

A simple and rapid micromethod for genomic DNA extraction from jugal epithelial cells

Application to human lymphocyte antigen typing in one large family of atopic/asthmatic probands

Aron Y, Swierczewski E, Lockhart A. A simple and rapid micromethod for genomic DNA extraction from jugal epithelial cells. Application to human lymphocyte antigen typing in one large family of atopic/asthmatic probands.

Allergy 1994; 49: 788–790. © Munksgaard 1994.

We describe a rapid and reliable micromethod for DNA isolation from buccal epithelial cells from the interior mouth mucosa. This convenient, noninvasive method could be applied to genetic typing in a small number of cells (about 2000 cells per cheek). We have shown that DNA released by this method is suitable for further amplification by polymerase chain reaction (PCR). Using this protocol, coupled with the PCR-RFLP (restriction fragment length polymorphism) method, we analyzed the allelic sequence diversity of the human lymphocyte antigen (HLA) class II genes in an extended family of 33 persons containing 14 asthmatic or atopic members. Six of eight DQA1 alleles, and 11 DQB1, 20 DPB1, and 10 DR haplotypes could be identified in a single DNA sample. Our results suggest that the DR53 group haplotype is frequently associated with allergic asthma and atopy. The micromethod described here may be useful in genetic epidemiology, especially in family studies involving small children.

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Key words: allergic/atopic family; DNA isolation; human lymphocyte antigen typing; jugal cells; micromethod.

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Accepted for publication 7 December 1993

We report a short and reliable method for DNA isolation from buccal epithelial cells collected by scraping the interior mouth mucosa. This method is especially suitable to prepare simultaneously a large number of samples from small numbers of cells (about 2000 jugal cells per cheek). We applied this method to study the allelic sequence diversity of the human lymphocyte antigen (HLA) class II genes in an extended family of three generations and 33 members. Some of these were asthmatic, atopic, or both.

HLA class II genes are highly polymorphic, and their products are functionally involved in the control of the immune response. These genes might be associated with susceptibility or resistance to asthma and allergic expression.

Material and methods

Clinical data

Of the 33 members of the family, 19 were healthy, asymptomatic subjects, and 14 were asthmatic,

atopic, or both. Among them, three clinical subsets were recognized: allergic asthma (seven subjects), allergic rhinoconjunctivitis (five subjects), and atopic dermatitis (six subjects). Two subjects had psoriasis.

Experimental data

DNA extraction. Jugal cells were collected with Cyto-brushes (Medical Packaging Corporation, Camarillo, CA, USA). Each cheek was treated separately for volume convenience. Cells were suspended in 2 ml conical polypropylene tubes containing 0.5 ml sterile 150 mM NaCl. Samples were kept at 4°C or –20°C, depending on the considered storage duration. A volume of 10 µl of 10 mg/ml proteinase K was added per tube, and the mixture was incubated at 56°C for 2 h. The mixture was extracted with an equal volume of 1:1 phenol/chloroform after vigorous mixing for 1 min. The samples were centrifuged for 5 min at 12000 rpm at room temperature in a

Sigma 210 M microcentrifuge. The upper aqueous phase was transferred to a new tube, extracted again with phenol/chloroform and centrifuged as previously described. The upper phase was saved and transferred to a new tube containing 1 V isopropanol. The samples were mixed gently by inverting the tubes, kept for 30 min at -20°C , and then centrifuged for 15 min at room temperature. The DNA pellets were washed in 70% ethanol and dried for 10 min by vacuum centrifugation. DNA was dissolved in 80 μl sterile distilled water. A volume of 160 μl cold absolute ethanol and 3 M $(\text{NH}_4)\text{CH}_3\text{COO}$, to a final concentration of 0.03 M, were added to the DNA solution. The DNA was allowed to precipitate for 30 min at 4°C . After a 15-min centrifugation at 12000 rpm, DNA was collected. The final pellets corresponding to the total amount of cells obtained from each subject were pooled and resuspended in 50 μl sterile distilled water.

In parallel, DNA from peripheral blood lymphocytes was extracted by the classical phenol/chloroform method (6).

HLA typing. The polymerase chain reaction/restriction fragment length polymorphism (PCR-RFLP) method was used in this study to evaluate genetic linkage between the asthma/allergy-associated phenotype and the HLA class II genes in a family having affected siblings. This procedure provides a simple and rapid way of defining the HLA DQ, DP, and DR alleles and haplotypes at the nucleotide level, eliminating the need for radioisotopes. The second exon of the DQA1, DQB1, DPB1, and DRB1 genes was selectively amplified by the PCR method with a Hybaid thermal reactor. This reaction was performed according to conditions already described (1). The sequences of the different primers used for DQA1, DQB1, and DPB1 genes have been given in previous publications (2, 7, 8). HLA DRB genes were amplified by using the primers GH46 and GH50 for the generic amplification, and the primers GAMP DRB1 and PL368 (4) to discriminate the different alleles of the DR3, 5, 6, and 8 haplotypes. PCR products were controlled in a 2% agarose gel stained with ethidium bromide. Amplification of the polymorphic exon of the DQA1, DQB1, and DPB1 loci and the three successive amplifications to discriminate the alleles associated with the different DRB haplotypes could be achieved with 30 μl of a single DNA sample.

The PCR products were genotyped by digestion of the amplified DNA products with allele-specific endonucleases recognizing allelic variations in the polymorphic exon. The endonucleases used were the following:

DQA1 gene: DdeI, RsaI, and FokI

DQB1 gene: AcyI, HinPI, Sau96I, and HaeIII

DPB1 gene: RsaI, BanII, FokI, EcoNI, DdeI, and Bst UI

DRB generic: HinfI, NsiI, and DdeI

DR3,5,6,8: AvaII, Asp700, HinPI, RsaI, and ApaI.

A volume of 8 μl PCR product was digested separately with four units of each enzyme under conditions recommended by the manufacturer (Boehringer, Mannheim, Germany), and the products were size-analyzed in an 8% polyacrilamide gel. The gel was stained in 1 $\mu\text{g}/\text{ml}$ ethidium bromide for 15 min and photographed under UV light. The different sizes of the fragments enable easy discrimination of different alleles. Six of the eight DQA1 alleles, 11 of the 12 DQB1 alleles, 20 of the 21 DPB1 alleles, 10 DR haplotypes, and 12 DRB1 alleles associated with DR3, DR5, DR6, and DR8 haplotypes could be identified in a single DNA preparation.

Results

Comparative yields of PCR products were obtained with template DNA prepared either from 4000 jugal cells or from peripheral blood lymphocytes (about 150000 cells) by the classical phenol/chloroform method (Fig. 1). As stated above, HLA typing was done by the PCR-RFLP method. No significant difference was found in the distribution of DQA1, DQB1, and DPB1 alleles between healthy and affected subjects (results not shown). In contrast, as shown in Table 1, the DR53 extended group haplotype was significantly associated with allergic asthma or atopic states and was absent in healthy subjects. Conversely, haplotype DR6 had a higher frequency in healthy subjects and could be associated with a protective effect.

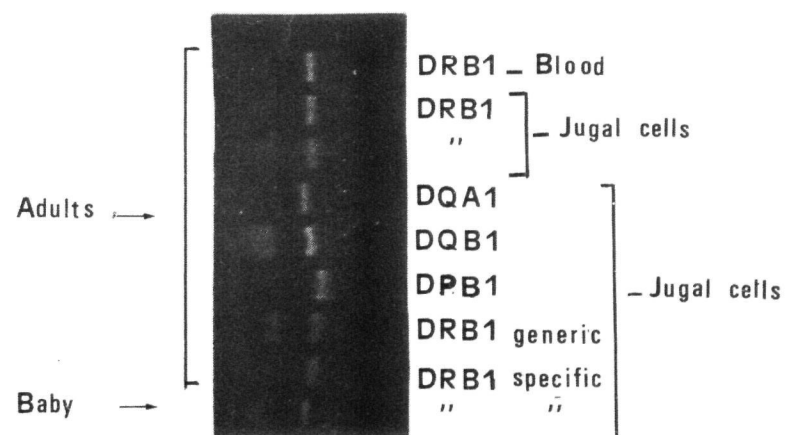


Fig. 1. Amplification of HLA class II sequences with target DNA extracted from peripheral blood lymphocytes (lane 1) and DNA from jugal cells (lanes 2–9). Lanes 1–3: DRB1 generic amplification. Lanes 4–8: amplification in the regions DQA1, DQB1, DPB1, and DRB1 (adult sample). Lane 9: DRB1 specific amplification (baby sample).

Table 1. Distribution of DR haplotypes in healthy and affected members of family

	H* (n° = 38)		Aa* (n° = 28)		P°°
	Haplotype no.	%	Haplotype no.	%	
DR1	2	5	0	0	NS
DR2	14	37	5	18	NS
DR3	3	8	4	14	NS
DR4	0	0	6	21	<0.0041
DR5	3	8	1	3	NS
DR6	14	37	4	14	<0.041
DR7	0	0	7	25	<0.0015
DR8	0	0	0	0	NS
DR9	0	0	0	0	NS
DR10	2	5	1	3	NS
DR53**	0	0	13	46	<0.000003

H* = Healthy subjects; Aa* = asthmatic and/or atopic; n° = number of chromosomes; DR53** group haplotype = DR4+DR7+DR9; P°° value determination by Fisher's exact test.

Discussion

Various methods are currently available to extract DNA from peripheral blood lymphocytes with phenol/chloroform (6). However, collecting large blood samples might in some cases be fraught with difficulties, especially in young children and in adults from different geographic and ethnic backgrounds, from whom collecting blood is not feasible for religious, psychologic, or technical reasons. Moreover, with micromethods utilizing whole blood rather than purified mononuclear cells, inhibition of PCR often occurs (3). The procedure described above releases DNA in a form suitable for PCR, while preserving the activity of Taq polymerase. Using this method, we were able to analyze rapidly the members of an extended family (33 members) in which haplotype DR53 was associated with asthma and atopy. Further studies now in progress in two larger groups of unrelated white subjects confirm these preliminary results. Indeed, data suggesting the existence of genetic linkage between candidate loci and atopy

have failed to be confirmed up to now (5). Notwithstanding the preliminary character of the latter results, the DNA isolation method described above, coupled to the PCR-RFLP method, can be successfully applied to genetic typing in a small number of cells obtained by a noninvasive sampling method. The search for major loci for atopy is a timely approach to allergic diseases (5). The method proposed here may prove to be particularly useful in genetic epidemiology, as in the large studies that will be required for solving the problem, and particularly suitable for family studies involving small children.

Acknowledgments

This work was supported by grants 91 FG/A1 and 92 FG/A3 from Le Comité National de Lutte contre les Maladies Respiratoires. We thank Dr B. Polla and Dr N. Frossard for helpful discussions.

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