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L'Ossido Nitrico come Marker T2 nell'Asma

Nitric Oxide as a T2 Marker in Asthma

El Óxido Nítrico como Marcador T2 en el Asma

La Farmacogenetica e la Farmacogenomica nella Terapia dell'Asma

Pharmacogenetics and Pharmacogenomics in Asthma Therapy

Farmacogenética y Farmacogenómica en el tratamiento del Asma

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SUMMARY

Notiziario Allergologico, 2024 Vol. 42, n. 2

Notiziario Allergologico

PDF VERSION

Notiziario Allergologico has been alive and well for over forty years. Today, it becomes international with a new layout that includes the translation of all content into three languages. The purpose remains unchanged if not implemented: to promote allergology culture by offering readers the possibility of an in-depth study and update on various allergology topics, also with a view to the future, thanks to the competence and authority of the authors of the articles published. The popular character of the articles contributes to making them accessible to a vast number of specialists, not only allergologists but also pulmonologists, paediatricians, dermatologists, etc.

ENGLISH

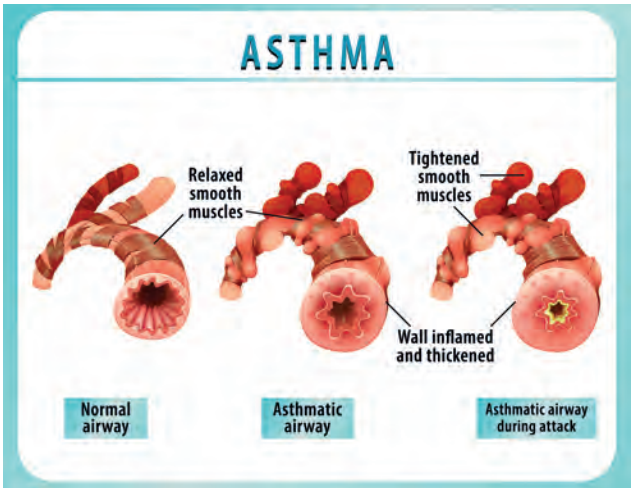
EDITORIAL

edited by **Gianni Mistrello**

Asthma is a chronic inflammatory airway disease characterised by an increase in bronchial responsiveness that causes symptoms such as wheezing and/or shortness of breath, chest constriction, and coughing associated with episodes of reversible bronchospasm. The incidence of the disease has increased significantly over time and millions of people suffer from it.

In this issue of the Notiziario, the focus is on this pathology, providing readers with original insights into the subject, thanks to contributions from Dr. Guida (Dept. of Clinical and Biological Sciences, University of Turin) and Prof. Cazzola (Dept. of Experimental Medicine, University of Rome Tor Vergata).

In particular, Dr. Guida began his article by describing in great detail the complex mechanism that leads to the production of nitric oxide (NO), a molecule that is normally present in the exhaled breath as it is produced by bronchial epithelial cells, and that seems to represent not only an initial form of defence, but also the result of an immune cascade following a series of exogenous stimuli (allergens, viruses, pollutants, stress factors, etc.). However, when its level in the exhaled breath exceeds 25 ppb (parts per billion), the subject in whom this value is measured presents a pre-inflammatory condition indicative of an inflammation of the bronchial district dependent on the accumulation in situ of a significant number of eosinophils. The pulmonologist has long since enriched his diagnostic toolkit with the use of an appropriate apparatus capable of accurately determining the amount of nitric oxide in exhaled air (FeNO). Based on the result of this investigation, the specialist can get a preliminary idea of the patient's bronchial inflammatory state and, if necessary, subject him/her to further multidisciplinary investigations to identify the type of asthma he/she is suffering from (e.g. allergic). The test is non-invasive and can therefore represent a valid alternative to others that are decidedly more invasive; it consists of a full-lung inhalation by the patient who must then exhale slowly, with a constant expiratory flow for a few seconds, into a mouthpiece connected to the meter that then provides the data. Given its simplicity and non-invasiveness, the test can also be carried out in



particular categories of patients such as children and can be very useful in monitoring the effectiveness of a therapy in progress and in modulating it, personalising the dosage and reducing any risks of side effects. Particularly interesting, at the conclusion of the author's contribution, is the update on the determination of FeNO in guiding asthma therapy with biological drugs.

Numerous studies have shown that generally drug-based therapies are only effective on a certain percentage of the population subjected to these therapies. These are the so-called "responders", while the remaining group belongs to the "non-responders", i.e. those patients with serious side effects. Several factors can play a role in determining the therapeutic efficacy of a drug. Among these, "person-to-person genetic variability" may be an essential factor behind the differences found in a group of patients undergoing a certain "drug treatment". Mutations in target genes or enzymes involved in drug metabolism may influence drug response. It is clear that the identification of appropriate approaches capable of predicting patient "responders" is of paramount importance in a personalised medicine perspective. In this sense, pharmacogenetics and pharmacogenomics can contribute greatly to improving the safety and efficacy of a drug. There is often a tendency to use the two terms interchangeably; in reality, pharmacogenetics is based on the testing of specific single genes selected a priori and highlights how any mutations in them may affect the response to a drug; pharmacogenomics, on the other hand, is an approach without predefined hypotheses that is applied at the level of the entire genome to identify the multigenic determinants involved in the response to a drug. The author of the second article, Prof. Cazzola, presents a very comprehensive overview of pharmacogenetics and pharmacogenomics studies applied to drugs used in asthma therapy. For each category of anti-asthma drugs, he describes the various genetic loci identified to date and how these might be associated with therapeutic responses. While acknowledging that the results of the studies to date are providing significant information on their potential diagnostic use, the author points out that they cannot yet be used in clinical practice to guide therapeutic decisions. The same applies to the biological drugs currently in use. Again, patients undergoing this therapy show a variable response, and it is possible that polymorphisms in genes coding for cytokines or their receptors may influence their efficacy. The article concludes with a reference to allergen-specific immunotherapy (AIT), which does not differ from other therapeutic approaches in terms of variability of therapeutic efficacy in treated patients. It has been observed that asthmatic patients with specific polymorphisms in genes coding for the cytokines IL-4 and IL-13 show increased production of allergen-specific IgE and increased severity of asthmatic symptoms. In the case of subjects allergic to *Alternaria*, patients carrying these polymorphisms respond less to AIT. In contrast, high levels of IL-10 transcripts in the blood of patients allergic to dust mites seem predictive of the efficacy of AIT.

I wish everyone a good read.

Nitric oxide in exhaled air: marker of T2 (Type-2) inflammation in asthma

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1. Introduction: the discovery of T2 (Type-2) inflammation

The "definition of bronchial asthma" as a "chronic inflammatory airway disease characterised by inflammatory cell infiltration, mediator release and structural airway remodelling" (1) was the result of progressive immunological, pathophysiological and biomolecular acquisitions. The characterisation of inflammation in asthma has followed developments in acquisitions concerning the mechanisms and cells involved in adaptive and natural immunity. Bronchial biopsies and bronchoalveolar lavage (BAL) of subjects with asthma showed, among the main changes, infiltration of eosinophils, lymphocytes, mast cells, and neutrophils in increased numbers compared to normal subjects (2). The isolation of clones of human Th1 and Th2 lymphocytes by Romagnani and co-workers in the early 1990s led to a large number of insights into the effects of Th1 and Th2 lymphocytes on the human immune system and to the subsequent paradigm that divides cytokines, molecules mediating communication signals between cells of the immune system, into type 1 (Th1-like) and type 2 (Th2-like). Several subsequent findings showed that Th1 and Th2 cytokines could be produced by cells other than CD4+ T lymphocytes, including CD8+ T lymphocytes, monocytes, NK cells, B lymphocytes, eosinophils, mast cells, basophils and other cells of innate immunity. Pathophysiologically, conditions involving a predominance of type 2 cytokines are called Type-2 or T2 (3).

2. T2 inflammation and "allergy"

In 1906, Clemens von Pirquet proposed the term "allergy". It was a process of sensitisation to a protein (allergen) causing an immediate hypersensitivity reaction following the re-administration of that substance that could lead to urticaria, angioedema, bronchospasm, and shock. Allergy could be passively transferred from an allergic to a non-allergic subject through that substance called "reagin", which the Ishizaka couple demonstrated in 1966 to be an immunoglobulin of isotype E (IgE). The cellular and molecular mechanisms involved in the regulation of human IgE synthesis are supported by interleukin-4 (IL-4), a Th2-like cytokine, whereas IFN-γ (Th1-like), negatively regulates IL-4-induced IgE synthesis (4). Thus, allergen-specific Th2 cells seem to play a crucial role in allergy by inducing IgE production via IL-4.

Type I hypersensitivity reactions are characterised by an effector phase, generally very rapid, during which IgE-mediated activation of basophils and mast cells leads to the release of inflammatory mediators and subsequent tissue damage. This is followed by a phase of late allergic inflammation, characterised mainly by eosinophilic inflammation, but in which other cells such as neutrophils, mast cells, and lymphocytes come into play. Th2 cells promote proliferation, differentiation, and activation of eosinophils by IL-5 (5).

More recent findings have led to the isolation and description of type 2 innate immunity lymphoid cells (ILC2), capable of secreting cytokines classically associated with Th2 lymphocytes but not directly involved in the allergic inflammatory cascade (6). ILC2s are mainly found at the interface sites

with the external environment, such as the bronchial submucosa, and can be activated by signals from the epithelium (alarmins). Once activated, ILC2 produce Th2 cytokines such as IL-4, IL-13, IL-5, but can also stimulate IgE production by B lymphocytes independently of T helper lymphocytes (polyclonal IgE).

2.1 T2 inflammation in allergic asthma

Allergic asthma is associated with a Th2-polarised response, and analysis of BAL and biopsy specimens in mouse models of allergic asthma demonstrates an influx of eosinophils into the airway mucosa, mucus overproduction and bronchial hyperresponsiveness (AHR) induced by Th2 cytokines such as IL-4, IL-5, IL-13 and IL-9. In the tissues of allergic asthmatics, not only is the increase in the number of cells expressing the high-affinity receptor for IgE (FcεRI) confirmed, but that of CD4+ T-cell infiltrates, mast cells and eosinophils co-localised with IL-4 and IL-5 (7).
The central role of eosinophils in the late allergic inflammatory response phase is evident in the delayed broncho-constrictive response that occurs 4-6 h after contact with the allergen, characterised by a prolonged AHR and pronounced eosinophilia. Airway eosinophils persist in the allergic asthmatic even for 7 days after allergen inhalation, being sustained by the action of chemotactic factors (8). Not only IL-5, but also IL-13 are involved in the regulation of allergen-induced

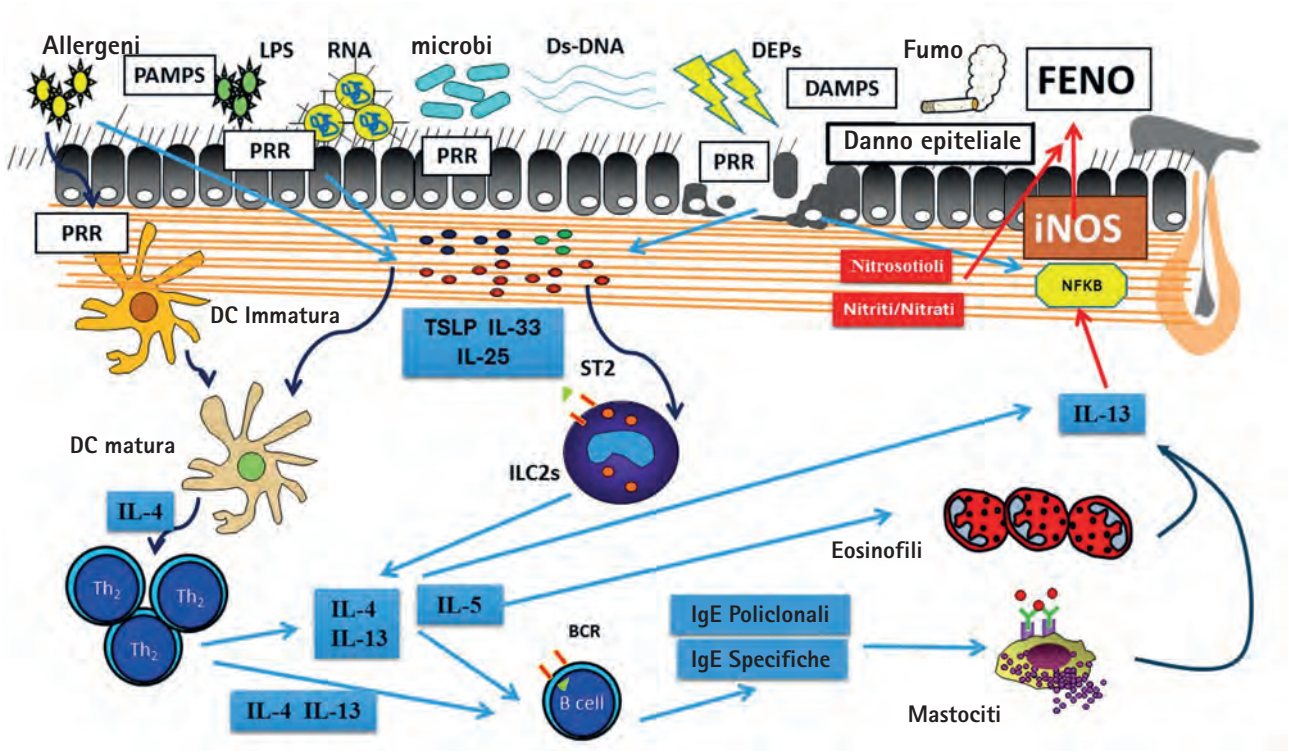
SUMMARY

Acronyms

- **AEC** airway epithelial cells;
- **AHR** airways hyperreactivity;
- **BAL** bronchoalveolar lavage;
- **DAMPs** damage-associated molecular patterns;
- **DEP** diesel exhaust particles;
- **FeNO** nitric oxide in exhaled air;
- **NO** nitric oxide;
- **NOS** nitric oxide synthase;
- **iNOS** inducible nitric oxide synthase;
- **cNOS** constitutive nitric oxide synthase;
- **CRS** chronic rhinosinusitis;
- **NP** nasal polyps;
- **PAMPs** pathogen-associated molecular patterns;
- **PRR** pattern recognition receptors;
- **RNS** reactive nitrogen species;
- **ROS** reactive oxygen species;
- **TRAP** traffic-related air pollution;
- **TSLP** thymic stromal lymphopoietin.

The measurement of nitrogen monoxide in exhaled air (FeNO) represents a simple, non-invasive and reproducible method for measuring inflammation in the airways defined as Type-2. In asthma, T2 inflammation is an expression not only of the classical allergic cascade mediated by IgE and eosinophils, but also of the activation of the bronchial epithelium, which responds to harmful stimuli from the external environment, such as pathogens and pollutants. The concentration of FeNO in asthma is therefore a sensitive index of T2 inflammation, which varies rapidly in response to therapy or disease exacerbation. Over time, the clinical application of FeNO in asthma has expanded due to its ability to adjuvant in diagnosis, predict exacerbations and measure adherence to therapy. Finally, FeNO has become an essential tool for characterising patients with severe asthma and appropriately initiating them to a biological drug as part of a personalised therapy strategy.

Figure 1 Mechanisms underlying FeNO increase in T2 inflammation.



delayed inflammatory responses, also modulating IgE production.

Early local activation of ILC2 capable of expressing IL-5 or IL-13 24 hours after allergen challenge has also been demonstrated. Furthermore, the increase in the number of ILC2 in BAL correlates with the concentrations of IL-33, an alarmin released from the epithelium after stimulation by certain allergens, e.g., alternaria, and capable of promoting IL-5-dependent reactive eosinophilopoiesis in the bone marrow (9).

2.2 T2 inflammation in "non-allergic" asthma

Between 10% and 33% of all asthma patients have a non-allergic (intrinsic) form of asthma, which has a later onset, a more severe clinical course in adults, and is significantly associated with nasal polyps in combination often with aspirin idiosyncrasy (ASA) (10). In both BAL and bronchial biopsies from subjects with intrinsic asthma, increased numbers of activated Th2 lymphocytes and eosinophils, as well as IL-5, were found. High levels of IgE and their receptors also indicate local IgE synthesis even in the absence of an allergen.

An innate Th2 cell-independent response is induced by exposure to air pollution, cigarette smoke, diesel exhaust particles, aspirin, and exercise. As a result of epithelial stimulation, ILC2 can produce high amounts of IL-5, which explains this severe eosinophilic inflammation. Contributing to this innate response is lipoxin A4, which modulates eosinophil apoptosis (11).

2.3 Dysregulation of epithelial barrier immunity and alarmins

Airway epithelial cells (AEC) constitute the main mechanism in the respiratory tract as the first line of defence against various infectious pathogens, allergens and physical insults. In recent years, it has emerged that the airway epithelium not only functions as a passive, physical barrier, but actively participates in the modulation of innate and adaptive immune responses (12).

AECs are able to recognise highly conserved biological structures shared by the same type of pathogenic microorganisms, called PAMPs, pathogen-associated molecular patterns. PAMPs, corresponding to lipids, proteins and nucleic acids (RNA and DNA) constitute exogenous danger signals, which AECs can recognise, distinguishing between "self" and "non-self", through specific receptors called PRRs (Pattern Recognition Receptors).

In response to microorganisms, respiratory viruses, air pollutants and allergens, the airway epithelium releases the so-called alarmins IL-25, IL-33 and thymic stromal lymphopoietin (TSLP). The alarmins are also molecules produced by the airway epithelium when the cell undergoes death and necrosis by producing endogenous danger signals, called DAMPs, and recognised by PRRs. In asthma, alarmins are able to recruit and activate DCs, ILC2 cells, mast cells, eosinophils, neutrophils and basophils, thereby promoting the production of Th2 cytokines, mainly IL-4, IL-5 and IL-13 (14) (Figure 1).

Interleukin 33 (IL-33) is a pro-inflammatory epithelial alarmin that is highly expressed in the airway mucosa in asthma in response to allergens and other antigens. It binds to its receptor ST2, inducing the expression of Th2 cytokines, mainly IL-4, IL-5 and IL-13 by Th2 cells and ILC2 cells.

TSLP is secreted in high amounts by AECs in response to epithelial disruption by allergens, viruses, diesel extract particles, microbes, favoured by the synergistic activity of IL-4 and IL-13 with loss of e-cadherin. TSLP binds to its receptor (IL-7 α/γc-chain) expressed in DCs and ILC2, leading to the release of Th2 cytokines. TSLP itself further alters the epithelial cell barrier during chronic exposure to, for example, dust mites (15).

3. Nitric oxide as a defense mediator of the innate immune response

Nitric oxide is a radical-free diatomic gas considered, until 1980, an environmental pollutant found in cigarette smoke and smog. The production of nanomolar quantities of NO by epithelial cells and macrophages in response to microbial stimuli and cellular damage contributes to oxidative stress in host defense against bacteria, viruses, fungi, parasites and cancer cells (16) (table 1).

NO is produced by iNOS, the expression of which depends, as already seen for epithelial cells, on the activation of the transcription factor NF-κB. The expression of iNOS in macrophages is induced by the activation of Toll-like receptors (TLRs) and is further increased by IFN-γ (17). The iNOS catalyses the conversion of arginine to citrulline, releasing diffusible gaseous NO. Within phagolysosomes, NO combines with H O22 or with -O2 - to produce peroxynitrite radicals (-ONOO), which contribute to the killing of microbes. These ions can cause nitrosylation of tyrosine (Tyr) resulting in the formation of 3-nitrotyrosine (3-NT). NO can also interact with thiols (organic compounds in which the oxygen atom has been replaced by a sulphur atom -SH) such as glutathione or albumin to produce S-nitrosotiols (RS-NO), or be metabolised into nitrite (NO2 -) or nitrate (NO3). Although ROI and NO are effective antimicrobial agents, they are non-specific and can also induce host tissue damage.

3.1 Nitric oxide in the respiratory epithelium: origin and physiological role

Nitric oxide is produced in the respiratory epithelium in response to the activation of NO synthase (NOS). Constitutive isoforms (cNOS, NOS-1 and NOS-3) are expressed in the airways, and in response to a calcium signal produce small amounts of NO (femtomoles). At low concentrations, NO has many physiological effects on the respiratory system, ranging from mild bronchodilation and anti-bronchoconstriction to pulmonary vasodilation and modulation of gas exchange (18). The direct actions of NO occur through the activation of enzymes such as guanylyl cyclase or the inhibition of cytochrome P-450, cytochrome oxidase and catalase. NO can also interact directly with

free radicals by inhibiting lipid peroxidation and reducing the generation of pro-inflammatory lipids.
NOS is not the only source of epithelial NO (19). Approximately 70–90% of NO is released from S-nitrosotiols, which are potent relaxants of the human airways. NO in exhaled air can also result from the protonation of nitrite to form nitrous acid (HNO2), which releases gaseous NO upon acidification. Nitrite can come either from food or saliva, through the reduction of nitrate to nitrite by oral bacteria.

3.2 The interdependence of the innate and adaptive immune system

The cells of the adaptive immune system require instructions from the innate system to establish an immune response towards a particular antigen. There is a direct activation of DCs triggered by PRR signals that convert resting DCs into potent antigen-presenting cells (APCs) immunogens capable of promoting the expansion and differentiation of pathogen-specific naïve T cells into appropriate effector cells (20). There is a second route of indirect activation of DCs through inflammatory signals produced by inflammatory/resident cells, which in turn are stimulated by PAMPs. Epithelia, in particular, have the unique ability to condition resting DCs (21). For instance, TSLP and TGF-β released by the lung epithelium condition pulmonary DCs just as GM-CSF, IL-1β, IL-33, osteopontin and IL-25 can be produced by airway epithelial cells and act indirectly on DCs. However, DCs exposed to pro-inflammatory signals from other cells (neutrophils, eosinophils, AECs) show only partial activation (maturation) and are poorly immunogenic.

3.3 Nitric oxide as an inflammatory mediator of the airway epithelial immune response

The third form of NOS is called inducible (iNOS or NOS-2) because different triggers that insult the respiratory epithelium can induce NO synthesis. iNOS releases large amounts (nM) of proinflammatory NO several hours after exposure, which can continue in a sustained manner (hours or days). Many stimuli are able to induce iNOS expression via NF-κB. Not only through recognition of microorganisms via TLR, but also under stimulation of pro-inflammatory cytokines, including TNF-α, interleukin-1β, IL-4, and IL-13. The high concentration of NO produced by the epithelium could serve as a defense mechanism in the airways. This has been corroborated especially in the upper airways, where very high levels of NO in the sinuses could contribute to the maintenance of sterility in this environment (22).
NO in inflammation may also be involved in hyperemia, oedema and hypotension. In addition, NO may reduce apoptosis of inflammatory cells such as eosinophils, reduce adhesion molecules, suppress inflammatory cell activation, and inhibit neutrophil chemotaxis and proliferative responses in human lymphocytes. Finally, overproduction of NO in the airways may also have a direct bronchodilator effect (16). However, the effect of inflammatory NO at the epithelial level can be considered harmful in many ways, precisely because of its ability to combine with reactive oxygen species (ROS) and reactive nitrogen species (RNS) such as peroxynitrite, which oxidise and damage the epithelial tissue itself and induce AHR. ROS and RNS can damage DNA, lipids, proteins, and carbohydrates and lead to altered cell functions and increased inflammatory reactions. For example, peroxynitrite and peroxynitrous acid ONOOH are potent cytotoxics and oxidants, capable of oxidising thiols, as well as cleaving DNA and inactivating enzymes and other proteins, particularly those important for the mitochondrial respiratory chain (18, 19).

3.4 Nitric oxide as an epithelial mediator of airway inflammation T2

Epithelial-derived nitric oxide represents a model of interconnection between the innate and adaptive immune systems, in fact representing an initial form of defense, but also the result of the immune cascade that draws in additional cells of innate immunity and conditions the adaptive response through modulation of DCs. For example, allergic exposure of the epithelium of an asthmatic subject leads to the production of cytokines such as IL-4 and IL-13, which in turn determine iNOS activation. In vitro experiments on human AEC have

Table 1 Functions and sources of NO in the airways

CELL	EPITHELIUM			MACROPHAGES
	ENZYMATIC		NON-ENZYMATIC	ENZYMATIC
Source	cNOS	iNOS	S-nitrosoproteins S-nitrosotiols nitrites and nitrates	iNOS
Quantity	femtomoles	nanomoles		nanomoles
Stimuli	PAMPs/PRRs DAMPs/PRR calcium/calmodulin	bacteria virus oxidants allergens LPS pollutants (chronic) cytokines: TNF-α, IL-1β, IL-4 and IL-13	DEP (acute) TRAP (acute) LPS (? acute)	PAMPs/TLR IFN-γ DEP
Target	**Enzymes:** ∧ guanylate cyclase ∨ cytochrome P-450 cytochrome oxidase catalase	DNA lipids proteins carbohydrates	ROS RNS S-nitrosotiols nitrites and nitrates	ROS RNS S-nitrosotiols nitrites and nitrates
Effects	**Smooth muscle** bronchial tone (bronchodilation)	**Smooth muscle** bronchodilation		**Pathogen** lysis opsonisation phagocytosis
	Endothelium vasodilation	**Endothelium** hyperemia edema ∨ adhesion molecules		
	Capillaries gas exchange	**Eosinophils** ∨ apoptosis		
	Peripheral neurotransmission neurons	**Neutrophils** ∨ chemotaxis		
	Lipids ∨ peroxidation	**Lymphocytes** ∨ proliferation		
		Epithelium airway defense sterility oxidation damage hyperresponsiveness cytotoxicity ∨ mitochondrial respiration		

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Table 2 FeNO values for clinical use in asthma

	Parameter	FeNO (ml/s)	meaning
Healthy Subjects		10–20 ppb	
Eosinophilia in sputum	>3%	>50 ppb (adults) >35 ppb (children)	Inflammation T2 (or T2High)
	<3%	<25 ppb (adults) <20 ppb (children)	Non-inflammation T2 (or T2low)
	To be interpreted	>25 <50 ppb (adults) >20 <35 ppb (children)	
Severe asthma		≥20 ppb	Inflammation T2 (or T2High)
FEV1	-10%	+4 ppb	Airway gauging
AHR		>40 ppb	Probable asthma diagnosis
		<25 ppb	Diagnosis of asthma unlikely
AEs	Naive from CCS	>60 ppb	Risk of asthma exacerbation
Response to CCS	In therapy with CCS	>28ppb	Risk of asthma exacerbation
		>50 ppb (adults) >35 ppb (children)	Likely answer
		<25 ppb (adults) <20 ppb (children)	Unlikely answer
		22 ppb	Reduction or suspension of ICS
Response to biologicals	omalizumab	>19.5 ppb	>Reduction of exacerbations
		>30.5ppb	>Improvement of FEV1
	dupilumab	≥25 ppb	Eligibility for treatment
		≥25 ppb	>Reduction of exacerbations
	mepolizumab	≥50 ppb	>Reduction of exacerbations
	benralizumab	≥50 ppb	>Reduction of exacerbations

shown that IL-13 is in fact behind the increased expression of iNOS and the subsequent production of NO by epithelial cells (23). Not only allergens, but also microbes, pollutants, oxidants and stress factors that interact with the bronchial epithelium are able to activate iNOS (figure 1).

3.5 The measurement of nitric oxide in exhaled air (FeNO)

In 1991, Gustafsson and colleagues discovered that NO of endogenous origin could be measured in the breath of humans and animals. Exhaled air contains detectable amounts of NO in the order of parts per billion (ppb-1:109). The most commonly used methods for measuring nitric oxide in exhaled air (FeNO) are chemiluminescence, electrochemical sensors and laser technology (16–19). NO measured with a chemiluminescence analyser is the gold standard

and uses photometric detection of the reaction between NO and ozone. The equipment is highly sensitive and has a very fast response time (0.5-0.7 sec). After rinsing the mouth, the measurement takes place with a deep inhalation of NO-free gas up to total lung capacity and a slow exhalation. Velum closure is mandatory and is achieved by using a positive pressure of 5–20 cm H2O against exhalation. A steady exhalation at a constant flow rate of 50 mL/s for about 10 seconds is recommended to reach the "steady state", and thus a stable concentration level in the exhaled breath (plateau) to which the measurement (FeNO50) corresponds. Several portable FeNO devices have been developed over time (24) using electrochemical or infrared sensor technology.

Healthy individuals have FeNO values (FeNO50) between 10 and 20 ppb; however, the fifth to 95th percentile FeNO values were found to be 3.5–39 ppb for individuals aged between 12 and 80 years. There are a number of factors that influence FeNO levels, such as gender, weight and height, diet (e.g., coffee), or intake of medications such as anti-inflammatory drugs. Active smokers have lower FeNO levels, while viral infections increase it. Spirometry may also influence FeNO results, so it should not be performed earlier (25).

3.6 FeNO cut-off for eosinophilic inflammation in asthma

The most well-documented association is between airway eosinophilia and FeNO. There is a good correlation between FeNO values and the objective assessment of eosinophilic inflammation in bronchial biopsies, the eosinophil count in BAL or sputum (S-EOS). An FeNO <26 ppb was associated with a differential S-EOS count of <3%, which is the recognised cut-off for talking about airway eosinophilia. The large study by Berry and colleagues reported that FeNO50 of 36 ppb had a sensitivity and specificity for S-EOS above 3%, 78%, and 72% respectively (26). The 2011 ATS guidelines recommend a mean FeNO of 20 ppb for subjects aged 6–11 years and 25 ppb for subjects aged 12–80 years as a marker of eosinophilic airway inflammation less likely in asthma, an average FeNO of 35 ppb for subjects aged 6 to 11 years and 50 ppb for subjects aged 12–80 years as being highly associated with eosinophilic airway inflammation, and FeNO values between 25 and 50 ppb (20–35 ppb in children) to be interpreted with caution depending on the clinical context (27) (table 2).

4. The two-compartment model for nitric oxide production: alveolar and bronchial NO

Guidelines recommend an exhaled flow of 50 ml/s for FeNO measurement. When NO measurements are acquired at other higher flow velocities, the flow of NO from the airway wall to the lumen (JawNO) and the fraction of gas-phase NO in the alveolar region (CANO) can be calculated. Basically, the measurement of exhaled NO at different flows (e.g., 50, 100, 200 ml/s) makes it possible to identify, by means of a mathematical model that constructs a regression line between the values measured at the different flows, an estimate of bronchial NO production (intercept of the line) or alveolar NO production (slope or slope of the line) (25). This type of measurement, although limited to clinical research, can explain many asthma-related pathophysiological phenomena.

4.1 Exhaled nitric oxide and airway calibre

The reduction in airway calibre reduces FeNO levels in the absence of inflammatory changes. This is a mechanical effect and is most likely due to the decrease in available epithelial surface area, which hinders the diffusion of NO from the airway epithelium into the lumen. A correction factor of +4ppb to the measured FeNO value was proposed for each 10% reduction below 100% of the expected FEV1. Furthermore, the impact of airway calibre on FeNO appears to be different if the more peripheral airways are involved, resulting in a doubled rate of FeNO reduction, or the proximal airways (28). The β2-agonists could induce divergent effects on FeNO values in asthmatic patients due to their effect on airway obstruction occurring at different lung depths.

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4.2 Nitric oxide and the airways: acute and chronic responses

Since NO is produced in the respiratory epithelium in response to NOS activation by various stimuli or insults, its role in acute lung injury has been hypothesised. ALI (acute lung injury) is a severe clinical/pathological condition caused by disruption of the endothelial and epithelial barriers of the lung and increased capillary permeability. It manifests clinically as an acute onset of diffuse bilateral pulmonary infiltrates and severe hypoxaemia. The underlying cause is infection, sepsis or trauma, which leads to an increase in proinflammatory cytokines (29). In both animal models and humans with ALI, there is increased endogenous NO production due to increased iNOS expression and activity. Systemic administration of LPS was associated with an increase in FeNO, but did not correlate with the severity of lung damage.

However, there are other situations in which FeNO may reflect an acute epithelial insult/damage. For example, exposure to traffic pollution can cause acute inflammation associated with an elevation of FeNO within 2 hours in young, healthy subjects (30). The diesel exhaust particles (DEP) consist of an elemental carbon core and may contribute to oxidative stress. Furthermore, in response to DEP, alveolar macrophages produce NO, which can combine with superoxide anion to produce peroxynitrite. Interestingly, the effects of traffic-related air pollution (TRAP) on the airways results in elevated alveolar NO levels, hence a response concentrated predominantly in the distal airways (31). Chronic exposure to pollutants or irritants can also lead to increased FeNO in both healthy and atopic children. In children, chronic exposure to black carbon was correlated with FeNO, whereas exposure on the morning of sampling was associated with oxidative stress in the airways, confirming 2 different mechanisms in the increase of NO in acute and chronic exposure.

5. Exhaled nitric oxide and asthma: clinical applications

The usefulness of measuring FeNO concentration in asthma has emerged over the last 20 years due to its function as a marker of airway inflammation and the non-invasive, reproducible, and sensitive technique of its measurement. Currently, FeNO is considered a biomarker that, in clinical practice, can help physicians in the diagnosis and management of asthma. Furthermore, FeNO is used in the phenotyping of severe asthma and can predict the response to biologics (16,19,25,32).

5.1 Exhaled nitric oxide and asthma: diagnosis and differential diagnosis

In clinical practice, the problem of overdiagnosis of asthma involving the taking of anti-asthmatic drugs, even in the absence of a confirmatory test, goes hand in hand with underdiagnosis or misdiagnosis resulting from the poor availability of routinely accessible tests (33).

Eosinophilic airway inflammation, or T2, although recognised as the main process involving the airway wall causing the development of flow restriction and increased AHR, has not actually been considered in the diagnostic work-up of asthma. However, measurement of FeNO can help diagnose asthma in adults with suggestive episodic or chronic symptoms. FeNO has the ability to predict AHR in patients complaining of respiratory symptoms, but only in patients not on steroid therapy. In fact, a cut-off value of 40 ppb offers the best compromise between sensitivity and specificity, whereas a cut-off value of 50 ppb has a high specificity (>90%) and supports the diagnosis of asthma. A FeNO value <40 ppb does not exclude asthma, and high FeNO levels do not define asthma. On the other hand, FeNO <25 ppb has a very high negative predictive value, thus being able to exclude the diagnosis of asthma (34) (table 2).

There are some clinical situations in which FeNO assessment in the diagnosis of asthma may be of great help: in children under 5 years of age; people who are difficult to diagnose due to confounding factors such as obesity, anxiety; smoking patients, the elderly and pregnant women. Furthermore, FeNO measurement could also be used to differentiate asthma variant cough (25, 34). The nasal polyps (NPs) impact asthma patients in terms of disease control and represent an independent risk factor for asthma that is difficult to

treat. NPs are known to be responsible for elevated FeNO values, even when they are not associated with asthma, and FeNO is significantly higher in severe asthma patients with CRSwNP than in patients without NPs (35).

5.2 Exhaled nitric oxide and asthma: symptom control, response to inhaled corticosteroids and exacerbations

The clinical application of FeNO in asthma gradually emerged after clinical studies demonstrated the efficacy of FeNO-guided ICS dosing on symptom scores, the number of exacerbations, and the use of rescue medication. Only more recently has GINA validated, at least in children and young adults, that FeNO-guided asthma treatment is useful for a significant reduction in the rate of exacerbations (1). To date, however, the optimal frequency of FeNO measurements and the possibility of predicting exacerbations during discontinuation or reduction of ICSs are not yet defined. After discontinuation of ICSs in patients with moderate asthma, an increase in FeNO > 60 ppb was able to predict loss of asthma control one week earlier, while during ICS therapy, a baseline FeNO value of 28 ppb was able to predict the first exacerbations with a probability of 76%. Another consequence is the importance of FeNO for identifying steroid reactivity, allowing the physician to avoid an empirical "steroid trial" or unnecessary long-term corticosteroid treatment. The ATS recommendations state that for FeNO >50 ppb (>35 ppb in children), a response to corticosteroids is likely. Conversely, achieving a low FeNO (22 ppb) predicts the likelihood of a reduction or discontinuation of ICS. A low FeNO (25 ppb, 20 ppb in children) in symptomatic patients, on the other hand, indicates unlikely responsiveness to corticosteroids (34) (table 2).

5.3 Exhaled nitric oxide for asthma phenotyping

The indisputable role of FeNO as a marker of type 2 airway inflammation is of particular clinical significance in the context of difficult-to-control asthma and severe asthma. Asthma that is difficult to control (difficult to treat) is defined as that clinical situation in which the patient does not achieve disease control despite GINA step 4 or 5 treatment (1). A period of treatment optimisation is necessary in this situation, controlling and correcting modifiable risk factors, comorbidities and ultimately confirming the diagnosis of asthma. One of the critical factors is to check patients' adherence to treatment; FeNO has the ability to assess adherence objectively, through a suppression test (25) that evaluates the decrease in FeNO during five consecutive days.

Once all confounding and complicating factors of asthma have been corrected, and despite this the disease remains uncontrolled until oral corticosteroids (OCS) have been taken for at least 50% of the previous year, a severe asthma (SA) picture is defined. In this context, FeNO has achieved several practical uses.

6. An update on the use of exhaled nitric oxide in guiding biological therapy of asthma

Over the past 20 years, monoclonal antibodies, called biological drugs, directed against the main effectors of T2 inflammation have been developed for the treatment of AEs. The efficacy of T2-specific agents in reducing AEs and the daily dose of OCS has been demonstrated in clinical trials (36), real-life studies (37) and registries (38). FeNO measurement is recommended by GINA (39) before starting direct biological T2 treatment in AS. The role of FeNO as a predictive biomarker of response to biologics or clinical monitoring in patients undergoing therapy has been explored.

6.1 Omalizumab

Omalizumab is a biological drug directed against IgE. Real-life experiences with omalizumab use extend to more than 5 years, and are often associated with a reduction in FeNO, on average from 47.3 to 37.1 ppb. FeNO levels >19.5 ppb may predict a better response to omalizumab in terms of AEs. Interestingly, the XPORT study revealed the ability of increased FeNO values after omalizumab discontinuation to predict exacerbations. A further role of FeNO is to predict a significant improvement in FEV1 in the subgroup of patients with severe allergic asthma showing a pre-treatment FeNO value ≥30.5 ppb. The inhibitory action of omalizumab on FeNO (35) in severe allergic asthma is taken into account

by treatment algorithms that, by integrating several biomarkers at baseline, suggest the preferential choice of a biologic (40).

6.2 Anti-IL-5 strategies

Biological drugs targeting IL-5 (mepolizumab) or IL-5 receptors (benralizumab) significantly reduce eosinophilic airway inflammation in AS, without affecting FeNO levels (25, 34). This result is apparently contradictory, as an elevated FeNO level ≥50 ppb is a very likely marker of eosinophilic airway inflammation in asthma and is predictive of response to anti-IL-5 therapies (34) in adults with severe eosinophilic asthma (41). Furthermore, FeNO retains the ability to discriminate the inflammatory phenotype at the time of exacerbation with mepolizumab treatment.

6.3 Dupilumab

FeNO has emerged as the biomarker that best fits the mechanism of action of dupilumab (36,37). It is able to interact with the IL-4Ra subunit of the IL-4/13 receptor by interfering with the T2 inflammatory cascade and inhibiting epithelial iNOS. Dupilumab provided a rapid and significant dose-dependent reduction in FeNO levels as early as week 2, and the effect of dupilumab resulted in a 48% reduction in FeNO from baseline by week 52, an effect achieved at 12 weeks. Early life experiences reported a large reduction in FeNO to normal levels as early as 4 weeks of treatment (34). The decreased risk of severe exacerbations and the extent of FEV1 improvement correlated progressively with baseline FeNO values (< 25, 25–50, > 50 ppb) (42). Although the threshold of 25 ppb FeNO is accepted as a valid predictor of dupilumab efficacy, a study that recorded FeNO and FEV1 during multiple visits in 32 patients showed that patients with the lowest FEV1 more frequently had FeNO levels <25 ppb, suggesting an association with airway calibre reduction, as already explained.

6.4 Tezepelumab

Tezepelumab targets TSLP. Recently approved for the treatment of both T2 and non-T2 severe asthma, it is effective in improving asthma control and reducing the rate of disease exacerbations regardless of T2 biomarker levels, including FeNO. However, patients with higher FeNO values appeared to show the best benefit from anti-TSLP treatment (43).

6.5 Biological switches and nasal polyposis

Failure to achieve a good clinical response with biologics in SA is a not uncommon observation, as is discontinuation of the drug. The need to switch to another biologic might be driven by biomarkers. Switching to mepolizumab caused a 50% drop in FeNO levels in a subgroup of patients not responding to omalizumab. It was hypothesised that treating patients with a high FeNO phenotype who do not respond to mepolizumab with benralizumab may lead to the depletion of IL-5R-expressing cells, such as eosinophils and basophils, which are a significant source of IL-13. Patients with high FeNO (≥25 ppb) with a biological history were more likely to become responder to dupilumab, leading to a response in 76% of patients (36,37).
Anti-IgE, anti-IL-5/IL-5R and anti-IL-4/IL-13R are currently approved and used biologics for the treatment of uncontrolled NP despite appropriate medical therapy and sinus surgery, and fulfil the criteria for the presence of T2 inflammation (44). Many experiences of treating severe asthma with comorbid NP have shown significant improvement in clinical sinonasal findings, sinus computed tomography images and nasal endoscopic scores (25). FeNO levels remain unchanged despite effective asthma treatment in NP patients treated with anti-IL5 and anti-IL-5R, while dupilumab is able to decrease FeNO values (45).

7. Conclusion: nitric oxide and the airways, T2 inflammation and beyond

The measurement of nitrogen monoxide in exhaled air (FeNO) is an approved method for clinical use in patients with bronchial asthma. It is a simple, completely non-invasive, reproducible, sensitive, and easy-to-obtain test that provides information on the inflammatory state of the airways and alveolar compartment. FeNO concentration in asthma is a sensitive index of airway inflammation that varies rapidly in response to anti-inflammatory therapy or disease exacerbation. The clinical application of exhaled NO measurement in asth-

ma is an important means of assessing adherence to therapy and its efficacy, disease activity, diagnosis, and predicting exacerbations over time. Its promising role in identifying a priori patients with severe asthma who are more responsive to a biological drug is being confirmed in real-life studies. The careful analysis of the pathogenesis of T2 inflammation and the close interconnections between innate and adaptive immunity are opening up new fields of study of the role of airway nitric oxide: discrimination between large and small pathways, the acute or chronic effect of pollutants or infectious agents, and the correlation between FeNO and airway calibre are just some of the new elements that will be incorporated into the clinical use of FeNO in the future.

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Pharmacogenetics and Pharmacogenomics in Asthma Therapy

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INTRODUCTION

Asthma is a complex disease characterised by chronic airway inflammation, bronchial hyperresponsiveness and variability of respiratory symptoms. The mainstay of current asthma pharmacotherapy includes the use of inhaled corticosteroids (ICS) with dose escalation as the severity and control of the disease worsens and/or the addition of other controller drugs, including inhaled long-acting β_2-agonists (LABAs), inhaled long-acting muscarinic antagonists (LAMAs) and leukotriene inhibitors (1). When asthma control is not achieved, it is useful to start an oral corticosteroid or a biological drug.

However, even when treatment has been optimised, drug response may vary considerably even among patients with similar clinical features. This variability is the result of several causes, including disease severity, adherence to treatment, concomitant medical conditions, environmental and infection exposure, age, drug interactions and definition of response (improvement in lung function, persistence of symptoms or exacerbations). However, it appears that a crucial role in inducing this inter-individual variability in response to anti-asthmatic drugs is mainly attributable to the characteristics of target genes or enzymes involved in drug metabolism, which are genetically influenced. Indeed, it has been estimated that between 60 % and 80 % of the variability in response to drug treatment is attributable to genetic factors (2).

The idea that genetic variants can influence drug response dates back several years and led to the introduction of the terms "pharmacogenetics" and "pharmacogenomics" to describe how genetics affects individual drug response (3). Pharmacogenetics focuses on polymorphisms in individual genes and how they influence the response to drugs, examining specific genes and their mutations that may impact the efficacy and safety of therapies. Pharmacogenomics uses whole-genome approaches to assess multigene determinants of drug response, allowing for a more complete and precise understanding of how genetic variations influence drug efficacy and safety in different individuals.

SUMMARY

Keywords

- **Pharmacogenetics** • **pharmacogenomics** • **asthma**
- **pharmacological treatment** • **inhaled corticosteroids** • **β2-agonists**
- **muscarinic antagonists** • **leukotriene inhibitors** • **biological drugs**
- **allergen-specific immunotherapy.**

Acronyms

- **SNP** single nucleotide polymorphism
- **ICS** inhaled corticosteroids
- **LABA** long-acting β_2-agonists
- **LAMA** long-acting muscarinic antagonists
- **CysLT** cysteinyl-leukotrienes
- **CYP** cytochrome P450 enzyme superfamily
- **AIT** allergen-specific immunotherapy
- **FEV1** forced expiratory volume in 1 second

SUMMARY

Pharmacogenetics and pharmacogenomics are fields of study that examine how genetic variations influence drug response. In asthma therapy, these approaches aim to personalise treatment to improve efficacy and reduce side effects. An increasing number of genetic loci have been associated with therapeutic responses to asthma drugs, but the individual effect of a single nucleotide polymorphism (SNP) is partial. Indeed, epigenetic changes can modify genetic effects in a time-, environment-, and tissue-specific manner; genes interact with each other in a network, and non-genetic components such as environmental exposure, gender, nutrients, and lifestyle can interact significantly with genetics to determine response to therapy. In theory, responses to inhaled corticosteroids in asthma are influenced by several genetic variants; however, replications of pharmacogenetic results are few, and we are still far from being able to use these results in clinical practice for the treatment of asthma. This is probably because the response to corticosteroids is too complex to be determined mainly by a few genetic variants. Pharmacogenetic studies on β_2-agonists have mainly focused on the ADRB2 gene. Three SNPs in particular – $Gly^{16}Arg$, $Gln^{27}Glu$ and $Thr^{164}Ile$ – are of functional significance. However, other less common genetic variants may also influence the individual response to salbutamol. Furthermore, haplotypes, which are combinations of variants in the ADRB2 gene, could play an important role. Many variants have been identified in genes coding for muscarinic receptors, especially in the M_2 receptors and, to a lesser extent, in the M_3 receptors. However, there is no consistent evidence that these polymorphisms have significant pharmacological relevance. Despite these studies, the reproducibility of pharmacogenetic results is often limited, and the bronchodilator response predicted on the basis of the identified SNPs does not always correspond to clinical reality. It is hypothesised that the genetics of the immune system may play a key role in allergen-specific immunotherapy. There is a solid body of evidence showing that sublingual immunotherapy effectively reduces the risk of asthma exacerbations in house dust mite allergic patients, especially in those with a genetic predisposition to asthma and/or an underlying T2 endotype.

All genes have variants such as single-nucleotide polymorphisms (SNPs) and copy number variations; however, identifying which of these variants influence drug response is complex (2). Three main approaches have been developed to address this issue (2). The first, based on candidate genes, focuses on analysing genetic variations within specific genes selected a priori on the basis of their known or hypothesised function with respect to a particular disease trait or phenotype. The second approach, without predefined hypotheses, involves studying the entire genome (genome-wide association study or GWAS), allowing millions of SNPs across the genome to be examined simultaneously. The third method, multi-omics, offers a broader and more integrative view of pharmacogenomics, combining different levels of large-scale molecular profiling, such as transcriptomics, proteomics and metabolomics, with data on genetic variants.

Pharmacogenetic and pharmacogenomic tests that predict drug safety and efficacy offer significant benefits to patients, healthcare professionals and industry, as they support the personalised medicine approach by using genetic information to guide therapeutic decisions. However, despite their potential, the clinical use of such tests remains limited compared to other specialities such as oncology and cardiovascular disease, as the application of pharmacogenetics and pharmacogenomics to the treatment of asthma is extremely complex (2). This is due to the fact that although numerous genetic loci related to asthma drug response have been identified, the effect of each individual SNP is limited due to the involvement of multiple proteins (4). Furthermore, epigenetic changes and gene-gene and gene-environment interactions further complicate these associations (4).

1. Genetic factors

Various genetic factors have been identified as exerting an influence on responses to asthma medication. Our research group has already examined these in depth in previous publications (2, 5, 6),

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Table 1-a Polymorphisms influencing the response to ICS

Gene variations in the corticosteroid pathway		
GENE	**SNP**	**RESPONSE**
CRHR1	rs242941 rs1876828	More pronounced increase in lung function in response to corticosteroids
	rs242941	Associated with a ΔFEV1 % predicted negative compared to homozygotes and heterozygotes of the major allele
NR3C1	ER22/23EK (rs6189 and rs6190)	Significant reduction in GRα transactivation capacity
	BclI (rs41423247)	Greater improvement in FEV1 at 4 hours with high-dose ICS
		Associated with corticosteroid resistance
	N363S (rs6195)	Associated with increased sensitivity to corticosteroids
	TthIII (rs10052957)	Associated with corticosteroid resistance
STIP1	rs6591838 rs223647 rs6591838 rs1011219	Improvement in lung function in response to corticosteroids, with the greatest change observed with rs6591838
FKBP4	rs4713916	Correlates with corticosteroid resistance, at least in Crohn's disease
DUSP1	rs881152 rs34507926	Associated with increased bronchodilator response and better asthma control in patients receiving regular ICS treatment
HDAC1	rs1741981	Significantly associated with less improvement in FEV1 in response to corticosteroid therapy in asthmatics
HDAC2	rs58677352	No relation to possible corticosteroid-induced changes in pulmonary function

From Cazzola et al. (5), modified.

to which we refer for a detailed bibliography. Other detailed literature reviews have also reported details of the putative associations of these genetic factors with response to asthma therapy (7-12), and these can be referred to for further bibliographical details. In the present article, we concentrate on summarising those aspects that we consider to be of most relevance or, at least, of greatest interest in guiding further research.

2. Inhaled corticosteroids

ICSs are a mainstay in the treatment of asthma, but patients' responses vary widely. Some derive no benefit, others experience severe side effects, while most are in between. The genetic substrate, in particular the characteristics of the genes targeted for pharmacological action or the enzymes involved in the metabolism of ICSs, emerges as a crucial factor in understanding this variability.

ICSs act mainly through interaction with the glucocorticoid receptor (GR), which is encoded by the NR3C1 gene. This connection activates a number of

processes, such as transrepression and transactivation, that help reduce inflammation in the airways. However, the reaction to ICSs can vary considerably from person to person, and this can be influenced by various genetic factors. In fact, it is estimated that about 70% of the observed differences in response to ICSs are due to genetic variations.

Several genetic polymorphisms have been identified that appear to influence the response to asthma treatment with ICS (Table 1). Some of these polymorphisms, such as rs9910408 and rs2240017 in the T-Box Transcription Factor 21 (TBX21) gene, which encodes the T-box expressed in T cells (T-bet) that regulates the development of naïve T lymphocytes, were associated with significant improvements in asthma symptoms and lung function in ICS-treated adults. In contrast, the rs28364072 polymorphism in the Fc fragment of IgE receptor II (FCER2) gene, which encodes for a low-affinity IgE receptor, appears to be related to an increased risk of severe asthma exacerbations despite ICS use. This polymorphism often requires an increase in the daily dose of ICS, as mutations in this gene can impair the ability to upregulate the IgE pathway, which itself is relatively resistant to corticosteroid-induced regulation.

The CRHR1 gene is responsible for encoding the CRH receptor 1 (CRHR1), to which corticotropin-releasing hormone (CRH) binds. This interaction occurs in the pituitary gland, where the CRHR1 receptor modulates the release of corticotropin (ACTH). ACTH, in turn, stimulates cortisol production and regulates endogenous corticosteroid levels. Variations in this gene could influence the response to ICSs by regulating endogenous corticosteroid levels. It is conceivable that genetic variants that result in lower levels of endogenous corticosteroids could favour a better response to ICSs.

Polymorphisms in the nuclear receptor subfamily 3 group C member 1 (NR3C1) gene, which codes for the mRNA that produces GR, result in changes in the structures of the secondary and tertiary domains of GR. These variations can interfere with the initiation of transcription and the stability of GR mRNA. In general, mutations in NR3C1 are associated with corticosteroid resistance. Such mutations can impair the formation of GR/corticosteroid complexes and reduce transcription, leading to transrepression of genes that govern protein synthesis as part of the cellular response to corticosteroids. This phenomenon represents a key mechanism contributing to individual variation in the response to ICSs in the treatment of asthma.

Polymorphisms in the stress induced phosphoprotein 1 (STIP1) gene have been associated with ICS-induced

Table 1-b Polymorphisms influencing the response to ICS

Genetic variation in the pharmacokinetics of corticosteroids		
GENE	**SNP**	**RESPONSE**
CYP3A	CYP3A4*22 (rs35599367)	Associated with a significant improvement in asthma control scores in patients treated with fluticasone propionate
	CYP3A5*3 (rs776746)	Related to asthma control scores in subjects treated with inhaled beclomethasone
MDR1 (ABCB1)	C1236T (rs1128503), G2677T/A (rs2032582) C3435T (rs1045642)	Possible influence on initial response to steroids in children with nephrotic syndrome
IPO13		Associated with reduced airway hyperresponsiveness among children with mild-to-moderate asthma not using long-term ICS

From Cazzola et al. (5), modified.

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Table 1-C Polymorphisms influencing the response to ICS

Other genetic variations that may alter corticosteroid activity

GENE	SNP	RESPONSE
NR1I2	rs3842689	Associated with non-response to corticosteroids
TBX21	rs2240017	Improved bronchial hyperresponsiveness or better bronchoprotection during ICS treatment
GLCCI1	rs37972	Significantly associated with lower response to ICS therapy in asthmatic patients
FBXL7	rs10044254	Associated with a better symptomatic response to ICS in children only
ORMDL3	rs2872507 rs72821893	Associated with response to ICS treatment in atopic subjects
VEGFA	rs2146323	Associated with response to ICS therapy in asthmatic patients
BBS9	rs2392165	Significantly associated with the use of ICSs in the treatment of wheezing and coughing, with an improved response to ICSs
FCER2	T2206C (rs28364072)	May predict low response to ICS in childhood asthma

From Cazzola et al. (5), modified.

changes in forced expiratory volume in 1 second (FEV1). Although the mechanisms contributing to the variation in corticosteroid response are still unknown, it is hypothesised that interactions with molecular chaperones of the heat shock protein (HSP) family, which function to maintain GR in a quiescent form, may be involved. Furthermore, polymorphisms in the protein kinase dual-specificity phosphatase 1 (DUSP1) gene, which encodes a protein that can inactivate p38 mitogen-activated protein kinase (MAPK) and reduce the expression of proinflammatory cytokines, appear to influence the relationship between ICS use and the bronchodilator response.

Other genes, such as the glucocorticoid-induced transcript 1 gene (GLCCI1), histone deacetylase 1 (HDAC1), orosomucoid-like protein 3 (ORMDL3) and vascular endothelial growth factor A (VEGFA) have been associated with the response to ICSs, highlighting the complexity of individual response to these drugs. However, variability in response to ICSs may not be entirely explained by common or rare genetic variants. The use of genetic variants as predictive biomarkers of response to ICSs may therefore not be as effective as in other diseases.

The metabolism of ICSs occurs mainly in the liver, where they are converted into inactive metabolites by a group of enzymes of the cytochrome P450 (CYP) family, namely CYP3A4 and CYP3A5, which are also present in the intestine and lung. Specific genetic variations can influence this metabolic process by modifying the response to ICSs. Genetic variations, such as the rs35599367 allele (CYP3A422) and the rs776746 allele (CYP3A53), reduce CYP3A4 and CYP3A5 activity, prolonging the anti-inflammatory effects of ICSs in the respiratory tract. They thus positively influence the efficacy of corticosteroids, enabling better asthma control.

P-glycoprotein 1 (P-gp), encoded by the multidrug resistance 1 gene (MDR1 or ABCB1), expels corticosteroids from cells by reducing intracellular concentrations and the response to ICS. Polymorphisms in MDR1, such as rs1128503, rs2032582 and rs1045642, affect corticosteroid treatment. Prolonged use of ICSs may increase P-gp expression, thus reducing the efficacy of systemic corticosteroids, which are also substrates of this glycoprotein. However, P-gp overexpression is only one of many mechanisms that may contribute to corticosteroid resistance in asthma.

Variations in the corticosteroid-inducible importin13 (IPO13) gene influence the nuclear translocation of GR and

the anti-inflammatory effects of corticosteroids. A specific genetic variation of IPO13 is associated with improved airway responsiveness in children with mild to moderate asthma treated with ICS, suggesting increased bioavailability of endogenous corticosteroids in the nucleus. However, further research is needed to determine whether these variations may influence ICS dosing.

3. Bronchodilator inhalers

Several SNPs are believed to influence bronchodilation, although many of them, identified in large populations, have only a slight impact on treatment response. Moreover, the literature is full of conflicting data and readers are referred to the individual articles for more in-depth discussion of these discrepancies.

4.1 β-agonists

β_2-agonists are effective bronchodilators because they relax airway smooth muscle by binding to β_2-adrenergic receptors (β_2-AR) on airway smooth muscle cells. These receptors are G-protein-coupled and are encoded by the ADRB2 gene on chromosome 5q31.32, an area linked to asthma-associated phenotypes. The ADRB2 transcript includes a cistrone

Table 2 Polymorphisms influencing the response to β_2-agonists

GENE	SNP	CLASS	VARIANT	RESPONSE
ADRB2	Gly^{16}Arg (A46G, rs1042713)	SABA	Arg^{16}	• Increased acute bronchodilation; • Impaired clinical response with regular use of SABA; • Increased susceptibility to desensitisation with regular use.
			Gly^{16}	• Faster response during acute asthma exacerbations; • Improved bronchodilation during regular use of SABA.
		LABA	Arg^{16}	• Worse therapeutic response to regular administration of salmeterol; • Increased reduction in bronchoprotection in response o LABA use; • Bronchoprotective effect of regular treatment with LABA on exercise-induced bronchoconstriction in subjects with unaffected asthma; • Decreased bronchodilation during the recovery period after short-term exercise.
			Gly^{16}	• Improved airway function in Gly^{16} similar to Arg^{16} with LABA + ICS; • Therapeutic response or tolerance to long-term formoterol + budesonide therapy similar to Arg^{16} and Gly^{16}Arg.

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Table 2 Polymorphisms influencing the response to β_2-agonists

GENE	SNP	CLASS	VARIANT	RESPONSE
ADRB2	$Gln^{27}Glu$ (C79G, rs1042714)	SABA	Glu^{27}	• It does not reduce the expression of β_2-AR; • Major improvement in PEFR, although many patients respond poorly; • Faster response during an acute asthma episode.
			Gln^{27}	• Significantly increased β_2-AR desensitisation, with a decrease in salbutamol bronchodilation; • Slower response during an acute asthma episode.
		LABA	Glu^{27}	• Improved response to LABA + ICS therapy in asthmatics over 50 years of age.
			Gln^{27}	• Better response to LABA + ICS therapy in asthmatics younger than 50 years.
ADRB2	$Thr^{164}Ile$ (C491T, rs1800888)	SABA		• Less bronchodilation; • Refractoriness to salbutamol in severe asthmatics.
		LABA		• Shorter duration of action for salmeterol; • More urgent or emergency room visits for asthma exacerbations; • Associated with hospitalisation for asthma exacerbations, but not in patients with less severe asthma or with more eosinophils.
ADCY9	$Ile^{772}Met$ (rs2230739)	SABA		• Associated with acute bronchodilation in asthmatic patients treated with ICS.
		LABA		• Associated with acute bronchodilation in patients treated for eight weeks with budesonide and formoterol.
ARG1	rs2781659	SABA		• Associated with acute bronchodilation.

Table 2 Polymorphisms influencing the response to β_2-agonists

GENE	SNP	CLASS	VARIANT	RESPONSE
NOS3	Asp^{298}Glu (rs1799983 TT)	LABA		• Increased FEV1 response to treatment with LABA + ICS.
THRB	rs892940	SABA		• Associated with bronchodilation.
SPATS2L	rs295137 TT	SABA		• Associated with bronchodilation.
ADCYAP1R1	rs34548976	SABA		• Associated with reduced bronchodilation.
ADRB2	Gly^{16}Gln^{27}	SABA		• Associated with reduced bronchodilation compared to patients with Arg^{16}Gln^{27}.
		LABA		• Improved bronchodilation, reduced frequency of hospitalisations and emergency visits, and reduced use of ICS/LABA in asthma.
	Arg^{16}Gln^{27}	SABA		• Bronchodilation reduced in patients with Gly^{16}Gln^{27} and increased in patients with Gly^{16}Glu^{27}; • Response to salbutamol in patients with Arg^{16}Gln^{27} significantly worse than in patients with Gly^{106}Gln^{27} and Gly^{16}Glu^{27}.
ADCY9 ADRB2	Ile^{772}Met and Arg^{16}Gln^{27}	LABA		• Additive therapeutic benefit in terms of percentage improvement in FEV1 after 8 and 12 weeks of treatment with budesonide + formoterol in asthmatics.

From Matera et al. (6), modified.

(5′ LC) that influences mRNA translation and cellular expression of ADRB2. Pharmacogenetic studies on β_2-agonists have focused on ADRB2, identifying several significant SNPs (Table 2).

The polymorphism at position 16 of β_2-AR (rs1042713), which involves the substitution of arginine (Arg^{16}) for glycine (Gly^{16}), affects responses to β_2-agonists. Individuals homozygous for Arg^{16} (Arg^{16}Arg) show a greater acute response to β_2-agonists than individuals homozygous for Gly^{16} (Gly^{16}Gly), but children homozygous for Gly^{16}Gly respond more rapidly during acute asthma exacerbations.

Arg^{16}Arg homozygotes are 5.3 times more likely to respond favourably to salbutamol than Gly^{16}Gly homozygotes, while Arg^{16}Gly heterozygotes show an approximately 2.3-fold better response than Gly^{16}Gly homozygotes. However, homozygous Arg^{16}Arg respond less favourably to prolonged and repeated doses of β_2-agonists. This suggests that regular salbutamol therapy may not be suitable for Arg^{16}Arg homozygotes,

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Table 3 Polymorphisms influencing the response to leukotriene inhibitors

GENE	SNP	RESPONSE
ALOX5	rs2115819	GG homozygotes have a better FEV1 esponse.
	Tandem repeats of the Sp1 binding domain	Lower exacerbation rate, better FEV1 esponse and less β_2-agonists as needed.
LTA4H	rs2660845	Improved clinical response to montelukast.
SLCO2B1	rs12422149	The clearance of montelukast is higher in patients with genotypes GA and AA than in genotype GG.
CysLTR1	927T>C	Related to aspirin intolerance.
	-634C>T	Significantly associated with the drug response rate of montelukast.
CysLTR2	rs912277 e rs912278	Improved response to leukotriene inhibitor therapy linked to increased cysteinyl-leukotriene concentration.
MLLT3	rs6475448	Best response to montelukast.

From et Zhao et al. (12), modified

who are more prone to desensitisation, while $Gly^{16}Gly$ homozygotes may have reduced basal ADRB2 expression. It should, however, be noted that in vitro studies have suggested that the $Gly^{16}Gly$ receptor is more susceptible to agonist-induced desensitisation than the $Arg^{16}Arg$ receptor.

Individuals with the $Arg^{16}Arg$ genotype have a worse therapeutic response to LABA than those with the $Gly^{16}Gly$ genotype, even when using ICS concomitantly. They are also more prone to exacerbations, especially if they are children or young adults. However, the addition of a LABA to a moderate dose of ICS improves airway function similarly for both genotypes, with no clinically significant differences in other parameters. The correlation between the response to LABA and the rs1042713 variant is more pronounced in children than in adults.

Rs1042714 is another important variant in which glutamine (Gln) is replaced by glutamic acid (Glu) at position 27 of β_2-AR. $Gln^{27}Gln$ homozygotes show a greater desensitisation to β_2-agonists and a reduced bronchodilator response to salbutamol than $Glu^{27}Glu$ homozygotes who, in contrast, show a greater improvement in peak expiratory flow (PEF) with β_2-agonists at short duration (SABA), presumably because this variation enhances the binding of β_2-AR to β_2-agonists. However, patients with the heterozygous $Gln^{27}Glu$ genotype generally show a greater salbutamol-induced improvement in lung function than those with the other genotypes. During acute asthma episodes, $Gln^{27}Gln$ individuals respond more slowly to β_2-agonists, followed by heterozygotes $Gln^{27}Glu$, while $Glu^{27}Glu$ individuals respond more rapidly. The combination of LABA and low to moderate doses of ICS produces better results in asthmatic patients who are less than 50 years old and have the Gln^{27} allele. In patients over 50 years of age, the response is better if the Glu^{27} allele is present. However, three randomised trials comparing $Gln^{27}Glu$, $Gln^{27}Gln$ and $Glu^{27}Glu$ found no correlation between these variants and lung function outcomes.

The rs1593054403 polymorphism in β_2-AR, which replaces threonine (Thr) with isoleucine (Ile) at position 164, is less common than the other two polymorphisms. This variation reduces the ability of the receptor to couple with the G protein and decreases the affinity for the ligand, making the receptor less sensitive to agonists. In patients with the rs1593054403 polymor-

phism, the response to β_2-agonists is reduced and the duration of action of salmeterol is shorter. Furthermore, some studies suggest that this polymorphism may reduce the efficacy of salbutamol in severe asthmatics. In an observational study, asthmatics with the rs1593054403 polymorphism treated with LABA needed more outpatient or emergency room visits for exacerbations than patients with the $Thr^{164}Thr$ genotype, while those with the $Thr^{164}Ile$ genotype needed fewer emergency room visits when not treated with LABA than homozygous $Thr^{164}Thr$. However, this difference was only observed in patients with less severe asthma or high eosinophil levels.

There are many other uncommon genetic variants, especially in solute transporter genes, which include membrane transport proteins involved in the movement of endogenous metabolites and xenobiotics. Genes of the β_2-AR G-protein-coupled pathway may also contribute to individual variability in the response to β_2-agonists. Among the various variants linked to the bronchodilator response are those in the adenylyl cyclase (ADCY9), corticotrophin hormone receptor 2 (CRHR2), arginase 1 (ARG1), thyroid hormone receptor 1 (THRB) and corticotropin-releasing hormone receptor-2 (CRHR2) genes. Lastly, the rs34548976 variant in the adenylate cyclase — activating polypeptide 1 receptor type 1 (ADCYAP1R1) gene was associated with reduced bronchodilation caused by neurohormonal mechanisms leading to underregulation of the β receptor2 -AR in highly stressed children.

Functional variations in the β_2-AR receptor may be influenced by a combination of polymorphisms rather than a single SNP. One study identified 12 haplotypes in the ADRB2 gene, but no single SNP can be used as a marker for these haplotypes. The four most common haplotypes are $Arg^{16}Gln^{27}$, $Gly^{16}Glu^{27}$, $Gly^{16}Gln^{27}$ and $Arg^{16}Glu^{27}$. There is a correlation between the Arg^{16} and Gln^{27} alleles and tachyphylaxis, while co-inheritance of Glu^{27} and Gly^{16} favours the Gly^{16} genotype. Cells with the $Gly^{16}Gln^{27}$ haplotype show less desensitisation; however, in asthma, this haplotype is associated with modest bronchodilation, whereas $Gly^{16}Glu^{27}$ leads to a better response. However, the response to salbutamol in patients with the $Arg^{16}Gln^{27}$ haplotype is apparently worse than in patients with the $Gly^{16}Gln^{27}$ and $Gly^{16}Glu^{27}$ haplotypes.

4.2 Muscarinic antagonists

Muscarinic receptor antagonists inhibit the activity of these receptors and are an effective therapy for inducing bronchodilation. Numerous polymorphisms have been identified in the genes of the different muscarinic receptor subtypes.

The human cholinergic receptor muscarinic 3 (CHRM3) gene, located on chromosome 1q43, is the most polymorphic of these genes, with over 1100 SNPs; however, no consistent evidence of pharmacological relevance of these SNPs has been found. The rs6962027 variant in the CHRM2 gene, located on chromosome 7q31-35, comprises six exons and has numerous transcription start sites. This variant involves a change from thymine (T) to adenine (A) in the last exon and is associated with a poor response to ipratropium in asthmatic patients. The rs1824024 variant in CHRM2 also plays an important role in regulating receptor responses, causing a significant reduction in the efficacy of muscarinic receptor antagonists. The SNP rs13247260 was significantly associated with the response to tiotropium in severe asthmatics.

The individual response to a treatment targeting a single type of G protein-coupled receptor (GPCR), such as a muscarinic receptor antagonist, may be influenced by genes encoding other GPCR receptors such as β_2-ARs; in particular, individuals homozygous for Arg^{16} in ADRB2 may be more sensitive to muscarinic receptor antagonists, demonstrating an epistatic impact of ADRB2 loci on CHRM genes.

4.3 Leukotriene inhibitors

Cysteinyl-leukotriene (CysLT) inhibitors act by blocking enzymes involved in the formation of leukotrienes or the binding of leukotrienes to CysLT1 receptors (CysLTR1); however, patients' response to these drugs may vary due to differences in their genes.

Indeed, the enzymes involved in the formation of leukotrienes are polymorphic, which means that they exist in different genetic variants (Table 3). These polymorphisms can influence the amount of leukotrienes produced. Variants in the genes encoding the enzymes involved, such as 5-LO (ALOX5), 5-lipoxygenase activator protein (ALOX5AP),

Table 4 Genetic variations in genes associated with the immune response and their impact on asthma and possibly allergen-specific immunotherapy (AIT)

GENES	GENETIC POLYMORPHISMS	EFFECTS ON THE IMMUNE RESPONSE	IMPACT ON AIT
HRH1	Different polymorphisms	Increased hypersensitivity to allergens, increased severity of allergic reactions.	Less effective response to AIT.
IL4	C-33T, C-589T	Increased production of allergen specific IgE, increased severity of asthmatic symptoms.	Lower response to AIT.
IL13	C-1112T, G+2044A	Increased production of allergen specific IgE, increased severity of asthmatic symptoms.	Lower response to AIT.
IL10	High transcript levels of IL10	Modulation of the immune response, reduction of inflammation.	Predictive of a good response to AIT.
IGHE	Several polymorphisms	High levels of specific IgE.	A specific IgE level above 10 kU/l indicates a good probability of AIT being effective.
IGH	IGHA and IGHG sequences	Increased uniformity of post-AIT sequences.	Used to monitor the effectiveness of treatment.
TRB	TRB sequences	Greater diversity in allergic persons than in non-allergic persons.	Difficulties in distinguishing new T cells from memory cells, promising as biomarkers for AIT.
ORMDL3	SNP rs7216389	Altered ORMDL3 mRNA levels, increased susceptibility to asthma exacerbations.	Reduced risk of asthma exacerbations in patients homozygous for this SNP.
MUC5B	Several B polymorphisms	Increased susceptibility to allergic asthma and increased severity of respiratory symptoms.	Variable response to AIT.
Tight junction genes (CDHR3)	Reduced expression of CDHR3	Increased airway permeability, increased vulnerability to allergens and increased susceptibility to allergic reactions.	Less effective AIT.

LTC4 synthesis (LTC4S) and the enzyme LTA4H (LTA4H), which converts LTA4 to LTB4, can influence patients' response to anti-leukotriene drugs. For example, certain ALOX5 polymorphisms may enhance responsiveness to leukotriene receptor antagonists and reduce the risk of asthma exacerbations. ALOX5AP polymorphisms are implicated in the response to such pharmacological agents, influencing responsiveness to bronchodilators. Increased synthesis of CysLTs is observed in 56% of patients with a variant in the LTC4S gene, suggesting that these patients may respond better to anti-leukotriene therapy; conversely, polymorphisms in LTA4H may increase the incidence of asthma attacks despite treatment with montelukast, probably due to higher levels of LTB4 in the blood.

The leukotriene receptors, CysLT1 and CysLT2, may also exhibit genetic variability. Variants of CYSLTR1 have been linked to aspirin intolerance and the need to use montelukast in aspirin-intolerant asthmatics. However, taken in isolation, CYSLTR1 polymorphisms do not appear to have a significant impact on the response to montelukast or zileuton. Relative to CYSLTR2 polymorphisms, the rs912277 and rs912278 variants increase the concentration of cysteinyl-leukotrienes, proposing a better response to treatment with leukotriene inhibitors.

Polymorphisms in the genes encoding MRP1 (ABCC1) and the organic anion transporter polypeptide or OATP (solute carrier organic anion transporter family, member 2B or SLCO2B1) may influence the response to montelukast and zileuton. The rs12422149 variant of SLCO2B1 is associated with a 30% reduction in the plasma concentration of montelukast; in contrast, the rs6475448 variant of the mixed-lineage leukemia translocated to chromosome 3 protein (MLLT3) gene correlates with a better response to montelukast.

4.4 Theophylline

Theophylline is still used in the treatment of asthma, although its role is more limited than in the past. It is extensively metabolised in the liver. The metabolisation process occurs mainly through enzymes of the cytochrome P450 family. In particular, CYP1A is primarily responsible for metabolising theophylline, while CYP2E1 plays a minor role. They act by following two different main pathways: CYP1A uses the N-demethylation pathway, while CYP2E1 follows the 8-hydroxylation pathway.

Genetic variations in these enzymes can significantly influence the rate at which theophylline is metabolised; for example, the rs2069514 polymorphism of the CYP1A2 gene increases the rate at which theophylline is eliminated from the body. In contrast, the rs762551 (CYP1A2*1F) polymorphism slows its metabolism by reducing CYP1A2 activity. This can lead to high levels of theophylline in the blood, increasing the risk of side effects such as nausea, vomiting, headaches and, in severe cases, convulsions and cardiac arrhythmias.

Although the CYP2E1 gene plays a minor role, genetic variations and interactions with other drugs that affect its activity can still be clinically relevant. In fact, some of these polymorphisms, such as -1055 C>T, -1027 T>C, -807 T>C, -1566 T>A, and -1295 G>C, may reduce CYP2E1 activity, thus affecting the liver's ability to metabolise theophylline.

5. Biologics

Patients eligible for treatment with biological drugs show a variable response to such therapy, but to date the cause of this variability is not entirely clear. It is plausible that polymorphisms in genes coding for cytokines or their receptors may influence the therapeutic response.

Omalizumab, an anti-IgE monoclonal antibody, is one of the biologics whose efficacy can be modulated by genetic polymorphisms in various genes involved in mechanisms of action. Two SNPs in the Fc Epsilon Receptor 1A (FCER1A) gene, rs2251746 and rs2427837, are associated with an increased likelihood of responding positively to omalizumab, likely due to higher levels of total and specific IgE. The rs2230199 variant of the complement 3 (C3) gene was linked to a greater response to omalizumab treatment in terms of reduced exacerbations, probably due to alterations in complement function. Three Fcγ receptor variants — rs1801274-G, rs3219018-C and rs396991-C — were associated with improved lung function. These SNPs may influence the stability of binding in the Fc region and the regu-

lation of IgG antibody responses.

With regard to mepolizumab, an anti-IL-5 monoclonal antibody, it was found that the intronic variant rs1021621 (A>G) in the POU class 2 homeobox 1 (POU2F1) transcription factor gene was associated with a 20% reduction in the rate of clinically significant exacerbations.

Some super-responders to benralizumab, a monoclonal antibody that specifically binds to the IL-5 receptor (IL-5Rα) on the surface of eosinophils and eosinophil progenitor cells, have been described as patients with high expression of eosinophil-related genes in peripheral blood. After treatment with benralizumab these super responders showed significant reductions in the expression of genes associated with eosinophilic inflammatory responses, including IL5RA. Therefore, IL5RA expression could be a useful biomarker of response, being more discriminating than eosinophil count.

6. Application of pharmacogenomics to allergen-specific immunotherapy

Allergen-specific immunotherapy (AIT), which is improperly called allergen vaccine in common parlance, is recommended for patients with allergen-induced asthma, comorbidity with allergic rhinitis and FEV1 in a stable state ≥70% of predicted value, but insufficiently controlled with ICS (1). Real-life evidence suggests that AIT can reduce the incidence of asthma attacks, decrease asthma medication use and slow disease progression.

However, similar to individual differences in drug response, AIT has shown significant variations in the magnitude of immune responses among individuals undergoing such therapy; although understanding of the distinctive immunological mechanisms of AIT in asthma remains limited, it is hypothesised that several factors may influence these variations, and the genetics of the immune system would play a key role (13).

Pharmacogenomics offers valuable tools to investigate the main genetic factors that may influence the response to AIT.

Genetic variations in genes that regulate the immune response, such as those associated with the production of pro-inflammatory cytokines or the expression of T-cell receptors, can influence the immune system's ability to tolerate allergens and respond appropriately to therapy (Table 4). One documented example concerns the gene coding for the histamine H1 receptor (HRH1) (14). Certain polymorphisms in this gene have been associated with increased hypersensitivity to allergens and increased severity of allergic reactions in asthma (15). Carriers of such polymorphisms have a less effective response to AIT than those without this genetic variant.

Certain genes involved in the regulation of airway inflammation, such as those coding for cytokines, chemokines or growth factors, may also influence the severity of asthma and the response to therapy by AIT. Asthmatic patients with specific polymorphisms in the IL4 gene (C-33T and C-589T) and in the IL13 gene (C-1112T and G+2044A) show increased production of allergen-specific IgE and increased severity of asthmatic symptoms (16). Furthermore, these patients show a lower response to AIT than those who do not possess these polymorphisms (17). In contrast, high levels of IL10 transcripts in the blood of patients allergic to house dust mites prior to AIT were predictive of the effect of therapy (18). Significantly higher levels of IgE are also found in individuals with certain polymorphisms in the IGHE gene (19). A specific IgE level above 10 kU/l before AIT is prescribed may indicate to the allergist that AIT has a good chance of being effective (20).

IGH transcript sequences, i.e. particular DNA sequences, have been examined on a large scale for specific reactions to allergens in blood (21). It was found that these sequences change after AIT, showing greater uniformity in the IGHA and IGHG sequences. Furthermore, analysis of IgG transcripts in patients allergic to ryegrass pollen, before and after three years of treatment with sublingual immunotherapy, showed an increase in IGHG2 and IGHG4 sequences. These changes could be used as indicators to monitor allergic diseases and the efficacy of treatments.

Similar research has been done for T-cell receptor beta (TRB) sequences and has shown a greater diversity in allergy sufferers than in non-allergy sufferers (21). However, as TRB genes do not undergo modifications that would allow one to distinguish new T-cells from memory cells, a clear distinction

is difficult. Nevertheless, thanks to advances in sequencing and computer analysis, this method gives hope for the identification of molecular biomarkers to assess the success of AIT.

A recent study was particularly intriguing because it demonstrated the efficacy of sublingual immunotherapy for house dust mite allergy in reducing the risk of asthma exacerbations in a specific group of genetically predisposed patients (22). These data suggest that individuals homozygous for the SNP rs7216389 in the ORMDL3 gene, who are known to have an elevated risk of exacerbations related to altered ORMDL3 mRNA levels, may benefit clinically from such treatment. This finding could influence future therapeutic strategies, moving towards a more personalised use of AIT based on specific genetic markers.

Finally, certain genetic variations may impact the reactivity of the respiratory tract to allergens and the magnitude of allergic responses, thus indirectly modulating the efficacy of AIT. For example, specific polymorphisms in the MUC5B gene correlate with increased susceptibility to allergic asthma and increased severity of respiratory symptoms (23). Individuals with such genetic variations manifest altered mucus production and impaired protective function of the respiratory epithelial barrier, making them more sensitive to allergens and more susceptible to allergic reactions. Similarly, genes responsible for the regulation of tight junctions between airway epithelial cells, such as those coding for occluding junction proteins, may influence epithelial barrier integrity and permeability to allergens, although the exact contribution of these genes to epithelial barrier defects and airway inflammation is not yet fully understood (24). Polymorphisms in these genes could impair the protective function of the airways, favouring allergen penetration and increasing susceptibility to allergic reactions. Indeed, it has been shown that reduced expression of the transmembrane protein cadherin-related family member 3 (CDHR3) gene may lead to increased permeability of airway epithelial layers and thus increased vulnerability to external factors (25).

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"Hidden" and rare food allergens in paediatric age

Hidden and Rare Food Allergens in Pediatric Age.

Tomei L. et al.
Nutrients. 2023; 15(6):1386. doi: 10.3390/nu15061386.

For those who suffer from food allergies, avoiding the trigger allergen can be a real challenge; in fact, accidental exposure to hidden allergens appears to be the cause of about 22% of all food allergic reactions (1). In this paper, the authors present an overview of the major rare and hidden food allergens, with reference to possible sources of exposure and cases in literature, while providing key information for prevention, risk assessment, diagnosis, and management of individuals with food allergies (Figure 1).

Concerning the risk of exposure through ingestion, attention should be paid to colours, such as carmine (E120), annatto (E160b), and tartrazine yellow (E102). Then there are preservatives, in particular sulphites (E220-228) and sodium benzoate (E211), contained not only in food but also in pharmaceuticals and cosmetics. Then there are thickeners, such as carboxymethyl cellulose (E446) and pectins (E440); these appear to be the cause of anaphylactic reactions in patients sensitised to cashews and pistachios. Several spices may give rise to allergic reactions, often as an effect of sensitisation to respiratory allergens; the authors report examples for black pepper, fenugreek (a plant extract of the grass of the same name, also known as *Trigonella (foenum-graecum),* which shows high cross-reactivity with peanut proteins), mustard (also present in some pre-packaged baby foods), and herbs such as sage, oregano and mint.

Allergy to cow's milk is one of the most common allergies in children, and exposure to milk proteins can occur through multiple routes; in the case of ingestion, these allergens have also been detected in soy-based infant powder formulations, in Sabin's vaccine (oral polio vaccine), and in most probiotics.

Foodstuffs can also hide other types of allergens, e.g., residues of antibiotics used in agriculture, as shown by the case of a child who had an anaphylactic reaction after eating blueberry cake. Other examples include food contaminated with latex from gloves used for food preparation; the so-called 'pancake syndrome' (caused by food prepared with flour contaminated with mites); allergens from the nematode *Anisakis simplex* in fish products, or other unusual allergens, such as in one case of allergy to a potato skin protein found in some sweets.

Among plant allergens, nsLTPs (non-specific lipid transfer proteins) are highlighted, which show high cross-reactivity even between botanically unrelated species; cofactors (e.g., exercise, NSAIDs, stress, etc.) often play a role in the reaction to these proteins. In the case of systemic reactions to fruit, GRPs (gibberellin-regulated proteins), present in fruits belonging to different families, such as peach, orange and pomegranate, should also be considered.

In the case of reactions related to meat consumption, the authors mention alpha-gal syndrome, pork-cat syndrome, and bird-egg syndrome. In addition, the increased availability of exotic products on the market may lead to exposure to new allergens or new cross-reactions, as in the cases of anaphylactic reactions to crocodile meat in two children with severe chicken and fish allergies (due to cross-reactivity between parvalbumin).

It is important to bear in mind that the processing of foods can induce changes in their allergenic potential, e.g., via a Maillard reaction. An example is the case of a child who developed hives after eating caramel from condensed milk, a food he normally consumed without any problems.

Inhalation may be another route of exposure to rare and hidden food allergens; interesting cases include reactions to lupine powder in a plant fertiliser, or gluten (from wheat flour) in a toy set for fingerprint identification. In addition, several drugs may contain lactose, which could carry milk proteins. The authors suggest checking the ministerial data sheet (SPC) of the drugs for the pres-

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ence of a specific food allergen to which the patient may have become sensitised. Other examples of excipients to watch out for are soy lecithin, e.g., present in benzylpenicillin, which may be contaminated with soy protein, and mannitol (e.g., in paracetamol for infusion). Rare cases of exposure to peanut proteins following blood transfusion are also known.

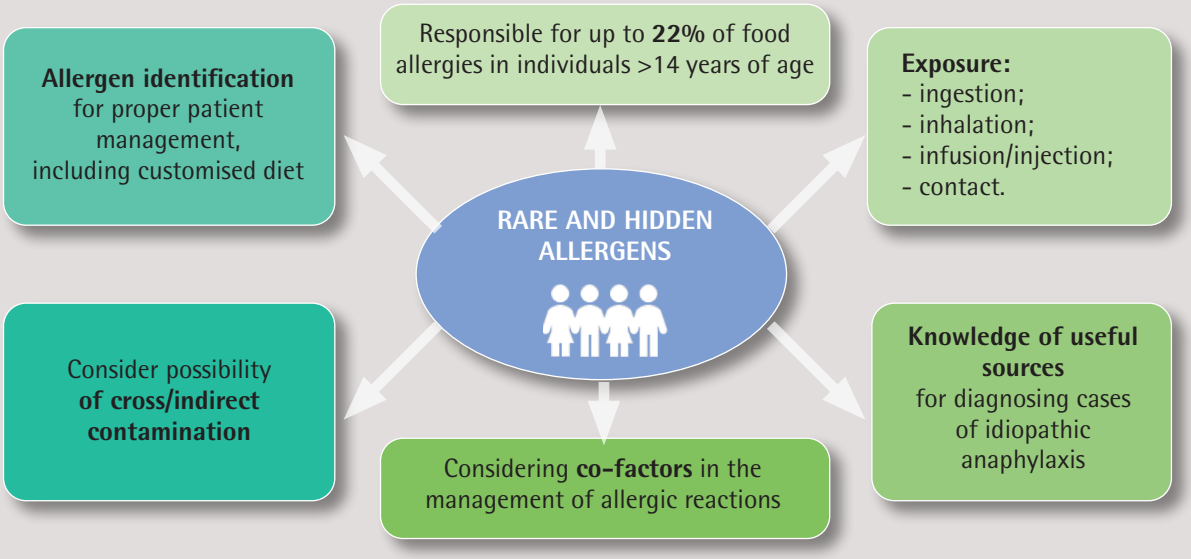
Exposure to food allergens can also occur through direct skin contact and cause hives or other skin symptoms. Wheat proteins, for example, are present in a variety of products, from facial cleansers to toys (such as modelling dough made from wheat flour), or even polyvinylpyrrolidone (PVP), which is present in medical products, personal care articles and food (E1201). It should also be considered that the penetration of allergens can be facilitated if the skin barrier is altered.

Finally, the authors emphasise the importance of cofactors, such as exercise (food-dependent exercise induced anaphylaxis), alcohol, menstruation, stress, NSAIDs and infectious diseases, and also to consider that some diseases, such as systemic mastocytosis, may have clinical manifestations similar to food-induced anaphylaxis.

A serious problem in the management of food allergies is the risk of cross (or indirect) contamination, which occurs when a certain allergen is passed from one food to another, for example through the use of inadequately washed kitchen utensils.

Although new therapeutic approaches for the treatment of food allergies are being studied (immunotherapy), to date the main strategy for managing a food allergy is to strictly avoid the allergen. The fear of accidental exposure to food allergens often leads to a monotonous, unbalanced diet and generates anxiety with negative consequences on the quality of life of the patient and

Figure 1 Rare and hidden allergens: general characteristics



family members. The identification of a rare and hidden allergen is an important diagnostic challenge in cases where the cause of the allergic reaction is unclear (e.g., idiopathic anaphylaxis), and is crucial for proper patient management in order to provide individual dietary advice, reduce risks and improve the quality of life of young patients with food allergy and their families.

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Sensitisation to cyclophilin, the pan-allergen that causes mysterious pollen allergies in children

IgE to cyclophilins in pollen-allergic children: Epidemiologic, clinical, and diagnostic relevance of a neglected panallergen

Matricardi et al. The Journal of Allergy and Clinical Immunology. 2024; 153(6):1586-1596.e2. doi: 10.1016/j.jaci.2024.01.030

Panallergens, such as profilins, polycalcins, pathogenesis-related protein-10 (PR-10), and non-specific lipid transfer protein (nsLTP) are allergenic molecules that exhibit high cross-reactivity with homologous allergens in even phylogenetically distant species, and may be associated with multiple sensitisations and comorbidities, such as asthma or oral allergy syndrome (OAS). A little-studied group of panallergens are the cyclophilins, ubiquitous and highly conserved proteins. Their allergenic properties were first recognised in 1995 (1); in addition to plants, allergenic members of this family have been identified in fungi (e.g., Mala s 6 from *Malassezia sympodialis*) and arthropods. However, their epidemiological, diagnostic and clinical relevance is largely unknown. Only one cyclophilin, the recombinant rMala s 6 protein, is included in molecular allergology tests (ALEX2).

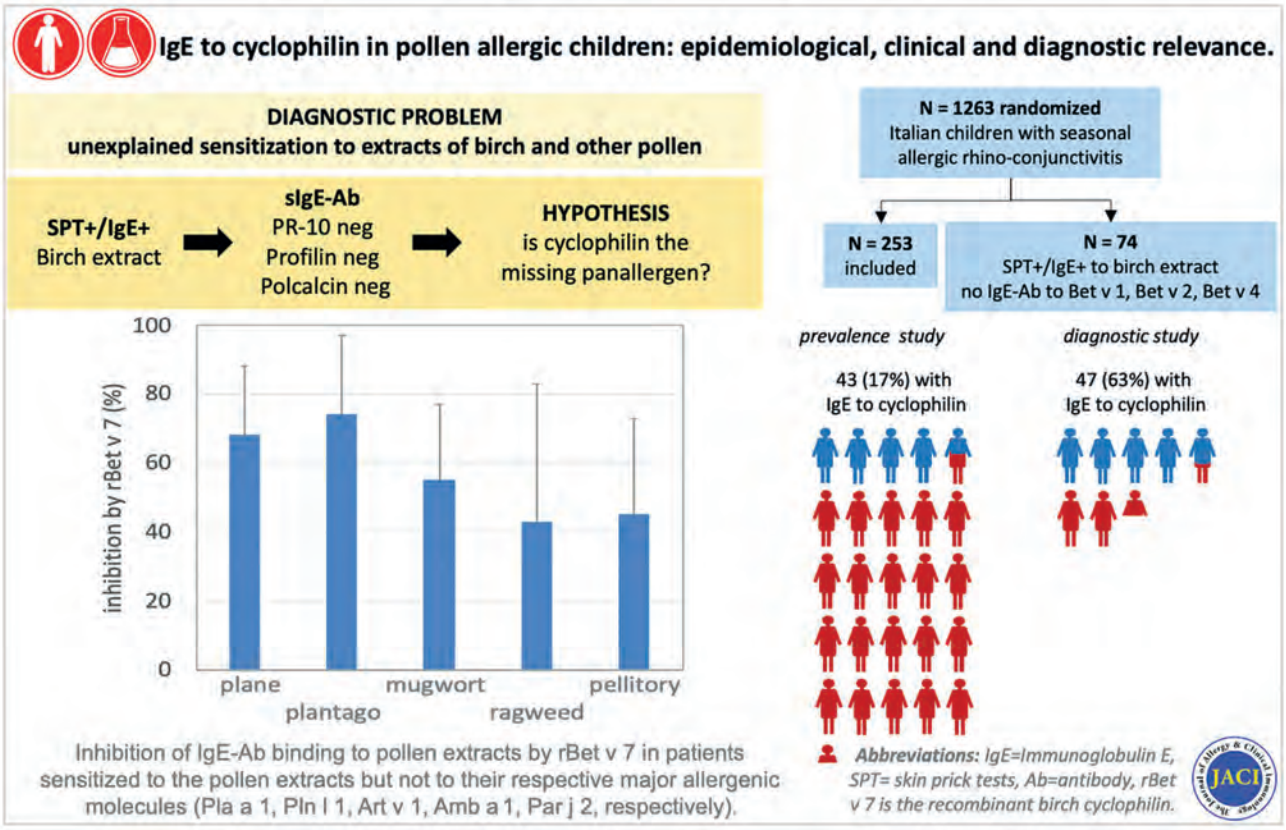
This article represents the first systematic study on prevalence, clinical and diagnostic relevance of cyclophilins in 1380 paediatric patients with pollen allergic rhinitis. In this study, it was observed that approximately 10% of the children showed positive SPT (skin prick test) with birch pollen extract in the absence of positivity towards the main allergen Bet v 1 (PR-10); furthermore, only half of these patients had IgE for Bet v 2 (profilin), Bet v 4 (polycalcin), or cross-reactive carbohydrate determinants (CCD). This finding led to the hypothesis of the existence of a 'panallergen X', i.e. a cross-reactive molecule to be identified, responsible for skin test positivity.

The authors, also in light of new literature data (2, 3), hypothesised that the mysterious 'panallergen X' might be a cyclophilin. They therefore conducted numerous analyses taking into account different subgroups of the cohort (Figure 1).

By means of correlation studies and IgE response inhibition assays using the Bet v 7 molecule (cyclophilin) in recombinant form, the authors observed that 17% of patients (43/253) were sensitised to Bet v 7, with 14 cases in which the IgE for Bet v 7 was > 10 kU/L, which is somewhat lower than for profilins (22%) but significantly higher than for polycalcins (3%). Among the 43 patients with IgE for Bet v 7, 60% suffered from asthma, 42% from OAS and the prevalence of allergic reactions to food was generally higher than in patients not sensitised to Bet v 7, a difference that became statistically significant for banana, cherry, lentils and pear. The authors then analysed the IgE sensitisation profile to other molecules with cross-reactivity implicated in OAS, such as profilins (Phl p 12), nsLTP (Pru p 3), as well as PR-10 (Bet v 1). Only 10 of the 43 patients with IgE for Bet v 7 had no IgE towards the other molecules; none of them suffered

Figure 1

IgE towards cyclophilin in pollen-allergic children: epidemiological, clinical and diagnostic significance (4).



from OAS, but 7 had allergic asthma. Statistical analysis revealed an association between each of the 4 allergens considered, but no association was found between Bet v 7 and OAS or between Bet v 7 and asthma. The authors also observed that more than 63% of the 74 patients sensitised to birch pollen extract, but not to the Bet v 1 / Bet v 2 / Bet v 4 allergens, had IgE towards rBet v 7, supporting the hypothesis that cyclophilin was indeed the 'panallergen X' responsible for the positivity. In addition, the 26 patients with high IgE for Bet v 1 suffered more frequently from asthma, OAS, atopic dermatitis and gastrointestinal allergic symptoms than patients without IgE for Bet v 1 / Bet v 2 / Bet v 4. The analysis of 'false positive' cases showed a high correlation between IgE levels to birch pollen extract and IgE to Bet v 7. Finally, the correlation and inhibition studies showed a positive correlation between IgE levels for rBet v 7 and IgE for extracts of other pollens such as olive, plantain, gramineae, plantain and ragweed, suggesting the presence of cyclophilins in these extracts as well, and thus highlighting the need for

further studies to investigate the content of cyclophilins in other allergenic sources.
The authors conclude by acknowledging some limitations of the study, such as having included only Italian children (thus prevalence data cannot be automatically extended to patients in geographic areas with different climate/vegetation, nor to adults), having considered only one cyclophilin (rBet v 7), not having investigated the role of mite or fungal cyclophilins, and not having conducted oral provocation tests.
In conclusion, the study shows that sensitisation to cyclophilin in pollen-allergic children may underlie positive reactions to pollen extracts of different species, especially in the absence of positivity to the allergens generally considered more clinically relevant. For this reason, molecular diagnostic guidelines should include this panallergen family, and IgE to cyclophilins should be tested in pollen-allergic and polysensitised patients before allergen-specific immunotherapy is prescribed.

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Oropharyngeal microbiota and its relevance in asthmatic children

Oral Microbiota and Pediatric Asthma Phenotype: A New Window for Biomarkers?

Ghedin E, Huang YJ. Am J Respir Crit Care Med. 2023; 208(2):119-121. doi: 10.1164/rccm.202305-0856ED

Asthma is a common chronic disease in children and can present with different clinical manifestations. Although the underlying mechanisms are not fully understood, it is known that several factors are involved and interconnected: from genetic predisposition, to atopy, to environmental factors. An increasing number of studies suggest that the microbiota may be involved in the relationships between environmental exposures, the immune system and the pathophysiology of asthma. In addition to the more studied gut microbiota, the microbial composition of the airways also appears to play a role in the development of childhood asthma. However, sampling of the lower airway microbiota (e.g. induced sputum, bronchoalveolar lavage, endobronchial biopsy) in children is complicated and impractical.
In this editorial, the authors present and critically discuss the data of a scientific study (Abdel-Aziz et al.), published within the same issue, which investigated the association between oropharyngeal microbiota and asthma or wheezing in children of school or pre-school age (1). The main objective of the research was to determine whether characterisation of the oropharyngeal microbiota (sampled by non-invasive methods) could help identify clinically relevant phenotypes in children with asthma or wheezing.
Abdel-Aziz and colleagues analysed 241 oropharyngeal swabs collected from 125 school-age patients (6-17 years) with asthma (86 severe; 39 mild/moderate) and 116 pre-school children (1-5 years) with severe (N = 65) or mild/moderate (N = 51) wheezing as part of the multicentre observational cohort study U-BIOPRED (Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes). The

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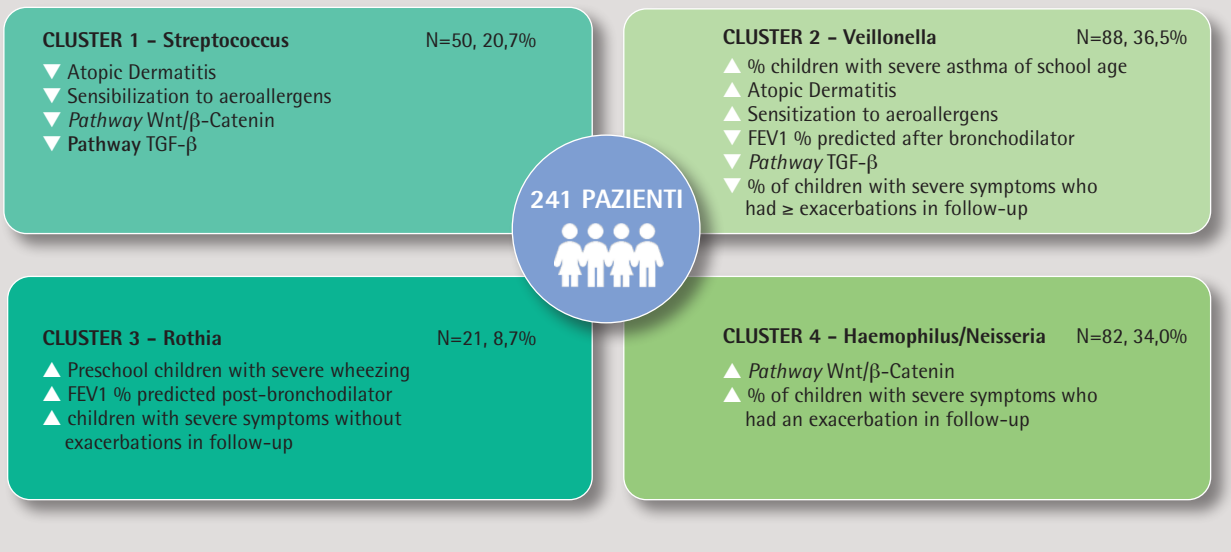
Figure 1 Summary of the characteristics associated with the four clusters



samples were characterised by 16S rRNA gene sequencing, and the data were used to compare the bacterial composition between the samples (β-diversity). The analysis made it possible to classify the children into four clusters, or groups, characterised by the dominance of different bacterial genera: *Streptoccoccus* in cluster 1, *Veillonella* in cluster 2, *Rothia* in cluster 3, and the genera *Haemophilus* and *Neisseria* in cluster 4. The clusters showed significant differences in atopic dermatitis, sensitisation to grass pollen and spirometry. Of all of them, cluster 2 (*Veillonella*) was the largest (88 patients) and had the highest proportion of school-age patients with severe asthma, atopic dermatitis and sensitisation to aeroallergens, especially grass pollen (Figure 1).

Furthermore, in order to identify possible biological pathways linked to the defined phenotypic clusters, the researchers analysed gene expression profiles by means of microarrays on the peripheral blood of 188 patients. Processing of the results led to the identification of two pathways that differed significantly between the different clusters: those of Wnt/β-catenin and the growth factor TGF-β. The former was found to be associated with cluster 4, the latter with cluster 2. Both pathways appear to be involved in airway remodelling in atopic asthma. Furthermore, children with severe asthma or wheezing were followed up for 12-18 months (follow-up), and cluster 2 was found to be associated with a higher frequency of exacerbations during this period (Figure 1). The grouping of children with asthma or wheezing according to oropharyngeal microbiota thus revealed differences in clinical characteristics between the groups, in the risk of exacerbations and in the transcriptomic pathways involved in airway remodelling.

In their article, the authors acknowledge the importance of the new evidence provided by the work of Abdel-Aziz et al., but also point out some limitations of the study (many also acknowledged by the authors themselves), such as the low diversity of the population analysed, the lack of information on in utero and perinatal exposure to antibiotics, the possible underestimation of the impact of corticosteroids, or the fact that taxonomic assignments are limited to gender.

Ghedin and Huang also highlight the need for further research to understand the mechanisms by which the microbiota may influence the clinical trajectories of asthma and see transcriptomics as the most suitable strategy for mechanistic insights. This approach, which characterises gene expression within complex samples, makes it possible to examine functional profiles in situ, and thus obtain useful information on the role of the various components present in the microbiome, including possible viruses.

In summary, analysis of the oropharyngeal microbiota can be used as a non-invasive approach to phenotyping children with asthma or wheezing. Further investigation of the oropharyngeal microbiota may lead to new insights into the pathophysiology and, potentially, to new diagnostic and therapeutic approaches for asthma.

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Food allergies and the gut microbiome

Manipulating the microbiome to enhance oral tolerance in food allergy

Gonzalez-Visiedo M et al. Cell Immunol. 2022; 382:104633. doi: 10.1016/j.cellimm.2022.104633

This review is part of a special volume devoted to oral tolerance (OT), an active process by which the immune system does not respond to food-borne antigens. Loss of OT, which leads to the development of food allergies, has been associated with several factors, most notably alterations in the function of regulatory T cells (Treg), with immune imbalance towards Th2 responses, and impairment of the integrity of the gut barrier. The gut microbiome also plays an important role in the acquisition and maintenance of OT; in this paper, the authors present experimental and clinical data on this topic and discuss current strategies aimed at inducing OT precisely through interventions on the microbiome.

Several studies on germ-free mice (free of micro-organisms, reared under sterile conditions) indicate a link between the absence of gut micro-organisms and lack of OT acquisition. Alterations in the composition and reduced diversity of bacterial strains in the gut have also been associated with loss of OT and development of food allergies. In the microbiome of allergic mice, an enrichment in bacteria of the genus *Citrobacter* (family *Enterobacteriaceae*) has been observed, and in clinical studies in neonates, a high *Enterobacteriaceae/Bacteroidaceae* ratio has been associated with subsequent food sensitisation. Several clinical studies have shown differences in the composition of the microbiome between allergic and non-allergic individuals; in particular, bacteria belonging to the *Clostridia* are those most commonly associated with OT.

But how does the microbiome interact with the immune system to promote and maintain OT? Several factors mediate this interaction, including short chain fatty acids (SCFAs), produced by the fermentation of undigested dietary fibres by gut bacteria. SCFAs, and in particular butyrate, can act as signalling molecules, interacting with receptors coupled to specific proteins (GPCRs; e.g. GPR33, GPR41, GPR43 and GPR109a) expressed in different cell types. The authors devote ample space to the action of SCFAs on different immune cells and schematise these interactions in Figure 1 of the original paper.

In particular, SCFA enhance the phenotype and suppressive function of dendritic cells and Treg, and the presence of *Clostridia* species, capable of producing SCFA, appears to promote the differentiation of a subset of Treg (RORyt+ Treg) involved in mucosal tolerance in the gut. Other studies suggest that SCFAs also act through inhibition of

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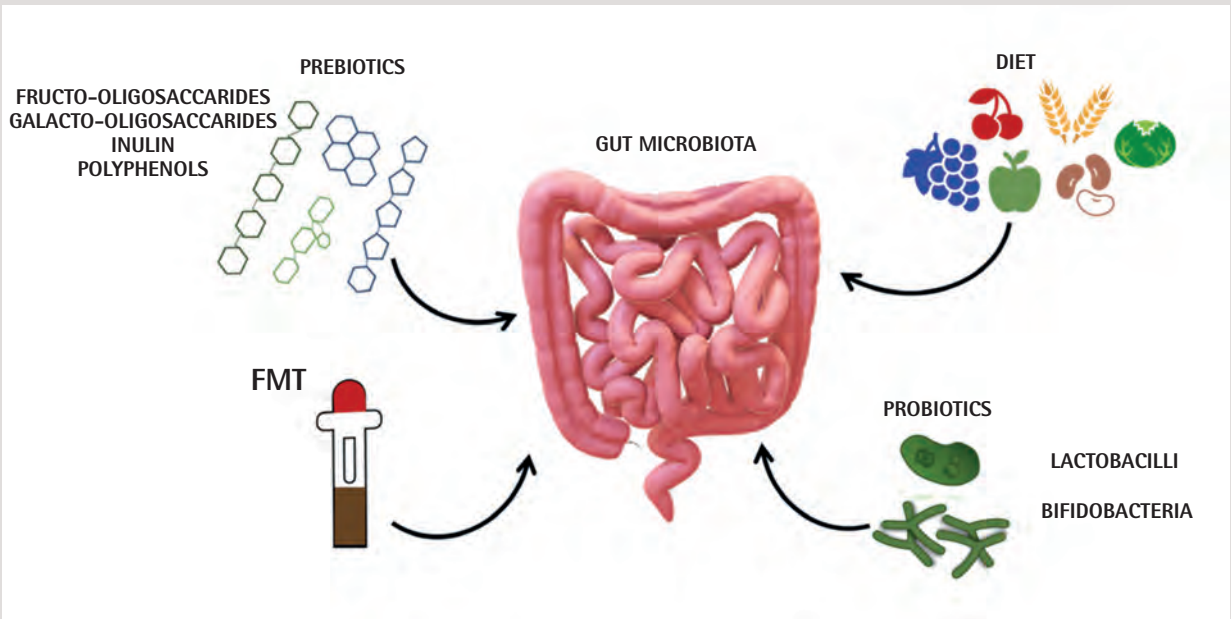
Figure 1 Main strategies for manipulating the gut microbiota



(modified from (1), CC BY 4.0, includes icons from unwing.com). FMT = fecal microbiome transplantation.

histone deacetylases (HDACs), leading to positive effects such as induction of IL-10+ plasma cells and down-regulation of IgE-specific receptor (FcεRI) expression on mast cells, thus inhibiting the triggering of allergic symptoms.
The authors then point out that certain immune cells, such as lymphoid innate cells (ILCs), may, in turn, have an influence on the composition of the microbiota and play a key role in preventing dysbiosis.
Microbiome interventions, which aim precisely to correct dysbiosis and specifically expand bacteria with beneficial properties, include dietary modifications and the administration of prebiotics and/or probiotics. Foods useful for enriching the microbiota with pro-tolerogenic bacteria include foods rich in non-digestible fibres, such as whole grains and bran, which promote the production of SCFA and the increase of commensal gut bacteria (*Bifidobacterium* and *Lactobacillus*). Glycated pea protein and fish oil have also been observed to induce an increase in these types of bacteria. However, with the exception of fish oil, high-fat diets, as well as diets high in protein and low in carbohydrates, appear to have a negative effect on SCFA-producing bacteria. Prebiotics, including oligosaccharides and various fermentable fibres, are defined as 'substrates that are selectively utilised by host microorganisms and confer a health benefit' (1). Prebiotics must therefore be: 1. resistant to gastrointestinal digestion; 2. fermentable by the microbiota; 3. capable of stimulating the growth/activity of beneficial gut microbes. Studies in mouse models have demonstrated the efficacy of prebiotics both in alleviating food allergy symptoms and as a preventive strategy during gestation to

induce a tolerogenic environment and protective microbiota in the offspring. Another useful approach is the direct administration of live microorganisms (probiotics), especially lactobacilli and bifidobacteria. Studies in mice have demonstrated the ability of such treatments to modulate immune responses and induce OT.

Clinical studies on prebiotics and probiotics are often aimed at treating cow's milk allergy. Positive results have been obtained by administering infants with a mixture of probiotics containing Lactobacillus rhamnosus and Lactobacillus casei, or a combination of extensively hydrolysed casein and L. rhamnosus-based formulas. However, further studies are needed to determine whether such therapy can be extended to other allergens. For example, in children with peanut allergy, co-administration of the probiotic L. rhamnosus and oral immunotherapy (OIT) showed no significant improvement in efficacy compared to children treated with OIT alone. Further research is needed to determine the reason for the different response to probiotic treatment in patients with allergies to different foods. Finally, an emerging strategy is faecal microbiome transplantation (FMT) from a healthy donor, whose protective effects have been suggested by studies in mouse models. In humans, a phase I clinical trial was also conducted to evaluate the safety and efficacy of orally encapsulated FMT in the treatment of peanut allergy in adult patients (18-40 years) (2).

In conclusion, the authors emphasise how the evidence gained from preclinical and clinical studies may pave the way for new investigations into the manipulation of the microbiome, and how future studies will in particular have to clarify whether this modulation of the microbiome can be associated with the co-administration of antigens, as is the case with oral immunotherapy.

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Provide information, create a profession

Edited by **Franco Frati**

Specialist in Paediatrics, Allergology and Clinical Immunology
Director of Lofarma Academy

We are pleased to present in the Notiziario Allergologico, in the area reserved for young researchers in allergology of the Lofarma Academy, the contributions of Dr. Richard Borrelli of the University of Turin and Dr. Giulia Garzi, of the La Sapienza University of Rome.

Kind regards,
Dr. Franco Frati, Director Lofarma Academy

In this issue:

FeNO and allergic rhinitis

Richard Borrelli
University of Turin

Exhaled nitric oxide (FeNO) is a non-invasive biomarker used in the evaluation and management of respiratory diseases such as allergic rhinitis and asthma, the measurement of which is a quantitative indicator of eosinophilic inflammation in the airways.

Allergic rhinitis is a condition characterised by inflammation of the nasal mucosa due to activation of a Th2 response by substances such as pollen, dust mites, animal epithelia and moulds; it manifests itself with symptoms such as rhinorrhoea, nasal itching, sneezing and nasal obstruction. Eosinophilic inflammation is a central component of this condition, and FeNO may reflect its presence and severity. Indeed, multiple studies have previously shown that elevated FeNO levels correlate with eosinophilic inflammation in allergic rhinitis (1).

Measurement of FeNO in such patients can guide the clinician in assessing response to treatment with intranasal corticosteroids; a significant reduction in FeNO levels in response to topical anti-inflammatory therapy has been demonstrated (2).

FeNO and asthma

Asthma is a chronic inflammatory airway disease characterised by reversible airway obstruction, bronchial hyperresponsiveness and remodelling of the bronchial tree. Typical symptoms include dyspnoea, coughing, chest wheezing and chest tightness. Eosinophilic inflammation is a hallmark of asthma, and measurement of FeNO has proven useful in its assessment.

In fact, FeNO can be considered an effective tool for monitoring airway inflammation and response to inhaled corticosteroid treatment; high FeNO levels are associated with

an increased risk of asthma exacerbations, while reduced levels following inhaled steroid therapy correlate with better symptom control.
Furthermore, trends in FeNO levels may predict response to steroid treatment, facilitating individualisation of therapy.

Advantages and disadvantages

One of the main advantages of FeNO is its non-invasive nature and the rapidity of the test, which makes it particularly suitable for paediatric use and in patients who have difficulty undergoing other diagnostic procedures.
However, there are limitations to consider: FeNO levels may be affected by variables such as smoking, respiratory tract infections, obesity, and the use of drugs that could alter results.
Furthermore, FeNO is not a diagnostic tool for specific diseases, but must be used in combination with other clinical and diagnostic evaluations.
In conclusion, FeNO represents a useful biomarker for the management of allergic rhinitis and asthma, offering crucial information on eosinophilic airway inflammation. Despite some limitations, its ease of use and ability to monitor response to treatment make it a valuable tool in clinical practice.

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FeNO determination in AIT monitoring

Giulia Garzi
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The measurement of nitric oxide (NO), which is produced by nitric oxide synthases (NOS) present in endothelial, epithelial and immune system cells of the upper and lower airways, has attracted the interest of many researchers and clinical practitioners in recent years. Indeed, as it is produced in high quantities during conditions of hypereosinophilia and type 2 inflammation by inducible nitric oxide synthase type 2 (iNOS2), it can be regarded as a marker of type 2 inflammation. In the management of asthma, the assessment of nitric oxide as a biomarker, measured as exhaled fractional nitric oxide (FeNO), is a validated and accurate tool to be used in both the diagnostic and therapeutic process, such as in the assessment of response to common drugs or in the possible prescription of biological drugs and specific immunotherapy (AIT) (1). Following the same approach, the measurement of nasal NO (nNO) is also being attempted in the diagnostic-therapeutic process of inflammatory diseases of the upper airways, such as in allergic rhinitis, chronic rhinosinusitis and even in a rare pathological condition such as primary ciliary dyskinesia. From the allergist's point of view, nNO could be useful to distinguish allergic from non-allergic rhinitis in combination with allergy testing. Several studies have shown that patients with allergic rhinitis have significantly higher FeNO values than healthy subjects and patients with non-allergic rhinitis, thus demonstrating the usefulness of this test in differential diagnosis. With regard to evaluating the effectiveness of various upper respiratory tract devices, studies on monitoring nasal inflammation are also being evaluated during AIT. To date, there are data confirming that its reduction is significantly associated with consensual reduction of other nasal parameters, such as nasal resistance to

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rhinomanometry according to rhinitis-specific questionnaires such as VAS and RQLQ.
Its determination in clinical practice is currently the subject of extensive study. In the future, the correct measurement of nasal NO values could open up great potential in terms of both research and clinical routine. Various research groups are considering interpreting those clinical conditions that may alter its value, such as nasal polyposis and other chronic upper airway diseases. While waiting for the nasal FeNO to be fully certified as well, we can use the classical expiratory FeNO in the monitoring of various anti-inflammatory Th2 therapeutics of the lower airways (asthma). Of the various therapeutic principles, specific immunotherapy is certainly the one that most distinguishes our speciality. While we are waiting for complete evidence regarding the use of FeNO in monitoring the therapy for allergic rhinitis, we can state, on the contrary, that there is certainty regarding the use of this modern diagnostic in Th2-type bronchial asthma in all its aspects: from monitoring the pharmacological treatment with modern biological drugs to monitoring the effectiveness of AIT

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The image chosen for the cover of this issue of the Notiziario shows a bed of rock fragments (rare earths) on which rests one of the most common technological objects (a smartphone), the production of which is tied to the use of these earths, or rather, certain components present in them. What are rare earths? They are particular rocks that contain a family of chemical elements, also known as lanthanides, which form a large group of boxes in Mendelev's periodic table (with atomic numbers from 57 to 71), with the exception of scandium and yttrium. By virtue of their extraordinary electrochemical, magnetic and optical properties, they play a decisive role in various strategic production sectors such as (to name a few) green and digital technology, renewable energy, electronics, electric cars and aerospace. These elements, in fact, can be found inside smartphones, in computer microprocessors, in LED and plasma TV screens, in electric car batteries and car windscreens, in electrical sensors, in optical fibres, in catalytic converters that underpin the production of wind turbines and photovoltaic panels, and in numerous other products. Given the fundamental role these elements play in a green and digital transition perspective, it is easy to imagine that the demand for rare earths is set to increase significantly in the coming years. Although they are defined as rare, in reality these elements are found in abundance, especially in certain countries such as China (with the largest deposits and a near-monopoly role), Africa (largely finished under Chinese influence), and Russia; one of the problems is to find them in such a concentration as to economically support their extraction. In fact, the latter is based on a *multistep* process that, besides requiring a lot of energy, is accompanied by a significant production of toxic waste with significant environmental consequences. It is clear that for countries, particularly Europe, committed to pursuing industrial strategies based on the ecological transition, it will be fundamental to identify alternative technologies that can compensate for the scarce presence of rare earths on the territory, and at the same time avoid finding themselves in conditions of almost total dependence on countries that are not always "friendly" in supplying them. In this sense, "our" mines could be represented by our own "rubbish", given that in Europe, 10 million smartphones alone are thrown away every year, and each smartphone contains more than 50 different types of elements.

For those wishing to delve deeper into the subject I recommend: "*China and the geopolitics of strategic minerals*" by Sophia Kalantzakos (Bocconi Edizioni).

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