ORIGINAL ARTICLE

Epidemiology and Genetics

Methylation risk scores for childhood aeroallergen sensitization: Results from the LISA birth cohort

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Abstract

Background: Epigenomic (e.g., DNA methylation [DNAm]) changes have been hypothesized as intermediate step linking environmental exposures with allergic disease. Associations between individual DNAm at CpGs and allergic diseases have been reported, but their joint predictive capability is unknown.

Methods: Data were obtained from 240 children of the German LISA cohort. DNAm was measured in blood clots at 6 ($N = 234$) and 10 years ($N = 227$) using the Illumina EPIC chip. Presence of aeroallergen sensitization was measured in blood at 6, 10, and 15 years. We calculated six methylation risk scores (MRS) for allergy-related phenotypes, like total and specific IgE, asthma, or any allergies, based on available publications and assessed their performances both cross-sectionally (biomarker) and prospectively (predictor of the disease). Dose–response associations between aeroallergen sensitization and MRS were evaluated.

Results: All six allergy-related MRS were highly correlated ($r > .86$), and seven CpGs were included in more than one MRS. Cross-sectionally, we observed an 81% increased risk for aeroallergen sensitization at 6 years with an increased MRS by one standard deviation (best-performing MRS, 95% confidence interval = [43%; 227%]). Significant associations were also seen cross-sectionally at 10 years and prospectively, though the effect of the latter was attenuated when restricted to participants not sensitized at baseline. A clear dose–response relationship with levels of aeroallergen sensitization could be established cross-sectionally, but not prospectively.

Conclusion: We found good classification and prediction capabilities of calculated allergy-related MRS cross-sectionally, underlining the relevance of altered

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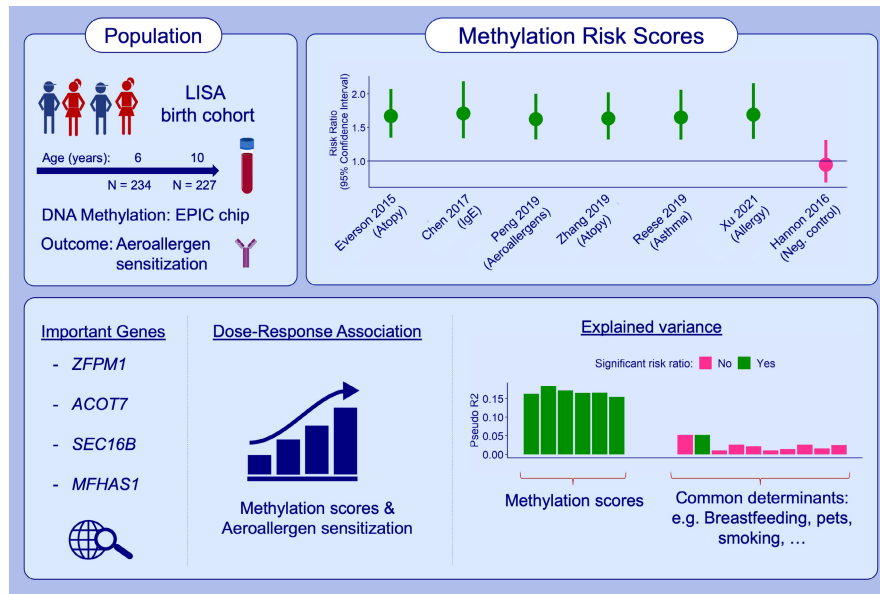gene-regulation in allergic diseases and providing insights into potential DNAm bio-markers of aeroallergen sensitization.

KEYWORDS
allergic diseases, DNA methylation, epidemiology, methylation risk scores, polygenic risk scores

GRAPHICAL ABSTRACT
We calculated six MRS for allergy-related phenotypes and present their association with childhood aeroallergen sensitization. All six allergy-related MRS were highly correlated and seven CpGs were overlapping between the MRS, all located in genes associated with allergic diseases. A clear dose–response relationship with levels of aeroallergen sensitization could be established cross-sectionally, but not prospectively.

1 | INTRODUCTION

The link between genetic variation and allergic diseases is already well established by several genome-wide association studies (GWAS).[1] However, non-genetic and environmental determinants, like birth order[2,3] or pet ownership,[4] have also been discussed and might explain further variance in allergic diseases (e.g., asthma and allergic rhino-conjunctivitis) through epigenetic mechanisms such as DNA methylation (DNAm).[5]

Over the past years, epigenome-wide association studies (EWAS) have identified differential DNAm at several CpG (addition of a methyl group to a cytosine in the context of CpG dinucleotides) sites to be associated with allergic phenotypes including atopy, defined as allergic reaction in skin prick test, high total (≥200 kU/L) or specific immunoglobulin E (IgE) (≥0.35 kU/L),[6–9] childhood asthma[10] or any allergic disease plus sensitization.[11]

Compared to large-scale GWAS, current EWAS often have limited sample size[12] with the maximum sample size in allergic phenotypes being 3493.[10] Age-, tissue-, and cell type-specific differences in DNAm patterns further limit the generalizability of results.[5] Additionally, identified CpGs usually have small effect sizes, similar to single genetic variants for common diseases.[13] Given the unknown generalizability and replicability of recent EWAS of allergy-related phenotypes, mainly due to small sample sizes, a proof of the applicability of their results in a predictive context is of great interest for methylation studies.

Following the same methodology as previously employed for polygenic risk scores (PRS),[14] methylation risk scores (MRS) could be used to evaluate the reproducibility of published atopy-related EWAS and their prediction accuracy cross-sectionally (as biomarkers of aeroallergen sensitization) and prospectively (as predictors of future aeroallergen sensitization). MRS have been reported as biomarkers for exposures like smoking,[15] as predictors of poor survival outcomes in hepatocellular carcinoma[16] and disease indicators for prostate cancer, even outperforming other known risk factors.[17]

Methylation risk scores are calculated by using external evidence from published EWAS and weighting the CpGs in the target cohort with the respective effect sizes from external EWAS on the same phenotype to calculate a weighted average. Thereby, small effects of single CpG sites are accumulated, which increases the statistical power and prediction accuracy.[14]

The objective of this study is to calculate MRS that are derived from published EWAS, in order to classify cross-sectional, and predict prospective, childhood aeroallergen sensitization in the prospective German LISA birth cohort. For this, we evaluated (1) the predictive accuracy of six different MRS in both cross-sectional and prospective models, (2) their overlap and correlations and (3) compared their associations and prediction accuracy to other known determinants of allergic sensitization and individual CpG sites.

2 | METHODS

2.1 | Study population

We used data from the prospective German birth cohort on the Influence of Life-style factors on Development of the Immune System and Allergies in East and West Germany (LISA), which

recruited 3097 full-term healthy newborns of European ancestry between 1997 and 1999 in four study centers (Munich, Wesel, Leipzig and Bad Honnef). The study was approved by the local ethics committees (Bavarian General Medical Council, Medical Council for North-Rhine-Westphalia and the University of Leipzig), and informed parental consent was given. More information can be found elsewhere.[18]

Allergen-specific serum immunoglobulin E (IgE) concentrations were assayed by the CAP-RAST FEIA system (Pharmacia Diagnostics) according to the manufacturer's instructions and in line with global recommendations.[19] An overall screening test was used to test allergic sensitization against aeroallergens at 6, 10, and 15 years. Our outcome was defined by a specific IgE threshold of >0.35 kU/L (Radio-Allergo-Sorbent-Test [RAST] class 1) to the screening test of common aeroallergens (Dermatophagoides pteronyssinus, cat, dog, rye, timothy grass, Cladosporium herbarum, birch, and mugwort). Further RAST classes were defined according to common cutoffs,[20] where RAST 0 implies no allergic sensitization and RAST 5 or 6 (combined into one category) is the highest possible value. Questionnaire-based information on symptoms of rhino-conjunctivitis (concurrent running nose and itching eyes) and wheezing in the previous 12 months was collected at the same time points.

We assessed potential determinants of allergic diseases, which have been shown to be associated with different allergic diseases or lung function, such as parental education,[21] breastfeeding,[21,22] birth order,[2] pet holding,[21] maternal smoking during pregnancy,[23] environmental tobacco smoke exposure[23] (ETS) or bronchitis infections[23] in early childhood, as well as PRS calculated as weighted scores from genome-wide significant GWAS hits for any allergic disease,[1,24] asthma,[25] dermatitis,[26] allergic rhinitis,[27] and total IgE.[28] Additional information on the study design and on the definition of determinants of allergic diseases can be found in the Appendix S1 (Table S1, Methods 1).

2.2 | DNA methylation (DNAm) data

Samples using genomic DNA (gDNA) from blood clots at 6 and 10 years were analyzed using the MethylationEPIC BeadChip (Illumina, Inc.). Paired samples were placed on the same chip to avoid batch effects among pairs. CpGs on sex chromosomes and those having missing values low intensities were excluded. We used functional normalization[29] to normalize the data and ComBat[30] to adjust for technical variation. After outlier removal, the final dataset includes information on 774,330 CpG probes for 461 DNAm samples, 234 at six and 227 at 10 years, with an overlap of 221 participants with DNAm data at both time points. Cell type proportions were estimated both with the Houseman method[31] using a new reference panel[32] and with the EpiDISH[33] package, which additionally includes eosinophil estimates. Further information on processing and quality control can be found in the Appendix S1 (Methods 2 and Figure S1).

2.3 | Calculation of MRS

We calculated MRS based on the effect estimates or other summary statistics for CpG sites that have previously been associated with allergic diseases[6,8,10,11] or additionally provided summary statistics[7,9] for associations with up to a raw p-value of .1 for each EWAS. A weighted sum of DNAm beta values, defined as estimated methylation level, was then transformed to z-scores, and MRS were produced for each respective EWAS and differing p-value thresholds. A literature review identified EWAS of phenotypes related to atopy or high IgE. Further publications for any kind of allergic disease were included, if they were conducted in a larger consortium framework (asthma[10] and any allergic disease[11]). Seven MRS were calculated, one for high IgE,[7] one for aeroallergen sensitization,[8] two for atopy, defined as high total IgE or positive skin-prick test and sensitization, respectively,[6,9] one for asthma[10] and one for any allergic disease[11] as well as one MRS for schizophrenia[34] as negative control. In all seven EWAS, DNAm was measured in whole blood. Varying p-value thresholds from 1×10^{-1} to the lowest reported p-value per EWAS were considered, resulting in several scores per EWAS with a decreasing number of CpGs for smaller p-values, similar to what is known as "thresholding" for PRS.[14] To correct for correlations between included CpG sites, co-methylated regions were calculated using the CoMeBack method,[35] which identifies co-methylated regions based on correlation and proximity of CpGs. In accordance with the original publication, we did this based on residuals corrected for Houseman cell type proportions of the LISA study. Only one CpG per co-methylated region was included in the final MRS, a procedure similar to "clumping" in PRS approaches.[14] All MRS were calculated as z-scores following a standard normal distribution. A more detailed description is further provided in the supplementary information (Methods 3).

2.4 | Statistical analysis

Associations between each MRS and aeroallergen sensitization were estimated using logistic and Poisson regression with robust standard errors. Poisson regression was used to assess risk ratios (RR), as aeroallergen sensitization was not a rare outcome in our sample and thus odds ratios would not resemble RR. All models were adjusted for sex, age, whether the blood was taken in the allergy season (March to August), as current pollen exposure might influence DNAm[36] as well as circulating IgE levels, and estimated cell type proportions using EpiDISH. We applied the following criteria to evaluate and compare the performance of different MRS: 1) RR and corresponding 95% confidence intervals (95% CI) were used to evaluate the strength and accuracy of the association with aeroallergen sensitization; 2) C-statistic, the area-under-the-curve and 3) explained variance (Pseudo R^2) were used to evaluate the prediction accuracy for aeroallergen sensitization. The different MRS were compared and evaluated under four different scenarios: Two cross-sectional

models assessing the association at 6 and 10 years and two prospective models, assessing the association between the MRS and subsequent aeroallergen sensitization (MRS at 6 and 10 years as predictor of aeroallergen sensitization at 10 and 15 years, respectively). As a sensitivity analysis, the prospective models were calculated in the non-sensitized population only, excluding all participants with sensitization at the time of DNA methylation measurement, thereby analyzing only those who could develop new sensitization between the two time points. We furthermore calculated the receiver operating characteristic (ROC) for the cross-sectional analyses to assess the diagnostic ability of our MRS.

The best MRS per EWAS were selected based on the highest c-statistic in the cross-sectional model at 6 years. Correlations between the seven "best MRS" (one per EWAS) and the corresponding CpGs were evaluated. All CpGs reported in the available EWAS were tested for replication in the LISA study, both with the Houseman (as done in the original EWAS[6–10]) and EpiDISH cell type proportions, with successful replication being defined as a p-value below .05 after adjusting for the total number of tested CpGs from all EWAS using the Benjamini–Hochberg correction.[37]

Associations between the MRS and the six RAST classes were investigated using boxplots to evaluate a potential dose–response relationship with increasing levels of aeroallergen sensitization and ordinal logistic regression analyses.

To compare the strength of association and prediction accuracy of the MRS to those of other common determinants of allergic diseases (including allergy-related PRS, Table S1) and the most common single CpGs, we calculated the explained variance and strength of association (RR and 95% CI) with aeroallergen sensitization and compared it to the performance of the MRS.

We further assessed the association of all MRS with allergic disease symptoms, namely rhino-conjunctivitis and wheezing, using the same approach as described above. In addition, we calculated correlations between the MRS and the different estimated cell type proportions to assess whether a specific cell type was overrepresented in the MRS. In a sensitivity analysis, we tested the impact of co-methylated regions on the robustness of MRS: Namely, we calculated MRS with and without application of the CoMeBack method and used a reference population instead of the LISA study to determine the co-methylated regions (see Gatev et al., 2020[35] for details).

All statistical analyses were run in R[38] V4.1.2.

3 | RESULTS

3.1 | Description of study participants

We included 461 samples, collected from 240 participants of the LISA birth cohort, in our analysis, both from six (N = 234) and ten (N = 227) years of age (Table 1), of which 221 were paired with DNAm data available at both time points (Figure S1). The sample included slightly more males than females (58% vs. 42%) and the prevalence of rhino-conjunctivitis symptoms increased, while that of wheezing

symptoms decreased, between 6 and 10 years. Relevant outcome measures used in the 6-year sample are aeroallergen sensitization at 6 years (prevalence: 32.6%, 74 cases) and at 10 years (44.9%, 105 cases). In the 10-year sample, aeroallergen sensitization at 10 years (44.5%, 101 cases) and at 15 years (37%, 84 cases) were analyzed in the main analysis. Differences seen between the two time points are due to sample removal, as originally all samples were paired and are presenting the same baseline characteristics. Baseline characteristics from our analysis sample (N = 240) are similar to the total study population of the LISA Munich cohort (N = 1464, Table S2).

3.2 | Methylation risk scores

Table 2 shows information on the seven EWAS, phenotype, age group, and sample size from which MRS were calculated. The EWAS reported between 13 and 395 significant signals and varied by age, from 4 to 18 years, and ethnicity, covering not only European but also Hispanic and multi-ethnic populations. The best MRS per EWAS were selected based on the highest c-statistic in the cross-sectional model at 6 years across all p-value thresholds that were tested (Figure 1 and Table S3). The best-performing MRS included two (Everson2015,[6] atopy) to 24 (Zhang2019,[9] atopy) CpGs for p-value thresholds ranging from 1×10^{-4} (Zhang2019,[9] atopy) to 1×10^{-13} (Peng2019,[8] aeroallergens). CpG sites and the corresponding weights for the best MRS are listed in Table S4.

The six allergy-related MRS were highly correlated with each other but not with the negative control (age 6 years: Figure S3, age 10 years: Table S5). A total of seven CpGs are included in more than one allergy-related MRS, with cg11699125 being the most common one included in all but one allergy-related MRS. All of these seven CpGs could be successfully replicated in LISA (Table 3) and were annotated to the genes ZFPM1, ACOT7, MFHAS1, and SEC16B using UCSC reference genes from the Illumina annotation file. Replication of reported EWAS signals (1501 in total) in LISA at six (N = 234) and ten (N = 227) years yielded 554 and 288 replicated hits correcting for Houseman and 111 and one replicated hits correcting for EpiDISH cell type estimates, respectively. Thus, we observe highly reduced replication rates when including eosinophils as a cell type confounder. Of note, of the published EWAS,[6–11] only one (including the cohorts in PR-GOAL and GALA-II)[7] controlled for eosinophils in their analyses. Complete results can be found in Table S6.

3.3 | MRS as cross-sectional biomarkers

Figure 1 and Table S3 present results from evaluating MRS that were calculated based on different p-value thresholds and EWAS for the cross-sectional (age 6 and 10 years) as well as prospective analyses (6–10 years and 10–15 years). To improve clarity, Figure 1A presents the mean MRS over all p-value thresholds per EWAS. All allergy-related MRS were significantly associated with aeroallergen sensitization in LISA (Figure 1A). Effect sizes were very similar

	Analysis sample (N = 240)	
	DNAm measured at 6 years (N = 234)	DNAm measured at 10 years (N = 227)
Male sex, % (n) [n missing]	57.69% (135) [0]	57.71% (131) [0]
Age at DNA methylation measurement [years], mean (sd)	6.072 (0.15)	10.155 (0.14)
High parental education[a], % (n) [n missing]	79.49% (186) [2]	79.74% (181) [2]
Aeroallergen sensitization[b], % (n) [n missing]		
At 6 years	31.62% (74) [0]	29.96% (68) [0]
At 10 years	44.87% (105) [0]	44.49% (101) [0]
At 15 years	37.18% (87) [63]	37.00% (84) [60]
Allergic symptoms in last 12 months, % (n) [n missing]		
Rhino-conjunctivitis	8.55% (20) [1]	14.98% (34) [4]
Wheezing	10.68% (25) [2]	7.93% (18) [2]
Blood taken in allergy season (Mar - Aug), % (n) [n missing]		
At 6 years	67.52% (158) [0]	66.96% (152) [0]
At 10 years	52.99% (124) [0]	51.10% (116) [0]
At 15 years	41.45% (97) [55]	39.65% (90) [52]
Polygenic risk scores[c], mean (sd)		
Any allergic disease	0.187 (0.95)	0.187 (0.93)
Asthma	0.147 (1.04)	0.155 (1.05)
Dermatitis	0.002 (0.99)	−0.014 (0.99)
Rhinitis	0.023 (0.97)	0.052 (0.95)
Total IgE	0.083 (0.97)	0.084 (0.97)
Family history of allergic diseases, % (n) [n missing]		
No parent	29.06% (68) [24]	28.19% (64) [24]
One parent	42.74% (100) [24]	43.17% (98) [24]
Both parents	17.95% (42) [24]	18.06% (41) [24]
Other known risk factors for allergy, % (n) [n missing]		
Smoking during pregnancy	7.26% (17) [7]	7.05% (16) [6]
Breastfeeding in first 4 months	83.33% (195) [1]	83.70% (190) [1]
Older siblings	49.57% (116) [0]	48.90% (111) [0]
ETS[d] in first 4 years	25.64% (60) [3]	25.11% (57) [3]
Bronchitis infection in first 3 years	64.96% (152) [2]	66.96% (152) [2]
Cat or dog in first 4 years	19.66% (46) [8]	19.82% (45) [9]

Note: If time point of measurement is not mentioned, data were obtained from questionnaire data filled out by the LISA parents at birth, 1, 2, or 4 years of age.

[a]Defined as more than 10 years of education.

[b]Defined by a specific IgE threshold of >0.35 kU/L (Radio-Allergo-Sorbent-Test (RAST) class 1).

[c]z-scores.

[d]Environmental tobacco smoke exposure.

between different MRS ranging from RR = 1.47 [95% CI: 1.19; 1.84] to RR = 1.81 [1.44; 2.27] in the cross-sectional model at 6 years and from RR = 1.12 [0.87; 1.44] to RR = 1.40 [1.19; 1.64] at 10 years (Table S3). Classification accuracy (Figure 1B, c-statistic) was about 0.7 for all allergy-related MRS and the best scores explain more than 15% of variance in aeroallergen sensitization, quantified with pseudo R^2, at 6 years and more than 12% at 10 years (Figure 1C). The negative control (MRS for schizophrenia) was not associated with aeroallergen sensitization in LISA. The ROC curves display similar patterns for allergy-related MRS and the negative control performs worst (Figure S2).

3.4 | MRS as prospective predictors

In the prospective models, all allergy-related MRS are significantly associated with aeroallergen sensitization, even though the effect estimates are smaller than in the cross-sectional models (Figure 1D,A).

TABLE 2 Overview of included EWAS, their phenotypes (allergy-related outcome) and age groups

EWAS	Phenotype	No. of significant CpGs with FDR-corrected p-values <.05 [with raw p-values <.01[a]]	Sample size (discovery +replication)	Age	Ethnicity	p-value threshold for best MRS[b]	No. of included CpGs (best MRS)	Cohorts	Chip
Everson2015[6]	Atopy status (IgE ≥200 kU/L)	13	367 + 464	18	European	1×10^{-8}	2	IoW +BAMSE	450K
Chen2017[7]	Log10(IgE)	200 [25 089]	879 (meta)	6–22	Hispanic	1×10^{-8}	3	PR-GOAL, GALA II	450K
Peng2019[8]	Environmental allergen sensitization (≥0.35 IU/ml to common aeroallergens)	395	739 (total)	Mean: 7.7 & 9.8	Multi-ethnic	1×10^{-13}	5	Project Viva, Generation R	450K
Zhang2019[9]	Atopic status (≥3 mm greater than negative control in SPT or IgE ≥ 0.35 kU/L for mix inhalant of food allergens)	35 [775]	376 + 267	10 & 18	European	1×10^{-4}	24	IoW +BAMSE	450K/ EPIC
Reese2019[10]	Childhood asthma	179	3493 (meta)	Mean range: 7.1 to 17.01	Multi-Ethnic	1×10^{-8}	9	BAMSE, CHOP, GALA II, ICAC, NFBC 1986, PIAMA, RAINE, STOPPA	450K
Xu2021[11]	Any allergic disease (asthma, eczema, rhinitis) PLUS sensitization against common aeroallergens (≥0.35 IU/ml)	21	1457 + 1436	4 & 8	European	1×10^{-9}	18	BAMSE, INMA, PIAMA +EDEN, ECA, INMA, PIAMA, Karelia	450K
Hannon2016[34]	Schizophrenia (negative control)	2519	675 + 847	Adults	European	1×10^{-5}	867	UCL case-control +Aberdeen case-control, MZ twins cohort	450K

[a]Some studies provided additional summary statistics for all CpG sites with p-values <.01, n/a indicates that these additional summary statistics were not provided.
[b]Best MRS per EWAS were selected based on the highest c-statistic in the cross-sectional model at 6 years across all p-value thresholds that were tested (see also Table S2, where all MRS are evaluated).
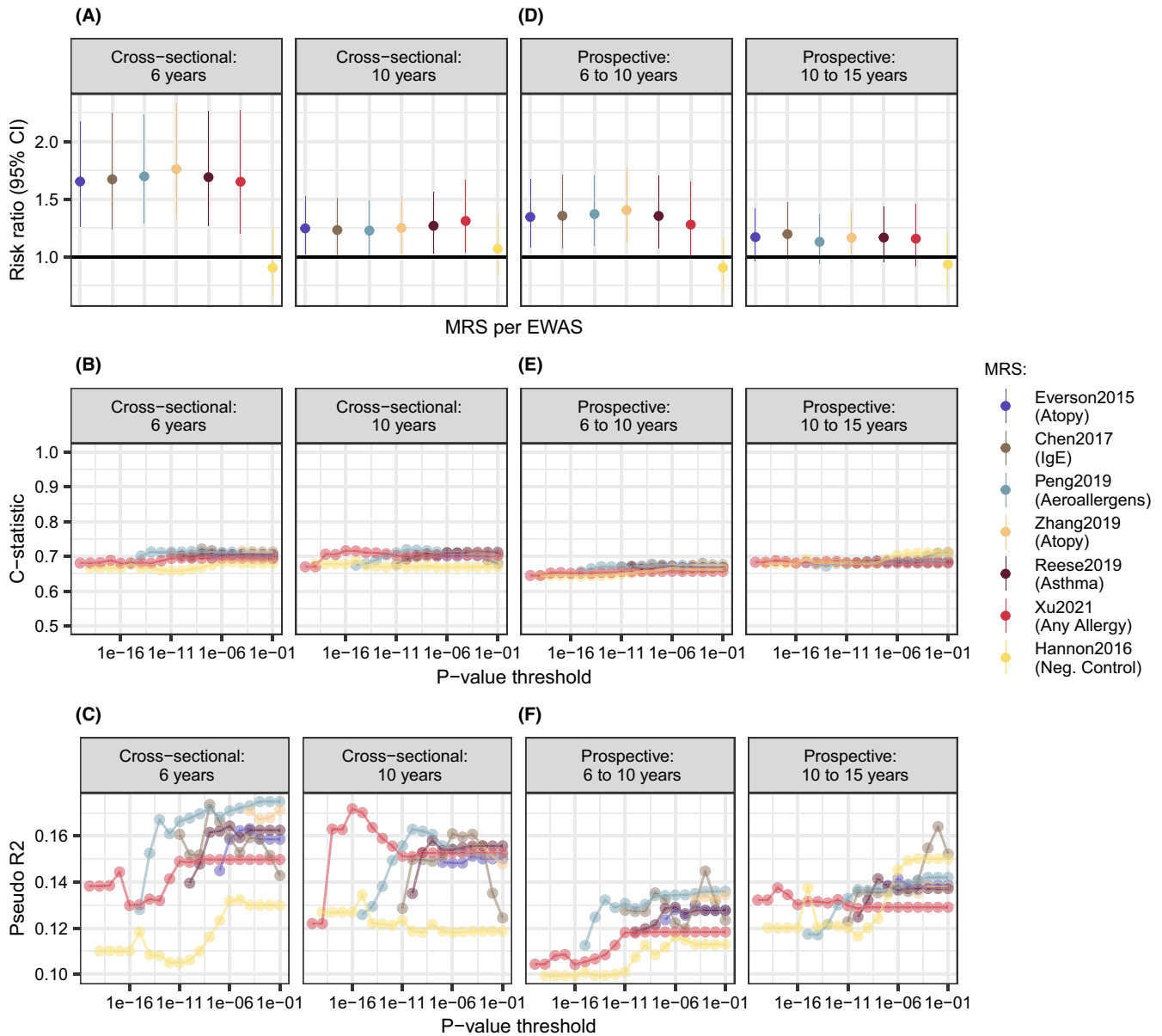
FIGURE 1 Predictive capabilities of MRS on aeroallergen sensitization. Four different models and criterions are displayed, assessing the cross-sectional and prospective impact of MRS as well as their (A) mean effect size per publication over all *p*-value thresholds, (B) performance and (C) explained variance for the different *p*-values thresholds (determining how many CpG sites were included in the MRS). All models are adjusted for sex, age, whether the blood was taken within the allergy season and cell type proportions. RR (A) were derived from Poisson regressions, whereas the other criterions (B&C) were calculated using logistic regression. Sample sizes for the four models were $n = 234$, $n = 227$, $n = 234$ and $n = 167$, respectively

The prediction accuracy and the explained variance of the prospective models was smaller than in the cross-sectional models. For example, the pseudo R^2 decreased from explaining roughly between 12% and 15% of the variance in the cross-sectional models to only 8%–12% in the prospective ones (Figure 1F). The c-statistic was also slightly lower with ~0.65 in the prospective models instead of ~0.7 in the cross-sectional models. In a sensitivity analysis, we analyzed whether prospective associations are observed because of participants that were already sensitized at the time of DNAm measurement. For this, we ran the prospective analyses restricted to the non-sensitized population only. Looking only at the non-sensitized population ($N = 160$

from 6 to 10, $N = 99$ from 10 to 15 years), the effect of MRS on prospective aeroallergen sensitization was further attenuated and no significant association was observed (Table S7). This might imply that DNAm is a consequence or biomarker of aeroallergen sensitization rather than a predictor of sensitization development.

3.5 | Dose–response relationship

Figure 2 shows a clear and significant positive trend between higher MRS and higher RAST classes in the two cross-sectional analyses,

TABLE 3 Overview of CpGs, which are present in more than one of the best-performing MRSs and their association with allergy-related outcomes in the original EWAS (A) and in the LISA cohort (replication, B).

CpG (gene)	Allergy-related outcome	cg04983687 (ZFPM1)	cg09249800 (ACOT7)	cg11699125 (ACOT7)	cg11988722 (intergenic)	cg12077460 (MFHAS1)	cg14011077 (intergenic)	cg17971251 (SEC16B)
A. Effect direction (p-values[a]) from original EWAS								
Everson2015[6]	Atopy	-(6.46E-09)	-(8.52E-09)					
Chen2017[7]	IgE	-(1.50E-12)	-(2.60E-11)	-(6.70E-07)				
Peng2019[8]	Aeroallergens	-(7.11E-15)		-(7.28E-12)	-(6.51E-14)			-(4.03E-11)
Zhang2019[9]	Atopy		-(1.17E-04)	-(4.34E-05)	-(9.04E-05)	-(2.98E-05)	-(2.83E-05)	-(1.46E-05)
Reese2019[10]	Asthma	-(1.33E-10)	-(1.19E-08)	-(7.54E-10)			-(7.02E-09)	-(9.52E-09)
Xu 2021[11]	Any allergy			-(5.84E-19)		-(3.73E-13)		
B. Beta coefficients (p-values[b]) in LISA								
LISA: 6 years	Aeroallergen sensitization	-11.61[c] (1.62E-03)	-14.71[c] (1.53E-02)	-11.94[c] (3.14E-02)	-21.59[c] (6.31E-04)	-19.65[c] (3.17E-03)	-40.89[c] (1.02E-02)	-26.84[c] (5.19E-04)
LISA: 10 years	Aeroallergen sensitization	-3.93 (1.62E-01)	-9.77[c] (2.79E-03)	-8.41[c] (2.47E-03)	-10.04[c] (2.35E-02)	-6.61 (4.27E-01)	-14.67 (1.22E-01)	-12.90[c] (8.01E-03)

Note: In A, effect direction (+/-) is reported instead of effect estimates because of the different allergy-related outcomes that were used in the original EWAS, which do not allow a direct comparison of effect estimates.

[a]Raw p-values corrected for confounding variables as per EWAS (check[6-11] for details).

[b]Raw p-values given corrected for Houseman cell type estimates.

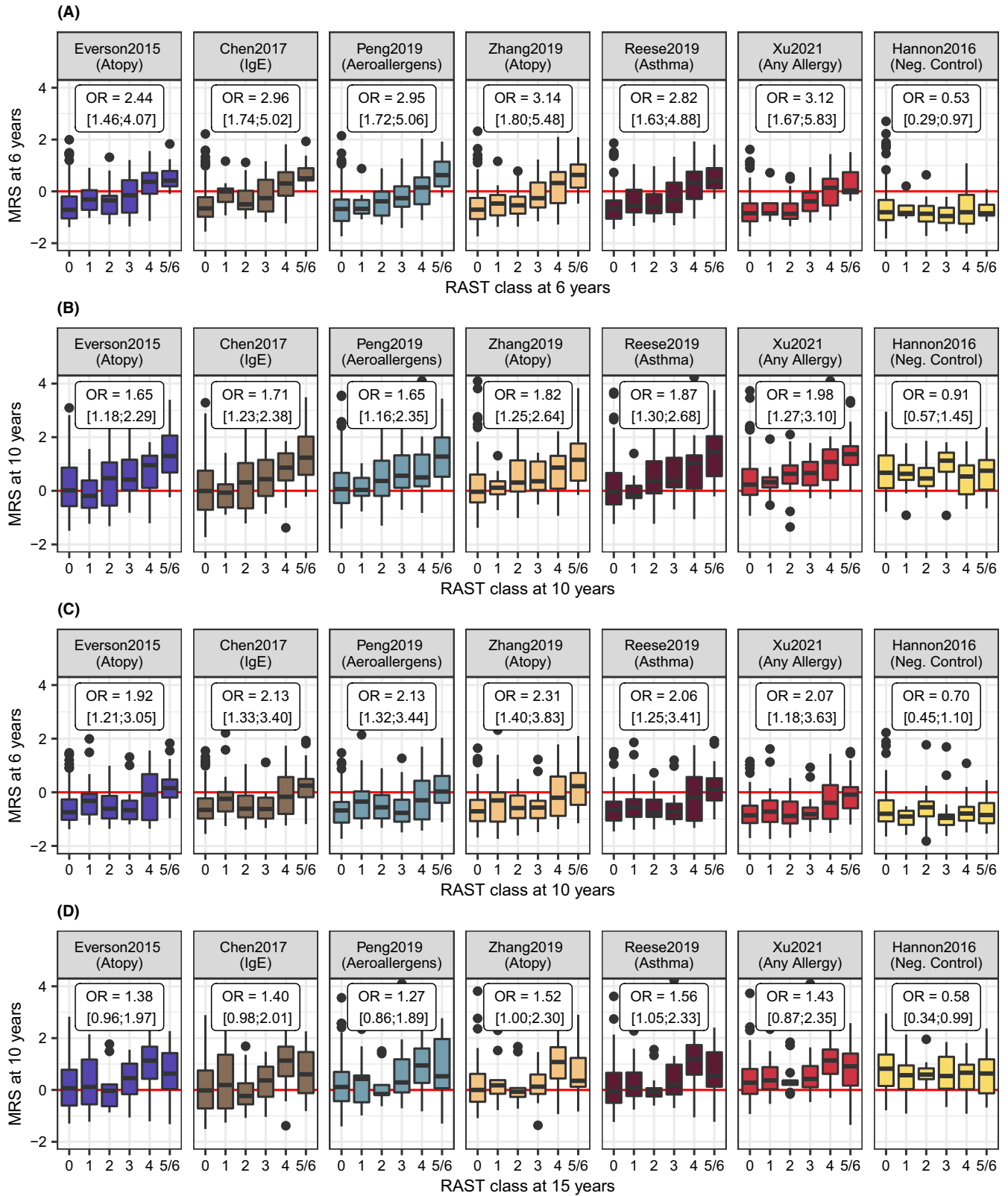[c]These CpGs were all successfully replicated in the LISA cohort (FDR threshold of 0.05).

FIGURE 2 Dose–response relationship of MRS z-scores and RAST classes of aeroallergen sensitization cross-sectionally at (A) 6 and (B) 10 years and prospectively from (C) 6 to 10 and (D) 10–15 years. The fifth and sixth RAST classes are combined due to the low sample size in the highest class. Odds ratios (OR) and 95% confidence intervals from ordinal logistic regression analysis of the association between RAST classes and MRS are displayed in their respective panels
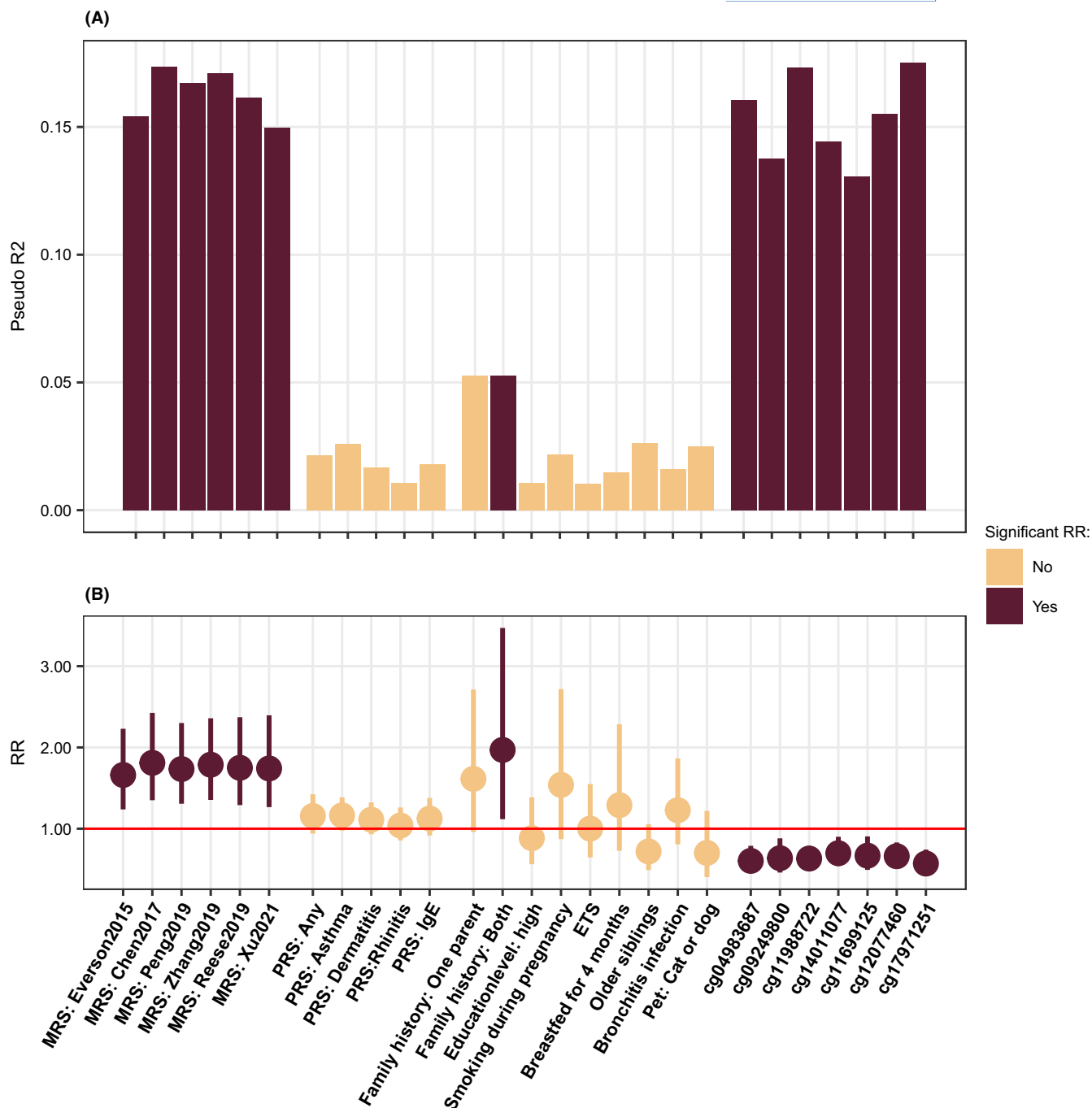
FIGURE 3 Prediction accuracy of MRS in comparison with other known risk factors. (A) Explained variance was assessed using logistic regression and (B) RR and 95% CI using Poisson regression with robust standard errors. For continuous variables (MRS, PRS, and CpGs z-scores were used; hence, the RR estimate can be understood per one standard error increase). All models were adjusted for sex, age and those with MRS and single CpGs additionally for cell type proportions and whether the blood was taken within the allergy season. Significance was determined in the Poisson model with a threshold of 0.05

except for the negative control (Figure 2A,B). This trend can be seen for all allergy-related MRS, independent of age group, ethnicity, or specific phenotype in the original EWAS. The trend was weaker in the prospective models (Figure 2C,D). The prospective trend from 6 to 10 years was significant for all allergy-related MRS, but with lower odds ratios than in the cross-sectional models. The prospective trend from 10 to 15 years was only significant for two of the six allergy-related MRS (Figure 2D and Table S8).

3.6 | Prediction accuracy of MRS in comparison with known determinants

As seen in Figures 1 and 3, the explained variance of allergy-related MRS is about 15% in the cross-sectional model at 6 years. Explained variance by other common determinants was lower, with family history of allergic diseases explaining around 5% and all others <3%, including PRS (Figure 3). Significant associations with aeroallergen

sensitization were only present for the MRS and having two parents with a history of allergic diseases. Of the seven CpGs, present in more than one MRS, all were significantly associated with a reduced risk for sensitization and the pseudo R^2 was similar to the MRS, especially for cg17971251 and cg11988722.

3.7 | Prediction accuracy of allergy-related MRS for other allergic symptoms

Associations between calculated MRS and allergic symptoms, such as rhino-conjunctivitis and wheezing, were weaker than associations with aeroallergen sensitization and those models explained less variance (pseudo R^2 <0.09 for rhino-conjunctivitis and <0.14 for wheeze) (Figure S4A,C). The prediction accuracy for rhino-conjunctivitis is similar to the accuracy for aeroallergen sensitization (c-statistic ~0.7). In contrast to this, the association between MRS and wheezing was stronger in terms of effect estimates and prediction accuracy. However, the higher RRs for wheezing and their wide CIs can also be attributed to the lower case numbers for allergic symptoms (Rhino-conjunctivitis: $n = 20$ and wheezing: $n = 25$ at 6 years), and these results should be interpreted cautiously (Figure S4 and Table S9). Results of best-performing MRS at 10 years and their association with symptoms of wheezing and allergic rhinitis can be found in Table S10.

3.8 | Correlations with cell type proportions

During bulk DNAm analysis, several different blood cell types with differing methylation profiles are analyzed. To assess whether a specific cell type is overrepresented in the MRS, we calculated correlations between the MRS and the different estimated cell type proportions. There was little correlation ($r \leq .3$) between the allergy-related MRS and estimated cell types apart from eosinophils ($r = [.53; .59]$), indicating that the MRS represent differential DNA methylation-related to aeroallergen sensitization independent of most immune cell types, apart from the known association with eosinophils[39] (Figure S5).

3.9 | Robustness of MRS to determination of co-methylated regions

In our main analysis, co-methylated regions were determined using the LISA cohort. Using a reference population instead of our own LISA cohort for the determination of co-methylation region (as described in[35]) or no filtering based on CoMeBack at all did not have an impact on our main results or the number of included CpGs (Tables S11 and S12). Individual CpGs included in the final MRS were correlated, despite application of the CoMeBack method, which only removes correlated CpG sites that are in close proximity to each other (Figure S6).

4 | DISCUSSION

In the present study, we calculated different MRS from available EWAS of atopy, high IgE, asthma, or any allergic disease and assessed their prediction accuracy for childhood aeroallergen sensitization using cross-sectional and prospective data on DNAm and sensitization from the German LISA study. We showed the superior performance of allergy-related MRS compared to well-known determinants of allergic diseases, like birth order, as well as their high correlation with each other. Seven CpGs were overlapping between the MRS, all located in previously reported genes associated with allergic diseases, and were successfully replicated in the LISA study. The best-performing MRS show a clear dose–response relationship with RAST classes of aeroallergen sensitization and explain more variance in aeroallergen sensitization than common determinants or PRS. However, we noticed differences between cross-sectional and prospective analyses, with the latter showing smaller effect sizes, lower prediction accuracy and less explained variance.

Our results fit with the accumulating evidence of improved disease definition using DNAm patterns and more specifically MRS as biomarkers for exposures[15] or predictor of diseases[16]

Looking at other determinants, MRS outperform them in explained variance, with about 15% of explained variance vs. <7% for the next best determinant. Similar values are achieved by the seven most commonly represented CpGs in the MRS. This highlights the role of DNAm as important allergy-specific factor. Even though other determinants of allergic disease have been widely established and are also included in clinical recommendations,[40] we could only observe significantly increased risk for the epigenetic factors and if both parents had a history of allergic diseases. Lack of associations with the other determinants could be explained by the relatively small sample size in this sub-sample of the LISA cohort. Furthermore, the low predictive capabilities of a PRS for asthma in childhood were published previously[41] and might underline our results of larger epigenetic associations as these lie on a level of omics closer to the phenotype.[42]

We found that the seven most important CpGs included in more than one MRS mapped to the genes *ZFPM1*, *ACOT7*, *MFHAS1*, and *SEC16B*, all of which have been reported in relation to allergic diseases.[43–46] The first three genes affect inflammatory responses through mast cell differentiation and development of cysteinyl leukotrienes. *ACOT7* has also been discussed as an important "cross-tissue allergy-associated methylation site" by one of the discovering EWAS.[11] The functional pathways of *SEC16B* have yet to be elucidated.

The MRS calculated for the 6-year data showed a stronger effect with aeroallergen sensitization at 6 years (cross-sectional analysis) than with aeroallergen sensitization at 10 years (prospective analysis; RR~1.7 vs. ~1.4). The RR were further attenuated and not significant anymore when analyzing only the non-sensitized population in both prospective models. This might indicate that the MRS are in fact not predictive of sensitization at a later time point but coincide with or follow aeroallergen sensitization and the prospective models only capture the effect of already sensitized participants at baseline.

However, the prospective analysis of the non-sensitized population is limited by a small sample size and thus limited statistical power. The positive trend between MRS and RAST classes seen in the cross-sectional models could not be seen in the prospective models, hence underlining the cross-sectional but not predictive nature of the association. A prospective prediction capability could have helped with early detection of allergic disease development, and future studies might evaluate the prospective capabilities of combining IgE and DNAm measurements to improve prediction of allergic disease development. Development of an enhanced predictive tool is of great interest in the context of personalized medicine and might include genetic and epigenetic aspects, as well as IgE as already available biomarker. Nevertheless, the observed cross-sectional classification capability of MRS underline the relevance of altered gene-regulation in allergic diseases, aligning with previous publications noting that DNAm changes are more often seen as a consequence rather than the cause of a disease and that especially SNP-CpG associations are not necessarily causal.[47]

We observed reduced replication rates of reported CpGs when adjusting for EpiDISH cell type estimates compared to the often used Houseman estimates (7.4% vs. 36.9% at 6 years). This might indicate that a high portion of previously seen associations may be attributable to eosinophils, which are not estimated in Houseman proportions. Notably, our MRS results remain significant even when adjusting for eosinophils, whereas EWAS replication is highly diminished.

In our study, we did not observe differences with ethnicity for our MRS in an population of European ancestry, as the MRS calculated from an EWAS of Hispanics with multiple racial backgrounds[7] performed just as well and sometimes even better than European-ancestry derived MRS. This aligns with the meta-analysis results from the EWAS on asthma conducted in the pregnancy and childhood epigenetics consortium,[48] which did not see any influence of ethnicity on detected CpG hits. We could, however, not evaluate the applicability of our MRS for non-European populations.

Taking into account, the relatively small sample sizes used in the applied EWAS ranging from only 367 samples in discovery[6] to 3493 used for meta-analysis,[10] the portion of replicated signals in the LISA study (38.8% at 6 years) indicates a good replicability of allergy-related EWAS results. Further, our MRS performed well over all included EWAS, independent of variation in ethnicity or age, ranging from childhood to young adulthood. EWAS results of allergy seem to be rather similar across childhood, indicated by replication of signals at 6 years, although only one EWAS obtained results in participants younger than 6 years,[11] while the others were mostly older (Table 2) and similar patterns in both cross-sectional models.

Robustness of our findings was also confirmed across the different phenotypes used in the published allergy-related EWAS. Although main phenotypes were similar, as our outcome is a direct categorization of aeroallergen sensitization measurements, even broader ones like total IgE[7] or any allergic disease[11] result in the same patterns. Especially the similar findings across EWAS of different phenotypes, for example, sensitization vs. asthma, might

hint in the direction of a general allergy phenotype,[49] also in terms of DNAm patterns. MRS were also associated with symptoms of allergic diseases in the LISA cohort, though associations with rhino-conjunctivitis and wheezing were weaker than for aeroallergen sensitization, likely due to the lower prevalence of these symptoms.

We recognize additional study limitations. We could not test the accuracy of MRS across different tissues (e.g., nasal epithelium), as there are, to the best of our knowledge, no respective large-scale EWAS available for calculating further MRS. However, previous publications could replicate their signals from whole blood in other allergy-relevant tissues.[10,50] Additionally, we extracted gDNA from blood clots, whereas other studies used whole blood, so predictive accuracy of proposed MRS might be even higher using identical sample processing methodology. Our MRS approach uses CoMeBack to remove correlated CpGs located in co-methylated regions from the MRS. Future studies should evaluate if the prediction accuracy of MRS can be further improved by considering all correlations between CpGs instead of only those located in close proximity to each other and does not account for trans-chromosomal correlations. Absence of significant associations between aeroallergen sensitization and established predictors of allergic diseases in our sub-cohort indicate a limited statistical power due to our relatively small sample size for this analysis. Therefore, future studies with larger sample sizes are needed to replicate our findings. However, the strong associations and prediction accuracy that we found for the MRS despite our relatively small study sample also demonstrates the applicability of this approach for small study populations and the robustness of previously reported EWAS results.

Strengths of this study include the objective assessment of aeroallergen sensitization in blood. This makes all of our main associations robust as neither aeroallergen sensitization diagnosis nor DNAm, estimated cell type proportions or sex are subject to recall bias. Moreover, the prospective design of the LISA study enabled us to compare repeated measures at two time points of DNAm with three time points of measured aeroallergen sensitization.

In summary, we established well-working MRS for aeroallergen sensitization, which outperform commonly known determinants in identifying the disease. The presented results confirm the association of DNAm at some CpGs with allergic diseases and underline the relevance of altered gene-regulation in allergic diseases. The results support replication and applicability of available EWAS results and pave the way for future analyses investigating the specific functions between methylation patterns as biomarkers of disease manifestation.

CONFLICT OF INTEREST

Dr. Celedón has received research materials from Pharmavite, GSK, and Merck in order to provide medications free of cost to participants in NIH-funded studies, unrelated to the current work. All other authors have no further conflicts of interest to declare.

AUTHOR CONTRIBUTION

A.H., M.S., and A.K. conceived the idea for the presented study. A.K. conducted the analyses and wrote the initial paper draft. A.H. conceptualized the methodology and supervised the analyses. R.W. and N.G. helped with data processing of DNAm data. E.T and T.E. provided input on the statistical analysis. M.W. answered questions regarding laboratory sample processing. J.C. wrote the initial code for calculation of MRS and H.Z. J.C.C and E.B. provided further summary statistics on the used EWAS results. A.H., M.S., and A.P. jointly supervised the present project. All authors revised and commented on the final manuscript version.

DATA AVAILABILITY STATEMENT

Due to data protection reasons, the datasets generated and/or analyzed during the current study cannot be made publicly available. The datasets are available to interested researchers from the corresponding author on reasonable request, provided the release is consistent with the consent given by the LISA study participants. Ethical approval might be obtained for the release and a data transfer agreement from the legal department of the Helmholtz Zentrum München must be accepted.

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REFERENCES

1. Ferreira MAR, Vonk JM, Baurecht H, et al. Eleven loci with new reproducible genetic associations with allergic disease risk. *J Allergy Clin Immunol.* 2019;143(2):691-699. doi:10.1016/j.jaci.2018.03.012
2. Nicolaou NC, Simpson A, Lowe LA, Murray CS, Woodcock A, Custovic A. Day-care attendance, position in sibship, and early childhood wheezing: a population-based birth cohort study. *J Allergy Clin Immunol.* 2008;122(3):500-506.e5. doi:10.1016/j.jaci.2008.06.033
3. Strachan DP. Hay fever, hygiene, and household size. *Br Med J.* 1989;299(6710):1259-1260. doi:10.1136/bmj.299.6710.1259
4. Chen CM, Rzehak P, Zutavern A, et al. Longitudinal study on cat allergen exposure and the development of allergy in young children. *J Allergy Clin Immunol.* 2007;119(5):1148-1155. doi:10.1016/j.jaci.2007.02.017
5. Long A, Bunning B, Sampath V, DeKruyff RH, Nadeau KC. Epigenetics and the environment in airway disease: asthma and allergic rhinitis. *Adv Exp Med Biol.* 2020;1253:153-181. doi:10.1007/978-981-15-3449-2_6
6. Everson TM, Lyons G, Zhang H, et al. DNA methylation loci associated with atopy and high serum IgE: a genome-wide application of recursive Random Forest feature selection. *Genome Med.* 2015;7(1):89. doi:10.1186/s13073-015-0213-8
7. Chen W, Wang T, Pino-Yanes M, et al. An epigenome-wide association study of total serum immunoglobulin E in Hispanic children. *J Allergy Clin Immunol.* 2017;140(2):571-577. doi:10.1016/j.jaci.2016.11.030
8. Peng C, Van Meel ER, Cardenas A, et al. Epigenome-wide association study reveals methylation pathways associated with childhood allergic sensitization. *Epigenetics.* 2019;14(5):445-466. doi:10.1080/15592294.2019.1590085
9. Zhang H, Kaushal A, Merid SK, et al. DNA methylation and allergic sensitizations: a genome-scale longitudinal study during adolescence. *Allergy.* 2019;74(6):1166-1175. doi:10.1111/all.13746
10. Reese SE, Xu CJ, den Dekker HT, et al. Epigenome-wide meta-analysis of DNA methylation and childhood asthma. *J Allergy Clin Immunol.* 2019;143(6):2062-2074. doi:10.1016/j.jaci.2018.11.043
11. Xu CJ, Gruzieva O, Qi C, et al. Shared DNA methylation signatures in childhood allergy: the MeDALL study. *J Allergy Clin Immunol.* 2021;147(3):1031-1040. doi:10.1016/j.jaci.2020.11.044
12. Bin L, Leung DYM. Genetic and epigenetic studies of atopic dermatitis. *Allergy Asthma Clin Immunol.* 2016;12:52. doi:10.1186/s13223-016-0158-5
13. Khera AV, Chaffin M, Aragam KG, et al. Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations. *Nat Genet.* 2018;50(9):1219-1224. doi:10.1038/s41588-018-0183-z
14. Hüls A, Czamara D. Methodological challenges in constructing DNA methylation risk scores. *Epigenetics.* 2019;15:1-11. doi:10.1080/15592294.2019.1644879
15. Elliott HR, Tillin T, McArdle WL, et al. Differences in smoking associated DNA methylation patterns in South Asians and Europeans. *Clin Epigenetics.* 2014;6(1):4. doi:10.1186/1868-7083-6-4
16. Villanueva A, Portela A, Sayols S, et al. DNA methylation-based prognosis and epidrivers in hepatocellular carcinoma. *Hepatology.* 2015;61(6):1945-1956. doi:10.1002/hep.27732
17. Neste LV, Partin AW, Stewart GD, Epstein JI, Harrison DJ, Criekinge WV. Risk score predicts high-grade prostate cancer in DNA-methylation positive, histopathologically negative biopsies. *Prostate.* 2016;76(12):1078-1087. doi:10.1002/pros.23191
18. Heinrich J, Brüske I, Cramer C, et al. GINIplus and LISAplus. Design and selected results of two German birth cohorts about natural course of atopic diseases and its determinants. *Allergol Sel.* 2017;1(1):85-95. doi:10.5414/ALX01455E

19. Ansotegui IJ, Melioli G, Canonica GW, et al. IgE allergy diagnostics and other relevant tests in allergy, a World Allergy Organization position paper. *World Allergy Organ J*. 2020;13(2):100080. doi:10.1016/j.waojou.2019.100080

20. Hoffman DR. Comparison of methods of performing the radioallergosorbent test: Phadebas, Fadal-Nalebuff and Hoffman protocols. *Ann Allergy*. 1980;45(6):343-346.

21. Hu C, Duijts L, Erler NS, et al. Most associations of early-life environmental exposures and genetic risk factors poorly differentiate between eczema phenotypes: the Generation R Study. *Br J Dermatol*. 2019;181(6):1190-1197. doi:10.1111/bjd.17879

22. Paternoster L, Savenije OEM, Heron J, et al. Identification of atopic dermatitis subgroups in children from 2 longitudinal birth cohorts. *J Allergy Clin Immunol*. 2018;141(3):964-971. doi:10.1016/j.jaci.2017.09.044

23. Luzak A, Fuertes E, Flexeder C, et al. Which early life events or current environmental and lifestyle factors influence lung function in adolescents? – Results from the GINIplus & LISAplus studies. *Respir Res*. 2017;18:138. doi:10.1186/s12931-017-0619-5

24. Ferreira MA, Vonk JM, Baurecht H, et al. Shared genetic origin of asthma, hay fever and eczema elucidates allergic disease biology. *Nat Genet*. 2017;49(12):1752-1757. doi:10.1038/ng.3985

25. El-Husseini ZW, Gosens R, Dekker F, Koppelman GH. The genetics of asthma and the promise of genomics-guided drug target discovery. *Lancet Respir Med*. 2020;8(10):1045-1056. doi:10.1016/S2213-2600(20)30363-5

26. Paternoster L, Standl M, Waage J, et al. Multi-ancestry genome-wide association study of 21,000 cases and 95,000 controls identifies new risk loci for atopic dermatitis. *Nat Genet*. 2015;47(12):1449-1456. doi:10.1038/ng.3424

27. Waage J, Standl M, Curtin JA, et al. Genome-wide association and HLA fine-mapping studies identify risk loci and genetic pathways underlying allergic rhinitis. *Nat Genet*. 2018;50(8):1072-1080. doi:10.1038/s41588-018-0157-1

28. Granada M, Wilk JB, Tuzova M, et al. A genome-wide association study of plasma total IgE concentrations in the Framingham heart study. *J Allergy Clin Immunol*. 2012;129(3):840-845.e21. doi:10.1016/j.jaci.2011.09.029

29. Fortin JP, Labbe A, Lemire M, et al. Functional normalization of 450k methylation array data improves replication in large cancer studies. *Genome Biol*. 2014;15(12):503. doi:10.1186/s13059-014-0503-2

30. Johnson WE, Li C, Rabinovic A. Adjusting batch effects in microarray expression data using empirical Bayes methods. *Biostat Oxf Engl*. 2007;8(1):118-127. doi:10.1093/biostatistics/kxj037

31. Houseman EA, Accomando WP, Koestler DC, et al. DNA methylation arrays as surrogate measures of cell mixture distribution. *BMC Bioinformatics*. 2012;13:86. doi:10.1186/1471-2105-13-86

32. Salas LA, Koestler DC, Butler RA, et al. An optimized library for reference-based deconvolution of whole-blood biospecimens assayed using the Illumina HumanMethylationEPIC BeadArray. *Genome Biol*. 2018;19:64. doi:10.1186/s13059-018-1448-7

33. Teschendorff AE, Breeze CE, Zheng SC, Beck S. A comparison of reference-based algorithms for correcting cell-type heterogeneity in Epigenome-Wide Association Studies. *BMC Bioinformatics*. 2017;18:105. doi:10.1186/s12859-017-1511-5

34. Hannon E, Dempster E, Viana J, et al. An integrated genetic-epigenetic analysis of schizophrenia: evidence for co-localization of genetic associations and differential DNA methylation. *Genome Biol*. 2016;17(1):176. doi:10.1186/s13059-016-1041-x

35. Gatev E, Gladish N, Mostafavi S, Kobor MS. CoMeBack: DNA methylation array data analysis for co-methylated regions. *Bioinforma Oxf Engl*. 2020;36(9):2675-2683. doi:10.1093/bioinformatics/btaa049

36. North ML, Jones MJ, MacIsaac JL, et al. Blood and nasal epigenetics correlate with allergic rhinitis symptom development in the environmental exposure unit. *Allergy*. 2018;73(1):196-205. doi:10.1111/all.13263

37. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Ser B Methodol*. 1995;57(1):289-300.

38. R Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing; 2019. https://www.R-project.org/.

39. Martin LB, Kita H, Leiferman KM, Gleich GJ. Eosinophils in allergy: role in disease, degranulation, and cytokines. *Int Arch Allergy Immunol*. 1996;109(3):207-215. doi:10.1159/000237239

40. Schäfer T, Bauer CP, Beyer K, et al. S3-Guideline on allergy prevention: 2014 update. *Allergo J Int*. 2014;23(6):186-199. doi:10.1007/s40629-014-0022-4

41. Dijk FN, Folkersma C, Gruzieva O, et al. Genetic risk scores do not improve asthma prediction in childhood. *J Allergy Clin Immunol*. 2019;144(3):857-860.e7. doi:10.1016/j.jaci.2019.05.017

42. Ritchie MD, Holzinger ER, Li R, Pendergrass SA, Kim D. Methods of integrating data to uncover genotype–phenotype interactions. *Nat Rev Genet*. 2015;16(2):85-97. doi:10.1038/nrg3868

43. Kitamura N, Mori A, Tatsumi H, Nemoto S, Hiroi T, Kaminuma O. Zinc finger protein, multitype 1, suppresses human Th2 development via downregulation of IL-4. *Int Arch Allergy Immunol*. 2011;155(Suppl 1):53-56. doi:10.1159/000327292

44. Ferreira GB, Overbergh L, van Etten E, et al. Protein-induced changes during the maturation process of human dendritic cells: a 2-D DIGE approach. *PROTEOMICS – Clin Appl*. 2008;2(9):1349-1360. doi:10.1002/prca.200800110

45. Zhong J, Wang H, Chen W, et al. Ubiquitylation of MFHAS1 by the ubiquitin ligase praja2 promotes M1 macrophage polarization by activating JNK and p38 pathways. *Cell Death Dis*. 2017;8(5):e2763. doi:10.1038/cddis.2017.102

46. Laffleur B, Duchez S, Tarte K, et al. Self-Restrained B cells arise following membrane IgE expression. *Cell Rep*. 2015;10(6):900-909. doi:10.1016/j.celrep.2015.01.023

47. Min JL, Hemani G, Hannon E, et al. Genomic and phenotypic insights from an atlas of genetic effects on DNA methylation. *Nat Genet*. 2021;53(9):1311-1321. doi:10.1038/s41588-021-00923-x

48. Felix JF, Joubert BR, Baccarelli AA, et al. Cohort profile: pregnancy and childhood epigenetics (PACE) consortium. *Int J Epidemiol*. 2018;47(1):22-23u. doi:10.1093/ije/dyx190

49. Anto JM, Bousquet J, Akdis M, et al. Mechanisms of the development of allergy (MeDALL): introducing novel concepts in allergy phenotypes. *J Allergy Clin Immunol*. 2017;139(2):388-399. doi:10.1016/j.jaci.2016.12.940

50. Hoang TT, Sikdar S, Xu CJ, et al. Epigenome-wide association study of DNA methylation and adult asthma in the agricultural lung health study. *Eur Respir J*. 2020;56(3):2000217. doi:10.1183/13993003.00217-2020

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