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Gene expression profiling of patients with latex and/or vegetable food allergy

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Abstract. – BACKGROUND: The prevalence of individuals allergic to latex, exhibiting cross-hypersensitivity with plant-derived food has been frequently reported as the so-called latex-fruit syndrome. Nonetheless, molecular mechanisms underlying allergy to latex and/or fruit are poorly understood.

AIM: The aims of this study were to identify candidate genes that may be associated with the pathogenesis of allergy to latex and/or vegetable food, and to assess if similar molecular pathways are involved in both types of hypersensitivity.

MATERIALS AND METHODS: DNA microarray analysis was performed to screen the molecular profiles of peripheral blood mononuclear cells isolated from patients with allergy to latex, to fruit, or with latex-fruit syndrome, and from control healthy subjects.

RESULTS: Molecular profiling identified an overlapping dataset of genes commonly regulated in all the atopic patients enrolled in this study, suggesting that similar molecular mechanisms are involved in the pathogenesis of allergy to the fruit and/or latex. Several regulators of the innate and acquired immunity reported to polarize the immunological response towards a Th2-mediated immune response were overexpressed in the patients. Evidences suggested that the expression of T-regulatory cells might be defective in allergic patients, as a consequence of a dysregulation of some inflammatory cytokines. Finally, several transcription factors that may be responsible for the Th1/Th2 imbalance were modulated in allergic patients.

CONCLUSIONS: This study identified relevant genes that may help to elucidate the molecular mechanisms underlying allergic disease. Knowledges of critical targets, along with transcription factors regulating gene activity may facilitate the development of new therapeutic options.

Key Words:

Latex allergy, Fruit allergy, Latex-fruit syndrome, Microarray, Th1/Th2 imbalance, Transcription factors.

Introduction

Natural rubber latex (NRL) extracted from the rubber tree *Hevea brasiliensis* is a potent allergen[1]. In the last decades, the use of latex-derived products has dramatically increased, and as a consequence, latex allergy has become an important health problem worldwide. Since the first report of latex allergy in 1927, several cases have been described, with clinical manifestations ranging from urticaria to bronchial asthma or anaphylactic shock[2-5]. Latex allergy prevalence in normal population is relatively low, under 1%. However, the prevalence of this peculiar hypersensitivity increases significantly in persons with prolonged latex exposure such as healthcare and rubber industry workers, as well as children and adult who have undergone multiple surgical procedures[6]. Subjects with fruit and vegetable allergy are also at risk due to cross-reactive allergens[7]. It is in fact estimated that about 30-50% of individuals who are allergic to NRL show an associated hypersensitivity to some plant-derived food, especially fruits, including avocado, banana, kiwi, mango among others. The cross-reactivity between NRL and various food allergens was established in the nineties as the so-called "latex-fruit syndrome"[8,9]. Although the reasons that certain individuals suffer particular hypersensitivities are unclear, there is evidence that both genetic and environmental factors influence susceptibility[10]. Analysis of genes contributing to allergic disorders have shown that susceptibility arises from complex multigenic interactions[11]. To date, few data are available regarding the molecular processes involved in latex, fruit, or latex-fruit allergies. Based on the cross-reactivity between fruit and latex allergens, and on the predisposition of latex allergic patients to develop fruit allergy (or vice versa), it could be hy-

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pothesized that similar pathways are involved in the pathogenesis of latex and vegetable food hypersensitivities.

In the last decade, microarrays have been developed for large-scale clinical research to identify genes involved in disease states. Recently, this technology has been successfully used to screen the genetic profiles of patients affected by atopic dermatitis, rhinitis, and asthma[12-14]. In line with the Th1/Th2 hypothesis, these studies identified predominantly transcripts encoding for Th2-derived cytokines, the hallmark signaling proteins implicated in allergic responses[15]. Furthermore, genes associated with noninflammatory pathways were also identified, suggesting that other mechanisms contribute to the pathogenesis[16].

The aim of this study was to identify genes that may be associated with the pathogenesis of allergy to latex and/or vegetable food. For this purpose, peripheral blood mononuclear cells (PBMCs) isolated from patients with allergy to latex, to fruit, or with latex-fruit syndrome, and from control healthy subjects were analyzed using DNA microarray. PBMCs can be easily and repeatedly isolated from patients and represent a convenient source of cells for medical research and diagnosis. Meaningful information related to pathogenetic processes associated with specific diseases can be deduced from the expression profiling of these cells.

Our results identified an overlapping dataset of genes commonly regulated in all allergic patients enrolled in this study. Such results may help to understand the molecular mechanisms and gene-expression patterns involved in food and latex allergy.

Materials and Methods

Subjects

Patients with latex, fruit or latex-fruit allergy symptoms demonstrated by accurate allergological evaluation (as described below) were enrolled from the Departement of Allergology of the Gemelli Hospital of Rome for a molecular profiling study. For this investigation, local Ethical Approval had been granted, and informed consent from patients obtained. We enrolled a total of 17 patients (median age 30 ys) of whom 9 were females. Patients were either allergic to the fruit (*group F: 5 patients*), to the latex (*group L: 6 patients*) or affected from the latex-fruit syndrome (*group L-F: 6 patients*). Furthermore, 4 non-atopic control subjects (1 man and 3 women, median age 33 ys) were included in this study. The 5 patients affected by fruit allergy showed no sensitization to latex (negative skin prick test and specific IgE). The most involved foods were peanut, peach and apple. The 6 patients affected by latex allergy showed no sensitization to any cross-reacting food and they all had positive skin prick tests with the latex extract and positive specific IgE. The diagnosis of latex allergy was then confirmed by the positivity of at least one challenge test. The 6 patients affected by latex-fruits syndrome showed sensitization to one or more cross-reacting foods. The most involved foods were kiwi, banana and peanut. Five ml of blood were collected on EDTA tubes from each individual. PBMCs were isolated on a Ficoll gradient (Histopaque-1077; Sigma Diagnostics, St. Louis, MO, USA) within 2 hours of blood collection, and stored at -80°C for further application.

Allergological Evaluation

The diagnosis of latex and food allergy was made through the clinical history and an allergological work-up as previously described[1,17,18]. Briefly, the diagnosis of latex allergy was assessed by skin prick tests with a standard latex extract (500 µg/ml; ALK Abellò, Madrid, Spain), and a 1-cm^2 piece of surgical glove latex material (prick-by-prick method). Glycerin solution and histamine (10 mg/ml) were used as negative and positive controls. Patch tests were performed to exclude a type IV delayed cell-mediated hypersensitivity to latex: results were checked 72 h after the patch has been placed, assessing positivity according to the North American Contact Dermatitis Group criteria[19]. Latex allergy was confirmed by specific provocation tests (cutaneous, mucous, sublingual, conjunctival, bronchial, nasal and vaginal tests). The diagnosis of allergy to fruit was performed using standardized cross-reactive vegetable food allergens (ALK Abellò, Madrid, Spain) and fresh food (prick-by-prick method). A double blind placebo controlled oral food challenge was also performed. Furthermore, specific anti-NRL or anti cross-reactive vegetable food immunoglobulins IgE were measured by means of fluorescent enzyme immunoassay (UniCAP System: Pharmacia. Uppsala, Sweden). Samples with specific IgE concentration of > 0.35 kU/l were regarded as positive. Furthermore, serum total IgE, ECP (UniCAP System: Pharmacia) were measured. ECP < 20 µg/l and total IgE < 200 kU/l were considered normal.

Microarray Analysis

Microarray Processing

Total RNA was extracted from PBMCs using the RNeasy mini kit, including DNase digestion on a RNeasy spin column (Qiagen, Inc, Valencia, CA, USA). RNA was quantified by UV spectrophotometer (Beckman DU800, Beckman Coulter, Inc., Fullerton, CA, USA) and quality was assessed on agarose gel. RNA was processed for the hybridization onto the Affymetrix Human Focus arrays, counting ≈8,500 transcripts, according to the manufacturer's recommended protocol. Briefly, 2 µg of each RNA was converted into double strands cDNA using a T7-$(dT)_{24}$ primer containing a T7 RNA polymerase promoter in the 5'end. The resulted cDNA was used as template to generate biotinylated cRNA during an overnight *in vitro transcription* reaction at 37°C. The labeled cRNA was purified and chemically fragmented. A total of 130 µl of hybridization sample cocktail containing 15 µg of cRNA was loaded onto each array. Chips were hybridized at 45°C for 16 hours in an oven set at 60 RPM. Finally, the arrays were washed and stained in the Affymetrix Fluidic Station and scanned twice using the Agilent Gene Array scanner 2500 (Affymetrix, Santa Clara, CA, USA).

Data Analysis

Scanned arrays were analyzed with Affymetrix MAS 5.0 and GeneSpring Expression Analysis version 7.3 softwares (Silicon Genetics, Redwood City, CA, USA). Raw intensity values from each chip were normalized using the 50th percentile of the measurements from that chip. Then, the averaged intensity of signals from biological replicates was calculated. Data filtration based on genes flagged "*present*" in at least 1 of the four experimental groups (groups L, F, L-F, and healthy controls) was performed. Molecular homogeneity of biological replicates belonging to each experimental group was verified by achieving the principal component analysis (PCA). Afterward, genes whose expression level increased or decreased more than 2 folds between one of the three allergic groups and the control healthy donor group were retained. Differentially expressed genes were identified by unpaired *t*-test (*p*-value cut-off 0.01; Benjamini-Hochberg false discovery correction), and further categorized according to their biological function using Gene Ontology (GO) annotations. Genes commonly regulated by all allergic patients were then emphasized by the means of Venn diagram representation. Microarray data presented here have been submitted to the NCBI's Gene Expression Omnibus (GEO) data repository (http://www.ncbi.nlm.nih.gov/geo/) and are accessible through the following accession number: GSE13619.

Real-time Quantitative PCR Validation

To validate microarray data, a subset of 8 candidate genes differently expressed between allergic and control patients was validated by the means of semi-quantitative real-time-PCR (Q-PCR) (LightCycler technology; Roche, Mannheim, Germany). Independent patients were enrolled for this analysis. Selected genes were chosen for their recognized involvement in allergic or inflammation processes. Oligonucleotide primers used for amplification were designed using Primer3 software: http://frodo.wi.mit.edu/. Primer forward and reverse sequences are recapitulated in Table I. One µg of RNA from each patient was reverse-transcribed by SuperScript III (Invitrogen, Carlsbad, CA, USA). PCR reactions were performed using the *LightCycler FastStart DNA Master Plus SYBR Green* kit with 10 minutes of pre-incubation at 95°C followed by 45 cycles of 10 seconds at 95°C, 7 seconds at 58°C and 8 seconds at 72°C. PCR products were then subjected to melting curve analysis to rule out synthesis of unspecific products. Crossing points (Cp) of real-time PCR curves were determined by the Lightcycler (3.5) software using the second derivative maximum method. For each sample, two independent amplifications were performed and the mean value was calculated for further analysis. The $2^{-\Delta\Delta Ct}$ method[20] was used to determine fold difference in gene expression using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as control.

Results

Molecular Profiling of Latex and/or Fruit Allergies

We enrolled a total of 17 patients (5 allergic to fruit, 6 allergic to latex, 6 with latex-fruit syndrome), and 4 healthy donors. Gene expression in individual PBMCs from all patients and controls were analyzed with a total of 21Affymetrix Human Focus arrays. We first used PCA, a dimensionality reduction method that produces a set of expression patterns known as principal components. This technique combines these pat-

Table I. Primer sequences employed in Q-PCR analysis for the validation of 8 selected genes that were found to be regulated differently in allergic patients compared to control individuals.

Gene	Symbol		5'-3' sequence	Amplicon (bp)
Defensin	DEFA1	F	TCCCAGAAGTGGTTGTTTCC	165
		R	AGCAGCAGAATGCCCAGAG	
Toll-like receptor 4	TLR4	F	TTTTGGGACAACCAGCCTAA	212
		R	TGCCATTGAAAGCAACTCTG	
Aquaporin 3	AQP3	F	TATCTACCCTCACCCCACGA	198
		R	ACACATACCTGCTGCCCATT	
Lactotransferrin	LTF	F	GGCTACTTCACTGCCATCCA	200
		R	GCTTCTCCTTTCAGCACCAG	
Charcot-Leyden crystal protein	CLC	F	TCAGAAGAGCCACCCAGAAG	218
		R	GACCACACGACGACCAAAG	
Viral oncogene v-FOS	FOS	F	AGAATCCGAAGGGAAAGGAA	246
		R	ATCAAGGGAAGCCACAGACA	
Interleukin 18 receptor accessory protein	IL18RAP	F	CAAGCAAGCTTGATGGACAA	143
		R	CGGACCACTGGGAAATCTTA	
Interleukin 1 receptor type 2	IL1R2	F	CTACGCACCACAGTCAAGGA	158
		R	TGAGGCCATAGCACAGTCAG	
Glyceraldehyde -3 phosphate dehydrogenase	GAPDH	F	ATGTTCGTCATGGGTGTGAA	173
		R	GTCTTCTGGGTGGCAGTGAT	

F: Forward primer; R: Reverse primer; bp: base pair.

terns to represent the behavior of all genes in a given sample, and allows to assess the molecular similarity between samples. By this way, we observed that allergic and control samples segregated into 2 independent clusters in a three-dimensional model, implying that allergic patients and healthy subjects have distinct molecular profiles (Figure 1). Among allergic patients, no significant subpopulation was identifiable, suggesting that similar molecular mechanisms are involved in the development of hypersensitivity to latex and/or fruits. Using a fold change expression analysis associated to a statistical validation, we identified genes significantly regulated in patients suffering from allergy compared to healthy donors. We emphasized 132 genes, 127 genes and 111 genes, respectively modulated in patients with allergy to latex, to vegetable food, and affected by the latex-fruit syndrome. We next assessed how gene expression was shared across multiple experimental conditions by the means of Venn diagram analysis (Figure 2). We found an overlapping dataset of 39 genes differently expressed in the three allergic populations (Table II). Furthermore, we identified 18 genes statistically regulated in both fruit and latex groups (Table III); 17 genes regulated in both fruit and latex-fruit groups (Table IV) and 23 genes regulated in both latex and latex-fruit groups (Table V). The expression of these genes significantly modulated in 2 cohorts of allergic patients was verified in the third allergic population, showing the same trend of regulation (i.e., all genes follow the same direction of regulation with similar fold change but do not achieve levels of statisti-

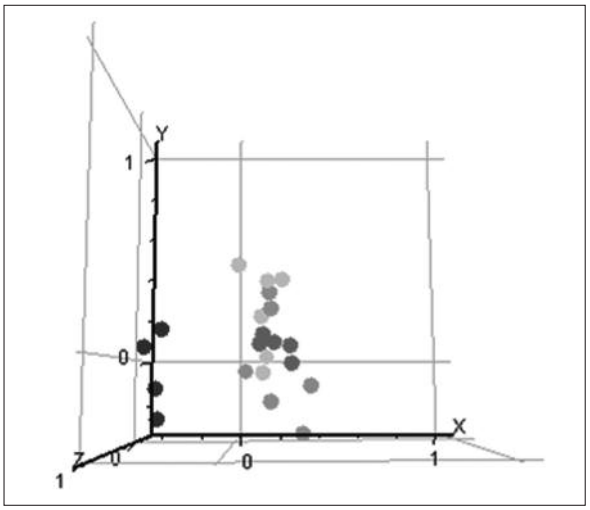

Figure 1. Principal component analysis (PCA) 3D view for gene expression profiles of samples of the four experimental groups (black: control healthy individuals; dark grey: patients allergic to latex; light grey: patients with latex-fruit syndrome; grey: patients with fruit allergy). Every dot represents a patient. The PCA representation emphasizes the clear segregation between the control individuals and the allergic patients.

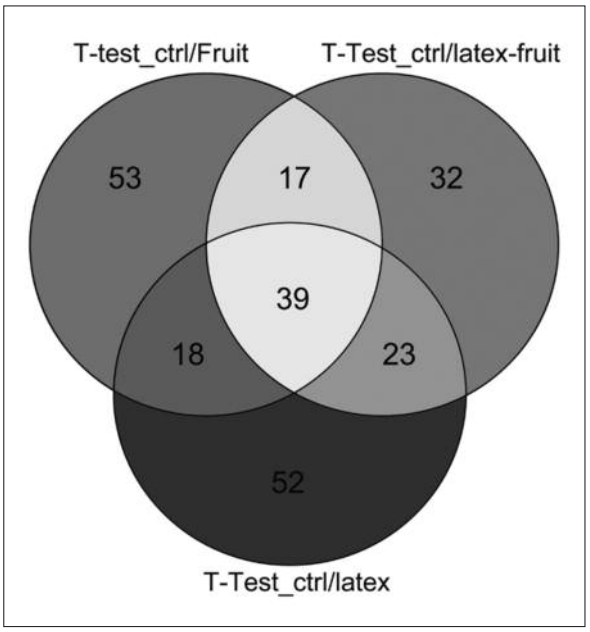

Figure 2. Venn diagram representation. This image highlights genes commonly regulated in the various sub-groups of allergic patients respectively to the control individuals. A dataset of 39 genes was commonly significant in the three groups of allergic patients compared to control individuals, whereas 17, 23 and 18 genes were statistically different in 2 groups of allergic patients; respectively, the fruit and latex-fruit groups, the latex-fruit and latex groups, and the latex and fruit groups.

cal significance). Thus, to simplify the complexity of this study, we considered all the previous lists as a unique dataset that could be regarded as a molecular signature of vegetable food and latex allergies. Selected genes were finally categorized into biological families according to their Gene Ontology annotations.

Among the genes whose expression was modulated in allergic patients, we identified several targets that are well documented in the pathogenesis of allergy, including regulators of the innate and adaptive immune responses. Antimicrobial peptides, such as *DEFA1, CAMP, LTF*, along with the pattern recognition receptor *TLR4* were transcriptionally activated in allergic patients. Two other genes, the eosinophil marker *CLC* and the mast cell inflammatory mediator *CPA3*, largely described for their role in hypersensitivity reaction were also overexpressed in allergic PBMCs. Candidate genes associated with susceptibility to asthma, including *HLA-DQA1* and *MS4A3* were also increased in latex and/or fruit allergic patients. Furthermore, DNA microarray analysis identified other mediators recently described for their implication in the pathogenesis of allergy, such as *MMP9* and *AQP3*.

Conversely, results showed a lower expression of the Th1-related cytokine *IL18RAP* in allergic patients. Furthermore, the proinflammatory cytokine *IL1A*, which functions as growth factor for Treg expansion was repressed. Finally, several transcription factors were down-regulated in patients allergic to vegetable food and/or latex, including *FOS* and *NFAT5*, both playing a central role in the transcription of inducible genes during the immune response.

Q-PCR Validation

To assess the validity of the microarray data, the expression of 8 genes chosen for their relevant pathophysiological significance in allergy were validated by Q-PCR (Figure 3). Analysis of the average expression for each of the 8 genes across patient samples matched the direction of expression measured by microarray technology. RT-PCR data confirmed the overexpression of DEFA1, LTF, AQP3, CLC, TLR4, IL1R2 genes in all allergic patients compared to control healthy individuals; while FOS and IL18RAP were repressed. Furthermore, as Q-PCR validation was performed with RNA extracted from independent patients (i.e., not enrolled for microarray study), it could imply that gene expression profiles obtained by microarray can be extrapolated to most of the patients with allergy to fruit and/or latex.

Discussion

Food and latex allergies represent an increasing concern in western countries. Nonetheless, the molecular mechanisms involved in the pathogenesis of both types of allergy are still poorly understood. In this study, patients with latex, fruit or latex-fruit allergy and healthy control subjects have been enrolled to screen their PBMCs gene expression profiles under steady-state condition. Overall, we demonstrated by the mean of principal component analysis that allergic patients enrolled in this study express a genetic profile distinguishable from that of healthy individuals. Among the different subpopulations of allergic patients, we did not find any statistical gene expression differences, suggesting that common molecular mechanisms are involved in latex and vegetable food allergies. Even if the correlation

Table II. Overlapping dataset of the 39 genes with statistically significant, more than 2-fold expression in all allergic patients (groups L; F; and L-F) compared with healthy donors.

Gene name (symbol)	GeneBank	Unpaired comparisons					
		L vs CTRL		F vs CTRL		L-F vs CTRL	
		FC	*p*-value*	FC	*p*-value*	FC	*p*-value*
Up-regulated genes							
Immune response							
Charcot-Leyden crystal protein (**CLC**)	NM_001828	4.6	9.73E-03	4.1	9.68E-03	3.5	4.99E-03
Lactotransferrin (**LTF**)	NM_002343	3.8	6.34E-03	5.6	6.05E-03	5.7	2.52E-03
Toll-like receptor 4 (**TLR4**)	NM_003266	2.1	1.00E-02	2	7.43E-03	2	3.10E-03
Signal transduction							
Membrane-spanning 4-domains. subfamily A. member 3 (**MS4A3**)	L35848	2.6	7.87E-03	3.3	3.89E-03	2.8	1.61E-04
Metabolism							
Flap structure-specific endonuclease 1 (FEN1)	BC000323	2.2	8.14E-03	2.3	5.47E-03	2.3	1.38E-03
NADH dehydrogenase 1 alpha subcomplex (NDUFA3)	NM_004542	2.4	2.99E-04	2.4	6.99E-03	2.3	2.70E-04
Pyrophosphatase (PP)	NM_021129	2.1	1.39E-03	2.1	2.37E-03	2.1	1.43E-03
Ubiquitin-conjugating enzyme E2M (UBE2M)	NM_003969	2.6	7.26E-03	2.7	5.51E-03	2.3	3.58E-03
Carboxypeptidase A3 (**CPA3**)	NM_001870	2.5	6.00E-03	2.9	3.89E-03	2.3	7.69E-03
Mitochondrial ribosomal protein S15 (MRPS15)	NM_031280	2.1	2.64E-04	2.3	2.30E-03	2.3	1.07E-04
Protein phosphatase 1. regulatory subunit 7 (PPP1R7)	BF718769	2.2	1.39E-03	2.2	4.17E-03	2	7.93E-03
Others							
Transcobalamin I (TCN1)	NM_001062	3.4	9.06E-03	3.9	7.82E-03	4.7	2.73E-03
Homer homolog 1 (HOMER1)	BE550452	2.8	4.76E-03	2.4	1.00E-02	2.3	7.40E-03

Table continued

Table II (Continued). Overlapping dataset of the 39 genes with statistically significant, more than 2-fold expression in all allergic patients (groups L; F; and L-F) compared with healthy donors.

Gene name (symbol)	GeneBank	Unpaired comparisons					
		L vs CTRL		F vs CTRL		L-F vs CTRL	
		FC	*p*-value*	FC	*p*-value*	FC	*p*-value*
Down-regulated genes							
Transcription regulation							
Nuclear factor of activated T-cells 5 (**NFAT5**)	NM_006599	-3.1	3.87E-04	-2.5	1.04E-03	-3.6	1.19E-03
Poly(A) binding protein. nuclear 1 (PABPN1)	AI130920	-3.1	3.80E-04	-2.1	2.36E-03	-3.5	1.04E-04
Serine/arginine repetitive matrix 2 (SRRM2)	AI655799	-2.8	6.01E-03	-2.0	4.52E-03	-3.0	3.26E-03
TAF9-like RNA polymerase II (TAF9L)	AF077053	-3.9	8.73E-04	-2.2	3.76E-04	-3.0	1.59E-03
Kruppel-like factor 12 (KLF12)	NM_016285	-2.4	5.16E-04	-2.2	1.23E-03	-2.2	3.09E-03
Homeo box A7 (HOXA7)	NM_006896	-2.6	4.32E-03	-2.0	3.11E-03	-2.5	8.43E-03
Jumonji. AT rich interactive domain 1A (JARID1A)	AF00713	-2.2	3.09E-02	-2.2	7.74E-03	-2.1	6.86E-03
Nuclear receptor subfamily 2. group C. member 1 (NR2C1)	NM_003297	-2.2	1.50E-03	-2.0	5.94E-03	-2.1	4.59E-03
HCF-binding transcription factor Zhangfei (ZF)	NM_021212	-2.9	7.65E-04	-2.9	5.51E-03	-2.7	2.52E-03
Zinc finger protein 83 (ZNF83)	M27877	-3.3	1.87E-03	-3.0	5.10E-03	-2.8	1.69E-03
Metabolism							
PRP4 pre mRNA processing factor (PRPF4B)	NM_003913	-4.0	2.92E-05	-3.4	3.11E-03	-4.5	1.99E-05
Heterogeneous nuclear ribonucleoprotein A1 (HNRPA1)	AW450929	-2.4	5.10E-03	-2.3	4.84E-03	-3.0	8.28E-03
Methyltransferase like 3 (METTL3)	BC001650	-3.0	1.25E-03	-2.2	2.36E-03	-2.3	8.06E-04
Cysteine sulfinic acid decarboxylase (CSAD)	NM_015989	-4.5	3.40E-03	-3.3	6.40E-03	-7.0	2.73E-03
Microtubule-actin crosslinking factor 1 (MACF1)	AB029290	-2.7	1.39E-03	-2.5	6.54E-03	-2.6	7.83E-04
Apoptosis							
Cisplatin resistance-associated overexpressed protein (CROP)	AW089673	-2.6	5.07E-05	-2.2	2.59E-03	-3.0	7.46E-05
Cisplatin resistance-associated overexpressed protein (CROP)	NM_006107	-5.7	2.92E-05	-3.9	3.68E-04	-5.6	2.87E-05
Death inducer obliterator 1 (DIDO1)	NM_080797	-3.3	3.00E-03	-2.3	2.60E-03	-2.5	8.90E-03
Defense response							
Lysosomal trafficking regulator (LYST)	U84744	-3.6	7.24E-04	-3.4	2.36E-03	-4.5	1.93E-04
Others							
Vesicle-associated membrane protein 1 (VAMP1)	AU150319	-2.8	1.48E-03	-2.1	6.38E-03	-3.2	4.90E-04
Amylase. alpha 1A (AMY1A)	NM_004038	-4.1	5.50E-04	-2.9	2.84E-03	-3.3	1.19E-03
Cyclin L1 (CCNL1)	NM_020307	-2.4	3.13E-03	-2.2	7.91E-05	-3.3	4.21E-05
Homo sapiens genomic DNA (KIAA0220)	AL049250	-6.1	3.46E-03	-4.5	1.00E-02	-5.9	3.05E-03
Natural killer-tumor recognition sequence (NKTR)	AI688640	-4.0	6.99E-03	-3.1	2.85E-03	-2.3	3.00E-04
Unknown							
Golgin-67 (GOLGIN-67)	AW006438	-5.2	8.10E-04	-3.9	5.54E-03	-4.3	1.19E-03
Leucine zipper like protein (LZLP)	NM_013344	-2.3	2.71E-04	-2.0	8.47E-04	-2.3	1.08E-03

Gene symbols in bold are detailed in the manuscript.

Table III. List of the genes with statistically, more than 2-fold expression in both fruit (F) and latex (L) patients compared to healthy donors.

Gene name (symbol)	GeneBank	Unpaired comparisons					
		L vs CTRL		F vs CTRL		L-F vs CTRL	
		FC	*p*-value*	FC	*p*-value*	FC	*p*-value*
Up-regulated genes							
Metabolism/catabolism							
Matrix metalloproteinase 9 (**MMP9**)	NM_004994	2,2	3.52E-03	2.6	6.45E-03	2.4	3.45E-02
A disintegrin and metalloproteinase domain 9 (ADAM9)	NM_003816	2.1	8.69E-03	2	7.80E-03	2.4	3.38E-02
Cytidine deaminase (CDA)	NM_001785	2.5	9.86E-03	2.7	4.13E-03	2.3	2.13E-02
Aldehyde dehydrogenase 1 family. member A1 (ALDH1A1)	NM_000689	2.4	7.17E-03	2.9	5.50E-03	1.7	2.46E-02
Phosphogluconate dehydrogenase (PGD)	NM_002631	2.1	1.54E-03	2.1	2.84E-03	2.4	3.52E-02
Phosphorylase, glycogen (PYGL)	NM_002863	2.1	3.12E-03	2	2.21E-03	2	2.21E-02
Nicotinamide nucleotide transhydrogenase (NNT)	U40490	2	8.62E-04	2.5	3.68E-04	2.3	3.69E-02
RNA binding motif protein 3 (RBM3)	NM_006743	2.1	1.19E-04	2.1	9.76E-04	2.2	2.86E-02
H1 histone family, member X (H1FX)	NM_006026	2.1	3.64E-03	2	8.14E-03	2	3.14E-02
Heterogeneous nuclear ribonucleoprotein H2 (HNRPH2)	NM_019597	2.3	2.26E-04	2.1	2.84E-03	2.3	2.17E-02
Signal transduction							
Cortistatin (CORT)	AL578583	2.5	7.70E-03	2.4	7.27E-03	2.3	4.27E-02
Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein (YWHAB1)	BC00135	2.6	2.75E-04	2.2	2.37E-03	2.3	3.12E-02
Amyloid beta (A4) precursor-like protein 2 (APLP2)	BC000373	2	2.75E-04	2.2	3.89E-03	2.3	3.70E-02
Transport							
Fatty acid binding protein 5 (FABP5)	NM_001444	2.1	3.33E-03	2.2	3.54E-03	2	3.38E-02
Coated vesicle membrane protein (RNP24)	NM_006815	2	9.05E-03	2	3.94E-03	2	1.94E-02
Apoptosis							
BCL2-associated X protein (BAX)	NM_004324	2.1	8.76E-03	2.1	4.52E-03	2.2	4.52E-02
Immune response							
Interleukin 1 receptor type II (**IL1R2**)	NM_004633	2.7	6.38E-03	2.8	9.50E-03	2.7	4.50E-02
Down-regulated genes							
DNA repair							
Polymerase (DNA directed) iota (POLI)	NM_007195	-2.1	1.01E-03	-2.3	6.05E-03	-1.4	4.76E-02

(Fold change values for the group of patients with latex-fruit syndrome (group L-F) are gven in the last two columns, showing the same trend of regulation, but not statistically validated. Gene symbols written in bold are detailed within the manuscript).

Table IV. List of the genes with statistically, more than 2-fold expression in both fruit (F) and latex-fruit (L-F) patients compared to healthy donors.

Gene name (symbol)	GeneBank	Unpaired comparisons					
		L vs CTRL		F vs CTRL		L-F vs CTRL	
		FC	*p*-value*	FC	*p*-value*	FC	*p*-value*
Up-regulated genes							
Transport							
Target of myb1 (TOM1)	NM_005488	2.3	4.12E-04	2.1	4.79E-03	2.8	1.20E-02
Hemoglobin, delta (HBD)	NM_000519	2.4	7.00E-03	2.9	9.80E-03	1.6	1.96E-02
Aquaporin 3 (**AQP3**)	N74607	2.2	5.51E-03	2.6	1.75E-03	2.7	2.30E-02
Bridging integrator 1 (BIN1)	U87558	2	8.00E-03	2.2	3.40E-03	2.6	6.70E-02
Immune response							
Cathelicidin antimicrobial peptide (**CAMP**)	U19970	3.3	5.60E-03	4.8	6.43E-03	2.8	5.60E-02
Carcinoembryonic antigen-related cell adhesion molecule 8 (CEACAM8)	M33326	7.4	3.00E-03	6.3	4.29E-03	3.2	2.50E-02
Defensin. alpha 1 (**DEFA1**)	NM_004084	4.3	5.85E-03	2.6	3.88E-03	2	6.70E-02
Cytoskeleton organisation							
Desmoplakin (DSP)	NM_004415	2.5	9.80E-03	2.8	2.04E-03	2.1	1.00E-02
Metabolism							
U5 snRNP-specific 40 kDa protein (HPRP8BP)	AL157420	2.2	2.95E-03	2	2.53E-03	2.6	5.40E-02
N-myristoyltransferase 2 (NMT2)	AW293531	2	9.76E-04	2	4.15E-04	2.5	8.90E-02
similarity to Pm5 (NOMO2)	AL512687	2.2	2.35E-03	2.2	7.83E-04	2.2	4.50E-02
Phosphatidylethanolamine N-methyltransferase (PEMT)	NM_007169	2.5	5.00E-03	2.2	6.70E-03	2.6	3.40E-02
IMP dehydrogenase 2 (IMPDH2)	NM_000884	2	2.37E-03	2	1.49E-04	2.5	6.50E-02
Other							
Hepatocellular carcinoma-associated antigen 112 (HCA112)	NM_018487	3.4	4.50E-03	3.3	1.94E-03	3.5	2.20E-02
Down-regulated genes							
Transcription regulation							
V-fos FBJ murine osteosarcoma viral oncogene homolog (**FOS**)	BC004490	-5.2	1.54E-03	-4.9	8.31E-03	-2	5.60E-02
Signal transduction							
Tripartite motif-containing 23 (TRIM23)	AI021991	-2.4	5.50E-03	-2.8	9.70E-03	-1.4	7.80E-02
Dual specificity phosphatase 1 (DUSP1)	NM_004417	-2.4	2.37E-03	-2.8	9.17E-04	-1.4	3.40E-02

(Fold change values for the group of patients allergic to latex (group L) are given in the last two columns, showing the same trend of regulation, but not statistically validated. Gene symbols written in bold are detailed within the manuscript).

Table V. List of the genes with statistically, more than 2-fold expression in both latex (L) and latex-fruit (L-F) patients compared to healthy donors.

Gene name (symbol)	GeneBank	Unpaired comparisons					
		L vs CTRL		F vs CTRL		L-F vs CTRL	
		FC	*p*-value*	FC	*p*-value*	FC	*p*-value*
Up-regulated genes							
Immune response							
Major histocompatibility complex, class II, DQ alpha 1 (**HLA-DQA1**)	BG397856	2.4	7.78E-03	2.4	7.54E-03	1.7	1.78E-02
Transport							
Sec23 homolog A (SEC23A)	NM_006364	2.1	8.68E-03	2.3	1.80E-03	1.9	2.68E-02
Metabolism							
Glutathione S-transferase M3 (GSTM3)	AL527430	2.1	9.51E-02	3	1.30E-03	2	1.38E-02
Nucleic acid							
Cullin 4B (CUL4B)	AI650819	2.2	1.19E-03	2	7.21E-03	2	1.21E-02
Exosome component 5 (EXOSC5)	NM_020158	2.1	1.39E-03	2.2	2.13E-03	2	2.99E-02
Down-regulated genes							
Transcription regulation							
Zinc finger protein 175 (ZNF175)	NM_007147	-2.4	6.99E-03	-2.0	7.55E-03	-1.9	1.41E-02
Polycomb group ring finger 3 (PCGF3)	AI692203	-2.6	1.25E-03	-3.6	3.96E-03	-3.1	1.35E-02
Fetal Alzheimer antigen (FALZ)	NM_004459	-2.0	7.63E-04	-2.3	5.58E-03	-1.8	1.10E-02
Immune response							
Interleukin 1, alpha (**IL1A**)	NM_000575	-3.3	3.61E-03	-3.0	4.99E-03	-2.1	1.12E-02
Major histocompatibility complex. class I, F (HLA-F)	BE138825	-2.2	9.88E-03	-2.5	6.84E-03	-1.9	4.65E-02
Interleukin 18 receptor accessory protein (**IL18RAP**)	NM_003853	-2.2	3.23E-03	-2.9	2.17E-04	-2.0	1.41E-02
Metabolism							
Titin (TTN)	NM_003319	-2.8	1.00E-02	-3.1	5.41E-03	-2.7	4.49E-02
Serine palmitoyltransferase. long chain base subunit 2 (SPTLC2)	U15555	-2.2	2.12E-03	-2.1	8.35E-03	-1.8	3.72E-02
O-linked N-acetylglucosamine transferase (OGT)	U77413	-2.2	3.67E-04	-2.1	4.55E-04	-1.8	4.76E-02
Ribosomal protein L10 (RPL10)	AW057781	-2.1	2.99E-04	-2.6	8.03E-05	-1.7	3.60E-02
Signal transduction							
Adrenergic. beta. receptor kinase 2 (ADRBK2)	NM_005160	-2.3	4.62E-03	-2.4	4.15E-04	-1.9	2.40E-02
Paired immunoglobin-like type 2 receptor beta (PILRB)	NM_013440	-2.4	2.64E-04	-2.8	1.04E-04	-2.0	1.50E-02
DEAD box polypeptide 17 (DDX17)	NM_030881	-4.3	9.65E-03	-5.2	3.41E-03	-2.7	6.20E-02
Others							
Plasminogen-like (PLGL)	BC005379	-3.0	1.54E-03	-2.1	7.49E-03	-2.0	4.70E-02
Translocated promoter region (TPR)	BF110993	-2.2	1.91E-03	-2.6	3.10E-04	-2.1	6.00E-02
Myomesin 2 (MYOM2)	NM_003970	-3.1	8.76E-03	-3.7	7.72E-03	-2.3	4.76E-02
NACHT. leucine rich repeat and PYD containing 1 (NALP)	AF310105	-2.1	1.44E-03	-2.4	4.23E-04	-2.0	6.80E-02
CDK5 regulatory subunit associated protein 3 (CDK5RAP3)	NM_025197	-2.0	2.26E-04	-2.5	4.21E-05	-2.0	2.45E-02

(Fold change values for the group of patients allergic to the fruits (group F) are given in the last two columns, showing the same trend of regulation, but not statistically validated. Gene symbols written in bold are detailed within the manuscript).

between latex and fruit allergies has been reported frequently, this is the first time, to our knowledge, that systematic molecular analysis has been performed, showing similar pathways involved in allergy to latex and/or fruit. Several genes identified in this study are known targets reported in inflammatory and allergical processes, regulating both the innate and adaptive immune responses.

DNA microarray profiling showed the up-regulation of several antimicrobial agents, such as the defensin alpha 1 (DEFA1) and the cathelicidin antimicrobial peptide (CAMP), that kill a wide range of microorganisms. A large body of evidences showed that in addition to acting as endogenous antibiotics, these peptides also display additional roles, such as regulating the inflammatory and immune responses, and chemoattracting immunocompetent cells to the sites of inflammation and infection. Defensins are indeed potent histamine-releasing factors that may provide important pathways for communication between neutrophils and mast cells [21]. Human cathelicidin causes also functional change in mast cells, by increasing their expression of toll-like receptor 4 (TLR4)[22]. In this study, we reported also the increase in expression of TLR-4 in allergic patients. TLR4 binds lipopolysaccharides (LPS) of gram-negative bacteria, leading to a complex signal transduction cascade with production of inflammatory cytokines, cell stimulation, and activation of T and B cells. In particular, the activation of TLR4 on mast cells induces Th2-associated cytokine release such as IL-5 and IL-13[23,24]. The expression of another antimicrobial protein, the lactoferrin (LTF) was increased in our allergic blood patients. LTF is an iron-binding glycoprotein that plays a central role in the interface between innate and adaptive immunity. Lactoferrin has a profound modulatory action on the adaptive immune system, promoting the maturation of T-cell precursors into competent helper cells and the differentiation of immature B cells into efficient antigen presenting cells[25,26]. In addition, LTF is a potent immunomodulator, affecting the Th1/Th2 cytokine balance[27]. Taken together, these results confirm a polarization of the Th2 immune-mediated response in allergic patients. This was corroborated by the overexpression of another Th2-associated gene, the eosinophil marker Charcot-Leyden crystal protein (CLC), previously identified in patients with allergic rhinitis[28]. Furthermore, the down-regulation of the IL18 receptor accessory protein (IL18RAP) gene, favouring the Th1 cell polarization in human diseases[29] confirmed the shift in the Th1/Th2 balance to favour the production of Th2 cytokines. This particular phenotype promotes mast cells degranulation as confirmed by the increase of the mast-cell carboxypeptidase A (CPA3) expression in allergic patients. CPA3 is stored in the secretory granules and is released, together with a range of other inflammatory mediators, upon mast-cell degranulation[30].

Current data suggest that T regulatory CD4+/CD25+ cells (Tregs) play also a key role in the allergic response. *In vitro* experiments showed that human peripheral blood Tregs may act to prevent inappropriate Th2 responses to environmental allergens[31]. In this study, we reported the down-regulation of the interleukin 1 alpha (IL1A) gene in allergic individuals, while the expression of the decoy interleukin 1 receptor type 2 (IL1R2) gene was increased. IL1 is a pleiotropic cytokine involved in host defense, and recently reported for its role in the expansion and proliferation of CD4+/CD25+ Tregs[32]. Taken together, these data suggest that IL1expression decrease might be involved in the inhibition of Treg-induced immunomodulation.

DNA microarray profiling of latex and fruit allergic patients allowed the identification of two genes associated with allergy-related traits, the HLA-DQA1 and the MS4A3 gene. To date, strong relationships between the immune response to several highly purified allergens and specific human leukocyte antigen (HLA)-DQ and -DR haplotypes have been reported[33]. Since agents triggering type I hypersensitivity reactions need to be recognized by HLA class-II restricted T-helper lymphocytes, the identification of HLA class-II alleles associated with some allergies may help to understand the pathogenesis. Regarding MS4A3, this gene was identified in both lymphoid and myeloid hematopoietic lineage and has been localized at human chromosome 11q12-13.1[34]. Interestingly, this locus has been reported as a candidate for asthma/atopy susceptibility genes[35]. Our results suggest that both HLADQA1 and MS4A3 may also be involved in vegetable food and latex allergy pathogenesis.

An increasing number of other mediators have been recently described in the regulation of allergy[12-14]. Among these, we identified the metalloproteinase 9 (MMP9) overexpressed in allergic patients. Previous studies have demonstrated that increased plasma levels of MMP9 were associated with the severity of chronic urticaria[36]. MMP9 also plays a role in chronic airway inflammation

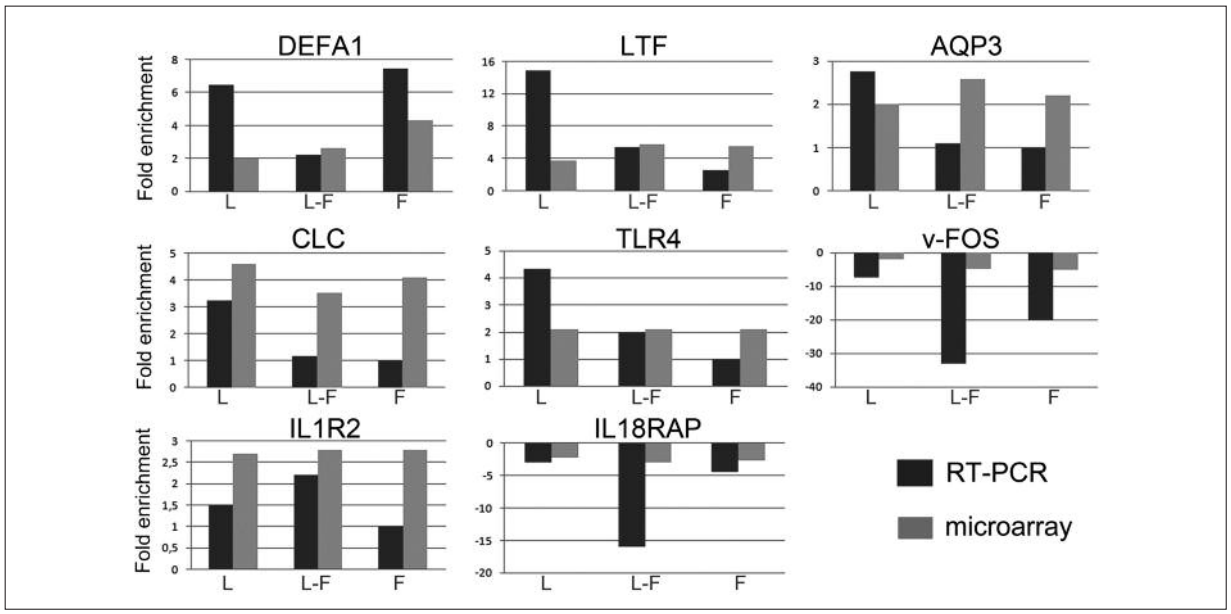

Figure 3. Confirmation of differential expression by Q-PCR. Semiquantitative Q-PCR was used to analyze differential gene expression of 8 selected candidates identified by microarray analysis as potential targets involved in allergic process. Gene expression was measured in all the three groups of patients (L: latex, F: fruit, L-F: latex-fruit) and compared to the expression in healthy individuals. Microarray and RT-PCR methods demonstrated good correlation of differential gene expression between allergic patients and control healthy volunteers.

and remodeling in asthma[37]. Our results showed also the upregulation of the water-permeable channel aquaporin 3 (AQP3), recently described as a potential target involved in atopic eczema[38].

The differentiation of naive T cells into Th2 cells producing a specific pattern of cytokines is tightly controlled and regulated by transcription factors and chromatin remodeling proteins. DNA microarray profiling showed a modulation of several transcription factors in allergic patients PBMCs. Among these, the protoncogene FOS regulating the expression of several pro-inflammatory cytokines, such as IL-5, IL-6, IL-10, IL-14, TNF-α, and GM-CSF, has still been reported as a candidate gene in the evolution of asthma[39]. Another key immune gene NFAT5 belonging to the transcription factor NFAT family might be involved in a regulatory mechanism eliciting different patterns of T cell responses[40].

Conclusions

This study provided advances in our understanding of the molecular mechanisms resulting in the polarization of theTh2-associated cytokines in allergic PBMCs. Furthermore, gene expression profiling showed that common signaling pathways are dysregulated in patients with allergy to latex and/or fruit, corroborating the latex and fruit allergens cross-reactivity. Identification of cytokines, signal transduction molecules and transcription factors responsible for Th2 development in atopic patients may contribute to a better knowledge of the physiopathology of the allergic process, and promote the development of new therapeutic options.

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