Genetic polymorphism analysis of NAD(P)H: quinone oxidoreductase 1 in different Iranian ethnic groups

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NAD(P)H: quinone oxidoreductase (*NQO1*) participates in the detoxification of numerous endogenous and foreign compounds. It has been shown that homozygous patients having T-allele exhibit negligible *NQO1* enzyme activity. Lack of *NQO1* activity might increase the risk of certain types of toxicity and cancer. It has been reported that this gene has a single nucleotide polymorphism at the site of codon 187 (nucleotide 609). It is indicated that *NQO1* polymorphism has been associated with susceptibility to several malignancies. The frequency of *NQO1* C609T polymorphism has not been reported among the Iranian population so far. We initiated a study to examine *NQO1* C609T genotype in different Iranian ethnic groups. We assessed the genotype patterns of *NQO1* among Iranian Fars, Mazandarani, Turk and Turkmen ethnic groups in eight regions. The *NQO1* C609T genotypes were determined by polymerase chain reaction–restriction fragment length polymorphisms analysis in 245 Iranian healthy individuals, 139 Fars (from five regions), 25 Mazandarani (from Babol), 42 Turk (from Urmia) and 39 Turkmen (from Bandar-Turkmen) ethnic groups. Their distributions for T-allele were similar to allele frequencies in Caucasians with the exception of the Mazandarani ethnic group (12%) and different from other Asians groups. Allele frequency of *NQO1* C609T for Yazd population was significantly different from Shiraz, Mashhad and Mazandarani (*P* value = 0.015, 0.009, 0.029).

Keywords: Ethic groups, Iran, *NQO1*, polymorphism.

NAD(P)H: quinone oxidoreductase (*NQO1*) participates in the detoxification of numerous endogenous and foreign compounds[1,2]. It has been shown that homozygous patients having T-allele exhibit negligible *NQO1* enzyme activity[3]. Lack of *NQO1* activity might increase the risk of certain types of toxicity and cancer[4–8]. It has been reported that this gene has a single nucleotide polymorphism (SNP) at the site of codon 187 (nucleotide 609)[8,9]. It is indicated that *NQO1* polymorphism has been associated with susceptibility to several malignancies. Frequency of homozygous individuals having T-allele has been reported to range from 1.5 to 20.3% among different ethnic groups[4]. It has also been reported[3] that acute and chronic side effects of cancer treatment might be involved in causing genetic variations of *NQO1*. There are numerous documented cases of cancer patients receiving chemotherapy with alkylating agents, who develop secondary myeloid leukaemia[3,12]. The frequency of *NQO1* C609T polymorphism has not been reported among the Iranian population so far. We initiated a study to examine *NQO1* C609T genotype in different Iranian ethnic groups. We found that the allele frequency of *NQO1* C609T genotype for Yazd population was different from Shiraz, Mashhad and Mazandarani.

We examined four Iranian ethnic variations for the prevalence of *NQO1* polymorphism in 245 healthy subjects, including Fars, Mazandarani, Turk and Turkmen groups (139 Farsis, 25 Mazandaranis, 42 Turks and 39 Turkmen). Among the four ethnic groups chosen, Farsis who speak Persian or Farsi are in majority (51%). The other three groups[11] are primarily Turkish people (including Azaris, 24%), Turkmen (2%) and Mazandaranis (4%). Farsis are distributed mostly in Central, East, North and Northeast Iran and Azeris in Northwest Iran. Turkmen are the minority group having common ancestors with Azeris located in the Northeast[11]. The Mazandaranis live in the North around the Caspian Sea[11]. The four groups who participated in this study were Turks from Azerbaijan state (Uremia city) in the northwest, and Farsis from five different locations in the South, Central and Northeast Iran (Shiraz, Esfahan, Yazd, Kerman and Meshhad cities). Turkmen were from a port city (Bandar-Turkmen) in the North and Mazandaranis were from Babol city, in the North. All the subjects who participated in this study were male and aged between 25 and 55 years. The study was approved by the National Institute for Genetic Engineering and Biotechnology (NIGEB), Iran. Blood samples were collected from individuals of each ethnic group. Informed consent of the participants was taken and they were all given a questionnaire to document their ethnicities. Samples were transferred immediately to the Central Laboratory of NIGEB.

Genomic DNA was extracted from the whole blood and stored at –20°C till further use. The samples were anonymized and subjected to PCR and RFLP to detect (C > T) at nucleotide 609, which creates a *Hin*fI restriction site. *NQO1* C609T genotyping was performed as described by Ozawa *et al.*[9] and Biramijamal *et al.*[12]. Initiation of denaturation for 5 min at 95ºC was followed by 35 cycles at 95 °C for 1 min, 57°C for 1 min, and 72°C for 45 s (Iranian patent number 30311)[12]. The PCR products were subsequently digested with 15 units of *Hin*fI restriction enzyme (Fermentas, Lithuania) and separated on a 3% agarose gel (Figure 1). PCR products with homozygous genotype (C/C) remained undigested, but those with heterozygous genotype (C/T) were cleaved to yield one or two fragments (154 and 164 bp) on 3% agarose gel. Selected PCR products were purified with PCR product purification kit (Roche) and sequenced directly by Big Dye™ fluorescent dye dideoxy sequenc-

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Table 1. *NQO1* genotypes in Fars, Mazandarani, Turk and Turkmen ethnic groups

Ethnic group	Population	Total	NQO1 C609T genotype			Ser allele frequency	*P* value of Pro/Ser allele of Mashhad/others	*P* value of Pro/Ser allele of Yazd/others	Hardy–Weinberg equilibrium
			Pro/Pro	Pro/Ser	Ser/Ser				
Fars	Shiraz	42	25 (59.5%)	13 (31%)	4 (9.5%)	0.25	0.702	0.015	1.2803
	Mashhad	25	13 (52%)	10 (40%)	2 (8%)	0.28		0.009	0.0015
	Kerman	24	17 (70.8%)	7 (29.2%)	0 (0%)	0.15	0.106	0.302	0.6986
	Yazd	25	21 (84%)	4 (16%)	0 (0%)	0.08	0.009	0.419	0.189
	Esfahan	23	17 (73.9%)	6 (26.1%)	0 (0%)	0.13	0.071		0.5177
Mazandarani	Babol	25	16 (64%)	6 (24%)	3 (12%)	0.24	0.648	0.029	2.9258
Turk	Urmia	42	29 (69%)	12 (28.6%)	1 (2.4%)	0.17	0.119	0.155	0.0335
Turkmen	Bandar–Turkmen	39	30 (76.9%)	8 (20.5%)	1 (2.6%)	0.13	0.032	0.394	0.2662

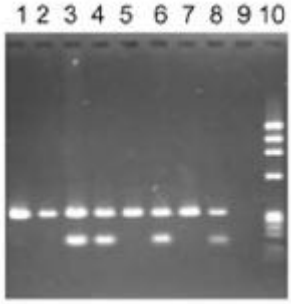

Figure 1. PCR assay to detect genetic polymorphism at *NQO1* locus. A 318 bp region of genomic DNA flanking exon 6 was amplified using primers NQO1-F (5′-ATT CTC TAG TGT GCC TGA G-3′) and NQO1-R (5′-AAT CCT GCC TGG AAG TTT AG-3′).The PCR product was then digested with *Hin*fI. Lanes 1, 2, 5, 7, Homozygous genotype (C/C); Lanes 3, 4, 6, 8, Heterozygous genotype (C/T); lane 9, Negative control; lane 10, 72–1353 bp molecular marker.

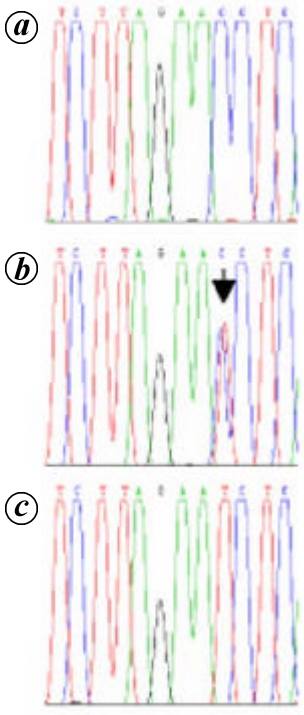

Figure 2. Electropherogram of DNA sequencing (5′→3′) showing a single base substitution (C→T) polymorphism at nucleotide 609 of *NQO1* gene. ***a***, Homozygous wild genotype (C/C). ***b***, Heterozygous genotype (C/T). ***c***, Homozygous genotype (T/T). (From Biramijamal F. *et al.*[12] with permission.)

ing and microcapillary electrophoresis using an ABI 310 Genetic Analyzer, according to the supplier's instructions (Applied Biosystems International; Figure 2). In addition, the samples were reanalysed by retrieving genomic DNA stocks, performing new PCR amplifications, and resequencing of new PCR amplification products.

The study group consisted of 245 healthy individuals. We assayed DNA samples from these individuals for the frequency of allelic polymorphism at position C609T (pro187Ser) in the *NQO1* gene. The frequency of the C609T-allele in Mashhad was 0.28, which is considerably higher than Turkmen and Esfahan (0.13), but comparable to the Shiraz and Mazanderani populations. In each group, the distribution of genotypes fits the Hardy–Weinberg equilibrium (Table 1).

Local inbreeding coefficient (F_S) of Shiraz, Mashhad Mazandarani and Turkmen populations (0.176, 0.007, 0.342, and 0.083 respectively) was positive, suggesting fewer heterozygotes than expected and indicating inbreeding. Also, negative F_S for Kerman, Yazd, Esfahan and Urmia populations (–0.169, –0.086, –0.149 and –0.027 respectively) suggests more heterozygotes than expected and indicating outbreeding (Table 2).

As shown in Table 3, the overall reduction in average heterozygosity is 2.82% of the total heterozygosity. It is indicative of little genetic differentiation among eight Iranian populations. Genetic divergence between human subpopulations is quite small. The average heterozygosity among the subpopulations is 0.286.

We found that the Mazandarani ethnic group has higher frequency (12%) of homozygous variant individuals (T-allele). Also, the prevalence of heterozygous individuals ranged from 20.5 to 40% (Table 1). Variation of T-allele among Fars ethnic groups was significantly different compared with Kerman, Yazd and Esfahan regions (Table 1).

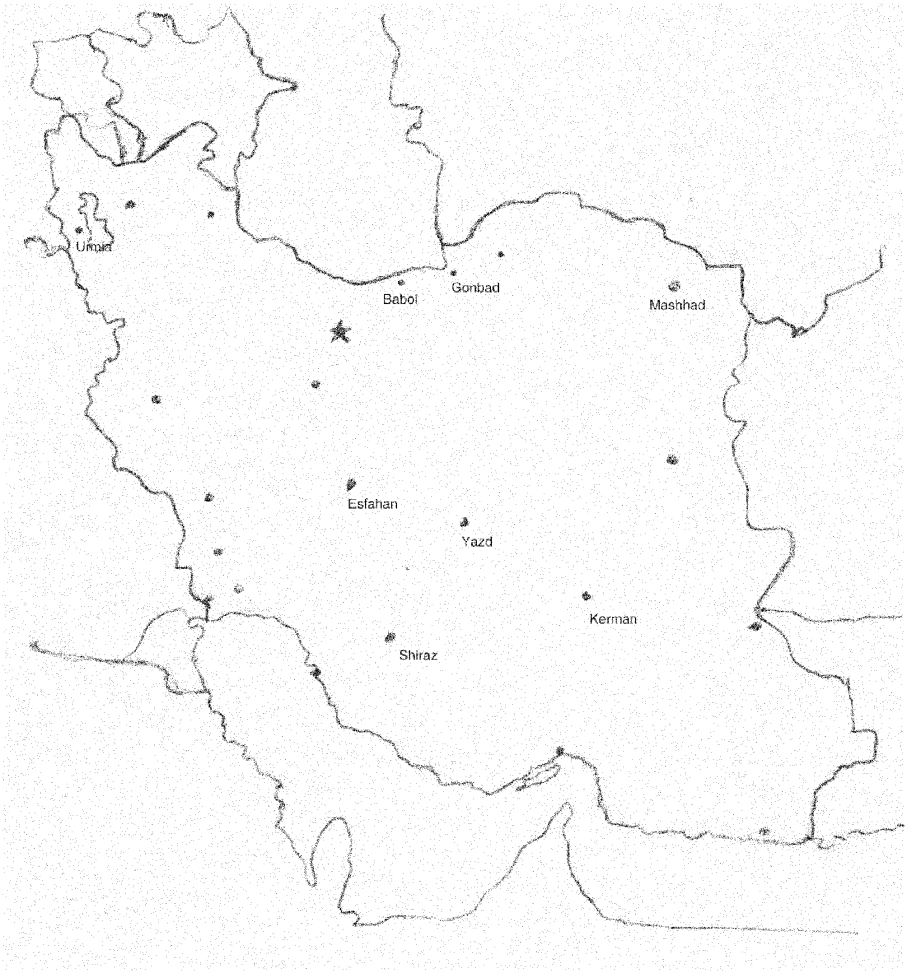

Figure 3. Map of Iran. Samples were collected from cities indicated on the map.

Table 2. Local inbreeding coefficient of each subpopulation (F_S) for different Iranian populations for *NQO1* loci

Ethnic group	Population	Total	F_S
Fars	Shiraz	42	+0.176
	Mashhad	25	+0.007
	Kerman	24	–0.169
	Yazd	25	–0.086
	Esfahan	23	–0.149
Mazandarani	Babol	25	+0.342
Turk	Urmia	42	–0.027
Turkmen	Bandar-Turkmen	39	+0.083

Table 3. Total heterozygosity (H_T), average heterozygosity among subpopulations (H_S), and fixation index (F_{ST}) for different Iranian populations for *NQO1* loci

Number of Iranian populations	H_T	H_S	F_{ST}
8	0.295	0.286	0.028

These results demonstrate that the variant allele (T-allele) of the *NQO1* gene among Iranian ethnic groups is similar to the Caucasian population reported by Kelsey *et al.*[10]. It is reported that the Asian population has the highest frequency of homozygous variant (20.3%) compared with the Caucasians (5%) and Mexican–Americans (15.5%). Data are different from the genotype frequency of T-allele of the *NQO1* C609T polymorphism, which has been reported in Korean, Chinese and Japanese individuals (18.8, 22.4 and 19.9% respectively)[10,13]. Frequency of T-allele shows significant differences among Fars ethnic groups from five regions, i.e. Shiraz, Mashhad, Kerman, Yazd and Esfahan (Table 1).

Our results on 245 healthy Iranian individuals indicate that the frequency of *NQO1* genotype is similar to that of the Caucasian population. However, further investigations on other ethnic groups are required to compare with our neighbouring countries.

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Technique to process xenogenic tissues for cardiovascular implantation – A preliminary report

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Processing of xenograft, a biomaterial in clinical cardiovascular surgery can influence implantation life and efficiency in desired function. Existing xenografts have a limited service time as they undergo degenerative changes. Degradation of xenografts occurs due to several deficiencies in processing and also due to *in vivo* enzymatic digestion and immune response. The objective of this study is to decellularize xenograft tissues and preserve their architecture using an efficient method. This method can produce decellularized tissues that are durable and sturdy to withstand physiological stresses due to circulation during cardiovascular surgeries. Porcine valves (40 pulmonary and eight aortic), bovine jugular veins (57 with and 41 without valves) and 29 bovine pericardia were treated chemically with different methods of decellularization. Anti-calcium treatment was performed to reduce mineralization and further processing of tissues was performed to prevent blood-protein seepage in the biological tissues during blood circulation. The most effective decellularization process was determined by a microscopic examination of the decellularized tissues. One per cent DCA and enzymatic treatment for 50 and 15 h respectively, decellularized the xenografts in an efficient manner. This decellularization along with enzymatic treatment was found to produce good results in comparison with other detergents. This can make them a suitable xenograft tissue for clinical use. Animal experiments can provide evidence of autologous cell seeding in the same processed tissues after implantation has taken place. These experiments can prove the ability of the tissue to grow cells in individuals inside whom the grafts are to be implanted.

Keywords: Anti-calcium treatment, calcific degeneration, decellularization, xenograft.

ALLOGRAFTS and xenografts have clinical advantages over non-biological synthetic biomaterials. Since allografts are rarely available, dependence on xenografts as implants during surgeries has grown exponentially over the years. Heart valves made of xenografts like porcine valve tissue

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