 0.75 | 1.14 |
| *C1QC* | 1.01 | < 0.001 | −0.05 | 0.84 | −0.37 | 0.19 | NA | NA | 1.05 | 1.37 |

A, attenuation index; NA, not represented on the adenovirus platform. Genes differing significantly from adenovirus ($q < 0.05$) are the three that do not satisfy the full four-comparator criterion.

# Gene sets selected against different comparators barely overlap

Selecting the top 100 up-regulated genes against healthy children, against viral illness and against bacterial illness produced three sets that shared almost nothing: 24 genes in common between the healthy- and viral-selected sets, 1 between the healthy- and bacterial-selected sets, and 1 between the viral- and bacterial-selected sets (Fig. 2c; Table S4). The healthy-selected genes were, on median, 45% attenuated against viral illness and 87% attenuated against bacterial illness. The bacterial-selected genes were 42% attenuated against viral illness, and the viral-selected genes were 77% attenuated against bacterial illness.

In other words, the transcriptome that distinguishes KD from one mimic is largely not the transcriptome that distinguishes it from another, and a signature derived against healthy children is composed mainly of genes that both mimics also induce.

# The residue that survives is small, and partly already explained

Eleven genes met the attenuation criteria against both in-cohort mimics (Table 4); of these, three differed significantly from adenovirus (TNFAIP6, IL1RN, IGF2BP3), two were not represented on the adenovirus platform (RAP1GAP, C1QC), and six were consistent across all four comparators (DEFA1, CAMP, ASGR2, GPER1, HIST1H4H, ABCA1). Strictly, therefore, 8 of 437 genes (1.8%) are KD-preferential in the sense defined here.

This residue is not a cytokine programme. It contains two antimicrobial peptides (DEFA1, CAMP), a complement subunit (C1QC), a scavenger receptor (ASGR2), an ATP-binding cassette transporter (ABCA1), a histone variant (HIST1H4H), a GTPase-activating protein (RAP1GAP) and an oestrogen-related G protein-coupled receptor (GPER1). The cathelicidin product of CAMP, LL-37, has already been shown to activate human coronary artery endothelial cells through TLR4–NF-κB–NLRP3 in KD [6], so the one member of this residue with a documented endothelial action is not a new axis.

## Discussion

# Principal findings

In a cohort that places KD beside its two main clinical mimics on a single platform, the KD blood transcriptome was found to be largely a shared febrile response. A score that separated KD from healthy children with an AUC of 0.99 separated it from bacterial illness with an AUC of 0.62. Half of the candidate genes and half of the secreted ligands were induced equally in viral and bacterial illness, and only 8 of 437 genes retained KD-preference across all four comparators. Sets of genes selected against different comparators were almost disjoint.

# Relation to previous work

These results do not contradict the high accuracy reported for KD signatures; they locate it. Wright and colleagues derived a 13-transcript signature against febrile controls rather than healthy children and reported an AUC of about 0.96 against febrile illness [3]. Herberg and colleagues, testing five signatures in parallel on a single NanoString platform, reported an AUC of 0.90 for KD versus other diseases and noted that applying signatures outside their designed context reduced performance [5]. Both observations are consistent with the geometry described here: a signature derived against the comparator it will face can perform well, whereas a signature derived against health is composed mostly of shared inflammation genes and cannot.

The interferon finding reproduces an observation made with a different platform and a smaller sample: Popper and colleagues reported a striking absence of interferon-stimulated gene expression in KD relative to adenovirus infection [4]. Our module analysis generalises this to a larger, mixed viral group and quantifies it (AUC 0.127), but also shows its limitation: interferon silence separates KD from viral illness, not from bacterial illness, and it is a negative marker rather than a KD-specific pathway.

# Implications for endothelial ligand hypotheses

A recurring proposal in KD bioinformatics is that a circulating ligand identified in whole blood activates the endothelium and drives coronary injury. The present analysis bears directly on how such claims should be built. Of the ligands most often nominated — IL1B, S100A8/A9/A12, RETN, NAMPT, MMP9, OSM — none was KD-preferential in this cohort; all were induced in viral or bacterial illness to a similar degree. A study that establishes endothelial activation by such a ligand is therefore establishing a mechanism of febrile inflammation, not a mechanism of KD. The only KD-preferential secreted ligands in the residue were the defensin/cathelicidin peptides, and the endothelial action of LL-37 in KD has already been demonstrated in vitro [6].

This does not make endothelial work less relevant; it changes what it can claim. A ligand axis can reasonably be presented as “a candidate amplifier of endothelial injury in KD” only if the ligand is shown to be elevated in KD relative to febrile mimics

at the protein level, and if the endothelial response is demonstrated with KD-relevant concentrations. Neither requirement is met by a transcriptome-only comparison with healthy children.

# Implications for reporting

Three practical consequences follow. First, the comparator should be stated as part of the result, not as a methodological detail: an AUC without its comparator is uninterpretable, because the same score ranges from 0.99 to 0.62 across the differential diagnosis. Second, attenuation against at least one viral and one bacterial comparator should be reported alongside fold change versus healthy children; the index used here is simple to compute from any two contrasts and requires no additional data. Third, gene-set overlap between comparator-specific selections should be reported when a signature is described as disease-specific, because an overlap of 1% indicates that the “specificity” belongs to the comparison.

# Limitations

Several limitations should be weighed. The analysis is confined to one discovery cohort and one array platform; although the four groups were collected together, which removes batch confounding between comparisons, it also means that the absolute attenuation values are cohort-specific. The viral and bacterial groups are aetiologically heterogeneous and the dataset does not identify individual pathogens, so attenuation against a specific virus (for example adenovirus) could differ; the adenovirus cohort we used for the fourth contrast is small (19 samples in the affected groups) and confidence intervals are correspondingly wide.

Absence of significance is not evidence of absence. We mitigated this by using a continuous attenuation index rather than a significance dichotomy, by requiring 50% attenuation as well as $q \geq 0.05$, and by showing that the classification is stable across attenuation thresholds from 0.3 to 0.7 (11 genes at $q \geq 0.05$, 7 at $q \geq 0.10$). Nevertheless, the count of KD-preferential genes should be read as an upper bound on a small number, not as a precise estimate.

Whole blood is a mixture, and the modules we scored are sensitive to leukocyte composition. The finding that the neutrophil module is higher in bacterial illness than in KD may reflect differential counts as much as differential expression per cell. This is a substantive limitation for mechanistic interpretation, but not for the clinical argument: a signature that cannot separate KD from bacterial illness is not rescued by knowing why. Finally, no protein measurements, functional assays or outcome data (coronary artery abnormalities, IVIG responsiveness) were available, so the study cannot address whether any of the surviving genes predicts treatment response.

## Conclusion

In a cohort that contains KD and its two main mimics, the KD blood transcriptome was found to be mostly a shared febrile response, and the apparent specificity of a KD signature was determined by the comparator chosen rather than by the disease. Reports of KD-specific transcripts, and of endothelial ligand axes built upon them, should be qualified by attenuation against viral and bacterial febrile illness.

## Declarations

**Ethics approval.**

Secondary analysis of de-identified public data; exempt from ethics review under the national regulation on ethics review of life-science and medical research involving humans (Article 32). A formal exemption was obtained from the institutional ethics committee.

**Consent for publication.**

Not applicable.

# Competing interests.

The authors declare no competing interests.

# Funding.

This study received no specific funding.

# Author Contribution

ML and JH conceived and designed the study, assembled the cohorts and wrote the first draft. JH performed the differential expression, feature selection and cross-validated modeling. CZ FH and WC contributed to the interpretation of the clinical contrasts and critically revised the manuscript. ZZ supervised the work, verified the analysis pipeline and revised the manuscript. ML and JH contributed equally. All authors read and approved the final manuscript.

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# Data Availability

We thank the investigators who deposited GSE73461, GSE73463, GSE68004 and GSE63881.

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## Figures

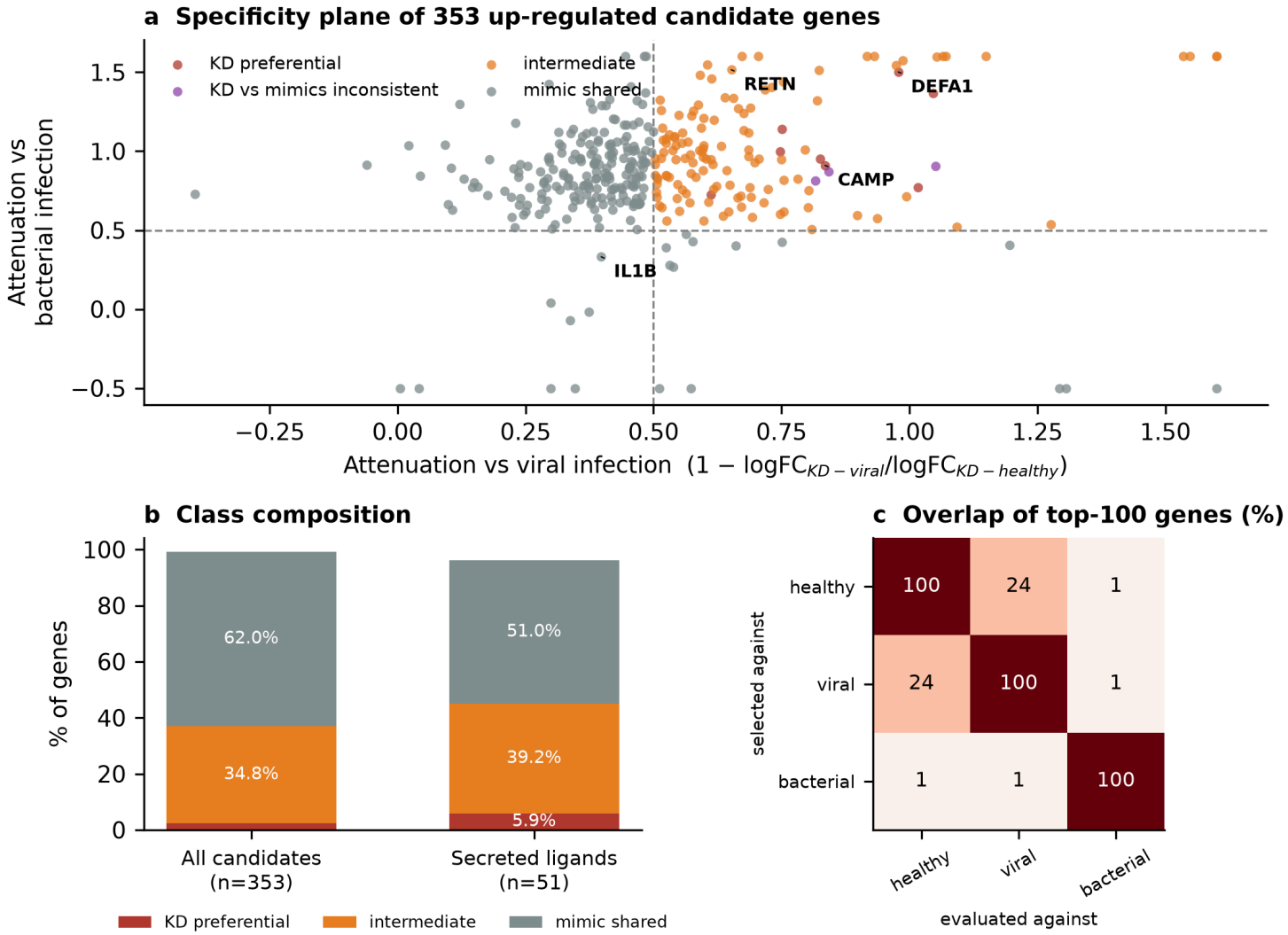


**Figure 2**

Specificity atlas of 437 candidate genes. **a** Attenuation against viral infection (x) and bacterial infection (y); values of 0 indicate full sharing with the mimic and 1 indicates no induction in the mimic. **b** Class composition of all up-regulated candidates and of the secreted ligand subset. **c** Percentage of genes shared between top-100 sets selected against each comparator.