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Whole Exome Sequencing Identifies Epithelial and Immune Dysfunction-Related Biomarkers in Food Protein-Induced Enterocolitis Syndrome

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ABSTRACT

Background: Food protein-induced enterocolitis syndrome (FPIES) is a food allergy primarily affecting infants, often leading to vomiting and shock. Due to its poorly understood pathophysiology and lack of specific biomarkers, diagnosis is frequently delayed. Understanding FPIES genetics can shed light on disease susceptibility and pathophysiology—key to developing diagnostic, prognostic, preventive and therapeutic strategies. Using a well-characterised cohort of patients we explored the potential genome-wide susceptibility factors underlying FPIES.

Methods: Blood samples from 41 patients with oral food challenge-proven FPIES were collected for a comprehensive whole exome sequencing association study.

Results: Notable genetic variants, including rs872786 (*RBM8A*), rs2241880 (*ATG16L1*) and rs2289477 (*ATG16L1*), were identified as significant findings in FPIES. A weighted SKAT model identified six other associated genes including *DGKZ* and *SIRPA*. DGKZ induces TGF-β signalling, crucial for epithelial barrier integrity and IgA production; *RBM8A* is associated with thrombocytopenia absent radius syndrome, frequently associated with cow's milk allergy; *SIRPA* is associated with increased neutrophils/monocytes in inflamed tissues as often observed in FPIES; *ATG16L1* is associated with inflammatory bowel disease. Coexpression correlation analysis revealed a functional correlation between *RBM8A* and filaggrin gene (*FLG*) in stomach and intestine tissue, with filaggrin being a known key pathogenic and risk factor for IgE-mediated food allergy. A transcriptome-wide association study suggested genetic variability in patients impacted gene expression of *RBM8A* (stomach and pancreas) and *ATG16L1* (transverse colon).

Conclusions: This study represents the first case–control exome association study of FPIES patients and marks a crucial step towards unravelling genetic susceptibility factors underpinning the syndrome. Our findings highlight potential factors and

The first three authors contributed equally to this article.

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pathways contributing to FPIES, including epithelial barrier dysfunction and immune dysregulation. While these results are novel, they are preliminary and need further validation in a second cohort of patients.

1 | Introduction

Food protein-induced enterocolitis syndrome (FPIES) is a non-IgE-mediated food allergy predominantly affecting young children. The symptoms include profuse vomiting, often lethargy and potentially shock usually 1–4 h after ingestion [1].

While FPIES involve systemic innate immune activation, their relation to food-specific exposure, gut inflammation and symptoms remains unclear [2]. Understanding disease mechanisms is crucial to develop specific diagnostic, prognostic, therapeutic and preventive strategies, currently unavailable in FPIES. The identification of diagnostic and prognostic biomarkers is a research priority by the FPIES patient/parent community; the lack of available tests and limited awareness often leads to missed or delayed diagnosis, compromising safety and quality of life [1].

Allergic diseases arise from intricate interactions between environmental and genetic factors. Studying patients' genetics can shed light on the pathophysiology and susceptibility to the disease. Twin studies on the heritability of food allergies suggest a genetic component [3]. Genetic studies in IgE-mediated food allergy have identified several genes loci associated with immune dysregulation and epithelial barrier dysfunction, including *FLG* ('Filaggrin gene') mutations [4]. These findings have allowed major developments in the recent years such as biologic therapy targeting key immune pathways for severe cases [5], or food allergy prevention by early food introduction in at-risk individuals [6]. Also, a growing list of inborn errors of immunity leading to heritable monogenic allergic disorders has been recently identified and coined as Primary Atopic Disorders (PAD); many of them associate food allergy due to immune dysregulation [7].

To our best knowledge, no studies have explored the association between genome variability and FPIES. This study aimed to investigate the potential genetic basis of FPIES by conducting a whole exome association study. The results revealed several single nucleotide polymorphisms (SNPs) and genes as new candidates for understanding the genetic susceptibility to FPIES. The identified SNPs and genes suggest potential involvement of immune dysregulation and epithelial barrier dysfunction as contributing mechanisms to FPIES. The discovery of new genetic susceptibility biomarkers holds promise for anticipating and improving the diagnosis and treatment of patients, contributing to a better understanding of the disease pathophysiology.

2 | Methods

2.1 | Sampling

This is an observational, multicentre, prospective study recruiting patients with a diagnosis of acute FPIES confirmed by a standardised oral food challenge (OFC) to the culprit food (BIO-FPIES study, ethics committee reference: 2017/396). OFC were conducted as a part of routine care across a network of tertiary hospitals from Spain and Italy. Informed consent from parents/guardians was obtained. Participants exclusion criteria were as follows: (i) systemic treatment with immunosuppressants or monoclonal antibodies, (ii) immunodeficiency, (iii) previous bone marrow transplant and (iv) other comorbidity that might interfere with the OFC outcome assessment or immune/inflammatory response.

The patient cohort consisted of 41 children aged 1–12 years and one adult. Blood (EDTA) samples were collected at baseline, prior to starting the OFC (see clinical characteristics in Table 1).

2.2 | Suitability for OFC

Contraindications to proceed with OFC on the day were assessed and OFC and blood extraction were postponed in the event of any of the following circumstances: (a) vaccination or systemic corticosteroids (oral, intramuscular and intravenous) in the previous 2 weeks, (b) proton pump inhibitors, first generation antihistamines (i.e., hydroxyzine [Atarax], chlorphenamine, ketotifen) in the previous 3 days, (c) second generation antihistamines (i.e., cetirizine, loratadine, desloratadine and fexofenadine), in the previous 5 days, (d) inhaled/nebulised salbutamol, montelukast, oral nedocromil, oral cromoglycate in the last 24 h. Physical examination is carried out to ensure that there are no significant abnormalities that could influence the OFC outcome assessment.

OFC were performed following current best practice recommendations [1] as described elsewhere [8]. Briefly, this involved incremental doses of the culprit food reaching a cumulative dose equal or above an age-appropriate portion. Subsequent doses were given only in the absence of significant symptoms suggesting a reaction. Age-appropriate portions were determined using national references. Patients were under observation for at least 4–6 h following their reaction onset. A telephone call was conducted 48 h postdischarge to enquire about delayed symptoms as this might impact on OFC outcome assessment. The OFC outcome as positive/negative/inconclusive and reaction severity as mild/moderate/severe were assessed following current consensus criteria [1]; Figure S1.

For genomic comparisons, control groups were drawn from the 1000 Genomes Project [9] (http://www.1000genomes.org; hereafter referred to as 1000G). The Iberian population (IBS; $n = 107$) was used as control group for the association tests in the discovery phase. In the validation phase, the candidate SNPs and genes underwent further testing using other European data sets: CEU ($n = 99$), TSI ($n = 111$), GBR ($n = 100$) and a merged European data set (combining IBS, CEU, TSI and GBR; $n = 417$).

2.3 | Sequencing

DNA was isolated from blood samples using the Wizard Genomic DNA Purification Kit (Promega). The concentration of the samples was analysed by fluorometric quantification with the Qubit system, the degree of purity by spectrophotometry with the

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Summary

- FPIES genetics reveals significant variants and associated genes; particularly, *DGKZ*, *RBM8A*, *SIRPA* and *ATG16L1*.
- Candidate genes are associated with TGF-β signalling, TAR syndrome, increased neutrophils/monocytes in inflamed tissues and IBD.
- Pathways involved in FPIES pathophysiology are related to epithelial barrier dysfunction and immune dysregulation.

NanoDrop system and the integrity of the DNA by TapeStation. Paired-end sequencing (2×100 bp) of the *SureSelectXT* libraries, previously enriched, indexed and multiplexed, has been performed on the NovaSeq 6000 platform (Illumina, Inc). The quality of FastQ files for sequenced samples was assessed using *FastQC* v0.11.9 [10]. Subsequently, the FastQ files were aligned with *BWA* 0.7.17-r1188 [11]. The alignment of sequence reads, clone sequences and assembly was carried out using the BWA-MEM algorithm against the human reference genome 38 (HG38). Mapping quality was evaluated using *Mosdepth* 0.3.2 [12], *Bamtools* 2.5.2 stats [13] and *Samtools* 1.15 stats [14] with assistance from the *MultiQC* 1.12 collector [15]. The resulting *Bam* files were then processed following best practices outlined in *Gatk* 4.2.5.0 [16] and annotated with the Combined Annotation Dependent Depletion (CADD) database GRCh38-v1.6.

2.4 | Statistical Analysis

Statistical power was computed using the *mitPower* tool (Pardo-Seco et al. [17]). For a nominal significance threshold of 0.05, the statistical power to detect an odds ratio (OR) greater than three with a SNP frequency of 0.25 in controls exceeds 80% for the sample size in this study. Similarly, for a frequency of 0.1 in controls, the OR required to achieve an 80% statistical power should be four or higher.

The databases were initially filtered based on frequencies, removing monomorphic, duplicated, indels or triallelic variants. Additionally, SNPs with a minimum allele frequency (MAF) below the 0.01 threshold and/or a genotyping rate below 99.5% were eliminated. Sex chromosomes were ignored from the analysis. Variants in Hardy–Weinberg disequilibrium ($p < 0.001$) were eliminated from the analysis, leaving a total of 45,302 SNPs for further analysis.

To address the potential over-representation of allelic data from duplicated or closely related individuals, a relationship analysis was conducted using identity-by-descent pairwise values, comparing them with theoretical values of family relationships.

A population analysis was performed to mitigate the influence of genomic interbreeding and reduce the confounding effect of potential population substructure on the false-positive rate. This analysis entailed referencing data from the 1000G and using the *ADMIXTURE* software [18] to estimate individual ancestry

TABLE 1 | Summary of the main clinical characteristics of the FPIES cohort.

	Participants (*n* = 38)
Gender—*n* (%)	
Female	15 (39.5)
Male	23 (60.5)
Age (months)—Median [IQR]	62.5 [51.5]
Age at first reaction (months)—Median [IQR]	9.50 [3.75]
FPIES culprit food—*n* (%)	
Milk	7 (18.4)
Vegetables	2 (5.3)
Egg	7 (18.4)
Fish	21 (55.3)
Fruit	1 (2.6)
Allergic comorbidities—*n* (%)	
IgE-mediated food allergy	7 (18.4)
Multiple food FPIES	4 (10.5)
Other non-IgE-mediated food allergy	2 (5.3)
Asthma	8 (21.1)
Allergic rhinitis	3 (7.9)
Atopic dermatitis	9 (23.7)
Family background—*n* (%)	
Atopy/allergic diseases	19 (50.0)
FPIES	1 (2.6)
Clinical manifestations at OFC—*n* (%)	
Vomiting	38 (100)
Lethargy	34 (89.5)
Pallor	36 (94.7)
Diarrhoea	3 (7.9)
Hypotension	10 (26.3)
Hypothermia	4 (10.5)
Hypotonia	3 (7.9)
Missing data	3 (7.9)
Increased neutrophil count at OFC—*n* (%)	
Increased compared to baseline	32 (84.2)
Missing data	1 (2.6)
Severity of reaction at OFC—*n* (%)	
Mild	2 (5.3%)
Moderate	19 (50.0%)
Severe	17 (44.7%)

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estimation through multilocus SNP data. Thirteen population datasets representing major ancestral groups, including Europeans, East Asians, sub-Saharan Africans and Native Americans, were considered. Each sample bar represents an individual, with the coloured proportions indicating the estimated ancestry derived from the unsupervised *ADMIXTURE* analysis; this analysis represents a number $K=4$ ancestral clusters, each suggesting one of the four main continental regions: Africa, Asia, Native America and Europe. A multidimensional scaling analysis (MDS) was carried out, using identity-by-state values to investigate patterns of genome variation within cohort genomes and reference populations.

Subsequent steps encompassed a single-point SNP allele association test, and a gene-based association testing. Allele statistical association was performed using X^2 exact test for variants with a minor allele frequency (MAF) exceeding 0.05 in healthy controls and Hardy–Weinberg equilibrium p value above 0.001 for both the case and the control cohorts. We calculated the genome inflation factor *lambda* for all cohorts used in the discovery and validation comparisons to prevent potential bias due to population stratification; these values were used to adjust p values (Table 2). The gene-based association test utilised the variant collapsing method Sequence Kernel Association test (SKAT) [19]. Variants were weighted based on their CADD values to account for their differing impacts on disease [20]. Genes with fewer than two variants were removed.

To address multiple testing and mitigate false positives, a Bonferroni correction was applied. Data curation, single-point analysis, relationship analysis and population analyses were conducted using *PLINK* v1.9 [21, 22], while graphical representations, such as MDS, were generated using R v4.2.2 software [23]. Gene-based association tests were computed using the *SKAT* R package [24].

The functional analysis of statistically significant genes was performed through an over-representation analysis using the *Clusterprofiler* R package [25] and using the biological processes from the Gene Ontology (GO) as reference database.

In addition, we explored genome-wide coexpression correlations to investigate gene–gene interactions using the *Correlation AnalyzeR* package (17). We utilised gene expression correlation data from samples of both healthy intestinal tissues ($n=5,356$) and stomach tissues ($n=320$) as a reference (see Supplementary Methods S1 for details).

To examine the biological impact of the variants detected associated with the FPIES condition, we conducted a transcriptome-wide association study (TWAS) analysis. We have integrated the summary statistics results from the exome association test and expression quantitative trait locus (eQTL) data from different tissues related to the digestive system, namely stomach, ileum, pancreas, muscularis oesophagus, mucosa oesophagus, gastroesophageal oesophagus, transverse colon and sigmoid colon [26] (see Supplementary Methods S1 for details). Gene set enrichment analysis for the trait-associated genes derived from *MetaXcan* was carried out with the Genotype Imputed Gene Set Enrichment Analysis (*GIGSEA*) package [27] and KEGG (Kyoto Encyclopedia of Genes and Genomes [28, 29]) as pathways reference database. A weighted multiple linear regression model was applied to account for redundancy in gene sets and $n=10,000$ permutations to assess the significance of regression coefficients.

3 | Results

3.1 | Clinical Characteristics of the FPIES Cohort

We recruited 41 patients with acute FPIES confirmed by a standardised OFC to the culprit food. After excluding three samples showing a non-European ancestry genetic background (see below), a total of 38 samples were included for the downstream analysis (Table 1). Details on the number of patients recruited per centre are included in the Table S1.

The final cohort comprised 15 females and 23 males (median age 62.5 months). OFC led to the classification of patients in severe ($n=17$), moderate ($n=19$) and mild ($n=2$) phenotype (Table 1). It is noteworthy that most reactions were triggered by fish (55.3%), although our cohort also included a significant proportion of cases with FPIES to other leading FPIES causes such as cow's milk (18.4%) and egg (18.4%). Other allergic comorbidities in different proportions were reported for our FPIES cohort, being atopic dermatitis (24%), asthma (21%) and IgE-mediated food allergy (18%) the most common coexisting conditions. In addition, we observed a remarkable proportion of patients with a familiar history of allergic diseases (50%). Finally, a significant increase in the number of neutrophils was detected at OFC for most of the patients (84%), which is in line with previous observations in FPIES patients.

3.2 | Population Genetic Characteristics of FPIES Patients

Upon filtering out indels, triallelic and monomorphic variants and variants with a genotyping rate $<90\%$, we successfully identified 141,103 biallelic SNPs. The cohort was merged with the 1000G database yielding an overlapping set of 75,817 SNPs. Family relationship analysis revealed that all study samples are unrelated (Figure 1A). We then performed a MDS analysis (Figure 1B,C) to identify outliers. MDS and ADMIXTURE (Figure 1D) analysis identified ancestral population clusters, with most samples falling within the European core with the GBR, CEU and IBS populations serving as European reference populations. Three samples showed a non-European ancestry genetic component and were therefore excluded for further analyses to maintain genetic homogeneity in cases and controls and mitigate population stratification impact.

3.3 | Single Nucleotide Polymorphism Association Test

Variants in Hardy–Weinberg disequilibrium (p value <0.001) were eliminated from the analysis. A total of 45,302 SNPs survived and were subsequently analysed. Then, we performed a single-point association test. The allele test (Figure 2A) revealed four SNPs surpassing the Bonferroni threshold: rs201740330 (p value $=3\times10^{-7}$; *OR2T32P* gene), rs200103703 (p value $=6\times10^{-7}$; *OR2T32P*), rs872786 (p value $=5\times10^{-7}$; *RBM8A*: 'RNA binding motif protein 8A') and rs9917044 (p value $=2\times10^{-6}$; *ZNF28*). rs2241880 (p

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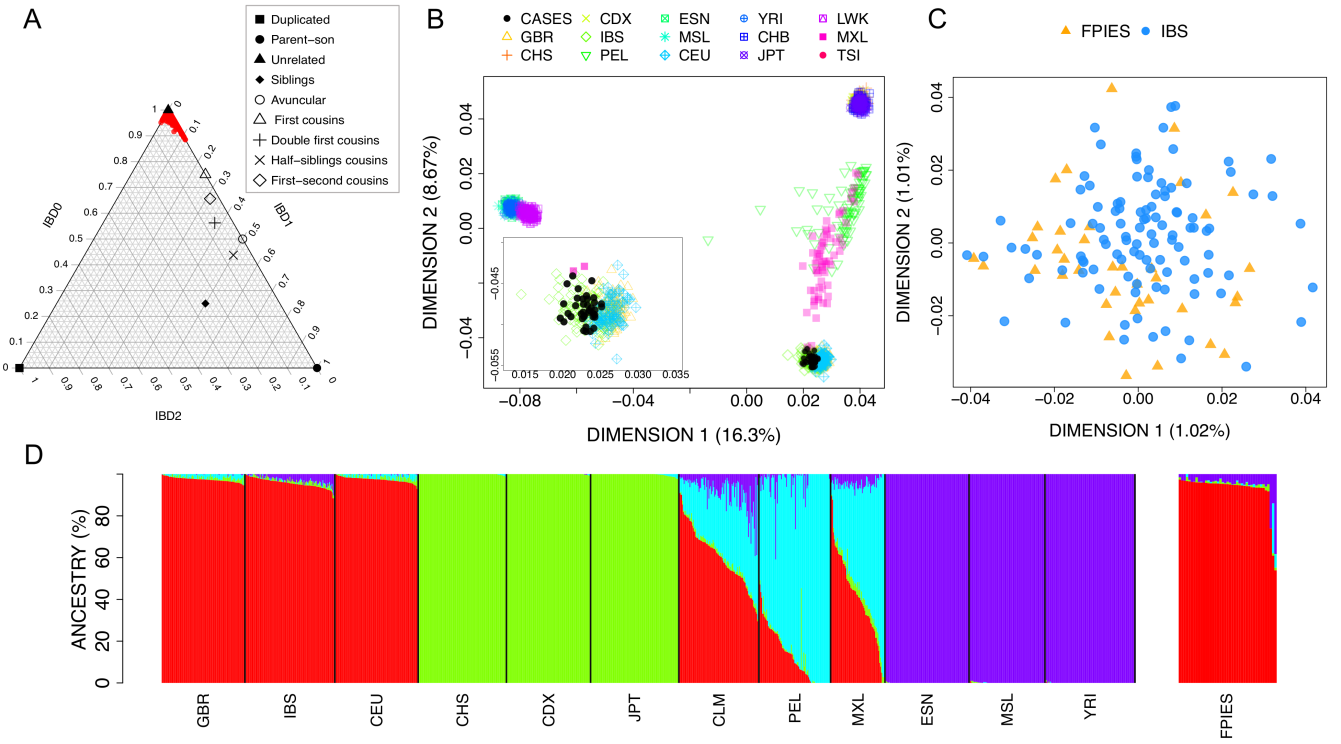

FIGURE 1 | Family relationships and ancestry analysis of exome data. (A) Family relationship: Ternary plot of theoretical (black dots) and FPIES (red dots) identity-by-descent values. (B) MDS plot of pairwise individual identity-by-state values of FPIES and 1000G data sets (inset: European cluster). (C) MDS plot for FPIES and IBS samples. (D) Admixture analysis considering FPIES cases and 1000G reference populations. Population codes in (C): https://www.coriell.org/1/NHGRI/Collections/1000-Genomes-Project-Collection/1000-Genomes-Project.

value = 1×10^{-4}; *ATG16L1:* 'Autophagy Related 16 Like 1 gene') falls close to the adjusted significance; and it is in high linkage disequilibrium (LD) ($r^2 = 0.93$) with rs2289477 (p value = 5×10^{-5}; *ATG16L1*) (Table 2). All candidate SNPs were validated using three additional European healthy control cohorts from the 1000G database (GBR, TSI and CEU), as well a merged European data set. Adjusted p values for the inflation factors maintained their statistical significance across all cohorts (Table 2).

3.4 | Gene-Based Association Test

A gene level analysis, using a CADD-weighted SKAT approach yielded significant results, initially identifying 24,098 genes. After applying a criterion requiring each gene to contain at least two SNPs, the list was reduced to 18,859 genes. Seven genes passed the Bonferroni correction threshold (Figure 2B), namely *DGKZ* ('Diacylglycerol Kinase Zeta gene'; p value = 1×10^{-18}), *TMEM99* (p value = 3×10^{-11}), *SIRPA* ('Signal Regulatory Protein Alpha'; p value = 1×10^{-7}), *SLC9B1P4* (p value = 5×10^{-7}), *PABPC1* (p value = 1×10^{-6}), *RBM8A* (p value = 1×10^{-6}) and *GnRHR2* (p value = 1×10^{-6}); *ATG16L1* was suggestively significant, nearly meeting the threshold for genome-wide significance (p value = 1×10^{-5}) (Table 2). From the point of view of FPIES, *DGKZ, SIRPA* and *RBM8A*, *ATG16L1* seem to be particularly interesting. All the genes were validated using additional European cohorts as reference controls (Table 2).

The enrichment analysis of the significant genes identified the nonsense-mediated mRNA decay (NMD) as the only statistically significant pathway (adjusted p value = 0.02), with two of the associated genes (*RBM8A* and *PABPC1*) involved.

In addition, *RBM8A* gene is located on chromosome 1q21. This region encompasses the epidermal differentiation complex, which includes the *FLG* gene (> 6,400 kilobases apart) [30]. Accordingly, and considering the association reported between risk of food allergy and *FLG* mutations, we investigated genome-wide coexpression correlations to study interactions between *RBM8A* and *FLG* genes in intestine and stomach. We found that *RBM8A* and *FLG* genes, despite displaying markedly different expression values in both intestinal and stomach tissues (p value = 2×10^{-16}; Figure 2C), exhibit significant relationship between their genome-wide coexpression correlations (Intestine: $R = -0.61$; Stomach: $R = -0.72$; p value = -2×10^{-16} in both cases); data inferred from *ARCHS* [4].

3.5 | Transcriptome-Wide Association Study

TWAS identified four significant eQTL-regulated genes (p value < 0.05) in different tissues as potential candidates involved in FPIES pathogenesis: *RBM8A* in stomach and pancreas, *ATG16L1* in the transverse colon *PIAS3* in pancreas and *RPIA* in esophagus mucosa (Figure 3A). *RBM8A* and *ATG16L1* showed predicted expression levels that were significantly higher in patients with FPIES than expected for these tissues (z-score = 5.1 and 4.6, respectively), whereas lower predicted expression values were detected for *PIAS3* and *RPIA* (z-score = −4.2 and −4.3, respectively) (Figure 3B).

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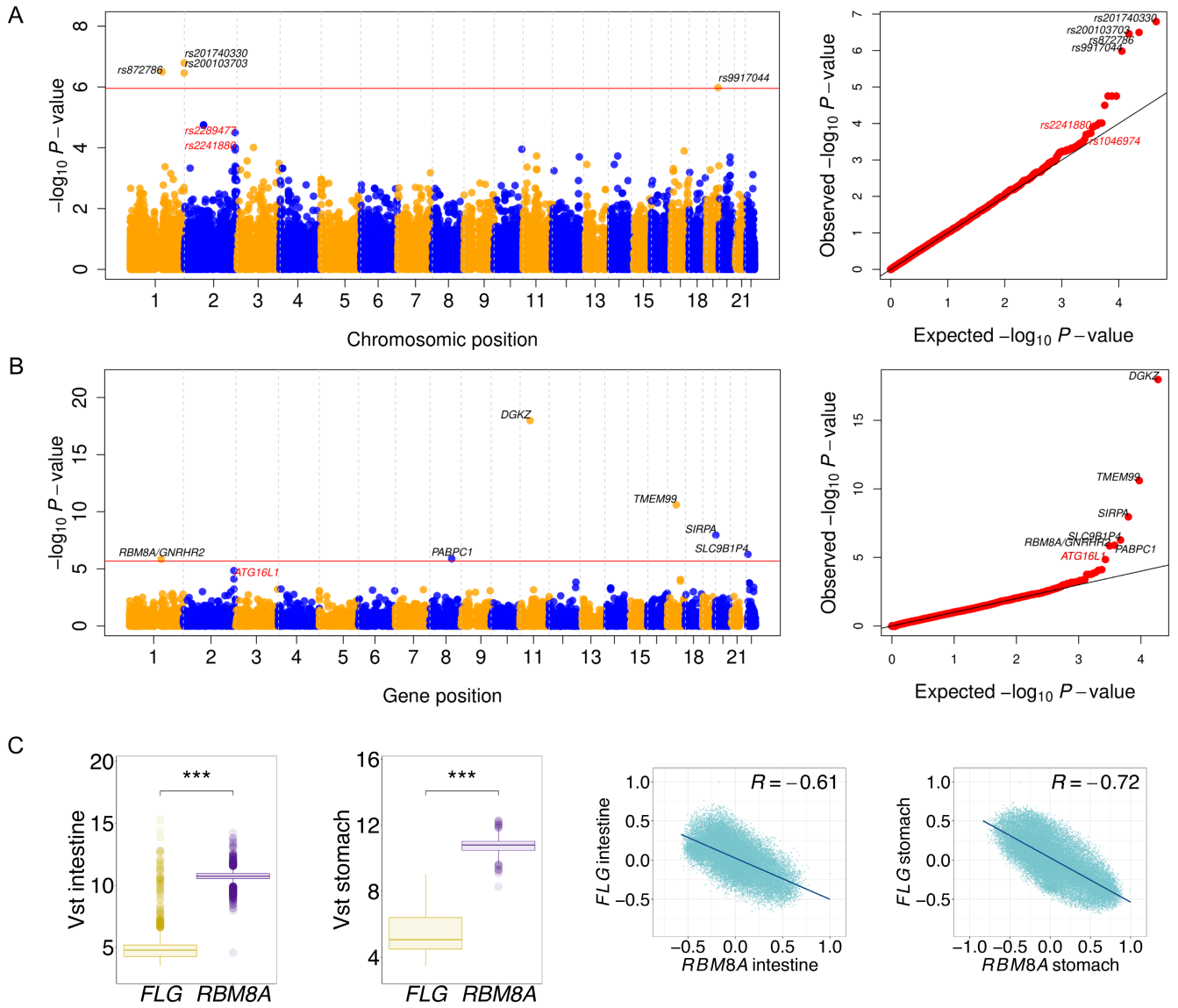

FIGURE 2 | Single-point and gene-based association tests. (A) Manhattan and QQ plot of *p* values for allelic association test; (B) Manhattan and QQ plot of CADD-weighted SKAT models. (C) Higher variance stabilizing transformation-adjusted (Vst) gene expression in *RBM8A* than *FLG*, and their genome-wide coexpression correlations; both in intestine/stomach from healthy subjects. Red line in (A) and (B): Bonferroni threshold. Labelled in red SNPs/genes in the limit of significance.

Further functional analysis of the imputed expression in these tissues highlighted several important pathways. Lysosomal activity related pathways were among the top significant processes in stomach and colon transverse. Top significant affected pathway in transverse colon was ATP-binding cassette (ABC) transporters. Other common significant pathways between stomach and transverse colon included different processes related to amino acids metabolism (Figure 3C).

4 | Discussion

We conducted a pioneering exome-wide case–control association study to comprehend the genetic basis of the FPIES syndrome. We identified (and further validated using three additional European ancestry healthy controls cohorts) different SNPs and gene candidates statistically associated with FPIES. These genetic factors could help to understanding the genetic susceptibility to this condition.

The protein encoded by *DGKZ* belongs to the eukaryotic diacylglycerol kinase family. *DGKZ* promotes transforming growth factor β (TGF-β) signalling [31]; TGF-β is a key tolerogenic cytokine which regulates intestinal epithelial barrier integrity by maintaining and restoring enterocyte' barrier function [32], and by regulating IgA production [33]. TGF-β and IgA are crucial to induce tolerogenic responses through allergen-specific immunotherapy to inhalant allergens [33]. Children with cow's milk-FPIES showed deficient TGF-β responses upon casein stimulation of peripheral blood mononuclear cells (PBMCs) and lower serum casein-specific IgA levels compared with milk-tolerant children [34]. Additionally, reduced expression of TGF-β Type I receptor has been reported on epithelial and mononuclear cells in the lamina propria of duodenal biopsies in

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TABLE 2 | *p* Values (and OR) for associated SNPs and genes.

	Discovery	Validation			
	IBS $\lambda = 1.04$; $n = 107$	**CEU $\lambda = 1.35$; $n = 99$**	**TSI $\lambda = 1.21$; $n = 111$**	**GBR $\lambda = 1.32$; $n = 100$**	**Europe $\lambda = 1.14$; $n = 417$**
SNP (chromosome region)					
rs201740330–A (1q44)	2×10^{-7} (5.3)	5×10^{-7} (7.4)	—*	2×10^{-5} (5.0)	2×10^{-12} (6.5)
Freq. (A)—FPIES	0.34	0.34	0.34	0.34	0.34
Freq. (A)—controls	0.09	0.07	0.05	0.09	0.07
rs200103703–C (1q44)	6×10^{-7} (4.1)	10×10^{-7} (6.8)	3×10^{-9} (9.1)	5×10^{-5} (4.4)	2×10^{-11} (5.9)
Freq. (C)—FPIES	0.34	0.34	0.34	0.34	0.34
Freq. (C)—controls	0.09	0.07	0.05	0.12	0.08
rs872786–T (1q21.1)	5×10^{-7} (5.0)	2×10^{-4} (3.4)	6×10^{-5} (3.3)	5×10^{-6} (4.3)	4×10^{-7} (3.7)
Freq. (T)—FPIES	0.68	0.68	0.68	0.68	0.68
Freq. (T)—controls	0.35	0.39	0.39	0.33	0.36
rs9917044–C (19q13.41)	2×10^{-6} (6.2)	8×10^{-4} (4.1)	3×10^{-4} (4.0)	8×10^{-3} (2.8)	3×10^{-6} (4.0)
Freq. (C)—FPIES	0.25	0.25	0.25	0.25	0.25
Freq. (C)—controls	0.05	0.08	0.08	0.11	0.08
rs2241880–A (2q37.1)	1×10^{-4} (2.9)	0.003 (2.5)	0.002 (2.5)	2×10^{-2} (2.1)	5×10^{-4} (2.5)
Freq. (A)—FPIES	0.67	0.67	0.67	0.67	0.67
Freq. (A)—controls	0.41	0.44	0.44	0.49	0.44
rs2289477–T (2q37.1)	5×10^{-5} (3.3)	0.001 (2.9)	8×10^{-4} (2.8)	0.009 (2.4)	2×10^{-4} (2.8)
Freq. (T)—FPIES	0.69	0.69	0.69	0.69	0.69
Freq. (T)—controls	0.41	0.44	0.45	0.49	0.45
Gene					
RBM8A (1q21.1)	1×10^{-6}	1×10^{-5}	2×10^{-5}	8×10^{-8}	9×10^{-8}
GnRHR2 (1q21.1)	1×10^{-6}	1×10^{-5}	2×10^{-5}	8×10^{-8}	9×10^{-8}
ATG16L1 (2q37.1)	1×10^{-5}	5×10^{-4}	4×10^{-4}	1×10^{-2}	2×10^{-4}
DGKZ (11p11.2)	1×10^{-18}	10×10^{-15}	3×10^{-11}	2×10^{-11}	1×10^{-20}
TMEM99 (17q21.2)	3×10^{-11}	5×10^{-13}	7×10^{-14}	1×10^{-10}	2×10^{-12}
PABPC1 (8q22.3)	1×10^{-6}	3×10^{-9}	2×10^{-8}	5×10^{-7}	6×10^{-8}
SLC9B1P4 (22q11.1)	5×10^{-7}	3×10^{-8}	3×10^{-6}	7×10^{-5}	3×10^{-10}
SIRPA (20p13)	1×10^{-7}	1×10^{-8}	2×10^{-9}	1×10^{-6}	3×10^{-13}

Note: Discovery phase: FPIES versus IBS. Validation phase: FPIES versus CEU, GBR, and TSI data sets, and a merged European data set (combining IBS, CEU, GBR and TSI data sets); all sourced from the 1000G. All *p* values were adjusted for the inflation factor *lambda*.
*MAF < 0.05. For SNPs, Freq. denotes allele frequency for the minor allele.

FPIES [35]. Skin and gut epithelial barrier dysfunction due to *FLG* mutations has been identified as a crucial pathogenic and risk factor for IgE-mediated food allergy, even in the absence of atopic dermatitis [4]. A disruption of the epithelial barrier function via impaired TGF-β signalling and reduced IgA neutralisation capability in the gut microenvironment might be plausible mechanisms in FPIES, potentially leading to increased antigen penetration to the submucosa and antigen-specific lymphocyte stimulation [36]. Given the fundamental role of the TFG-β pathway in epithelial barrier function and generation of IgA responses, *DGKZ* might play a key role in the pathophysiology of FPIES.

ATG16L1 has been found to be associated with inflammatory bowel disease (IBD) [37]. Thus, *ATG16L1* is involved in autophagy, a complex cellular process crucial for intestinal homeostasis, that is dysregulated in IBD [38]. IBD has been associated with FPIES in adults [39]. A barrier function defect and immune dysregulation are key to IBD pathophysiology, and Crohn's disease is associated with an increased

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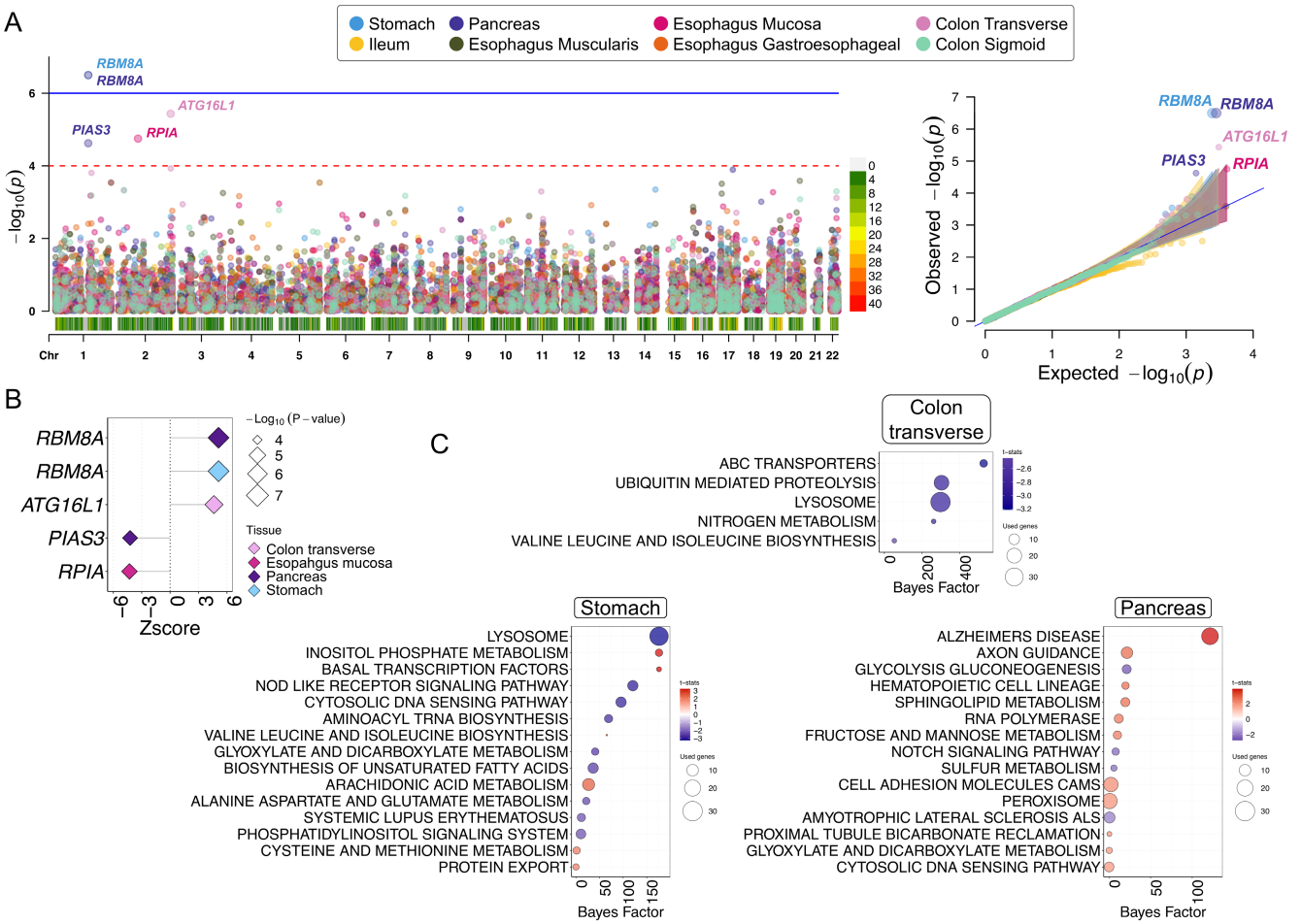

FIGURE 3 | TWAS analysis based on EWAS FPIES data. (A) Manhattan and QQ plot representations of the TWAS analysis in various tissues. The colour scale represents SNP density in the chromosomes. Blue and red dashed line thresholds refer to p values 10^{-6} and 10^{-4}, respectively. (B) TWAS z-score of the most significant genes. (C) Enrichment analysis for genes identified in the TWAS analysis conducted in the stomach, pancreas and transverse colon.

Th17 response, the key signature in FPIES [40]. The genetic association with *ATG16L1* in both FPIES and IBD, and the increased Th17 signalling, suggest common mechanisms in FPIES and IBD.

SIRPA is an immunoinhibitory receptor primarily expressed by myeloid lineage of immune cells, including neutrophil, monocytes, macrophages and dendritic cells. Numerous publications propose an association between elevated gene signatures of monocytes and neutrophils and IBD [41]. *SIRPA*, as other neutrophil/monocyte-associated genes, show upregulation in inflamed tissues, most likely due to the presence of an increased number of neutrophils/monocytes expressing SIRPα protein [42]. Although, the pathophysiology of FPIES remains incompletely elucidated, acute FPIES reactions involve profound innate immune activation including neutrophils, monocytes, eosinophils and lymphocytes [43]. Increase in neutrophils, and decrease in eosinophil and lymphocyte counts in peripheral blood has been reported, which might suggest migration of this type of cells to the gut tissue [43]. Innate immune dysregulation via the *SIRPA* might be a novel potential factor contributing to this picture.

Epithelial barrier defects resulting from *FLG* mutations are a major risk factor for the development of atopic eczema, IgE-mediated food allergy, eczema-associated asthma and allergic rhinitis [4, 44]. Interestingly, we have found a significant relationship between *FLG* and *RBM8A* genome-wide coexpression patterns. This may indicate a functional link between both genes through the existence of crucially correlated and anticorrelated gene clusters. The functional connection between *RBM8A* and FPIES might point towards shared pathogenic mechanisms related to IgE-mediated food allergy. Additionally, the *RBM8A* gene has been found to be associated with cow's milk allergy. For instance, a deletion located at chromosome band 1q21.1, and other mutations fallen in *RBM8A*, are often associated with thrombocytopenia with Absent Radius (TAR) syndrome. TAR associates cow's milk allergy in up to two-thirds of individuals, and susceptibility to recurrent bouts of gastroenteritis [45–47]. An association between cow's milk allergy and carriers of 1q21.1 deletion without TAR syndrome has been also documented [45]; this observation could result from incomplete penetrance of 1q21.1 deletion and other mutations in *RBM8A* associated to TAR [45].

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Regarding other genes identified in this work as associated with FPIES (*GnRHR2*, *PABPC1*, *SLC9B1P4* and *TMEM99*), minimal information is available related to their biological function or potential role in disease.

The enrichment analysis of significant genes revealed the NMD pathway as statistically significant. NMD eliminates mRNAs with premature translation–termination codons and plays a role in pre-mRNA splicing, although its specific relevance to FPIES remains uncertain.

TWAS pinpointed *RBM8A* (stomach and pancreas), and *ATG16L1* (transverse colon) as the most promising candidates, each exhibiting predicted expression values higher in FPIES than expected. The functional assessment of imputed gene expression in these tissues underscored pathways linked to lysosomes (in the transverse colon and stomach), ABC transporters (in the transverse colon) and amino acid metabolism—all of which have previously been associated with IBD and other intestinal diseases [48]. Indeed, lysosomes are also key elements of the autophagic machinery.

The study acknowledges certain limitations, including a small sample size and variations in patient age, potentially affecting statistical power (and therefore precluding the possibility to identify variation with lower effects) and result accuracy. In addition, other allergic diseases, relatively common in our FPIES cohort, might be a confounder factor in our results. However, no association has been reported between the genes identified in this work and other allergic diseases (beyond the abovementioned *RBM8A* gene and milk allergy). To our knowledge, no formal epidemiological studies have quantified the genetic heritability. Two sets of identical twins with FPIES with close similarities in clinical manifestations and age of onset have been reported, suggesting a strong genetic component [49]. Given the global disparities in FPIES characteristics across the globe [1], including regarding food culprits, further research should explore our findings in a diverse international cohort.

5 | Conclusions

Our investigation represents the first case–control exome association study in FPIES, revealing gene and SNP candidates. These insights initiate genomic exploration in FPIES and provide further evidence of a role of genetics in the condition, suggesting involvement in epithelial barrier dysfunction and immune dysregulation—major pathogenic factors identified in IgE-mediated food allergy [4] and IBD [40]. While our results are novel, they are still preliminary and need additional validation in a second cohort of patients.

Author Contributions

A.G.-C., A.S., F.M.-T. and M.V.-O. conceived, designed and provided financial support to the study. M.V.-O. and L.A. analysed the clinical data. A.F., A.M., A.P., C.G.-M., E.G., F.M., G.Z.-I., G.M., I.C., J.D.M.-G., L.A., L.E., L.V., M.F.-R., M.T.-P., M.P., M.J.T., N.L.H.-M., P.G.-D., S.A., S.B., S.I., S.Q., S.V.-C., T.B., T.G., V.O.-A. and V.P. were involved in sample recruitment. A.C.-M., A.S., A.G.-C., J.P.-S. and X.B. analysed the data. A.C.-M., A.S., A.G.-C., J.P.-S. and M.V.-O. wrote the initial draft of the article. All the authors revised and contributed to the final version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

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