Immunobiology 228 (2023) 152724

Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.com/locate/imbio

PDE4D gene variants and haplotypes are associated with asthma and atopy in Brazilian children

Hatilla dos Santos Silva [a], Helena Mariana Pitangueira Teixeira [a], Luciano Gama da Silva Gomes [a], Álvaro A. Cruz [b], Neuza Maria Alcantara-Neves [a], Maurício Barreto [c,d], Camila Alexandrina Figueiredo [a], Ryan dos Santos Costa [a,*]

[a] *Instituto de Ciências da Saúde, Universidade Federal da Bahia (UFBA), Salvador, Brazil*
[b] *Fundação ProAR, Bahia, Brazil*
[c] *Centro de Integração de Dados e Conhecimento para Saúde (CIDACS), Fiocruz, Salvador, Bahia, Brazil*
[d] *Instituto de Saúde Coletiva, Universidade Federal da Bahia, Salvador, Brazil*

ARTICLE INFO

Keywords:
Variants
Phosphodiesterase
cAMP
Asthma
PDE4D
Atopy
IL-10

ABSTRACT

PDE4D (Phosphodiesterase 4D) gene encodes a hydrolase of cyclic AMP. *PDE4D* genetic variants have been associated with asthma susceptibility. Therefore, this study aimed to investigate the association between *PDE4D* variants (and haplotypes) with asthma and atopy in a Brazilian population. The study comprised 1,246 unrelated participants from the SCAALA (Social Changes Asthma and Allergy in Latin America) program. Genotyping was performed using the Illumina 2.5 Human Omni bead chip. Multivariate logistic regression was used to investigate the association between *PDE4D* variants and asthma/atopy phenotypes in PLINK 1.09 software. Twenty-four SNVs in *PDE4D* were associated with atopy or asthma. The rs6898082 (A) variant increased asthma susceptibility (OR 2.76; CI 99% 1.26–6.03) and was also related to a greater *PDE4D* expression in the GTEx database. Also, the variant rs6870632 was further associated with asthma in meta-analysis with a replication cohort. In addition, the variants rs75699812 (C), rs8007656 (G), and rs958851 (T) were positively associated with atopy. Moreover, these variants formed an atopy risk haplotype (OR 1.82; CI 99% 1.15–2.88). Also, these variants were related to lower levels of IL-10. Functional *in silico* assessment showed that some *PDE4D* SNVs may have an impact on gene regulation and expression. Variants in the *PDE4D* are positively associated with asthma and allergy markers. It is possible that these variants lead to alteration in *PDE4D* expression and therefore impact immunity and pulmonary function.

1. Introduction

Asthma is a chronic respiratory disorder that affects approximately 358 million people worldwide and is considered the most prevalent chronic condition in children. (GBD, 2015; Ferrante and La Grutta, 2018) In Brazil, the prevalence of asthma symptoms is estimated at 23.4% among children aged 6–7 years old. (Solé et al., 2014) Asthma is characterized as a pulmonary obstructive disease, in which the inflammation causes airway hyperresponsiveness, thus eliciting symptoms such as chest tightness, cough, shortness of breath, and wheezing. (Global Initiative for Asthma, 2021) Asthma in children is traditionally associated with atopic conditions, with the immune response to common aeroallergens proposed as an immunopathogenic mechanism (Hammad and Lambrecht, 2021).

In susceptible individuals, atopic conditions are triggered by common environmental allergens such as house dust mite, cockroach and animal dander. Sensitization involves allergens being processed by dendritic cells and presented to CD4+ T cells, which differentiate into a Th2 (T-helper 2) lymphocytes that mainly produce IL(Interleukin)-4, IL-5, IL-9, and IL-13. (Hammad and Lambrecht, 2021; Morianos and Semitekolou, 2020) Moreover, Th2 cytokines are related to the induction of eosinophilia, mast cell hyperplasia, and allergen-specific B cells to produce immunoglobulin E (IgE) antibodies. (Morianos and Semitekolou, 2020) Upon an allergen re-exposure, crosslinking of IgE antibodies to the high-affinity FcεR on the surface of mast cells and basophils leads to its activation and release of several inflammatory mediators

* Corresponding author at: Instituto de Ciências da Saúde, Universidade Federal da Bahia, Avenida Reitor Miguel Calmon, s/n, Vale do Canela, CEP-40110-100 Salvador, Bahia, Brazil.
E-mail address: ryan.costa@ufba.br (R.S. Costa).

https://doi.org/10.1016/j.imbio.2023.152724
Received 30 January 2023; Received in revised form 20 July 2023; Accepted 30 July 2023
Available online 3 August 2023

(Morianos and Semitekolou, 2020).

Asthma and atopy are both considered heterogeneous and complex conditions in which the contribution of genetic background in its susceptibility has been demonstrated by the association of several genetic *loci* with asthma and atopic markers. (Morii, 2023; Portelli et al., 2015; Willis-Owen et al., 2018) Among such *loci*; *PDE4D* (Phosphodiesterase type 4D) gene was identified as an asthma susceptibility gene in a Genome-Wide Association Studies (GWAS), in which the results were replicated using independent populations. (Himes, 2009) Also, one of the variants associated was posteriorly related to severity of airway hyperresponsiveness in asthma patients (El-Husseini et al., 2023).

PDE4D is a member of cyclic nucleotide phosphodiesterases (PDEs) superfamily of cAMP-degrading specific enzymes. The PDE4D protein plays a role downstream in the pathway of β2 adrenergic receptor (β2AR), which promotes an increase in cAMP levels by stimulating adenylyl cyclase activity. (Trian et al., 2011) In airway smooth muscle (ASM) cells elevated cAMP levels activate PKA (protein kinase A) leading to a bronchodilator effect that is therapeutically utilized to manage asthma symptoms. Therefore, PDE4D is the main phosphodiesterase subtype involved in the negative regulation of bronchial contractility. (Méhats, 2003) Studies have shown that individuals with asthma or atopy have a greater expression and activity of PDE4D in both ASM and immune cells than individuals without such conditions.(Trian et al., 2011; Landells et al., 2000) Furthermore, PDE4D is significantly upregulated in PBMCs of children with atopic dermatitis when compared to age-matched healthy control (Nousbeck et al., 2023).

The PDE4D activity is inherently related to T-cell function and has a significant impact on T regulatory cells (Tregs). Research has revealed that PDE4D is less expressed in Tregs compared to T conventional cells, indicating its importance in Treg function (Hua et al., 2015; Dolina et al., 2022). These findings support the role of cAMP in the inhibitory function of Treg cells, mediated by cAMP-dependent signaling pathway through several effector mechanisms, such as IL-10 production. The immunomodulatory function exhibited by IL-10 in atopy and asthma is well established, especially by influencing immune responses promoting immunological tolerance and regulating effector responses associated with asthma and atopy (Zhang et al., 2022; Bodor et al., 2012). Other important T-reg cAMP-dependent mechanisms are related to the direct suppression of antigen presentation cells (APCs) and T effector cells, limiting the differentiation into Th subsets, such as the Th2. Also, dendritic cells have their Th2 priming ability dependent on low cAMP levels. (Lee, 2015) Nevertheless IL-10 production can also be seen in other immune cells, such as dendritic cells and monocytes.

Variants in genes involved in the adrenergic pathway, including in *PDE4D*, have already been shown to influence asthma susceptibility (Himes, 2009; Ding, 2013). Some of these studies were conducted in Brazil, although none have analyzed the *PDE4D* (Teixeira et al., 2017; De Paiva et al., 2014). Thus, this study aimed to investigate the association between SNVs (Single Nucleotide Variants) on *PDE4D* gene with asthma symptoms and atopy in a Brazilian population. We performed a SNV and haplotype-based genetic association analysis considering a case-control design. Also relation between associated SNVs and cytokines production in whole blood culture was assessed. Finally, we performed a replication in an independent asthma Brazilian cohort followed by a meta-analysis including both populations.

2. Material and methods

2.1. Study population and design

The study comprised 1,246 unrelated children aged 4–11 years old enrolled in SCAALA (Social Change, Asthma, Allergy in Latin American) Brazilian cohort (Alcantara-Neves et al., 2012; Figueiredo, 2009). The project was approved by the ethics committee of the Federal University of Bahia (registry 003-05/CEP-ISC) and by the National Council of Ethics in Research (CONEP, resolution number 15 895/2011).

2.2. Asthma and atopy definition

A Portuguese-adapted version of the ISAAC questionnaire was used to classify the children into case/control. Cases were defined by at least one of the following parameters in the last 12 months: clinical diagnosis of asthma; occurrence of wheezing with exercise; four or more episodes of wheezing; waking up at night because of wheezing.

Serum IgE against the most common aeroallergens found in Salvador such as mites (*Dermatophagoides pteronyssinus* and *Blomia tropicalis*) and cockroaches (*Periplaneta americana* and *Blattella germanica*), were evaluated as previously described by Alcantara-Neves and collaborators (2012) (Zhang et al., 2022). Children who had positive IgE test for at least one allergen were defined as atopic.

2.3. Spontaneous IL-10 production

Whole blood cells were cultivated (1:4) in supplemented RPMI at 37 °C, 5% of CO_2 for 24 h in order to detect IL-10 production and 5 days for IL-5 and IL-13. The optimal condition for each cytokine detection in whole-blood culture was previously determined via a standardization process in our laboratory (Figueiredo, 2009). Supernatant fluid was collected, and cytokines levels were determined using Pharmingen BD antibody pairs and recombinant standards (Pharmingen, San Diego, Ca, USA), by capture ELISA following the manufacturer's instructions (Figueiredo, 2009).

2.4. DNA extraction and genotyping

DNA was extracted from peripheral blood using a commercial kit (Flexigene DNA Kit, Qiagen, Hilden, Germany) following the protocol recommended by the manufacturer. Genotyping was performed using the Illumina 2.5 Human Omni bead chip. Genetic markers in *PDE4D* were extracted considering the location 58,264,865 to 59,784,925 on chromosome 5 (GRCh37). Quality control was posteriorly applied in markers using the exclusion filters: genotyping rate < 0.9; Hardy-Weinberg Equilibrium deviation ($p < 0.0001$); minor allele frequency (MAF) < 0.05.

2.5. Statistical analysis

Association analysis was conducted in PLINK 1.09 software by multiple logistic regression for each genetic variant, including age, sex, helminth infection, and genetic ancestry as covariates. Considering that Brazilian populations are highly admixed, Principal Component Analysis (PCA) was performed to generate the two first PCs, which were included in the regression to control potential genetic ancestry influence. Analysis was carried out in three inheritance models (additive, dominant and recessive) and considered significant if the p-value was <0.01. Furthermore, an adaptive permutation approach was used to solve the problem of multiple tests and control the false discovery rate (Purcell et al., 2007), since we analyzed variants separately.

Linkage Disequilibrium (LD) plot was obtained using the Haploview software. Haplotype-based association analysis was performed using the R package haplo.stats (Sinnwell et al., xxxx) by a multiple logistic regression. A sliding window approach (2 – 5 SNVs per window) was performed considering a window framework grouping neighboring SNVs, the framework slid across the region shifting one SNV at time. All comparisons considered the protective haplotype as the baseline. A graphical assessment of the haplotype analysis was generated using the R package snp.plotter (Luna et al., 2007). Haplotype analysis was carried out considering the genetic model of the associated variants.

The normality of cytokine levels was assessed, thus, cytokine production was further compared among genotypes according to Mann-Whitney or Kruskal-Wallis tests. Only variants associated with asthma or atopy were investigated with regard to IL-5, IL-13, and IL-10 production. Also, an alpha of 0.01 was used as the cutoff for significance

results.

2.6. In silico analysis

GTEx database was used to investigate the impact of the variants on gene expression. Therefore, it permits to identify eQTL according to a genotype and relative expression of the gene (GTEX Consortium and et al., 2015).

2.7. Replication and meta-analysis

Variants with significant p-values in the SCAALA cohort and genotyped in the Brazilian replication cohort (Program for Control of Asthma in Bahia - PROAR) were tested by using multiple logistic regression analysis as described before. In brief, this is a case-control designed cohort including adult individuals with doctor-diagnosed mild or severe asthma and paired control individuals without asthma (Lima-Matos et al., 2018). Regression analysis for both asthma and atopy, included as covariates age, sex, and first principal component to adjust for population stratification.

Meta-analysis was carried out in METAL software, which performs weighted analysis based on summary statistics of genetic association studies. For joint analysis, variant effect (Odds Ratio), standard error, p-value and effective sample sizes were used from both cohorts. Effective sample sizes were calculated by using the formula recommended by authors ($N_{effetive} = 4/(1/N_{cases} + 1/N_{controls})$ in order to correct for unbalanced case-control ratio (Willer et al., 2010).

3. Results

3.1. Study population

Characteristics of the study population are summarized in Table 1. We observed greater proportions of children with asthma in the younger group. The proportion of atopy was also greater in asthma than in non-asthma. No difference was found in gender and helminth infection between groups.

3.2. Description of the PDE4D variants

In the *PDE4D* region 1,180 SNVs were genotyped. Of these, 454 variants were excluded by MAF, and one variant was due to genotyping call rate. After quality control, 727 variants were included in the analysis. Table 2 provides genetic and functional information for the associated SNVs with asthma or atopy using Haploreg (Ward and Kellis, 2012) and RegulomeDB (Boyle, 2012) databases.

3.3. Single marker and haplotype-based association of PDE4D with asthma

Twelve SNVs were significantly associated with asthma symptoms (Table 3). Using an additive model, the variants rs9968728-C and rs298024-A were negatively associated, while rs1824154-A, rs6896215-G, and rs16889869-G increased asthma risk. All variants associated in additive model were also associated in the dominant model, except the rs9968728-C (Table 3). In addition, rs33950471-G and rs16889878-A were positively associated with asthma in the dominant model. On the other hand, rs2112910-A, rs6870632-A, and rs12188950-T were negatively associated with asthma. Regarding the recessive model, rs34762247-G was negatively associated, while rs6898082-A increased asthma risk (Table 3). Fig. S1 shows the pairwise LD plot of associated SNVs.

Regarding the haplotype analysis, eight haplotypes were associated with asthma in the additive model (Table 5, Fig. S2.A) and nineteen using the dominant model (Fig. S1.B). No association was found using the recessive model (Table 5, Fig. S3.A). The haplotype ATA (rs6896215-rs9968728-rs1824154), which was composed of two risk alleles, showed to increase twice the chance of asthma in the additive model (Table 5). Also, the haplotype AGC (rs1824154-rs16889869-rs298024) which is formed only by risk alleles, was positively associated with asthma in the additive model. The haplotypes GCAGG (rs33950471-rs2112910-rs1824154-rs6870632-rs16889869) and AC (rs16889878-rs298024) were risk haplotypes to asthma in dominant model. The haplotype AGCAG (rs6896215-rs33950471-rs2112910-rs1824154-rs6870632), with just one protective allele, was associated with risk of asthma (Table 6).

Table 1
Characteristics of the SCAALA sample according to asthma status and the variables included in the study.

	Non-asthma (n = 941)		Asthma (n = 273)		p-value †
Age					
≤ 5	314	33.4%	132	48.4%	**< 0.001**
6–7	336	35.7%	88	32.2%	
≥ 8	291	30.9%	53	19.4%	
Sex					
Male	506	53.8%	150	54.9%	0.732
Female	435	46.2%	123	45.1%	
Atopy	324	34.4%	133	48.7%	**< 0.001**
Helminth Infection	206	22.3%	67	25.0%	0.342

†p-value obtained from the chi-square test.

3.4. Single marker and haplotype-based association of PDE4D with atopy

Twelve SNVs were associated with atopy (Table 4). The variants rs13177163-A, rs159625-T, rs75699812-C, and rs80075656-G were associated with the risk of atopy in both additive and dominant models. The rs10472114-C was also positively associated with atopy using the additive model only. The variants rs194368-C, rs4700340-T, and rs958851-T were positively associated with atopy in the dominant model. Notably, the variants rs159625-T and rs194368-C are in strong linkage disequilibrium (LD; $r^2 = 0.94$, Fig. S1). In addition, the SNVs rs4699942-T, and rs112873625-C were associated as protective factors to atopy in the additive model and are in strong LD ($r^2 = 1$, Fig. S1). The rs17782374-C was associated with an increased risk for atopy in the additive model. This variant was also associated in the recessive model, increasing almost three times the risk for atopy. In a similar way, rs9942415-A was also associated with atopy in the recessive model.

Considering the atopy haplotype-based analysis, 27 haplotypes were associated in the additive model (Fig. S2.C), and 17 of them were associated with atopy using the dominant genetic model (Fig. S2.D). Table 5 summarizes the most significant associated haplotypes with a p-value<0.001. No association was observed regarding the recessive model (Fig. S3.B). The haplotype AGT (rs13177163-rs4699942-rs112873625) was associated as a risk haplotype (Table 5), as mentioned before, rs4699942 and rs112873625 are in the same LD block (Fig. 1). The overlapping haplotypes CGTGG (rs75699812-rs17782374-rs80075656-rs10472114-rs13177163), and CGTGG (rs17782374-rs80075656-rs10472114-rs13177163-rs4699942) had three risk alleles and were significantly associated with an elevated chance of atopy (Table 4). In the dominant model, the CTG (rs75699812-rs958851-rs80075656) haplotype was also associated with an increased risk of atopy (Fig. 2).

3.5. Association of PDE4D SNVs with cytokines production

Homozygous genotypes for variants rs112873625-C and rs4699942-T were related to higher levels of IL-10 when compared to other genotypes (Fig. 1A-B). Individuals carrying at least one variant allele of rs75699812-C had lower IL-10 production than individuals with the wild genotype (Fig. 1C). The same occurred for rs8007656-G and rs958851-T (Fig. 1D-E). No other SNV was associated with IL-10

Table 2
Genetic and functional description of *PDE4D* SNVs.

Variant	A1/A2	Freq.	HWE	Position (HG 19)	Annotation †	RegulomeDB ‡	Haploreg §
rs10472114	C/T	0.16	0.35	59,035,558	Intronic	5	MC, eQTL
rs112873625	C/T	0.42	0.40	59,074,408	Intronic	6	MC
rs12188950	T/C	0.11	0.06	59,783,317	Intronic	4	PHM, EHM, DNAse, PBR, MC, eQTL
rs13177163	A/G	0.14	0.10	59,054,145	Intronic	5	PHM, EHM, PBR, MC
rs159625	T/C	0.14	0.10	59,161,352	Intronic	5	PHM, EHM, MC
rs16889869	G/A	0.30	0.60	58,755,580	Intronic	7	PHM, EHM
rs16889878	A/G	0.23	0.72	58,768,140	Intronic	4	EHM, DNAse, PBR
rs17782374	C/T	0.16	0.20	58,663,831	Intronic	4	EHM, DNAse, PBR, MC
rs1824154	A/G	0.31	0.37	58,737,707	Intronic	5	EHM
rs194368	C/T	0.14	0.08	59,179,484	Intronic	7	no
rs2112910	A/C	0.47	0.53	58,727,409	Intronic	6	EHM, DNAse, MC
rs298024	A/C	0.32	0.94	58,941,676	Intronic	6	EHM, MC, eQTL
rs33950471	G/T	0.24	0.72	58,449,784	Intronic	7	EHM, MC
rs34762247	C/A	0.22	0.04	59,629,365	Intronic	5	EHM, DNAse, MC, eQTL
rs4699942	T/G	0.42	0.40	59,073,475	Intronic	5	EHM, MC, eQTL
rs4700340	T/C	0.14	0.79	59,001,077	Intronic	5	PHM, EHM, DNAse, MC, eQTL
rs6870632	A/G	0.43	0.41	58,753,267	Intronic	5	MC
rs6896215	G/A	0.10	0.60	58,418,501	Intronic	4	PHM, EHM, PBR, MC
rs6898082	A/G	0.20	0.19	58,406,404	Intronic	6	EHM, MC
rs75699812	C/T	0.07	0.32	58,628,006	Intronic	6	EHM, MC
rs80075656	G/T	0.07	0.34	58,707,869	Intronic	7	MC
rs958851	T/G	0.20	0.62	58,640,143	Intronic	7	EHM, MC
rs9942415	A/G	0.19	0.26	58,771,680	Intronic	6	EHM, MC
rs9968728	C/T	0.27	0.05	58,418,981	Intronic	2b	PHM, EHM, DNAse, MC

A1: Minor allele; A2: Major allele; HWE: Hardy Weinberg Equilibrium.
EHM - Enhancer Histone Mark; eQTL - expression Quantitative Trait Loci; MC - Motifs Changed; PBR - Proteins Bound Region; PHM - Promoter Histone Mark.
† NCBI (National Center for Biotechnology Information); ‡ RegulomeDB; § Haploreg.

Table 3
Significant associations between *PDE4D* SNVs and asthma.

SNV	Genotype	Control	Case	Model	OR (CI 99%)	P-value†
rs12188950	CC	78.55% (714)	84.35% (221)	Additive	0.63 (0.39–0.998)	0.0100
	CT	19.47% (177)	14.5% (38)	Dominant	**0.58 (0.35–0.98)**	**0.0099**
	TT	1.98% (18)	1.15% (3)	Recessive	0.60 (0.12–3.05)	0.3175
rs16889869	AA	50.32% (471)	42.44% (115)	Additive	**1.32 (1.001–1.74)**	**0.0079**
	AG	40.71% (381)	46.49% (126)	Dominant	**1.48 (1.02–2.14)**	**0.0066**
	GG	8.97% (84)	11.07% (30)	Recessive	1.32 (0.73–2.38)	0.2072
rs16889878	GG	60.21% (569)	52.38% (143)	Additive	**1.34 (0.997–1.81)**	**0.0077**
	GA	34.71% (328)	41.39% (113)	Dominant	**1.47 (1.02–2.13)**	**0.0063**
	AA	5.08% (48)	6.23% (17)	Recessive	1.31 (0.61–2.82)	0.4571
rs1824154	GG	49.74% (470)	42.49% (116)	Additive	**1.33 (1.01–1.75)**	**0.0082**
	GA	40.95% (387)	46.15% (126)	Dominant	**1.48 (1.02–2.14)**	**0.0072**
	AA	9.31% (88)	11.36% (31)	Recessive	1.36 (0.76–2.45)	0.1655
rs2112910	CC	25.82% (244)	34.43% (94)	Additive	0.82 (0.63–1.06)	0.0397
	CA	51.01% (482)	43.59% (119)	Dominant	**0.64 (0.43–0.94)**	**0.0020**
	AA	23.17% (219)	21.98% (60)	Recessive	0.97 (0.62–1.5)	1
rs298024	CC	44.76% (423)	54.95% (150)	Additive	**0.74 (0.55–0.99)**	**0.0068**
	CA	44.34% (419)	36.26% (99)	Dominant	**0.65 (0.45–0.94)**	**0.0028**
	AA	10.9% (103)	8.79% (24)	Recessive	0.8 (0.42–1.5)	0.3750
rs33950471	TT	59.15% (559)	50.55% (138)	Additive	1.34 (1.00–1.8)	0.0103
	TG	35.24% (333)	42.49% (116)	Dominant	**1.46 (1.01–2.11)**	**0.0073**
	GG	5.61% (53)	6.96% (19)	Recessive	1.38 (0.67–2.88)	0.2692
rs34762247	AA	62.01% (586)	59.71% (163)	Additive	0.92 (0.67–1.25)	0.5652
	AC	31.96% (302)	38.1% (104)	Dominant	1.06 (0.73–1.55)	0.8571
	CC	6.03% (57)	2.2% (6)	Recessive	**0.29 (0.08–0.98)**	**0.0044**
rs6870632	GG	30.08% (284)	38.6% (105)	Additive	0.82 (0.63–1.07)	0.0479
	GA	50.53% (477)	43.01% (117)	Dominant	**0.67 (0.46–0.99)**	**0.0086**
	AA	19.39% (183)	18.38% (50)	Recessive	0.95 (0.59–1.52)	0.7500
rs6896215	AA	81.9% (774)	74.36% (203)	Additive	**1.56 (1.05–2.33)**	**0.0031**
	AG	17.14% (162)	24.54% (67)	Dominant	**1.65 (1.07–2.55)**	**0.0034**
	GG	0.95% (9)	1.1% (3)	Recessive	1.42 (0.25–8.15)	0.6
rs6898082	GG	64.41% (608)	63.24% (172)	Additive	1.16 (0.85–1.59)	0.28
	GA	32.52% (307)	29.04% (79)	Dominant	0.99 (0.68–1.47)	1
	AA	3.07% (29)	7.72% (21)	Recessive	**2.76 (1.26–6.03)**	**0.0007**
rs9968728	TT	52.87% (497)	59.71% (163)	Additive	**0.75 (0.56–0.999)**	**0.0096**
	TC	37.66% (354)	34.07% (93)	Dominant	0.71 (0.49–1.03)	0.0159
	CC	9.47% (89)	6.23% (17)	Recessive	0.61 (0.30–1.24)	0.0803

†Logistic regression adjusted for sex, age, helminth infections, and principal components 1 and 2.

Table 4
Significant associations between *PDE4D* SNVs and atopy.

SNV	Genotype	Control	Case	Model	OR (CI 99%)	P-value†
rs10472114	TT	73.82% (561)	66.95% (310)	Additive	**1.37 (1.01–1.85)**	**0.0053**
	TC	24.47% (186)	29.59% (137)	Dominant	1.37 (0.97–1.93)	0.0205
	CC	1.71% (13)	3.46% (16)	Recessive	2.1 (0.77–5.73)	0.0432
rs112873625	TT	29.58% (226)	37.8% (175)	Additive	**0.78 (0.61–0.98)**	**0.0054**
	TC	52.36% (400)	48.81% (226)	Dominant	0.71 (0.51–0.99)	0.0101
	CC	18.06% (138)	13.39% (62)	Recessive	0.74 (0.48–1.15)	0.0906
rs13177163	GG	76.83% (587)	68.68% (318)	Additive	1.41 (1.03–1.94)	0.0049
	GA	21.47% (164)	29.59% (137)	Dominant	**1.52 (1.07–2.16)**	**0.0022**
	AA	1.7% (13)	1.73% (8)	Recessive	1.02 (0.31–3.36)	1
rs159625	CC	76.18% (582)	68.47% (317)	Additive	**1.39 (1.01–1.91)**	**0.0082**
	CT	21.99% (168)	30.02% (139)	Dominant	**1.52 (1.07–2.16)**	**0.0023**
	TT	1.83% (14)	1.51% (7)	Recessive	0.80 (0.23–2.71)	0.6000
rs17782374	TT	72.91% (557)	67.17% (311)	Additive	**1.38 (1.02–1.85)**	**0.0056**
	TC	25.26% (193)	28.08% (130)	Dominant	1.33 (0.95–1.88)	0.0260
	CC	1.83% (14)	4.75% (22)	Recessive	**2.75 (1.07–7.07)**	**0.0033**
rs194368	TT	76.12% (577)	69.48% (321)	Additive	1.31 (0.95–1.8)	0.0389
	TC	22.03% (167)	29.22% (135)	Dominant	**1.43 (1.003–2.03)**	**0.0089**
	CC	1.85% (14)	1.3% (6)	Recessive	0.68 (0.19–2.49)	0.3830
rs4699942	GG	29.58% (226)	37.8% (175)	Additive	**0.78 (0.61–0.98)**	**0.0054**
	GT	52.36% (400)	48.81% (226)	Dominant	0.71 (0.51–0.99)	0.0101
	TT	18.06% (138)	13.39% (62)	Recessive	0.74 (0.48–1.15)	0.0906
rs4700340	CC	76.7% (586)	69.76% (323)	Additive	1.40 (1.01–1.94)	0.0110
	CT	22.12% (169)	28.08% (130)	Dominant	**1.44 (1.01–2.06)**	**0.0080**
	TT	1.18% (9)	2.16% (10)	Recessive	1.62 (0.45–5.84)	0.2800
rs75699812	TT	88.35% (675)	83.59% (387)	Additive	1.64 (1.07–2.51)	0.0043
	TC	11.39% (87)	15.33% (71)	Dominant	**1.62 (1.03–2.56)**	**0.0074**
	CC	0.26% (2)	1.08% (5)	Recessive	4.81 (0.54–42.64)	0.0147
rs80075656	TT	88.32% (673)	83.33% (385)	Additive	**1.69 (1.11–2.59)**	**0.0018**
	TG	11.55% (88)	15.37% (71)	Dominant	**1.63 (1.04–2.57)**	**0.0045**
	GG	0.13% (1)	1.3% (6)	Recessive	11.74 (0.71–194.1)	–
rs958851	GG	67.32% (513)	59.83% (277)	Additive	1.33 (1.00–1.77)	0.0108
	GT	29.53% (225)	36.72% (170)	Dominant	**1.41 (1.02–1.96)**	**0.0079**
	TT	3.15% (24)	3.46% (16)	Recessive	1.25 (0.52–3.00)	0.4571
rs9942415	GG	67.23% (513)	63.42% (293)	Additive	1.26 (0.95–1.66)	0.0439
	GA	29.75% (227)	30.74% (142)	Dominant	1.2 (0.86–1.67)	0.1589
	AA	3.01% (23)	5.84% (27)	Recessive	**2.25 (1.01–4.99)**	**0.0064**

†Logistic regression adjusted for sex, age, helminth infections, and principal components 1 and 2.

production in our study. None of the investigated variants were associated with IL-5 or IL-13 production in whole blood.

3.6. Functional annotation prediction

A correlation between the rs6898082-A and increased *PDE4D* expression in whole blood was observed in the GTEx database (Fig. S4). No other SNV was associated with *PDE4D* expression (Data not shown).

3.7. Replication and meta-analysis

Four (rs12188950, rs4699942, rs6870632 and rs958851) of the 24 variants were genotyped and available in the replication cohort and were further tested for association by considering the same phenotypes from the discovery population. None of the investigated variants were replicated. However, in the meta-analysis conducted with both populations (SCAALA and PROAR), the rs6870632 was significantly associated with a lower chance of asthma (OR: 0.71; CI 99%: 0.54–0.94; p: 0.0076).

4. Discussion

Limited research has been conducted, particularly in Latin-American populations, regarding the association between variants in *PDE4D* and asthma and atopy, despite previous findings in this area. (Himes, 2009; Ding, 2013) Here, we investigated the association between genetic variants in *PDE4D* gene and asthma and atopic conditions in Brazilian children.

We identified six SNVs significantly associated with a greater chance of having asthma and ten with atopic phenotype. Considering that some atopy and asthma-associated variants might impair gene regulation promoting a greater expression of *PDE4D*, the mechanism whereby those variants functionally impact could be related to PDE4D activity. A higher expression of *PDE4D* is observed in activated T cells, which is inversely correlated with cAMP levels. (Peter, 2007) Thus, this finding may explain its impact on increased *PDE4D* transcription that potentially relates to lower cAMP production and consequently increased asthma and atopy susceptibility by suppressing immunoregulatory mechanisms (Bodor et al., 2012; Lee, 2015; Rueda et al., 2016).

The variants rs6898082, rs16889869, rs33950471, rs1824154, rs6896215, and rs16889878 were positively associated with asthma. The AA genotype of rs6898082 increased almost three times the chance of having asthma symptoms. Furthermore, this same genotype was related to increased *PDE4D* expression in whole blood. The rs6898082, rs1824154 and rs6896215 are located in an enhancer region in fetal lung tissue and shown to modify the sequence of motifs that interact with transcription factors (TF) (Ward and Kellis, 2012).

The variants rs33950471, rs6896215 and rs16889869 are located on regions marked as enhancers in T helper cells. The rs6896215 is also in a CEBPB (glucocorticoid-responsive TF) binding region, and the alternative allele also impacts in a higher affinity the FOXP1 TF, transcription factors involved in lung organogenesis and immune response (Roos and Nord, 2012; Pniewska, 2014). In this sense, *FOXP1* is also differentially methylated in patients with asthma which can be related to changes in *FOXP1* expression observed in asthmatics (Pniewska, 2014; Hoang et al., 2020). Therefore, recognizing that FOXP1 is critical for T-cell development and function, including effector in regulatory T-cells (Ren et al., 2019), it is possible that variants in putative response gene (i.e. *PDE4D*) influencing the interaction between the genome and the epigenetic machinery may play a role in asthma susceptibility.

Table 5
Most significant ($p < 0.001$) associations between *PDE4D* haplotypes with asthma by logistic regression adjusted for sex, age, helminth infections, and principal components 1 and 2.

Model	Haplotype									Frequency		OR (CI 99%)	p-value
					rs6896215	rs9968728	rs1824154	rs16889869	rs298024	Control	Case		
Additive	–	–	–	–	A	C	–	–	–	0.29	0.23	REF	–
	–	–	–	–	G	T	–	–	–	0.09	0.14	1.83 (1.17–2.88)	0.0006
	–	–	–	–	–	C	G	–	–	0.17	0.14	REF	–
	–	–	–	–	–	T	A	–	–	0.17	0.26	1.83 (1.18–2.84)	0.0004
	–	–	–	–	–	–	–	A	A	0.27	0.2	REF	–
	–	–	–	–	–	–	–	G	C	0.23	0.29	1.65 (1.14–2.4)	0.0006
	–	–	–	–	A	C	G	–	–	0.17	0.14	REF	–
	–	–	–	–	A	T	A	–	–	0.13	0.21	1.96 (1.21–3.18)	0.0004
	–	–	–	–	–	–	G	A	A	0.25	0.2	REF	–
	–	–	–	–	–	–	A	G	C	0.18	0.24	1.68 (1.13–2.52)	0.0009
Dominant	rs6896215	rs33950471	rs2112910	rs1824154	rs6870632	rs16889869	rs16889878	rs298024	rs12188950				
	–	–	–	–	–	–	G	A	–	0.29	0.21	REF	–
	–	–	–	–	–	–	A	C	–	0.18	0.22	1.66 (1.12–2.46)	0.001
	–	T	A	G	–	–	–	–	–	0.39	0.33	REF	–
	–	G	C	A	–	–	–	–	–	0.09	0.16	2.05 (1.31–3.21)	0.0001
	A	T	A	G	–	–	–	–	–	0.37	0.29	REF	–
	A	G	C	A	–	–	–	–	–	0.07	0.13	2.11 (1.25–3.57)	0.0002
	–	T	A	G	A	–	–	–	–	0.35	0.31	REF	–
	–	G	C	A	G	–	–	–	–	0.09	0.16	1.99 (1.26–3.14)	0.0001
	A	T	A	G	A	–	–	–	–	0.33	0.27	REF	–
	A	G	C	A	G	–	–	–	–	0.07	0.13	2.03 (1.19–3.45)	0.0006
	–	T	A	G	A	A	–	–	–	0.35	0.31	REF	–
	–	G	C	A	G	G	–	–	–	0.08	0.14	1.92 (1.19–3.11)	0.0005

Table 6
Most significant ($p < 0.001$) associations between *PDE4D* haplotypes with atopy by logistic regression adjusted for sex, age, helminth infections, and principal components 1 and 2.

Model	Haplotype								Frequency		OR (CI 99%)	p-value
	rs75699812	rs17782374	rs80075656	rs10472114	rs13177163	rs4699942	rs112873625	rs159625	Control	Case		
	T	T	–	–	–	–	–	–	0.85	0.81	REF	–
Additive	C	C	–	–	–	–	–	–	0.05	0.08	1.83 (1.17–2.86)	0.0005
	–	–	–	–	G	T	–	–	0.44	0.38	REF	–
	–	–	–	–	A	G	–	–	0.12	0.17	1.57 (1.11–2.22)	0.0009
	T	T	T	–	–	–	–	–	0.84	0.81	REF	–
	C	C	G	–	–	–	–	–	0.05	0.08	1.83 (1.17–2.87)	0.0006
	–	T	T	T	–	–	–	–	0.73	0.65	REF	–
	–	C	G	T	–	–	–	–	0.04	0.07	1.96 (1.17–3.29)	0.0008
	–	–	–	–	G	T	C	–	0.44	0.38	REF	–
	–	–	–	–	A	G	T	–	0.12	0.17	1.57 (1.11–2.22)	0.0009
	T	T	T	T	–	–	–	–	0.72	0.65	REF	–
	C	C	G	T	–	–	–	–	0.04	0.07	2.09 (1.25–3.49)	0.0002
	–	T	T	T	G	–	–	–	0.73	0.64	REF	–
	–	C	G	T	G	–	–	–	0.04	0.07	1.95 (1.17–3.26)	0.0008
	T	T	T	T	G	–	–	–	0.72	0.64	REF	–
	C	C	G	T	G	–	–	–	0.04	0.07	2.08 (1.24–3.48)	0.0003
	–	T	T	T	G	T	–	–	0.36	0.29	REF	–
	–	C	G	T	G	G	–	–	0.02	0.04	2.93 (1.31–6.56)	0.0006
Dominant		rs75699812	rs958851	rs80075656	rs4700340	rs13177163	rs159625	rs194368	Control	Case		
	–	T	G	T	–	–	–	–	0.81	0.77	REF	–
	–	C	T	G	–	–	–	–	0.05	0.08	1.82 (1.15–2.88)	0.0008

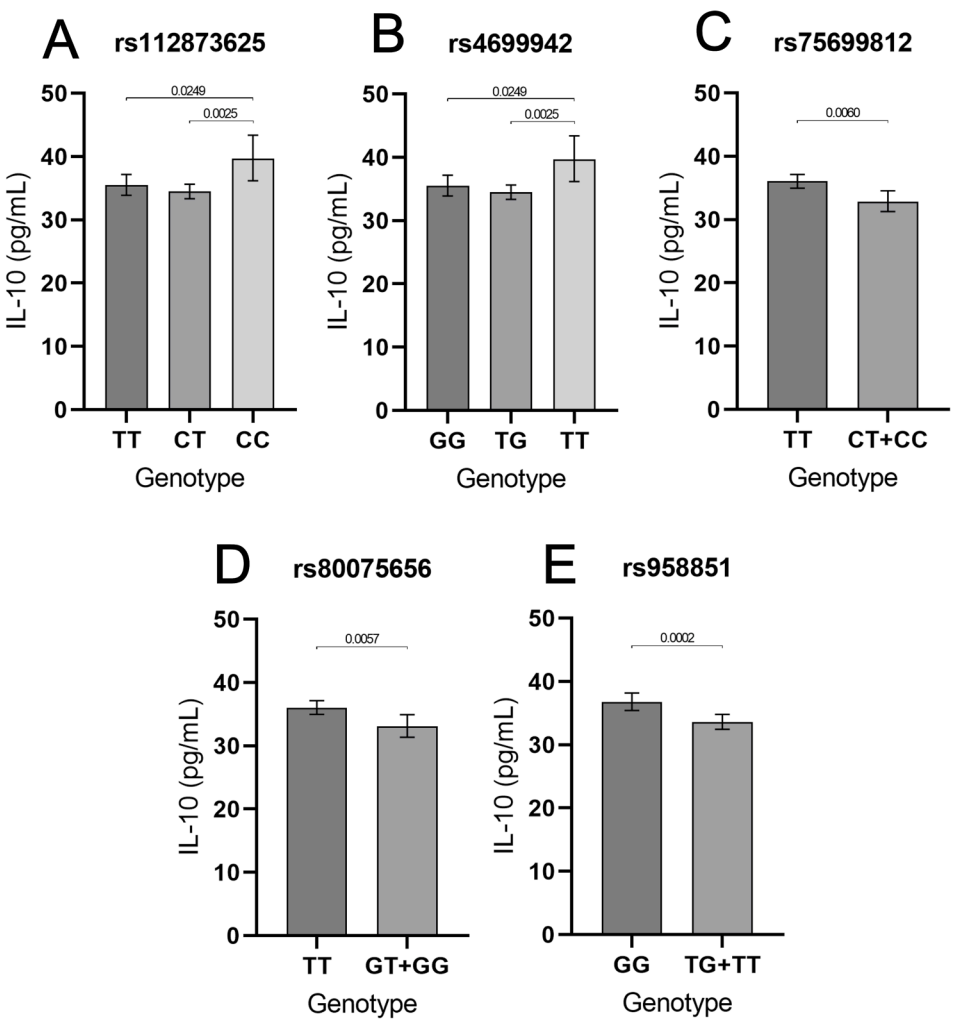

Fig. 1. IL-10 production (pg/mL) according to genotypes of (A) rs112873625, (B) rs4699942, (C) rs75699812, (D) rs8007656, and (E) rs958851. Bars represent the geometric means, and vertical bars represent 95% CIs. Graph generated using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com".

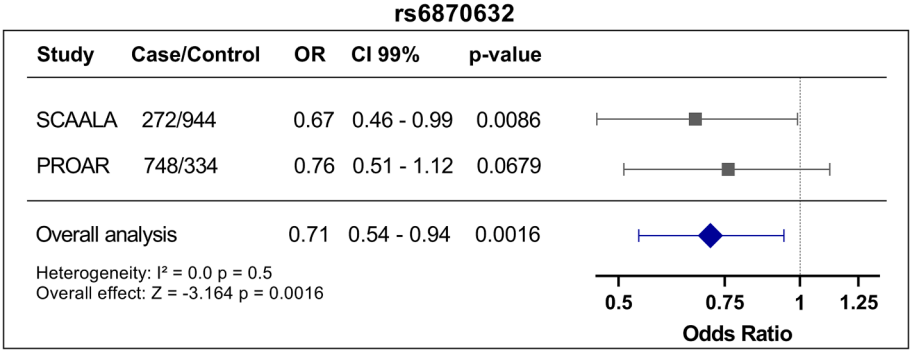

Fig. 2. Replication and meta-analysis of association between rs6870632 and asthma in the dominant model. Graph generated using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com".

The presence of rs958851, rs17782374, rs159625, rs4700340, rs13177163, rs75699812, and rs80075656 variants increased the chance to atopy. All variants were related to the enhancer, promoter, DNAse peak, or protein binding sites. The variant rs17782374 altered relative affinity to RXR-α (Retinoid X receptor alpha). There is some evidence that individuals with IgE-mediated food allergy exhibit a differential DNA methylation pattern at key genes involved in Th1/Th2 differentiation in T CD4+ cells, including *RXRA*. (Imran, 2022) The RXR-α is known to interact with the VDR-Vitamin D complex acting as corepressors to regulate gene expression, in this way, it was shown that vitamin D resistant cancer cells were characterized by *PDE4D* hypomethylation and consequent overexpression (Lai et al., 2020), reinforcing the role of VDR-RXR-α pathway in regulating PDE4D gene expression. Also, rs75699812 is related to altered binding of FOXP1,

which can be related to the same molecular mechanism presented for rs6896215. The rs159625 and rs4700340 variants were both located in PU.1 TF motifs, which might change the interaction with such protein that is associated with Th2 response profile differentiation. (Yashiro et al., 2015) Moreover, a correlation between rs4700340 and greater expression of *PDE4D* is reported by a blood eQTL database (Westra, 2013).

With regard to haplotype analysis, the combination of a few risk alleles was observed, and these combinations were related to increasing asthma and atopy susceptibility. For example, the GCAGG (rs33950471-rs2112910-rs1824154-rs6870632-rs16889869) whose frequency is higher in asthmatic when compared to non-asthmatic subjects. Also, a haplotype composed of rs958851, rs75699812, and rs80075656 is more observed in atopic than non-atopic individuals.

Additionally, we could see that rs958851, rs75699812, and rs80075656 variants, associated with a higher chance of having atopy, were related to lower levels of the immunomodulatory cytokine IL-10. Another possible mechanism is related to dendritic cell activity, in which low concentrations of cAMP are crucial to induce a Th2 profile by stimulating the activation of lymphocytes, which also express high levels of PDE4D (Peter, 2007; Chinn and Insel, 2020). Therefore, variants that increase the PDE4D activity can lead to a reduction in imbalance in the immunoregulatory network, increasing the likelihood of asthma and atopy.

Alternatively, other six variants were related to a lower chance of having asthma, while other two SNVs were negatively associated with atopy. The variants rs12188950, rs298024, rs2112910, rs34762247, rs6870632, and rs9968728 were associated as protective factors for asthma symptoms. Consistently, rs6870632 showed an association as a protective factor to asthma in the meta-analysis that included both populations, SCAALA and PROAR. These variants are located on regulatory regions in multiple tissues based on Haploreg data. The rs12188950 lies on promoters and enhancer regions in several cells, including in primary T helper naive cells and fetal lungs. The presence of the variant allele reduces the affinity with the TF RFX5 based on *in silico* prediction (Ward and Kellis, 2012). Moreover, RFX5 is known as an important TF in immune system development by promoting the expression of other genes. (Xia, 2012) In the same direction, rs6870632 changes the relative affinity to the nuclear factor of activated T-cells (NFAT), resulting in weaker binding of this TF on DNA (Ward and Kellis, 2012).

The rs112873625 and rs4699942 were associated with protection for atopy and increased production of IL-10. Also, both SNVs are in complete LD and were related to changing the sequence of DNA motifs in Haploreg. One of the motif sequences changed by rs4699942 is a binding site for IK-2 (Ikaros 2). The IK-2 is expressed in many T cells, but the modulation activity is best known for in T regulatory cells, being important for Treg suppressive function (Kim et al., 2015). In accordance with this finding, rs4699942 is associated with a lower expression of *PDE4D* in a large meta-analysis study of eQTLs in blood (Westra, 2013).

It is possible that variant affecting the binding to TF modulates the expression of *PDE4D*. Since a reduced expression of *PDE4D* increases the cAMP levels, in effectors T lymphocytes, high levels of cAMP promote the inhibition of nuclear factor-kappaB (NF-κB) and nuclear factor of activated T cells activation (NFAT), resulting in the suppression of inflammatory cytokines production, such as IL-2 (Jimenez, 2001). Moreover, Treg cells activity is cAMP-dependent, in which its turn can induce IL-10 production and cAMP can also be released into the cytoplasm of other lymphocytes and dendritic cells through GAP junctions (Jimenez, 2001). Accordingly, the arctigenin, a PDE4D inhibitor, is able to reduce IgE production and Th2 cytokines in PMBC of allergic individuals (Cao et al., 2022). Another plausible mechanism is related to the effect of cAMP in dendritic cells, as it was previously shown an increased IL-10 production by dendritic cells when treated with a PDE4 inhibitor, which attenuates their Th2-promoting ability (Bros et al., 2016). In addition, arctigenin is related to inhibition of mast cell-mediated allergic responses, which could be associated with other immunological mechanisms (Kee and Hong, 2017). We propose that genetic variants that reduce the activity and/or expression levels of PDE4D may have a biological effect similar to pharmacological inhibition of the enzyme, resulting in increased anti-inflammatory activity.

Our study has several limitations, including the absence of functional assays such as evaluating PDE4D expression or enzyme activity. However, the findings can contribute to a more comprehensive understanding of the molecular changes associated with asthma and allergic diseases. It is possible that some variants contribute to the reduction of *PDE4D* expression, additional studies should be conducted in order to address this. This, in turn, may enhance the development or rescue of pharmacological therapies that target the modulation of PDE4D activity. Therefore, our study reinforces the importance of reevaluating asthma and allergy treatments, particularly by considering the identification of genetic biomarkers associated with specific endotypes. This approach could potentially improve efficacy while reducing side effects or adverse reactions.

5. Conclusions

In summary, SNVs and their haplotypes in PDE4D are significantly associated with asthma and allergy. Therefore, these variants may play a crucial role in asthma and atopy pathophysiology, possibly by affecting the regulation of gene expression and, consequently, the production of the phosphodiesterase enzyme. These variants may be modulating inflammation by impairing IL-10 production and also modifying lung development. Further studies are necessary to better understand the mechanism whereby PDE4D variants affect asthma and allergy.

Funding

This study was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001, Brazilian National Research Council (CNPq) and Foundation for Research Support of the State of Bahia (FAPESB).

CRediT authorship contribution statement

Hatilla dos Santos Silva: Conceptualization, Writing – original draft, Formal analysis, Methodology, Writing – review & editing, Data curation, Investigation, Validation. **Helena Mariana Pitangueira Teixeira:** Writing – review & editing, Conceptualization, Data curation, Writing – original draft, Methodology, Investigation, Validation. **Luciano Gama da Silva Gomes:** Data curation, Formal analysis, Methodology, Software, Validation. **Álvaro A. Cruz:** Methodology, Investigation, Validation. **Neuza Maria Alcantara-Neves:** Funding acquisition, Writing – review & editing, Resources, Project administration, Conceptualization, Data curation, Methodology, Investigation. **Maurício Barreto:** Writing – review & editing, Funding acquisition, Conceptualization, Project administration, Resources, Data curation, Methodology, Investigation. **Camila Alexandrina Figueiredo:** Supervision, Resources, Data curation, Writing – review & editing, Methodology, Project administration, Investigation, Funding acquisition. **Ryan dos Santos Costa:** Supervision, Resources, Data curation, Conceptualization, Writing – review & editing, Methodology, Project administration, Investigation, Funding acquisition, Validation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

We thank all participants and all the collaborators of the Laboratory of Immunopharmacology and Molecular Biology (IMUNOBIO) for all their support, and the Universidade Federal da Bahia for the structure to develop the work. Moreover, we thank the SCAALA (Social Change of Asthma and Allergy in Latin America) cohort for making this study possible.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.imbio.2023.152724.

References

Alcantara-Neves, N.M., Veiga, R.V., Dattoli, V.C.C., Fiaccone, R.L., Esquivel, R., Cruz, Á. A., Cooper, P.J., Rodrigues, L.C., Barreto, M.L., 2012. The effect of single and multiple infections on atopy and wheezing in children. J. Allergy Clin. Immunol. 129 (2), 359–367.e3.

Bodor, J., Bopp, T., Vaeth, M., Klein, M., Serfling, E., Hünig, T., Becker, C., Schild, H., Schmitt, E., 2012. Cyclic AMP underpins suppression by regulatory T cells. Eur. J. Immunol. 42 (6), 1375–1384.

Boyle, A.P., et al., 2012. Annotation of functional variation in personal genomes using RegulomeDB. Genome Res. 22, 1790–1797.

Bros, M., Montermann, E., Cholaszczyńska, A., Reske-Kunz, A.B., 2016. The phosphodiesterase 4 inhibitor roflumilast augments the Th17-promoting capability of dendritic cells by enhancing IL-23 production, and impairs their T cell stimulatory activity due to elevated IL-10. Int. Immunopharmacol. 35, 174–184.

Cao, M., Liu, C., Srivastava, K.D., Lin, A., Lazarski, C., Wang, L.u., Maskey, A., Song, Y., Chen, X., Yang, N., Zambrano, L., Bushko, R., Nowak-Wegrzyn, A., Cox, A., Liu, Z., Huang, W., Dunkin, D., Miao, M., Li, X.-M., 2022. Anti-IgE effect of small-molecule-compound arctigenin on food allergy in association with a distinct transcriptome profile. Clin. Exp. Allergy 52 (2), 250–264.

Chinn, A.M., Insel, P.A., 2020. Cyclic AMP in dendritic cells: A novel potential target for disease-modifying agents in asthma and other allergic disorders. Br. J. Pharmacol. 177, 3363–3377.

De, A.C.Z., Paiva, de, F.A.L., Marson, Ribeiro, J.D., Bertuzzo, C.S., 2014. Asthma: Gln27Glu and Arg16Gly polymorphisms of the beta2-adrenergic receptor gene as risk factors. Allergy, Asthma Clin. Immunol. 10, 1–9.

Ding, L., et al., 2013. Rank-based genome-wide analysis reveals the association of Ryanodine receptor-2 gene variants with childhood asthma among human populations. Hum. Genomics 7, 1–17.

Dolina, J.S., Lee, J., Moore, E.L., Hope, J.L., Gracias, D.T., Matsutani, T., Chawla, A., Greenbaum, J.A., Linden, J., Schoenberger, S.P., 2022. Developmentally distinct CD4+ Treg lineages shape the CD8+ T cell response to acute Listeria infection. Proc. Natl. Acad. Sci. 119 (10).

El-Husseini, Z.W., Vonk, J.M., Van den Berge, M., Gosens, R., Koppelman, G.H., 2023. Association of asthma genetic variants with asthma-associated traits reveals molecular pathways of eosinophilic asthma. Clin. Translat. Allergy 13, e12239.

Ferrante, G., La Grutta, S., 2018. The burden of pediatric asthma. Front. Pediatr. 6, 1–7.

Figueiredo, C.A., et al., 2009. Spontaneous cytokine production in children according to biological characteristics and environmental exposures. Environ. Health Perspect. 117, 845–849.

GBD 2015 Chronic Respiratory Disease Collaborators. Global, regional, and national deaths, prevalence, disability-adjusted life years, and years lived with disability for chronic obstructive pulmonary disease and asthma, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *The Lancet. Respiratory Medicine*, **5**, 691 - 706 (2017).

Global Initiative for Asthma. *Global strategy for asthma management and prevention.* ginasthma.org/gina-reports (2021).

GTEX CONSORTIUM, *et* al. The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans. *Science.* **348,** 648-660 - (2015).

Hammad, H., Lambrecht, B.N. The basic immunology of asthma. *Cell*, **184**, 1469-1485 (2021).

Himes, B.E., et al., 2009. Genome-wide association analysis identifies PDE4D as an asthma-susceptibility gene. Am. J. Hum. Genet. 84, 581–593.

Hoang, T.T., Sikdar, S., Xu, C.-J., Lee, M.K., Cardwell, J., Forno, E., Imboden, M., Jeong, A., Madore, A.-M., Qi, C., Wang, T., Bennett, B.D., Ward, J.M., Parks, C.G., Beane-Freeman, L.E., King, D., Motsinger-Reif, A., Umbach, D.M., Wyss, A.B., Schwartz, D.A., Celedón, J.C., Laprise, C., Ober, C., Probst-Hensch, N., Yang, I.V., Koppelman, G.H., London, S.J., 2020. Epigenome-wide association study of DNA methylation and adult asthma in the Agricultural Lung Health Study. Eur. Respir. J. 56 (3), 2000217.

Hua, J., Davis, S.P., Hill, J.A., Yamagata, T., 2015. Diverse gene expression in human regulatory T cell subsets uncovers connection between regulatory T cell genes and suppressive function. J. Immunol. 195 (8), 3642–3653.

Imran, S., et al., 2022. Immuno-epigenomic analysis identifies attenuated interferon responses in naïve CD4 T cells of adolescents with peanut and multi-food allergy. Pediatr. Allergy Immunol. 33, e13890.

Jimenez, J.L., et al., 2001. Phosphodiesterase 4 inhibitors prevent cytokine secretion by T lymphocytes by inhibiting nuclear factor-κB and nuclear factor of activated T cells activation. J. Pharmacol. Exp. Ther. 299, 753–759.

Kee, J.-Y., Hong, S.-H., 2017. Inhibition of mast cell-mediated allergic responses by Arctii Fructus extracts and its main compound arctigenin. J. Agric. Food Chem. 65 (43), 9443–9452.

Kim, H.-J., Barnitz, R.A., Kreslavsky, T., Brown, F.D., Moffett, H., Lemieux, M.E., Kaygusuz, Y., Meissner, T., Holderried, T.A.W., Chan, S., Kastner, P., Haining, W.N., Cantor, H., 2015. Stable inhibitory activity of regulatory T cells requires the transcription factor Helios. Science 350 (6258), 334–339.

Lai, G.-R., Lee, Y.-F., Yan, S.-J., Ting, H.-J., 2020. Active vitamin D induces gene-specific hypomethylation in prostate cancer cells developing vitamin D resistance. Am. J. Phys. Cell Phys. 318 (5), C836–C847.

Landells, L.J., Spina, D., Souness, J.E., O'Connor, B.J., Page, C.P., 2000. A biochemical and functional assessment of monocyte phosphodiesterase activity in healthy and asthmatic subjects. Pulm. Pharmacol. Ther. 13, 231–239.

Lee, J., et al., 2015. Cyclic AMP concentrations in dendritic cells induce and regulate Th2 immunity and allergic asthma. PNAS 112, 1529–1534.

Lima-Matos, A., Ponte, E.V., de Jesus, J.P.V., Almeida, P.C.A., Lima, V.B., Kwon, N., Riley, J., de Mello, L.M., Cruz, Á.A., 2018. Eosinophilic asthma, according to a blood eosinophil criterion, is associated with disease severity and lack of control among underprivileged urban Brazilians. Respir. Med. 145, 95–100.

Luna, A., Nicodemus, K.K., 2007. snp.plotter: An R-based SNP/haplotype association and linkage disequilibrium plotting package. Bioinformatics 23, 774–776.

Méhats, C., et al., 2003. PDE4D plays a critical role in the control of airway smooth muscle contraction. FASEB J. 17, 1831–1841.

Morianos, I., Semitekolou, M., 2020. Dendritic cells: critical regulators of allergic asthma. Int. J. Mol. Sci. 21, 7930.

Morii, W., et al., 2023. A genome-wide association study for allergen component sensitizations identifies allergen component–specific and allergen protein group–specific associations. J. Allergy Clin. Immunol.: Global 2, 100086.

Nousbeck, J., McAleer, M.A., Irvine, A.D., 2023. Peripheral blood gene expression profile of infants with atopic dermatitis. JID Innov. 3, 100165.

Peter, D., et al., 2007. Differential expression and function of phosphodiesterase 4 (PDE4) subtypes in human primary CD4+ T cells: predominant role of PDE4D. J. Immunol. 178, 4820–14483.

Pniewska, E., et al., 2014. Exacerbating factors induce different gene expression profiles in peripheral blood mononuclear cells from asthmatics, patients with chronic obstructive pulmonary disease and healthy subjects. Int. Arch. Allergy Immunol. 165, 229–243.

Portelli, M.A., Hodge, E., Sayers, I., 2015. Genetic risk factors for the development of allergic disease identified by genome-wide association. Clin. Exp. Allergy 45 (1), 21–31.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D., Maller, J., Sklar, P., de Bakker, P.I.W., Daly, M.J., Sham, P.C., 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. 81 (3), 559–575.

Ren, J., Han, L., Tang, J., Liu, Y., Deng, X., Liu, Q., Hao, P., Feng, X., Li, B., Hu, H., Wang, H., Oliver, P.M., 2019. Foxp1 is critical for the maintenance of regulatory T-cell homeostasis and suppressive function. PLoS Biol. 17 (5) e3000270.

Roos, A.B., Nord, M., 2012. The emerging role of C/EBPs in glucocorticoid signaling: lessons from the lung. J. Endocrinol. 212, 291–305.

Rueda, C.M., Jackson, C.M., Chougnet, C.A., 2016. Regulatory T-cell-mediated suppression of conventional T-cells and dendritic cells by different cAMP intracellular pathways. Front. Immunol. 7, 216.

Sinnwell, J.P., Schaid, D.J. Haplo.stats: Statistical analysis of haplotypes with traits and covariates when linkage phase is ambiguous. https://cran.r-project.org/package=haplo.stats.

Solé, D., Camelo-Nunes, I.C., Wandalsen, G.F., Mallozi, M.C., 2014. Asthma in children and adolescents in Brazil: contribution of the International Study of Asthma and Allergies in Childhood (ISAAC). Rev. Paul. Pediatr. 32, 114–125.

Teixeira, H.M.P., Alcantara-Neves, N.M., Barreto, M., Figueiredo, C.A., Costa, R.S., 2017. Adenylyl cyclase type 9 gene polymorphisms are associated with asthma and allergy in Brazilian children. Mol. Immunol. 82, 137–145.

Trian, T., Burgess, J.K., Niimi, K., Moir, L.M., Ge, Q.i., Berger, P., Liggett, S.B., Black, J.L., Oliver, B.G., Morty, R.E., 2011. β2-agonist induced cAMP is decreased in asthmatic airway smooth muscle due to increased PDE4D. PLoS One 6 (5), e20000.

Ward, L.D., Kellis, M., 2012. HaploReg: A resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. Nucleic Acids Res. 40, 930–934.

Westra, H.J., et al., 2013. Systematic identification of trans eQTLs as putative drivers of known disease associations. Nat. Genet. 45, 1238–1243.

Willer, C.J., Li, Y., Abecasis, G.R., 2010. METAL: fast and efficient meta-analysis of genomewide association scans. Bioinformatics 26, 2190–2191.

Willis-Owen, S.A.G., Cookson, W.O.C., Moffatt, M.F., 2018. The genetics and genomics of asthma. Annu. Rev. Genomics Hum. Genet. 19 (1), 223–246.

Xia, J., et al., 2012. SIRT1 deacetylates RFX5 and antagonizes repression of collagen type I (COL1A2) transcription in smooth muscle cells. Biochem. Biophys. Res. Commun. 428, 264–270.
Yashiro, T., Kubo, M., Ogawa, H., Okumura, K.o., Nishiyama, C., Nakano, H., 2015. 1 suppresses Th2 cytokine expression via silencing of GATA3 transcription in dendritic cells. PLoS One 10 (9) e0137699.
Zhang, J., Zou, Y., Chen, L., Xu, Q., Wang, Y., Xie, M., Liu, X., Zhao, J., Wang, C.-Y., 2022. Regulatory T cells, a viable target against airway allergic inflammatory responses in asthma. Front. Immunol. 13.