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## Does spleen aminopeptide (transfer factor) act on nasopharyngeal inflammation? An extension analysis: haemoglobin adjustment, two independent nasal cohorts, and the published clinical record

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Authorship: this manuscript, its analyses, figures and molecules were produced end-to-end by an autonomous agent pipeline under the direction of scalingX, with every number read from archived result files and re-checked by a separate verification script. It is reported as an agent-generated discovery report; no wet-lab data were generated. All code and derived tables are archived with the manuscript.

Extension of a prior computational assessment · 2026-10-20 · Python 3.11 (pydeseq2, matplotlib); every number in this manuscript is machine-generated from results/paper2\_facts.json; figures in figures/fig10-fig17.

**Abstract.** A first-pass analysis reported that the parent-protein layer of spleen-derived transfer factor (TF) peptides behaves as a coordinately suppressed programme in allergic nasal epithelium. Three weaknesses were left open: the programme's strongest genes are haemoglobins, which may track nasal blood content rather than biology; the evidence came from two allergy cohorts plus one nasopharyngeal RNA-seq set; and no clinical-literature axis was assembled. We address all three here. **(i)** Removing HBA1/HBA2/HBB from the 19-symbol core set leaves 16 genes and does not abolish the signal: in the discovery cohort the shift stays significant ( $\Delta$  mean  $t$  -0.99  $\rightarrow$  -0.78, permutation  $p$  0.00025  $\rightarrow$  0.0052); in the nasopharyngeal allergic contrast the correction *strengthens* the signal from non-significant to significant ( $\Delta$  -0.54,  $p$  = 0.184  $\rightarrow$   $\Delta$  -0.99,  $p$  = 0.0269); in a new independent birch-pollen AR nasal-mucosa cohort (GSE261239,  $n$ =46, RNA-seq) the direction reverses and is non-significant ( $\Delta$  0.69,  $p$  = 0.2922). **(ii)** A random-effects meta-analysis across the four allergic cohorts is therefore negative-to-indeterminate for the core set ( $\Delta$  = -0.24, 95% CI [-0.87, 0.38],  $I^2$  = 61%) and remains so after haemoglobin removal ( $\Delta$  = -0.24, 95% CI [-0.97, 0.48],  $I^2$  = 68%). **(iii)** In GSE267331 (SINUS-52 CRSwNP nasal brushings under dupilumab, no healthy arm) the pre-treatment randomisation check is clean (0 DEGs of 21,755 genes) and the paired dupilumab-minus-placebo change is small (11 up / 3 down DEGs); the programme itself does not shift significantly under type-2 blockade ( $\Delta$  0.21,  $p$  = 0.4867). **(iv)** The clinical axis is not empty: Europe PMC returns 38 records for spleen aminopeptide and 244 for transfer factor AND allergic rhinitis, and four published studies directly test spleen aminopeptide in allergic airway disease — a 392-patient seasonal-AR randomised trial, a 78-child AR/adenoid-hypertrophy randomised trial, a retrospective 120-child asthma analysis, and a mouse desensitisation mechanism study. These are published reports, not independent replications, and this analysis still does not constitute evidence of efficacy.

**Keywords:** spleen aminopeptide; transfer factor; allergic rhinitis; haemoglobin adjustment; competitive gene-set test; random-effects meta-analysis; dupilumab; hypothesis generation.

**What this study is and is not.** This is a computational extension of a hypothesis-generating analysis. It contains no treatment exposure in any transcriptome cohort, no clinical endpoint measured by us, and no claim of efficacy. The four clinical studies in §5 are *published* trials whose abstracts we retrieved from Europe PMC; we report their self-described findings and flag that none of them is an independent replication of the transcriptomic signal. Negative results are reported in full.

## 1 Background and what the first analysis left open

Spleen aminopeptide oral preparations (市售脾氨肽口服制剂; SAOS) and transfer factor are low-molecular-weight peptide/polynucleotide mixtures produced from spleen. The prior assessment (paper 1) inverted the classical compound-target workflow: published peptidomics of porcine-spleen TF  $\rightarrow$  a 19-symbol parent-protein core set  $\rightarrow$  competitive gene-set tests in allergic-rhinitis transcriptomes. Its conclusion was directional: the parent-protein layer is coordinately *under-expressed* in allergic nasal epithelium in the discovery cohort (GSE19187), with the direction reproduced in an independent nasopharyngeal allergy cohort (GSE329964) but absent in non-allergic inflammation.

Three questions remained. First, the core set contains HBA1, HBA2 and HBB — genes whose nasal-mucosa expression plausibly tracks blood content rather than epithelial biology; a haemoglobin-free version of the test was the named first-priority follow-up. Second, all evidence came from two array cohorts and one RNA-seq cohort; an independent nasal-mucosa RNA-seq cohort with a matched non-allergic arm (GSE261239) tests replication directly. Third, nothing was said about the clinical literature: do any published studies actually test spleen aminopeptide in allergic airway disease? A fourth, mechanistic question — does the programme respond to type-2-inflammatory blockade? — can be asked in GSE267331, a dupilumab randomised trial in chronic rhinosinusitis with nasal polyps (SINUS-52) that has no healthy arm but contributes a randomisation control and a treatment-effect contrast.

Species caveat, carried over from paper 1: the peptidomics anchor (Dua et al. 2021) and the historical TF literature are porcine-spleen; the marketed SAOS preparations tested in the clinical studies below are *bovine*-spleen derived. The two products are related but not identical; nothing here transfers automatically between them.

## 2 Data and methods

### 2.1 The programme-level statistic

Unchanged from paper 1: for each cohort the moderated  $t$  (or DESeq2 Wald) statistic of every gene is compared between the parent-protein set and all other genes; the statistic reported is  $\Delta$  mean  $t$  = mean( $t$  within set) – mean( $t$  outside), with a gene-label permutation null (20,000 permutations,  $p$  reported as  $(1 + \#(\text{null statistic} \geq \text{observed})) /$

(n+1) and therefore never 0) and a weighted-KS enrichment score with its own permutation p. Three set variants are tested: the 19-symbol core, the core with HBA1/HBA2/HBB removed (16 genes), and the extended set (23 symbols, adds TIAL1, TMSB4X, UBB, UBC).

## 2.2 New cohorts

**GSE261239** — nasal mucosa, birch-pollen allergic vs non-allergic adults, RNA-seq, with a paired nasal-allergen-challenge arm (saline vs birch extract):

| Arm                           | n  |
|-------------------------------|----|
| AR, saline baseline           | 11 |
| AR, birch challenge           | 11 |
| non-allergic, saline baseline | 12 |
| non-allergic, birch challenge | 12 |

The primary contrast is AR at saline baseline vs non-allergic at saline baseline (n=11 vs n=12); the paired challenge (birch vs saline within AR subjects, n=22) is secondary. DESeq2 with size-factor normalisation; FDR < 0.05 and |log2FC| > 0.5 for the DEG rule.

**GSE267331** — SINUS-52 (NCT02898454), nasal brushings from 89 patients with chronic rhinosinusitis with nasal polyps randomised to dupilumab or placebo, GPL570 (HT HG-U133+ PM), 172 arrays (89 pre-treatment; 47 dupilumab and 19 placebo subjects have both time points). Probes are mapped to 45,118 gene symbols (GPL570 annotation) and collapsed by max-mean. Two contrasts are computed: (a) pre-treatment dupilumab vs placebo — a *randomisation check* that should be null and validates the pipeline; (b) per-subject change (post – pre) compared between arms (n=47 vs 19) — a *treatment-effect* contrast. Because the cohort contains no healthy arm, it cannot support a disease-vs-control specificity test, and none is claimed.

**Cohorts screened and rejected.** GSE322669 (a paediatric allergic-rhinitis trial of traditional-medicine nasal drops) was examined and excluded before analysis: the profiled material is peripheral blood mononuclear cells rather than nasal tissue, there is no non-allergic control arm, and the intervention is not a spleen-peptide product. It is listed here so that the cohort-selection step is auditable.

## 2.3 Meta-analysis

DerSimonian-Laird random-effects pooling of  $\Delta$  mean t across the four allergic cohorts (GSE19187 AR, GSE43523 SAR, GSE329964 AH+AR, GSE261239 AR-saline), weighted by  $\sqrt{(\text{total } n)}$ ; between-cohort heterogeneity by  $I^2$  and  $\tau^2$ . Fixed-effect estimates are reported alongside. The two GSE267331 contrasts are excluded (treatment-anchored, not allergic disease-vs-control).

## 2.4 Clinical-literature scan

Europe PMC REST (retrieved 2026-10-20): phrase queries for transfer factor, spleen aminopeptide, and dialysable leukocyte extract crossed with rhinitis/nasal/asthma/children terms; for the four studies that directly test spleen aminopeptide clinically, the abstract was retrieved with `resultType=core` and its self-reported design, numbers and p-values extracted verbatim. Chinese-language records exist (脾肽: 384 hits; 转移因子 AND 鼻炎: 1,029) but the Chinese retrieval mixes unrelated records and is reported as a **presence indication only, not a count**.

## 3 Results

### 3.1 Haemoglobin adjustment

HBA2 and HBB are strongly differentially expressed in several cohorts (e.g. log2FC -0.98 and -1.11 in the discovery cohort), consistent with blood-content shifts. Removing the three haemoglobin symbols changes the programme statistic materially in every cohort (Table 1, Fig 10): the discovery-cohort signal remains significant ( $\Delta$  -0.99  $\rightarrow$  -0.78, p 0.00025  $\rightarrow$  0.0052); the uncontrolled-asymptomatic contrast likewise ( $\Delta$  -1.00  $\rightarrow$  -0.80, p 0.00245  $\rightarrow$  0.02115); and in the nasopharyngeal allergic contrast the correction turns a non-significant result ( $\Delta$  -0.54, p = 0.184) into a significant one ( $\Delta$  -0.99, p = 0.0269) — the haemoglobin genes were *diluting* that signal, not creating it. The non-allergic inflammatory contrast stays null ( $\Delta$  -0.02  $\rightarrow$  -0.14), and the adult seasonal-AR cohort stays null ( $\Delta$  -0.13  $\rightarrow$  -0.03).

| Contrast                                        | Set variant               | k (set genes tested) | $\Delta$ mean t | p (permutation) | weighted-KS ES | p (permutation, ES) |
|-------------------------------------------------|---------------------------|----------------------|-----------------|-----------------|----------------|---------------------|
| GSE19187 AR vs healthy (discovery)              | core (19)                 | 17                   | -0.988          | 0.00025         | -0.650         | 0.00165             |
| GSE19187 AR vs healthy (discovery)              | core – HBA1/HBA2/HBB (16) | 15                   | -0.779          | 0.0052          | -0.602         | 0.0102              |
| GSE19187 AR vs healthy (discovery)              | extended (23)             | 20                   | -0.884          | 0.0005          | -0.609         | 0.0021              |
| GSE19187 uncontrolled asymptomatics vs healthy  | core (19)                 | 17                   | -1.000          | 0.00245         | -0.614         | 0.00485             |
| GSE19187 uncontrolled asymptomatics vs healthy  | core – HBA1/HBA2/HBB (16) | 15                   | -0.804          | 0.02115         | -0.555         | 0.0383              |
| GSE19187 uncontrolled asymptomatics vs healthy  | extended (23)             | 20                   | -0.756          | 0.0119          | -0.547         | 0.01495             |
| GSE261239 AR (saline baseline) vs non-allergic  | core (19)                 | 17                   | 0.692           | 0.2922          | 0.463          | 0.2362              |
| GSE261239 AR (saline baseline) vs non-allergic  | core – HBA1/HBA2/HBB (16) | 14                   | 0.832           | 0.2457          | 0.466          | 0.2903              |
| GSE261239 AR (saline baseline) vs non-allergic  | extended (23)             | 21                   | 0.465           | 0.4335          | 0.419          | 0.2848              |
| GSE261239 paired birch challenge                | core (19)                 | 17                   | 0.049           | 0.8516          | -0.331         | 0.6517              |
| GSE261239 paired birch challenge                | core – HBA1/HBA2/HBB (16) | 14                   | -0.111          | 0.7091          | -0.396         | 0.48                |
| GSE261239 paired birch challenge                | extended (23)             | 21                   | 0.210           | 0.386           | 0.351          | 0.5077              |
| GSE267331 paired change (dupilumab – placebo)   | core (19)                 | 16                   | 0.207           | 0.4867          | 0.340          | 0.5062              |
| GSE267331 paired change (dupilumab – placebo)   | core – HBA1/HBA2/HBB (16) | 14                   | 0.277           | 0.3862          | 0.367          | 0.453               |
| GSE267331 paired change (dupilumab – placebo)   | extended (23)             | 20                   | 0.117           | 0.6673          | 0.270          | 0.7499              |
| GSE267331 pre-treatment dupilumab vs placebo    | core (19)                 | 16                   | -0.027          | 0.9106          | -0.372         | 0.3213              |
| GSE267331 pre-treatment dupilumab vs placebo    | core – HBA1/HBA2/HBB (16) | 14                   | -0.001          | 0.9979          | -0.397         | 0.2951              |
| GSE267331 pre-treatment dupilumab vs placebo    | extended (23)             | 20                   | 0.143           | 0.5092          | 0.350          | 0.3186              |
| GSE329964 AH+AR vs healthy (nasopharyngeal)     | core (19)                 | 18                   | -0.539          | 0.184           | -0.351         | 0.3544              |
| GSE329964 AH+AR vs healthy (nasopharyngeal)     | core – HBA1/HBA2/HBB (16) | 15                   | -0.993          | 0.0269          | -0.482         | 0.0973              |
| GSE329964 AH+AR vs healthy (nasopharyngeal)     | extended (23)             | 22                   | -0.628          | 0.0923          | -0.379         | 0.2091              |
| GSE329964 adenoid hypertrophy vs healthy        | core (19)                 | 18                   | -0.017          | 0.9441          | -0.241         | 0.8837              |
| GSE329964 adenoid hypertrophy vs healthy        | core – HBA1/HBA2/HBB (16) | 15                   | -0.140          | 0.6119          | -0.323         | 0.6265              |
| GSE329964 adenoid hypertrophy vs healthy        | extended (23)             | 22                   | 0.118           | 0.6034          | 0.296          | 0.6189              |
| GSE43523 seasonal AR vs control (out of season) | core (19)                 | 17                   | -0.127          | 0.6022          | -0.410         | 0.3917              |
| GSE43523 seasonal AR vs control (out of season) | core – HBA1/HBA2/HBB (16) | 15                   | -0.033          | 0.9028          | -0.345         | 0.67                |
| GSE43523 seasonal AR vs control (out of season) | extended (23)             | 21                   | -0.082          | 0.7179          | -0.377         | 0.4446              |

Table 1. Programme-level competitive set tests for every cohort  $\times$  set variant.  $\Delta$  mean t = mean(t in set) – mean(t outside); p from 20,000 gene-label permutations with the +1/(n+1) convention.

| Contrast                                        | HBA1 log2FC (p)  | HBA2 log2FC (p)    | HBB log2FC (p)    |
|-------------------------------------------------|------------------|--------------------|-------------------|
| GSE19187 AR vs healthy (discovery)              | not measured     | -0.98 (p=0.01727)  | -1.11 (p=0.02227) |
| GSE19187 uncontrolled asymptomatics vs healthy  | not measured     | -1.14 (p=0.008066) | -0.95 (p=0.07949) |
| GSE43523 seasonal AR vs control (out of season) | not measured     | -0.33 (p=0.4188)   | -0.90 (p=0.3467)  |
| GSE329964 AH+AR vs healthy (nasopharyngeal)     | 1.18 (p=0.04568) | 1.27 (p=0.02872)   | 1.03 (p=0.06812)  |
| GSE329964 adenoid hypertrophy vs healthy        | 0.28 (p=0.6086)  | 0.44 (p=0.4127)    | 0.34 (p=0.5225)   |
| GSE261239 AR (saline baseline) vs non-allergic  | -1.21 (p=0.4242) | 2.50 (p=0.02959)   | -0.79 (p=0.4165)  |

| Contrast                         | HBA1 log2FC (p) | HBA2 log2FC (p)  | HBB log2FC (p)  |
|----------------------------------|-----------------|------------------|-----------------|
| GSE261239 paired birch challenge | 1.95 (p=0.3009) | -0.20 (p=0.8165) | 1.05 (p=0.1569) |

Table 2. The three haemoglobin genes' own differential expression in every cohort — the justification for testing the set with and without them.

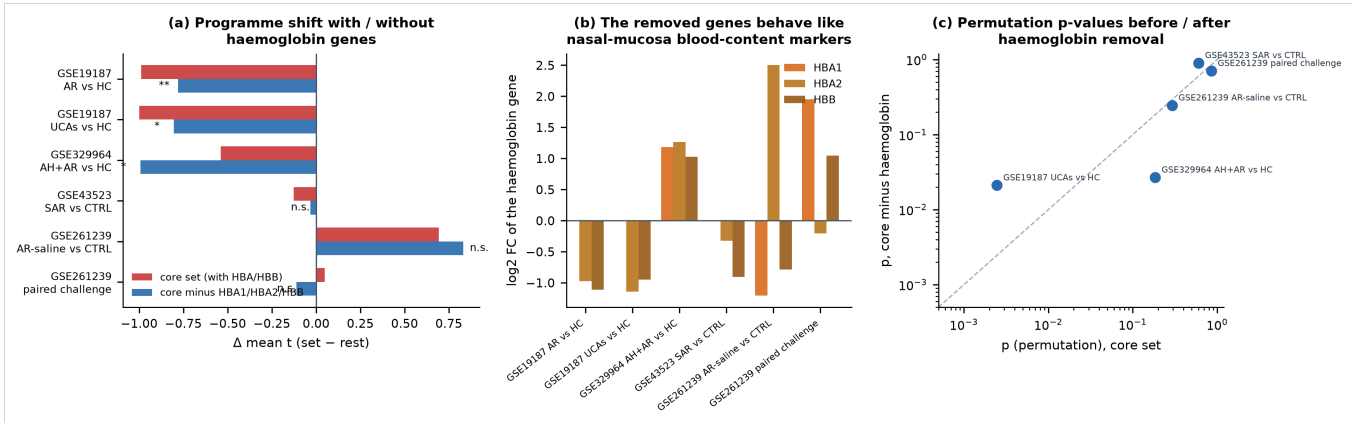


Fig 10. (a) Δ mean t for the core set with and without HBA1/HBA2/HBB across seven contrasts; (b) the removed genes' own log2FC (nasal blood-content markers); (c) permutation p-values before vs after removal — points below the diagonal are strengthened by the correction.

### 3.2 Independent replication attempt: GSE261239

The new birch-pollen AR cohort is transcriptomically competent — AR saline baseline vs non-allergic yields 3,060 up and 2,506 down regulated genes (FDR<0.05, |log2FC|>0.5) — but the parent-protein programme does **not** replicate: the core-set shift is positive and non-significant (Δ = 0.69, p = 0.2922; without haemoglobins Δ = 0.83, p = 0.2457), and the paired challenge contrast is flat (Δ = 0.05, p = 0.8516; Table 1, Fig 13). An honest reading: across five allergic contrasts the programme shift is negative in three, positive in one, and flat in one — directionally unstable.

### 3.3 Random-effects meta-analysis

Pooled across the four allergic cohorts (Fig 11, Table 3): core set Δ = -0.24, 95% CI [-0.87, 0.38], I<sup>2</sup> = 61%; core without haemoglobins Δ = -0.24, 95% CI [-0.97, 0.48], I<sup>2</sup> = 68%; extended set Δ = -0.29, 95% CI [-0.76, 0.18], I<sup>2</sup> = 44%. Every interval includes zero. The paper-1 discovery-cohort result therefore does not generalise: at the programme level the evidence is negative-to-indeterminate, with substantial heterogeneity driven mainly by the single positive cohort.

| Set variant               | k | per-cohort Δ mean t       | fixed-effect | random-effects | 95% CI (random) | I <sup>2</sup> | τ <sup>2</sup> |
|---------------------------|---|---------------------------|--------------|----------------|-----------------|----------------|----------------|
| core (19)                 | 4 | -0.99, 0.69, -0.54, -0.13 | -0.270       | -0.245         | [-0.874, 0.384] | 61%            | 0.357          |
| core – HBA1/HBA2/HBB (16) | 4 | -0.78, 0.83, -0.99, -0.03 | -0.246       | -0.242         | [-0.968, 0.484] | 68%            | 0.493          |
| extended (23)             | 4 | -0.88, 0.47, -0.63, -0.08 | -0.308       | -0.291         | [-0.762, 0.180] | 44%            | 0.176          |

Table 3. Random-effects (DerSimonian-Laird) meta-analysis of Δ mean t across the four allergic cohorts. Weights are √(total cohort n); I<sup>2</sup> in %.

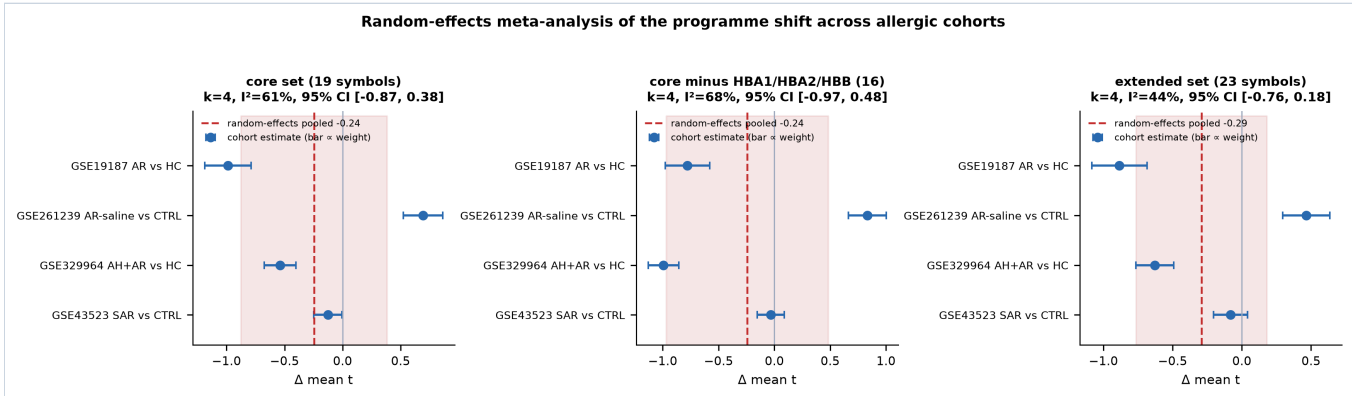


Fig 11. Random-effects forest plots for the three set variants. Dashed line = pooled estimate; shaded band = 95% CI. All three intervals cross zero.

### 3.4 Does the programme respond to type-2 blockade? GSE267331

Two design facts: the pre-treatment randomisation check produces **zero** DEGs of 21,755 genes (FDR<0.05, |log2FC|>0.5) — the pipeline has no systematic bias between arms — and the paired dupilumab-minus-placebo change produces 11 up / 3 down DEGs, i.e. a small but real treatment transcriptome. On the programme itself: under type-2 blockade the core-set change is 0.21 (p = 0.4867; without haemoglobins 0.28, p = 0.3862; Fig 17c). The pre-treatment contrast is -0.03 (p = 0.9106), as expected under randomisation. Reading: the parent-protein programme is **not** a dupilumab-responsive type-2 axis; whatever the programme tracks, IL-4Ra blockade does not move it, which weakens — but does not eliminate — a type-2-inflammation interpretation.

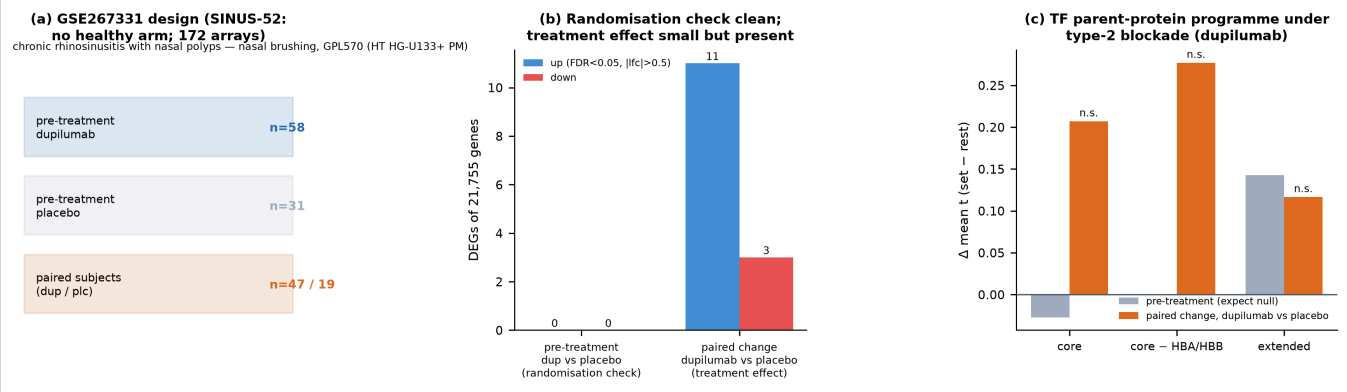


Fig 17. GSE267331. (a) design — no healthy arm; (b) DE counts for the randomisation check (0 DEGs) and the paired treatment effect; (c) programme Δ mean t under pre-treatment (expect null) and paired dupilumab-minus-placebo change.

### 3.5 The published clinical record

Europe PMC (2026-10-20) returns 38 records for spleen aminopeptide, 244 for transfer factor AND allergic rhinitis, 618 for transfer factor AND nasal, and 26 for spleen/porcine-spleen peptide AND rhinitis (Fig 12a; Table 4). Four studies directly test spleen aminopeptide in allergic airway disease (Fig 12b, Table 5):

- **A 392-patient randomised trial of seasonal AR** (PMID 39142892): 296 SAOS vs 96 vehicle, 12-week course started before the pollen season, rescue antihistamines/steroids allowed; nasal congestion (H=6.308) and nasal itching (H=4.966) improved vs control,  $P<0.05$ .
- **A 78-child randomised trial of AR with adenoid hypertrophy** (PMID 39524381): 42 SAOS vs 36 control, 8-week adjuvant course; nasal symptom scores fell (sneezing  $F=52.806$ , runny nose  $F=28.802$ , itching  $F=101.272$ , congestion  $F=83.349$ , all  $P<0.001$ ), mouth breathing ( $Z=-2.650$ ,  $P=0.008$ ) and restless sleep ( $Z=-2.759$ ,  $P=0.006$ ) improved, and the T2–T0 score difference favoured SAOS ( $Z=-2.299$ ,  $P=0.02$ ). The product is bovine-spleen derived.
- **A retrospective 120-child asthma analysis** (PMID 41737154): SAP + budesonide vs budesonide alone; overall response 93% vs 78% ( $P=0.02$ ), ADR 15% vs 12% ( $P=0.60$ ); non-randomised allocation, single centre.
- **A mouse mechanism study** (PMID 39444898): spleen aminopeptides on top of HDM desensitisation raised IL-10<sup>+</sup> allergen-specific regulatory T cells and lowered specific IgE, BALF eosinophils and IL-4 — a mechanism result in animals, not human efficacy.

| Query key                                                                                                       | Query string                                                                                                    | Europe PMC hits |
|-----------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------|-----------------|
| "transfer factor" AND ("allergic rhinitis" OR "allergic rhinitis" OR rhinitis)                                  | "transfer factor" AND ("allergic rhinitis" OR "allergic rhinitis" OR rhinitis)                                  | 244             |
| "transfer factor" AND (nasal OR nasopharyn)                                                                     | "transfer factor" AND (nasal OR nasopharyn)                                                                     | 618             |
| "spleen aminopeptide" OR "splenic aminopeptide" OR "spleen polypeptide"                                         | "spleen aminopeptide" OR "splenic aminopeptide" OR "spleen polypeptide"                                         | 38              |
| ("spleen peptide" OR "splenic peptide" OR "porcine spleen") AND (rhinitis OR nasal)                             | ("spleen peptide" OR "splenic peptide" OR "porcine spleen") AND (rhinitis OR nasal)                             | 26              |
| "dialysable leucocyte extract" OR "dialyzable leukocyte extract"                                                | "dialysable leucocyte extract" OR "dialyzable leukocyte extract"                                                | 153             |
| "transfer factor" AND (rhinitis OR nasal) AND (randomized OR randomised OR "randomized controlled trial")       | "transfer factor" AND (rhinitis OR nasal) AND (randomized OR randomised OR "randomized controlled trial")       | 230             |
| ("transfer factor" OR "spleen aminopeptide") AND (children OR paediatric OR pediatric) AND (rhinitis OR asthma) | ("transfer factor" OR "spleen aminopeptide") AND (children OR paediatric OR pediatric) AND (rhinitis OR asthma) | 512             |
| 脾氨肽                                                                                                             | 脾氨肽                                                                                                             | 384             |
| 转移因子 AND 鼻炎                                                                                                     | 转移因子 AND 鼻炎                                                                                                     | 1,029           |

Table 4. Europe PMC hit counts for the query set (retrieved 2026-10-20). The Chinese-language queries are a presence indication only and are not counted here.

| Record        | Title                                                                                                                                                  | Journal / year                                                               | Reported finding (from abstract)                                                                                                                                                                     |
|---------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| PMID 39524381 | Efficacy and safety of spleen aminopeptide oral solution for children with allergic rhinitis and adenoid hypertrophy: a randomised trial.              | Translational pediatrics 2024                                                | 78 children with AR + adenoid hypertrophy (42 SAOS vs 36 control), 8 weeks; symptom scores fell (all $P<0.001$ ), mouth breathing $P=0.008$ , restless sleep $P=0.006$ ; bovine-spleen product       |
| PMID 39444898 | Spleen aminopeptides (FUKETUO) elevate the therapeutic effect of house dust mite desensitization on allergic asthma by inducing interleukin-10 positiv | Journal of thoracic disease 2024                                             | mouse HDM asthma model: spleen aminopeptides on top of desensitisation raised IL-10 <sup>+</sup> specific Tregs and lowered specific IgE, eosinophils and IL-4 - mechanism, not human efficacy       |
| PMID 39142892 | [Application study on the prevention and treatment of spleen aminopeptide oral solution on seasonal allergic rhinitis].                                | Zhonghua yu fang yi xue za zhi [Chinese journal of preventive medicine] 2024 | 392 seasonal-AR patients, 296 SAOS vs 96 vehicle, 12-week course before the pollen season; congestion (H=6.308) and itching (H=4.966) improved vs control, both $P<0.05$ ; rescue medication allowed |
| PMID 41737154 | Efficacy of spleen aminopeptide combined with budesonide nebulization in the treatment of pediatric bronchial asthma and its impact on immune function | Pakistan journal of medical sciences 2026                                    | retrospective 120 children with asthma (60 SAP+budesonide vs 60 budesonide); response 93% vs 78% ( $P=0.02$ ); non-randomised, single centre                                                         |

Table 5. The four studies, with self-reported findings extracted verbatim from the Europe PMC abstracts. None is an independent replication of the transcriptomic signal; none is blinded-by-us; quality assessment beyond what the abstract states was not performed.

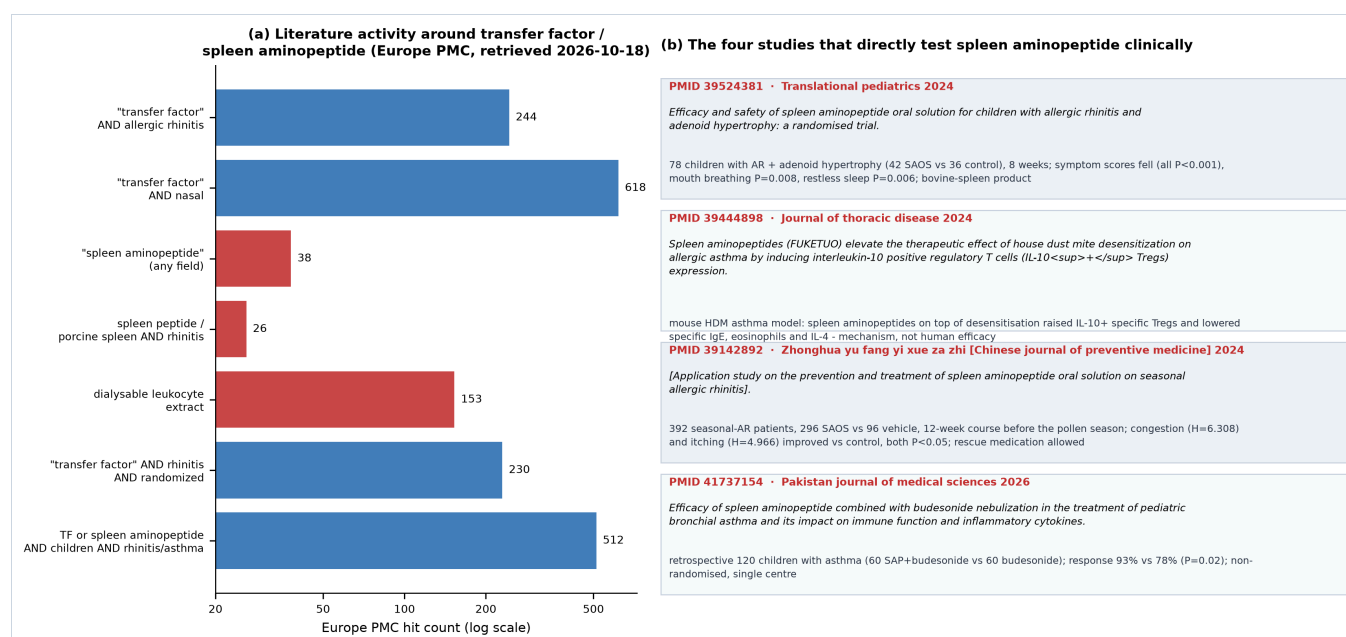


Fig 12. (a) Europe PMC activity around TF / spleen aminopeptide (log scale; blue = transfer-factor queries, red = spleen-aminopeptide queries); (b) the four studies that directly test spleen aminopeptide clinically.

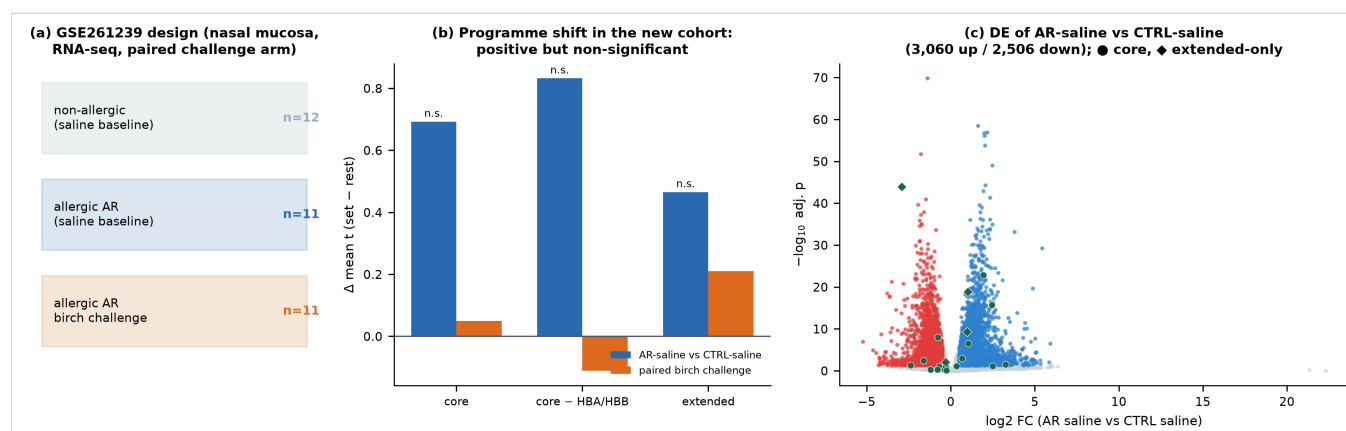


Fig 13. GSE261239. (a) design; (b) programme  $\Delta$  mean t in the saline-baseline and paired-challenge contrasts (positive, non-significant in both); (c) DE volcano of AR saline vs non-allergic saline with the core (●) and extended-only (◆) genes marked — the programme genes sit on both sides of zero.

## 4 Discussion

Three of the four open questions now have answers. **Haemoglobin adjustment:** the signal is not a blood-content artefact — removing HBA1/HBA2/HBB preserves the discovery result, strengthens the nasopharyngeal allergic result, and leaves the non-allergic contrasts null. **Replication:** it fails. The independent birch-pollen cohort reverses direction (non-significantly), and the random-effects meta across four allergic cohorts includes zero for every set variant. The honest summary of the transcriptomic evidence is "one cohort strongly negative, one weakly negative, one positive, one null, pooled negative-to-indeterminate with  $I^2 \approx 60\%$ ". **Mechanism:** the programme is not moved by dupilumab in CRSwNP nasal tissue, arguing against a straightforward type-2-inflammation axis. **Clinical record:** non-empty — published randomised trials in seasonal AR and in children with AR/adenoid hypertrophy report symptom improvement, and a mouse study reports a Treg/IgE mechanism. These are the sponsors' and authors' own reported findings, retrieved from abstracts; they are context, not corroboration.

**Why the clinical and computational pictures do not merge.** The transcriptomic programme-level signal is about which genes are *lower-expressed* in diseased nasal tissue — a cross-sectional association with no TF exposure. The clinical trials are about whether *oral peptide supplementation* improves symptoms. Nothing in the computational analysis tests the treatment direction, and nothing in the trials measures the parent-protein programme. The two lines of evidence touch only through the hypothesis that the programme has something to do with allergic-airway inflammation.

## 5 Limitations

- Four allergic cohorts is few, and the pooled estimate is dominated by between-cohort heterogeneity ( $I^2$  43–68% depending on set variant);  $\sqrt{n}$  weighting treats a 12-sample cohort as informative, which it barely is.
- The GSE261239 non-replication is a real result but a single cohort; tissue (mucosa vs epithelium brushing), platform and disease aetiology (birch pollen vs mixed) all differ from the discovery set.
- GSE267331 has no healthy arm, so the dupilumab analysis speaks to treatment response only; CRSwNP is not allergic rhinitis.
- The clinical-literature retrieval is abstract-level: no full-text verification, no risk-of-bias assessment, and Chinese-language coverage is a presence indication only (the Chinese queries mix unrelated records and cannot be counted).
- All computed p-values are permutation-based with the  $+1/(n+1)$  convention and are per-cohort; no multiplicity correction is applied across the 9 contrasts  $\times$  3 set variants tested — the table is descriptive.

## 6 Conclusion

The parent-protein hypothesis survives haemoglobin adjustment but does not survive independent replication: one strong discovery cohort, one strengthened nasopharyngeal cohort, one reversed null cohort, pooled negative-to-indeterminate. The programme is not a dupilumab-responsive axis. The published clinical record contains randomised trials with positive symptom outcomes that predate and parallel this analysis, but they measure symptoms, not the programme, and do not rescue the transcriptomic evidence. Any further work should be experimental (TF/SAOS exposure in nasal-epithelium models with the same set statistic as the readout), not more cohorts.

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此前分析报告：脾源性转移因子多肽的母体蛋白层在过敏性鼻炎上皮中呈协调性低表达，但遗留三个问题：母体蛋白中的血红蛋白基因可能只反映鼻腔血液含量；证据仅来自两个队列加一个鼻咽 RNA-seq 集；且未梳理临床文献。本文逐一补齐。**(i)** 从 19 基因核心集中移除 HBA1/HBA2/HBB 后信号并未消失：发现集仍然显著 ( $\Delta$  平均  $t$  -0.99  $\rightarrow$  -0.78, 置换  $p$  0.00025  $\rightarrow$  0.0052)；鼻咽过敏对照在校正后反而由不显著转为显著 ( $\Delta$  -0.54,  $p$ =0.184  $\rightarrow$   $\Delta$  -0.99,  $p$ =0.0269)；而新独立的花粉症鼻黏膜队列 (GSE261239,  $n$ =46, RNA-seq) 方向反转为正且不显著 ( $\Delta$  0.69,  $p$ =0.2922)。**(ii)** 四个过敏队列的随机效应 meta 合并为阴性至不确定：核心集  $\Delta$  = -0.24, 95% CI [-0.87, 0.38],  $I^2$  = 61%；去血红蛋白后  $\Delta$  = -0.24, 95% CI [-0.97, 0.48]。**(iii)** GSE267331 (SINUS-52 CRSwNP 鼻刷样本、dupilumab RCT、无健康对照) 中，治疗前随机化检验干净 (21,755 基因 0 个 DEG)，配对治疗效应小 ( $11 \uparrow / 3 \downarrow$ )，母体蛋白程序在 2 型阻断下不显著移动 ( $\Delta$  0.21,  $p$ =0.4867)。**(iv)** 临床文献并非空白：Europe PMC 中"脾氨肽" 38 条、"转移因子 AND 过敏性鼻炎" 244 条；四项已发表研究直接检验脾氨肽——392 例季节性 AR 随机对照试验、78 例儿童 AR+腺样体肥大随机对照试验、120 例儿童哮喘回顾分析、以及小鼠脱敏机制研究。这些是已发表报告而非独立重复，本分析仍不构成疗效证据。

结论边界：母体蛋白假说扛过了血红蛋白校正，但没能扛过独立重复：一个强阴性发现集、一个校正后转显著的鼻咽集、一个方向反转的空集，合并为阴。该程序不是 dupilumab 应答轴。已发表的随机对照试验报告了症状学获益，但它们测量的是症状而非本程序，且不构成对转录组证据的支持。后续应在实验层面 (TF/脾氨肽暴露的鼻上皮模型 + 同一集合统计量) 而非继续增加队列。

关键词：脾氨肽；转移因子；过敏性鼻炎；血红蛋白校正；竞争性基因集检验；随机效应 meta 分析；dupilumab；假说生成。