Methods

Study Population

Healthy controls (HCs) fulfilled the criteria previously described [1]. Briefly, HCs should have no symptoms or history of asthma, other pulmonary diseases, rhinitis, CRSwNP, NSAID intolerance, or atopy and should have negative skin tests for a standard battery of aeroallergens. T2-asthma was diagnosed according to GINA guidelines [2, 3]. CRSwNP and N-ERD were diagnosed according to EPOS criteria [4]. Skin prick tests were performed as previously described [5] according to The European Academy of Allergy and Clinical Immunology (EAACI) recommendations [6]. Patients were divided into asthmatics, asthmatics with CRSwNP, and N-ERD. Patients were treated according to GINA guidelines [2]. Nevertheless, none were receiving oral corticosteroids or biologicals thus avoiding influences on gene expression.

Gene expression analysis

Table SI. Primer pairs used in the qPCR analysis.

GENE	Primer pairs 5'-3'sequences	
	Forward	**Reverse**
ALOX15	ACCTTCCTGCTCGCCTAGTGTT	GGCTACAGAGAATGACGTTGGC
CLC	ATCAAAGGGCGACCACTTGCCT	CCCATACTCACGGCTGTTCATG
CYSLTR2	ATGTCACCAGCATCAGGAGTGC	TGTGACACTGCCGTTCTGCTCA
HRH4	CGATACCTGTCAGTCTCAAATGC	GCCCATTCACTAAGAAGGCCAG
SMPD3	AACTGCTCCTCTGACGACAA	GTGTCCAGCAGAGTACCGAT

Statistical analysis

A p-value < 0.05 indicated statistical significance. The sample size was determined using statistical power and false positive report probability considerations to minimize the risk of Type I error. The normality distribution was evaluated using the Kolmogorov-Smirnov test. Proportions were compared using the Chi-squared test. ANOVA with DMS or Games-Howell post hoc tests was used to compare continuous parametric data. Krustal-Wallis (K-W) test was used for non-parametric data. Pearson's correlation coefficient (r) was used to test possible relations between variables. Receiver operating characteristic (ROC) analysis was used to assess the biomarkers' diagnostic performance, and cut-off values were calculated from ROC data using the Jouden index [7]. Sensitivity, specificity, positive (+), or negative (-) likelihood ratio (LR) were calculated to assess the diagnostic value of a test. Logistic regression analysis was used to predict disease phenotype based on potential biomarkers individually (simple

logistic regression) or in combination (multiple logistic regression). Age, sex, and atopy were used as potential confounding variables for the adjustment of the p-values.

References (supplementary material)

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Figure legends

Figure S1. (A) Heatmap of RNAseq data [1] was plotted and computed with Morpheus [8]. Color representation was performed using expression values transformation subtraction of row median, divided by row median absolute deviation, with extreme values -1 and +1. Green represents downregulated genes, and red represents upregulated genes. **(B)** RNAseq data analysis of genes selected (pink squares in panel A) for the validation study.