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DNA Banking of Primary Immunodeficiency Disorders in Iran

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ABSTRACT

Primary immunodeficiency disorders are a heterogeneous group of genetic disorders, with different modes of inheritance, consisting of more than 100 different types. We constructed the DNA banking of primary immunodeficiency disorders for the first time in Iran. The DNA of 31 immunodeficient patients and their families (total of 92 samples) were collected, as the first step for construction of DNA banking. DNA was isolated from whole blood by salting out method. Among our patients, Common variable immunodeficiency was the most common disorder, followed by X-linked agammaglobulinemia, Ataxia-telangiectasia, Chronic granulomatous disease, Severe combined immunodeficiency, Hyper IgM syndromes, and Leukocyte adhesion defects. DNA banking is a useful method for further detection of mutation in immunodeficient patients and prenatal diagnosis for presence or absence of the disorder in the fetus which can be confirmed by molecular genetics testing.

Key words: DNA banking; Iran; Primary Immunodeficiency Diseases

LETTER

Primary immunodeficiency disorders (PID) are relatively rare disorders, characterized by unusual susceptibility to infections and predisposition to the development of autoimmune diseases and malignancies.[1-4]

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More than 100 different disorders have so far been identified and categorized in PID classification[1,2] PID constitutes a wide group of genetic disorders, with different modes of transmission. Although the responsible genes for some of these disorders have been identified, there are still some mysteries about these disorders.[1-4] The mutation data for the majority of diseases are available in the internet with a special database management system.[5] DNA technology can help in diagnosis of genetic diseases by detecting the defective gene. DNA sample collections are used for various purposes, namely for clinical, research and industrial uses, carrier detection, presymptomatic diagnosis, and prenatal diagnosis by family investigation.

We constructed the DNA banking of PID for the first time in Iran. This DNA bank could further be used for molecular genetic testing, detection of mutation of these diseases and prenatal diagnosis.

Thirty one immunodeficient patients (20 male and 11 female; mean age: 15.8±9.7) with members of their families (total of 92 cases) were referred to Children's Medical Center during March 2003- June 2005 (Table 1). The diagnosis of PID was based on standard criteria.[1]

Table 1. Characteristics of primary immunodeficient patients in this study.

N.	Sex	Age (years)	Diagnosis	Extraction of DNA from the family
1	M	16	CVID	brother, 2 sisters
2	F	17	CVID	father, mother, 2 brothers, sister
3	F	7	AT	father, mother
4	M	23	XLA	mother
5	F	21	CVID	mother
6	M	11	AT	father
7	M	48	CVID	-
8	M	19	XLA	father, mother
9	F	17	HIGM	father, mother, sister
10	F	3	SCID	mother
11	M	34	XLA	mother
12	F	23	CVID	mother
13	M	30	XLA	mother, 2 sisters
14	F	17	AT	mother
15	M	12	XLA	mother
16	M	19	CGD	father, mother
17	M	12	CVID	father, mother
18	F	6	CVID	father, mother
19	M	9	CVID	father
20	M	13	HIGM	father, mother, sister
21	M	11	CGD	father, mother
22	F	3	SCID	father, mother
23	M	13	CVID	father, mother, sister
24	F	3	SCID	father, mother
25	M	14	CGD	father, mother, brother
26	M	7	LADs	father, mother
27	M	18	CGD	father, mother
28	M	19	AT	father, mother
29	M	25	CGD	father, mother, 2 sisters
30	F	13	AT	father, mother
31	M	6	XLA	mother

CVID: Common variable immunodeficiency; AT: Ataxia-telangiectasia; XLA: X-linked agammaglobulinemia; HIGM: Hyper IgM syndrome; SCID: Severe combined immunodeficiency; CGD: Chronic granulomatous disease; LADs: Leukocyte adhesion defects; M: Male; F: Female

The samples were collected following approval of the local Ethics Committee and after informed consent of the patients. DNA was isolated from whole blood, using a "Salting out" method.[6] The method described in salting out involves digesting eukaryotic cells with proteinase K in the presence of EDTA as anticoagulant, solubilizing membranes and denaturing proteins with a detergent such as SDS, and saturation with NaCl 6M.

Common variable immunodeficiency was found the most common disorders (9 cases), followed by X-linked agammaglobulinemia (6 cases), Ataxia-telangiectasia (5 cases), Chronic granulomatous disease (5 cases), Severe combined immunodeficiency (3 cases), Hyper IgM syndromes (2 cases), and Leukocyte adhesion defects (1 case) (Table 1). Purity of DNA in terms of RNA and protein contamination is of vital importance. The method has been applied for DNA isolation from whole blood cell of immunodeficient patients. Such DNA molecules have optimal A230/A260 and A260/A280 ratios of 0.4±0.04 and 1.80±0.05 is essentially free of proteins and RNA, respectively. Occasionally some DNA preparations have been found to be turbid as a result of co precipitation of other macromolecules. DNA is more resistant to environmental decay than proteins or other biochemical molecules. The extraction methods typically produce high yields of purified genomic DNA with size between 100-200 kilo bases.

After establishment of Iranian Primary Immunodeficiency Registry in August 1999, we tried to organize a DNA banking for these patients. This could help us to construct the mutation database for the patients.[7] The American National Bioethics Advisory commission defined a DNA bank in 1999 as a facility that stores extracted DNA, transformed cell lines, frozen blood or other tissue and biological materials for future DNA analysis. The last few years have witnessed an important expansion of human DNA sampling and data collecting. This activity is very important for genetic research, clinical care and future treatment. Health care also improved as the result of research activities and modern research depends on access to biological samples, including DNA. Thus the potential benefits justify the establishment of DNA banks, however the possibility of misuses imposes a responsibility for proper management and protection of the subjects' interests.[8] There are several types for DNA collections; in anonymous collections the biological materials are originally collected without identifiers and are impossible to link the findings to their sources.

In identifiable collections biological materials are unidentified for research purposes but can be linked to their sources through the use of a codes system.

In identified collections, identifiers such as patients' name, number or clear pedigree and location are attached to the biological materials.[9] Banking of DNA is useful to help identification of missing family members, providing for inheritance issues, prenatal diagnosis, and to identify several inherited genetic disorders.[10]

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