

Microsatellite alterations suggestive of organ-specific asthma and atopy in exhaled breath condensate

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Several genetic linkage studies demonstrated the existence of organ-specific disease susceptibility genes which may induce asthma and atopy (1). In particular, chromosomes 6p and 14q seem to host asthma/atopy susceptibility genes (2).

Microsatellite alterations (MAs) in these regions were detected in sputum cells of asthmatics (3). The MAs can serve as a valid tool to discriminate between asthma and COPD patients (4). MAs are undetectable in nasal cytology samples from patients with allergic rhinitis as bronchial asthma MAs are specific for disease target organ (5).

The possibility to analyze MAs in exhaled breath condensate (EBC) was demonstrated. The method was validated by the comparative study of the EBC DNA MAs and paired lung tissue DNA, allowing the use of this sample in lung disease genetic studies (6).

The aim of the study was to investigate MAs of chromosomes 6p and 14q in EBC DNA of a group of asthmatics with atopy (AAs), a group of nonatopic

asthmatics (ANAs) and a group of nonasthmatic atopics (As), and to verify whether atopy and asthma have different genetic traits.

Twenty-eight consecutive AAs, 13 ANAs, 15 As and 15 healthy subjects (HS) were enrolled (Table 1). The diagnosis of asthma and the assessment of its severity were performed according to GINA. Peripheral whole blood (WB) and EBC were collected from each patient. DNA from WB and EBC was extracted by using QIAmp DNA mini Kit (Qiagen, Mican, Italy).

Five fluorescence labeled markers located at chromosomes 14q (D14S258, D14S588, D14S292) and 6p (D6S2223, D6S263) were used to evaluate LOH and MI. DNA was amplified by the PCR.

The samples were analyzed by capillary electrophoresis on ABI PRISM 310 (Applied Biosystems, Monza, Italy).

Good quality DNA in terms of integrity and amount (mean quantity: 20 ng/μl) was obtained from all EBC samples. None of the HS exhibited genetic alteration of the five markers tested supporting Paraskakis' hypothesis

that these markers are disease-specific (4). MAs in WB-DNA were absent.

The microsatellites D14S292 and D6S2223 drive the susceptibility to atopy and asthma, respectively, other microsatellites are involved in both phenotypes. The increased number of MAs is associated with a declined lung function, severe asthma and high degree of atopy. The AAs exhibited the highest percentages of MAs at 6p and 14q.

The highest number of MAs was found in the EBC DNA of AAs confirming the results obtained from sputum DNA (4). In accordance with Parashakis et al. (4) the correlation between the number of MAs at 6p and 14q and asthma severity was observed. This is particularly true for microsatellite D14S258.

The ANAs showed high percentages of exhaled MAs at D6S2223, a locus which characterizes intrinsic asthma susceptibility and is not involved in atopy.

Our study is the first one to investigate MAs at 6p and 14q MAs in the EBC DNA of As. Seventeen percent of MAs

Exhaled microsatellite alterations at chromosomes 6p and 14q are suggestive of organ-specific asthma and atopy.

Table 1. Anthropometric and clinical characteristics of the subjects

	Asthma with atopy	Asthmatics nonatopic	Atopics without asthma	Healthy subjects
Subjects	28	13	15	15
Female/male	18/10	9/13	9/6	7/8
Age (years)	43.9 ± 9.2	45 ± 4.3	41 ± 5.3	44 ± 5.4
IgE (IU/ml)	251.7 ± 36.9	36.7 ± 20.3	241.4 ± 32.5	41.7 ± 24.7
Blood eosinophil cell/μl	533.6 ± 110.70	24.7 ± 4.5	251.65 ± 25.79	39.6 ± 35.3
FEV1 (% of predicted)	71.7 ± 11.1	72.1 ± 7.1	90.8 ± 9.1	120.1 ± 6.2
FVC (% of predicted), stage (n):	90.8 ± 4.7	92.1 ± 2.8	95 ± 9.0	119 ± 3.4
I	6	5		
II	8	3		
III	7	2		
IV	7	3		

Data are presented as mean ± SD unless otherwise stated.

IgE, immunoglobulin E; FEV₁, forced expiratory volume in one second; FVC, forced vital capacity % pred.

were found in this group. D14S292 is the most altered microsatellite in atopics, it has the most pronounced association with total serum IgEs.

In conclusion, our preliminary results suggest that microsatellites D14S292 and D6S2223 can play a role in atopy and asthma susceptibility, respectively. Atopy and asthma share somatic/acquired genetic alterations whose number increases with the severity of asthma and atopy worsening. These alterations can also derive from either isolated atopy or asthma, or from the association of the two phenotypes.

The genetic analysis of the EBC DNA can play an important role in clinical practice since it may allow identification of subjects susceptible to asthma among patients with atopy. Moreover, noninvasiveness of the method makes it easily accepted by subjects and suitable for a large screening.

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