Frequency of the G6PD nt 1311 C/T polymorphism in English and Iranian populations: relevance to studies of X chromosome inactivation

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Abstract

X chromosome inactivation is widely studied using DNA sequence polymorphisms and DNA methylation as a surrogate measure of inactivation, but the correlation of methylation with inactivation is not perfect. Thus, it may be better to study sequence polymorphisms expressed in the mRNA. A recent paper reported use of a silent C/T polymorphism at nt 1311 of the G6PD cDNA, and this polymorphism was reported to have a frequency of 40% in all ethnic groups. We have screened 218 English and 50 Iranian subjects by PCR and restriction digestion; 53/218 (24%) British and 22/50 (44%) Iranian subjects were heterozygous. Thus, X inactivation studies using this polymorphism may be useful in some populations, including Iran, but much less so in the UK.

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Keywords: G6PD; nt 1311 C/T polymorphism; X chromosome inactivation

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X chromosome inactivation studies are used to detect female carriers of a number of X linked diseases, as well as to detect clonality in diseases arising from somatic mutation. The application of DNA methylation as a surrogate

measure of X inactivation has proved powerful when used in combination with DNA sequence polymorphisms. However, there are potential problems because methylation is not always perfectly correlated with inactivation.¹

It has been proposed that the silent C/T polymorphism at nt 1311 of the G6PD cDNA² would be particularly useful for these studies^{3,4} because it is expressed in mRNA, giving a direct measure of gene activity. Prchal *et al*⁵ reported the frequency of the polymorphism to be around 40% in all ethnic groups. However, in other reports the frequency appeared to vary considerably between different groups.^{5,6}

We have studied the frequency of the C/T polymorphism in England and Iran. DNA was obtained from 218 English females, comprising normal subjects and patients referred to St George's Hospital, London, as well as from 50 Iranian normal female volunteers. Exons 10 and 11 of the X linked G6PD gene were amplified by PCR using nested primers.⁵ By incorporating two mismatched bases into the antisense primer, a *Bcl*I site (TGATCA) is created during PCR if a T is present at nt 1311. PCR products were digested with *Bcl*I and separated by electrophoresis on 4% NuSieve agarose.

As shown in fig 1, heterozygotes showed two distinguishable bands of 207 and 184 bp corresponding to the C and T alleles respectively. A total of 53/218 English subjects (24.3%) were found to be heterozygous and four were homozygous for the T allele; 22/50 female Iranian subjects (44%) were found to be heterozygous and 28/50 subjects showed the C band only. The difference between the English and Iranian groups is significant ($p < 0.01$).

Our results are summarised, together with previously published data on T allele frequency, in table 1. Beutler and Kuhl⁶ have reported the frequency of the T allele in a variety of ethnic groups. A significantly higher T allele frequency was found in Indians and a significantly lower frequency in Orientals, but other differences were non-significant. The "mixed Middle Eastern" population⁵ pools people from Morocco to India!

The use of sequence polymorphisms which are expressed in the mRNA may have distinct advantages over indirect studies of X chromosome inactivation by DNA methylation. The G6PD nt 1311 polymorphism will be useful in populations with a significant heterozygote frequency, including Iranians. It will be much less

Figure 1 Gel electrophoresis of PCR products after *Bcl*I digestion. Lane 1: size marker. Lane 2: undigested second round PCR product. Lanes 3-7: PCR products digested with *Bcl*I. Lane 3: homozygous (C/C) subject. Lane 4: homozygous (T/T) subject. Lanes 5-7: heterozygous (C/T) subjects.

Table 1 T allele gene frequencies

Ref	Population	No of chromosomes studied	T allele gene frequency	p value
6	Oriental	59	0.051	$p < 0.05$
6	Central/South American	30	0.100	NS
6	White	68	0.132	NS
6	Sicilian	18	0.167	NS
6	White Jewish	41	0.220	NS
6	African	20	0.25	NS
6	Indian	20	0.45	$p < 0.05$
5	Mixed "Middle Eastern"	36	0.14	
This paper	English	436	0.137	
	Iranian	100	0.22	$p < 0.05$

useful in populations like that in the UK, owing to the low frequency of heterozygotes.

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