**RESEARCH ARTICLE**

**CYP2E1*5B, CYP2E1*6, CYP2E1*7B, CYP2E1*2, and CYP2E1*3 Allele Frequencies in the Iranian Population**

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**Abstract**

**Background:** CYP2E1 encodes an enzyme which is mainly involved in bioactivation of several potential carcinogens such as N-nitrosamines. CYP2E1 polymorphisms have been reported to be associated with cancer. The aim of this study was to evaluate genotype distributions and allele frequencies of five CYP2E1 polymorphisms.

**Materials and Method:** Two hundred healthy individuals of an Iranian population from southwest were included in this study. PCR-restriction fragment length polymorphism and Tetra-ARMS PCR methods were applied for CYP2E1 genotyping. Afterward, data were analyzed.

**Results:** The allele frequencies for *5B, *6, *7B, *2, and *3 were calculated to be 1.5%, 16%, 28.5%, 0%, and 2.75% respectively. Results of this study showed that no significant differences in genotype and allele frequencies of five single nucleotide polymorphisms with respect to the gender and tribes. The chi-square test showed that the genotype frequencies of CYP2E1*5B were similar to Caucasians, but the distribution of CYP2E1*6 genotypes was similar to Asians. The frequencies of CYP2E1*2 (0%) and CYP2E1*3 (2.75%) alleles were within the range for Caucasians and Orientals. In the case of CYP2E1*7B, the data was limited. Accordingly, the results were only compared with Europeans and the comparison showed significant differences. **Conclusions:** In conclusion, ethnic and geographic differences may explain discrepancies in the prevalence of CYP2E1 polymorphisms.

**Keywords:** Cytochrome P450 - polymorphism - genotyping - allele frequency - Iranian population

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**Introduction**

The only member of human Cytochrome P450 E subfamily is CYP2E1 (Zhuge et al., 2003). Human CYP2E1 gene is located in 10q24.3-qter region of chromosome 10. It contains 9 exons, 8 introns, and a typical TATA box and it spans 11,413 base pairs of genomic DNA (Umeno, 1988). CYP2E1 plays a key role in the metabolic activation of many low molecular weight carcinogens (e.g., benzene, N-nitrosamines, carbon tetrachloride chloroform and vinyl chloride) and producing reactive oxygen species (e.g., superoxide anion radical, hydrogen peroxide), which can affect target tissue and ultimately lead to carcinogenesis (Hou et al., 2007; Guo et al., 2010; Feng et al., 2012).

CYP2E1 has several polymorphisms. Studies reported that its functional polymorphisms are associated with increased and decreased susceptibility to many cancer types, including esophageal cancer, lung cancer, nasopharyngeal carcinoma, and colorectal cancer (Cai et al., 2005; Sangrajrang et al., 2006). CYP2E1*5B (rs2031920) and CYP2E1*6 (rs6413432) single nucleotide polymorphisms (SNPs) have been studied frequently. CYP2E1*5B is a PstI polymorphism, caused by a G>C change at position 1293 in the 5-flanking region of CYP2E1 gene. G and C alleles were named c1 and c2, respectively. It has been shown that CYP2E1*5B can affect the transcription of CYP2E1 gene in vitro (Hayashi et al., 1991). The correlation between this polymorphism and oral, pharyngeal, liver, gastric and lung cancers has been found (Boccia et al., 2007). CYP2E1*6 polymorphism (position T7632A) was identified in intron 6 of the gene by using the restriction enzyme Dral (Persson et al., 1993). Common homozygote (TT), heterozygote (TA), and rare homozygote (AA) genotypes of CYP2E1*6 were
named DD, DC, and CC, respectively. CYP2E1*6 doesn’t affect gene transcription, but it is likely to affect CYP2E1 catalytic activity (Uematsu et al., 1991). CYP2E1*7B (rs6413420; G-71T) was also found in the promoter region of the gene. CYP2E1*7B affects the transcription of the gene and causes 1.8-fold increase in the transcriptional activity of CYP2E1 (Fairbrother et al., 1998). Therefore, it is expected that CYP2E1*6 and CYP2E1*7B influence cancer risk. CYP2E1*2 (rs72559710) and CYP2E1*3 (rs55897648) were identified in exon 2 and exon 8 of the gene, respectively. CYP2E1*2 that causes an R76H amino acid exchange, lowers enzyme synthesis and catalytic activity. The additional polymorphism, CYP2E1*3, was detected, causing the substitution of Valine to Isoleucine at position 389, but without any effect on enzyme synthesis and catalytic activity (Hu et al., 1997).

Inter-individual differences in the expression level, which can result in tumor development, have been connected with its polymorphisms. The allele frequencies of these polymorphisms differ remarkably among different human populations (Bolt et al., 2003). These polymorphisms are well characterized in different populations, but little is known about the Iranian ethnic group. This study could provide valuable data for association studies between these polymorphisms and cancer susceptibility. Therefore, the genotype and allele frequencies of CYP2E1 single nucleotide polymorphisms, including *5B, *6, *7B, *2, and *3 were presented, estimated and analyzed in this study. The present study was aimed to provide basic information about the allele and genotype distribution of CYP2E1 polymorphisms.

Materials and Methods

Study population

Samples from 200 genetically unrelated, healthy individuals blood donors were taken which consisted of 100 males between the ages of 1 and 80 (mean age 41.9±22.3) and 100 females between the ages of 3 and 84 (mean age 40.87±19.97) were examined in this study. Blood samples were collected at the University hospital of Jundishapur and an accredited medical diagnostic laboratory in Ahvaz city (southwest of Iran). The population study was composed of two ethnic subgroups, including non-Arabs (66%) and Arabs (34%). Since these two groups of individuals were seen homogenous in relation to the allelic and genotypic frequencies and both were in Hardy-Weinberg equilibrium, they were considered as one group.

This investigation was approved by the Ethics Committee of Jundishapur University of Medical Sciences.

Genotype analysis

Genomic DNA was extracted from 100 µl of whole blood using the Diatom DNA Kit (IsoGene, Russia) according to the manufacturer’s instructions. The extracted DNA was visualized on 1% agarose gel and stored at −20°C until genotyping was performed. Two different methods were used to detect five single nucleotide polymorphisms (SNPs) of CYP2E1, CYP2E1*5B, CYP2E1*6, and CYP2E1*7B. Polymorphisms were determined by polymerase chain reaction (PCR) based on the restriction fragment length polymorphism (RFLP) method with designed primer pairs. The PCR products of CYP2E1*5B, CYP2E1*6, and CYP2E1*7B were digested with the restriction enzymes PstI (Vivantis, Malaysia), Drai (Roche, Germany), and DdeI (Fermentas, UK), respectively. PCR products and restriction fragments were visualized by electrophoresis in 1.5% and 2.5% agarose gels, respectively. The information about sequence of primer pairs, amplification conditions, size of PCR products and digested products with restriction enzymes is listed in Table 1.

Tetra-ARMS PCR (amplification refractory mutation system) method was applied for genotyping of CYP2E1*2 and CYP2E1*3 because they don’t have a restriction site by which alleles of CYP2E1 can be distinguished from one another. The fragment of the CYP2E1 gene that contains either *2 or *3 polymorphism was amplified by the two outer primers and the inner primers which amplified the two allelic states (Ye et al., 2001). Tetra-ARMS PCR products were visualized by electrophoresis in 2.5% agarose gel for CYP2E1*2 and CYP2E1*3 regions. The details about PCR condition and primer sequences are listed in Table 2. Several samples were randomly selected for direct sequencing of amplified products to validate the results of genotyping by PCR/RFLP and Tetra-ARMS

| SNP          | Primer sequence               | PCR Condition                  | PCR product (bp) | RFLP product (bp) |
|--------------|-------------------------------|-------------------------------|------------------|-------------------|
| CYP2E1*5B    | F:ACCCCAATGGGTTCTCTGTC R:TCATTCTGTCTTCTAACTGGCAAT | 95 -5min, 95 -30sec, 52 -45sec, 72 -30sec x 35cycles 72 -5min | 576 | 282, 294 |
| CYP2E1*6     | F:AGGGCTCGTCAGTCTCTGAAA R:AAGGCAGGAGGATGACTTGA | 95 -5min, 95 -30sec, 63 -45sec, 72 -30sec x 35cycles 72 -5min | 685 | 309, 376 |
| CYP2E1*7B    | F:CTGGAGTTCCCGTTGTCTA R:GGGTTAAGGACTTGGGAAT | 95 -5min, 95 -30sec, 57.6 -45sec, 72 -30sec x 35cycles 72 -5min | 547 | 301, 246 |
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**Table 2. Tetra-ARMS Conditions for the CYP2E1*2 and CYP2E1*3**

| SNP          | Outer primer sequence | Inner primer sequence | Annealing Temperature |
|--------------|-----------------------|-----------------------|-----------------------|
| CYP2E1*2     | F:GTGGCTTAGAGCCCGACCTCTC  R:AACGCGGGCGCCGCTGAGCACTCA  | F:TTGAGCCGTGCAACCAACACCAGGG  R:CACGCTGACGTGTCCTGCACTCA  | 53                     |
| CYP2E1*3     | F:AGAGGTGGAGGAGCTGAAGCC  R:CCACCTCAAAACACTTCTTCAAGCGCA  | F:ACAAACAGAAGTGCAAGTGGACGCACTCA  R:AGCTTTGATTCTTCCAGGCAACAGCA  | 62                     |

**Figure 1. Chromatogram of Corresponding Sequence for CYP2E1*7B Polymorphism Visualization with Chromas Program. CYP2E1*7B is represented by a -71G->T substitution in the promoter region of the gene. Homozygote individuals with dominant alleles (GG genotype) present only one peak.**

**Figure 2. Chromatogram of Corresponding Sequence for CYP2E1*3 Polymorphism Visualization with Chromas Program. CYP2E1*3 is a G10059A base substitution in exon 8 of the gene. Homozygote individuals with dominant alleles (GG genotype) present only one peak.**

**Statistical analysis**

The statistical analysis by χ²-test was done to determine if the genotype frequencies of every polymorphism fit the Hardy-Weinberg equilibrium. Also, the estimated genotype frequencies were compared between the two genders and between Arabs and non-Arabs populations. Finally, the results of this study were compared with other populations by the same test. Differences with P-values<0.05 were considered statistically significant.

**Results**

CYP2E1*5B (rs2031920), CYP2E1*6 (rs6413420), and CYP2E1*7B (rs6413420) polymorphisms were examined by digestion with PstI, Dral, and Ddel enzymes in 200 subjects from the general population in Ahvaz city. The genotype frequencies of CYP2E1*5B polymorphism were found to be 97% for G/G (*1A/*1A) and 3% for G/C (*1A/*5B). Allele frequencies of CYP2E1*5B were 98.5% for G and 1.5% for C. In the case of CYP2E1*6 polymorphism, the genotype frequencies were determined as 69% for T/T (*1A/*1A), 30% for T/A (*1A/*6), and 1% for A/A (*6/*6). The allele frequencies of this polymorphism were 84% T and 16% A. Investigation of CYP2E1*7B polymorphism yielded the genotype frequencies as 50% for G/G (*1A/*1A), 43% for G/T (*1A/*7B), and 7% for T/T (*7B/*7B). The allele frequencies of CYP2E1*7B were 71.5% G and 28.5% T. The genotype frequency of CYP2E1*2 polymorphism was found as 100% for G/G (*1A/*1A), but no individual with G/A or A/A genotype was identified. The genotype frequencies of CYP2E1*3 were determined as 94.5% for G/G (*1A/*1A) and 5.5% for G/A (*1A/*3), while A/A genotype wasn’t detected in the population. The allele frequencies of CYP2E1*3 were 97.25% and 2.75% for G and A, respectively.

The genotype distributions for *5B, *6, *7B, and *3 polymorphisms fitted the Hardy-Weinberg equilibrium and P-value>0.05 was calculated for four polymorphisms (Table 3). For every SNP, there were no significant differences in genotype frequencies between the two genders and between Arabs and non-Arabs populations. The estimated P-values by χ²-test were >0.05 for all studied polymorphisms (P-values of gender and ethnic
Table 4. Comparison between the Genotype and Allele Distributions of CYP2E1*5B in Iranian Population and Other Populations

| Population          | N^  | CYP2E1*5B P-value* |
|---------------------|-----|-------------------|
|                     |     | genotype frequency (%) |
|                     |     | *1A/*1A  *1A/*7B  *7B/*7B |
| Asian               |     | (GG) (G) (C)       |
| Iranian (present study) | 200 | 97.0 3.0 0.0       |
| Chinese             | 122 | 51.6 43.5 4.9 <0.0001 |
| Taiwanese           | 231 | 58.0 35.1 6.9 <0.0001 |
| Thai (Kongruttanachok et al., 2001) | 297 | 63.6 34.7 1.7 <0.0001 |
| Japanese            | 241 | 68.0 29.0 3.0 <0.0001 |
| Indian (Rejali et al., 2009) | 350 | 98.0 2.0 0.0 0.31 |
| European            |     |                   |
| Spanish             | 200 | 89.5 10.5 0.0 <0.0001 |
| French              | 172 | 91.6 4.7 0.0 0.29  |
| British             | 155 | 96.8 3.2 0.0 0.88  |
| German              | 297 | 94.9 4.4 0.7 0.11  |
| Polish              | 316 | 94.4 5.6 0.0 0.08  |
| Italian (Bocca et al., 2008) | 245 | 93.4 6.2 0.0 0.43  |
| American            |     |                   |
| Mexican             | 92  | 70.6 28.4 1.1 <0.0001 |
| Brazilian           | 212 | 88.2 11.8 0.0 <0.0001 |
| American (Liu et al., 2001) | 399 | 96.2 3.8 0.0 0.6   |
| Chilean             | 148 | 71.0 27.0 2.0 <0.0001 |
| Mexican Mestizos    | 239 | 72.0 23.8 4.2 <0.0001 |
| Mexican Huichols    | 99  | 21.2 54.1 24.2 <0.0001 |
| Turkish (Ulusoy et al., 2007) | 206 | 96.1 3.9 0.0 0.54  |

*P-values express whether Iranian population is similar to respective populations.

Table 5. Comparison between the Genotype and Allele Distributions of CYP2E1*6 in Iranian Population and Other Populations

| Population          | N^  | CYP2E1*6 P-value* |
|---------------------|-----|-------------------|
|                     |     | genotype frequency (%) |
|                     |     | *1A/*1A  *1A/*7B  *7B/*7B |
| Asian               |     | (TT) (TA) (AA) |
| Iranian (present study) | 200 | 69.0 30.0 1.0 |
| Taiwanese           | 320 | 57.2 38.4 4.4 0.0013 |
| Tainians            | 123 | 71.5 25.2 3.3 0.26 |
| Japanese            | 241 | 52.3 40.2 7.5 <0.0001 |
| Kazakh              | 107 | 72.0 27.1 0.9 0.4 |
| Ugur (Wang et al., 2009) | 149 | 66.4 29.6 4.0 0.14 |
| Chinese             | 103 | 55.3 36.9 7.8 <0.0001 |
| Indian (Rejali et al., 2009) | 350 | 72.2 26.9 0.9 0.16 |
| European            |     |                   |
| French (Bouchardy et al., 2000) | 172 | 87.8 11.6 0.6 <0.0001 |
| Caucasians          | 1360| 85.4 13.8 0.8 <0.0001 |
| British             | 155 | 83.2 16.1 0.7 <0.0001 |
| German (Neuhaus et al., 2004) | 236 | 83.1 16.5 0.4 <0.0001 |
| Italian (Bocca et al., 2008) | 245 | 91.8 7.8 0.4 <0.0001 |
| American            |     |                   |
| Chilean             | 129 | 63.6 31.0 5.4 <0.0001 |
| Mexican (Konishi et al., 2003) | 104 | 72.1 24.0 3.9 0.26 |
| Brazilian (Rossi et al., 2006) | 251 | 86.9 12.7 0.4 <0.0001 |
| Turkish (Omer et al., 2001) | 153 | 84.3 15.0 0.7 <0.0001 |
| Turkish (Ulusoy et al., 2007) | 206 | 84.0 15.5 0.5 <0.0001 |
| Turkish (Kayaalti et al., 2010) | 163 | 85.3 14.1 0.6 <0.0001 |

*P-values express whether Iranian population is similar to respective populations.

were determined as 0.41 and 0.97 for *5B, 0.36 and 0.11 for *6, 0.25 and 0.43 for *7B, and 0.12 and 0.41 for *3, respectively. \( \chi^2 \)-test wasn’t performed for genotype

Table 6. Comparison between the Genotype and Allele Distributions of CYP2E1*7B in Iranian Population and Other Populations

| Population          | N^  | CYP2E1*7B P-value* |
|---------------------|-----|-------------------|
|                     |     | genotype frequency (%) |
|                     |     | *1A/*1A  *1A/*7B  *7B/*7B |
| Asian               |     | (GG) (G) (C)       |
| Iranian (present study) | 200 | 50.0 43.0 7.0 |
| European            |     |                   |
| North European      | 115 | 89.6 10.4 0.0 <0.0001 |
| British (Yang et al., 2001) | 155 | 90.3 9.7 0.0 <0.0001 |
| German (Thier et al., 2002) | 56  | 85.7 14.3 0.0 <0.0001 |
| German (Rejali et al., 2009) | 299 | 92.6 7.4 0.0 <0.0001 |
| Swedish (Ernstgard et al., 2004) | 37  | 91.9 8.1 0.0 <0.0001 |
| Turkish (Ulusoy et al., 2007) | 206 | 86.9 12.6 0.5 <0.0001 |
| Turkish (Kayaalti et al., 2010) | 163 | 86.5 13.5 0.0 <0.0001 |

*P-values express whether Iranian population is similar to respective populations.

Discussion

Iran is a country which has a population with different ethnic identities and different languages. CYP2E1 polymorphisms shows inter-ethnic and inter-racial differences, significant contribution to individual differences in susceptibility to cancer development. Estimation probabilities of cancer development both for individuals and ethnic groups can be performed by using SNPs as genetic markers (Danko and Chaschin, 2005). Therefore, the identification of polymorphisms in different populations such as Iranians could be useful for assessing genetic cancer susceptibility. The present results are the first to evaluate genotype and allele frequencies of CYP2E1 polymorphisms. The next step after the initial data is to study the association of these polymorphisms with cancer. If such a conclusion is true, particularly among industrial workers, this is recommended because it is possible that the pattern of polymorphism is different. Therefore, whether a polymorphism is a crucial or has no significant differences, a specific test will be performed specifically among the high risk groups.

CYP2E1 polymorphisms could also affect susceptibility to adverse drug reactions (ADRs) (Costa et al., 2012). Accordingly, this study provides valuable information not only for further investigation of association between CYP2E1 polymorphisms and susceptibility to several types of cancer but also for the study of adverse drug reactions.

Since SNPs of autosomal chromosomes can be changed by effects of many factors in the evolutionary history, the investigation of single nucleotide polymorphisms provide valuable data regarding relations between populations (Kayaalti and Soylemezoglu, 2010). Alhavz city lies in the southwest of Iran between Iraqi border and the Zagros Mountains. Centuries ago, Arabs migrated from neighboring countries to Iran. In the current study, the genotype distributions were compared between Arabs and non-Arabs. CYP2E1 polymorphisms had similar
allele frequencies for two ethnic subgroups of the study population. Therefore, two subgroups might have the same cancer susceptibility.

**CYP2E1** genotype distribution was also compared between Iranian and other populations. The genotype distribution of **CYP2E1*5B** was similar to Europeans such as German (P-value=0.11) (Neuhaus et al., 2004), British (P-value=0.88) (Yang et al., 2001), French (P-value=0.29) (Bouchardy et al., 2000), and Poland Caucasians (P-value=0.08) (Gajecka et al., 2005), but it was different from Italian (P-value=0.03) (Boccia et al., 2008). There were no significant differences between this study and studies on American (P-value=0.6) (Liu et al., 2001) and Turkish (P-value=0.54) (Ulusoy et al., 2007) populations. But, the results of this population differed significantly from those of Chilean (Quinones et al., 2001), Mexican Mestizos, Mexican Huichols (Gordillo-Bastidas et al. 2010), and Brazilian (Marques et al., 2006) (P-value<0.0001). The genotype frequencies of **CYP2E1*5B** showed significant differences between Iranian and other Asians, including Japanese (Sugimura et al., 2006), Thai (Sangrajrang et al., 2006), and Northeastern Chinese (Guo et al., 2012) (P-value<0.0001). On the other hand, the results were similar to Indian (P-value=0.31) (Ruwall et al., 2009) (Table 4).

There was a significant correlation between c2/c2 genotype and head and neck cancer in Asians, but not in Caucasians (Tang et al., 2010). The other study found that c2/c2 homozygote genotype was associated with the increased risk of colorectal cancer in Caucasians (Zhou et al., 2010). The other study found that c2/c2 homozygote genotype was associated with the increased risk of colorectal cancer in Caucasians (Zhou et al., 2010). Therefore, we expect the same association study results in Iranian population because **CYP2E1*5B** genotype distribution was similar to Caucasians.

When the genotype distributions of **CYP2E1*6** were compared between Iranian population and other Asians, no significant difference was found between this study and studies on Tamilians (P-value=0.26) (Soya et al., 2005), Thai (P-value=0.12) (Sangrajrang et al., 2006), Kazakh (P-value=0.2), Uygur (P-value=0.14) (Wang et al., 2009), Indian (P-value=0.16) (Ruwall et al., 2009), and Northeastern Chinese (P-value=0.06) (Guo et al., 2012). In contrast, **CYP2E1*6** genotype frequencies were significantly different from Chinese Han (P-value<0.0001) (Wang et al., 2009), and Japanese (P-value<0.0001) (Sugimura et al., 2006) populations. χ2-test showed that the genotype distribution of **CYP2E1*6** differed from American countries, including Chilean and Brazilian (P-value<0.0001) (Quinones et al., 2001; Rossini et al., 2006). But the results were similar to Mexicans even though two populations are ethnically different (P-value=0.26) (Konishi et al., 2003). In comparison with Europeans such as Caucasians (Garte et al., 2001), British (Yang et al., 2001), French (Bouchardy et al., 2000), German (Neuhaus et al., 2004), and Italian (Boccia et al., 2008), the genotype frequencies of **CYP2E1*6** were significantly different (P-value<0.0001). There was also a significant difference between Iranian and Turkish populations (P-value<0.0001) (Ulusoy et al., 2007; Kayaalp and Soylemezoglu, 2010) (Table 5).

A meta-analysis showed a protective effect of Dral C/D and C/C genotypes for lung cancer in the Asian population (Wang et al., 2010). On the other hand, homozygote genotype of Dral polymorphism was associated with head and neck cancer in Asians (Tang et al., 2010). Thus, this polymorphism may have an impact on cancer susceptibility. Accordingly, the risk of cancer development in Iranian population might be similar to other Asian countries.

The genotype frequencies of **CYP2E1*7B** were compared between this study and studies done on other populations. The results of Chi-square test showed that genotype distribution of **CYP2E1*7B** was significantly different from European populations, including German (Neuhaus et al., 2004), British (Yang et al., 2001), and Swedish (Ernstgard et al., 2004) (P-value<0.0001). Also, the results were significantly different from those of Turkish population (P-value<0.0001) (Ulusoy et al., 2007; Kayaalp and Soylemezoglu, 2010) (Table 6).

In the case of **CYP2E1*7B**, the data was limited. Accordingly, the results were only compared with Europeans and the comparison showed significant differences.

Statistical analysis by the same test didn’t show any association between **CYP2E1*5B**, **CYP2E1*6** and, **CYP2E1*7B** polymorphisms.

For **CYP2E1*2** polymorphism, all subjects carried the common homozygote genotype (GG) and the GA or AA genotypes were not detected. **CYP2E1*2** and **CYP2E1*3** polymorphisms occur at very low frequencies in both Caucasians and Orientals (Ingelman-Sundberg, 2001). Thus, Iranian population is expected to have the same susceptibility to cancer in relation to xenobiotic effects. Similarly, Iranians exhibit low frequency of the **CYP2E1*2** (exon 2) and **3** (exon 8) alleles. This results emphasizes that the coding sequence of **CYP2E1** gene is highly conserved and **CYP2E1** isoenzyme is an important physiologically (Hu et al., 1997).

In conclusion, ethnic and geographic differences may explain discrepancies in the prevalence of **CYP2E1** polymorphisms. Genotype distribution studies could provide valuable information to help further investigations of association between polymorphisms and several types of cancer and other diseases. Studies in larger groups are recommended to confirm our results. A larger data base may allow for a more precise estimate of the population frequency for these polymorphisms among normal samples.

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