SNP Communication

Ethnic Differences of two Non-synonymous Single Nucleotide Polymorphisms in CDA Gene

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Summary: Cytidine deaminase, encoded by the CDA gene, catalyzes anti-cancer drugs gemcitabine and ara-C into their respective inactive metabolites. In CDA, two functionally significant non-synonymous polymorphisms, 79A>C (Lys27Gln) and 208G>A (Ala70Thr), have been found and their minor allele frequencies (MAFs) were reported in Japanese and Chinese patients and a relatively small numbers of healthy volunteers in Caucasians and Africans. In this study, we determined the MAFs of both polymorphisms in 200 healthy volunteers of Koreans, along with 206 Japanese, 200 Chinese-Americans, 150 Caucasian-Americans and 150 African-Americans to reveal ethnic differences. MAFs of 79A>C (Lys27Gln) were 0.153 in Koreans and 0.327 in Caucasian-Americans, 0.204 in Japanese, 0.155 in Chinese-Americans and 0.087 in African-Americans. MAFs of 208G>A (Ala70Thr) were 0.005 in Koreans and 0.022 in Japanese and the minor allele was not detected in Chinese-Americans, Caucasian-Americans or African-Americans. Thus possibly, MAF of 208G>A in Japanese is likely to be somewhat higher than in Koreans and Chinese-Americans. These data would provide fundamental and useful information for pharmacogenetic studies on cytidine deaminase-catalyzing drugs.

Keywords: CDA; allele frequency; non-synonymous single nucleotide polymorphisms; ethnic-difference

Cytidine deaminase is an enzyme involved in the pyrimidine salvage pathway and catalyzes the deamination of cytidine and deoxycytidine into their uridine compounds.1) Anti-cancer nucleoside analogs, cytosine arabinoside (ara-C) and gemcitabine are known to be inactivated by this enzyme. Cytidine deaminase is encoded by the CDA gene located at chromosome 1p36.2-p35.

Two non-synonymous single nucleotide polymorphisms (SNPs) 79A>C (Lys27Gln) and 208G>A (Ala70Thr) and their functional significance have been reported. The recombinant enzyme with Gln27 showed reduced activity with increase in Km for gemcitabine.2)
However, the minor allele of this SNP has been shown associated with higher enzymatic activity for gemcitabine based on tests using lysates of red blood cells taken from Caucasian cancer patients.\textsuperscript{3,4} In line with this, the minor allele is associated with decreased response, shorter time to progression and overall survival and lower frequencies of grade 3 and 4 neutropenia in Caucasian non-small cell lung cancer patients treated with gemcitabine and cisplatin.\textsuperscript{5} As for 208G $\rightarrow$ A (Ala70Thr), the mutant enzyme expressed in yeast has reduced activity for both ara-C and cytidine.\textsuperscript{5} Plasma of the patients with the minor allele had reduced activity for gemcitabine and cytidine and 208A was shown associated with reduced clearance of gemcitabine as well as increased frequencies of grade 3 and 4 neutropenia in Japanese cancer patients.\textsuperscript{5,6}

Minor allele frequencies (MAFs) of the two SNPs have been reported in a few papers on Japanese and Chinese patients and relatively small numbers of healthy volunteers in Caucasians and Africans. In this study, we determined MAFs of both polymorphisms by newly developed pyrosequencing protocols in 200 healthy volunteers of Koreans. In addition, 206 Japanese, 200 Chinese-Americans, 150 Caucasian-Americans and 150 African-Americans were also genotyped to compare MAFs in order to reveal ethnic differences.

Korean genomic DNA samples from 200 healthy volunteers (189 males and 11 females) with average age of 24.6 years old (ranging from 20 to 53) were collected for genotyping analysis at the INJE pharmacogenomics research center (Inje University College of Medicine, Busan, Korea). DNAs were obtained from Epstein-Barr virus-transformed lymphoblastoid cells prepared from 206 healthy Japanese volunteers at the Tokyo Women’s Medical University under the auspices of the Pharma SNP consortium (Tokyo, Japan). DNA from 200 healthy Chinese-Americans was extracted from cord blood samples purchased from AllCells (Emeryville, CA, USA). Peripheral blood samples from healthy Caucasian- and African-American volunteers (150 each) were purchased from the Tennessee Blood Service Corporation (Memphis, TN, USA) and DNA was extracted as described previously.\textsuperscript{9} Written informed consent was obtained from all subjects. Ethical review boards of all participating organizations approved this study.

CDA genotypes were determined by pyrosequencing. First, polymerase chain reaction (PCR) was performed to amplify regions containing each target polymorphic site from approximately 25–100 ng of genomic DNA using 0.02 units/μl of Ex-Taq (Takara Bio Inc., Shiga, Japan) with 0.2 mM each of dNTP mixtures and 0.2 μM primers as follows: biotin-ATGGCCAGAAGCGTCCT and CGCCTTCTCTGTACATCTT for 79A $\rightarrow$ C and biotin-CCACCTTGGAGTTAACC and TGTGAAGGAGATTG for 208G $\rightarrow$ A. PCR conditions were 94°C for 5 min, followed by 50 cycles of 94°C for 30 sec, 55°C for 45 sec and 72°C for 20 sec, and then a final extension at 72°C for 7 min. Generation of the single-stranded fragment and annealing of the sequencing primers were described previously.\textsuperscript{9} The sequencing primers used were GGGCAGTAGCTGACT for 79A $\rightarrow$ C and ACGGCCCTCTGGAT for 208G $\rightarrow$ A. Genotypes were determined using the PSQ 96MA (Biotage AB, Uppsala, Sweden) and PSQ 96 SNP reagent set (Biotage AB). The dispensation orders were ATGACTGCT for 79A $\rightarrow$ C and CAGCTGTC for 208G $\rightarrow$ A. The accuracy of genotyping results by pyrosequencing was validated by direct sequencing using at least 5 genomic DNA samples each for wild-type, heterozygote and homozygote of both SNPs (excluding homozygous 208A which was validated by 2 other genomic samples we have). Hardy-Weinberg equilibrium analysis was performed with SNPAllyze version 3.1 (Dynacom Co., Yokohama, Japan). Statistical significance for the differences in MAFs between the Asian populations was analyzed by the Fisher’s exact test using Prism 5.0 (La Jolla, CA, USA).

Genotyping of the 79A $\rightarrow$ C and 208G $\rightarrow$ A SNPs was successfully performed for all samples from the five populations by pyrosequencing (Fig. 1), except for 14 Caucasian-American and 4 African-American samples, which gave small lightning peaks. Genotypes of these samples were clearly determined for confirmation by direct sequencing.\textsuperscript{6} All obtained genotypes were in Hardy-Weinberg equilibrium. The genotypes and MAFs of the two non-synonymous SNPs are summarized in Table 1.

As for 79A $\rightarrow$ C (Lys27Gln), MAF was high in Caucasian-Americans (0.327), medium in three Asians (0.153–0.204) and low in African-Americans (0.087). MAF in Korean healthy volunteers (0.153) was slightly lower than that in Japanese (0.204) and comparable to that in Chinese-Americans (0.155). MAF in healthy Japanese was similar to MAFs in our previous report\textsuperscript{5,0} on 256 Japanese cancer patients (MAF = 0.207) suggesting that this polymorphism is not related to cancer-susceptibility. MAF in Chinese-Americans was also similar to that (0.121) in the 286 Chinese patients containing 87 acute leukemia patients.\textsuperscript{10} MAF of Caucasian-Americans was comparable to those in previous studies with smaller sample numbers: 0.363 in Europeans (n = 95)\textsuperscript{11} and 0.298 in Caucasian-Americans (n = 60).\textsuperscript{21} While MAF in African-Americans in the current study was similar to that in the previous report for 60 African-Americans (0.108),\textsuperscript{8} our results differed from MAF (0.035) in Kenyans plus Ghanaians (n = 85).\textsuperscript{11}

Regarding 208G $\rightarrow$ A, the minor allele was detected in Koreans and Japanese but not in Chinese-Americans, Caucasian-Americans and African-Americans. Although the difference did not reach significance (p = 0.0640), MAF in the Korean healthy volunteers (0.005) was lower than in the Japanese (0.022), which was slightly lower.
than in the 256 Japanese cancer patients (0.037). The 208A allele was not detected in Chinese-Americans. By the Fisher's exact test, significant difference in MAF was found between Japanese and Chinese-Americans ($p = 0.0038$) in the current study. However, since the number of subjects with 208A was very small in Koreans ($n = 2$) or none in Chinese-Americans, differences should be confirmed using a larger number of subjects. In the previous paper, the 286 Chinese patients containing 87 acute leukemia patients also had a very low MAF (0.005). The 208G $>$ A was not detected in Caucasian-Americans or African-Americans in this study, in contrast to Kenyans plus Ghanaians (0.131). This SNP is very important for gemcitabine treatment since 3 out of 4 Japanese patients with gemcitabine-induced life-threatening toxicity had homozygous 208A. However, the current study on MAFs suggests that the clinical importance of this SNP is ethnic-dependent, maybe even within the Asian populations.

In conclusion, we determined the genotypes and MAFs of CDA non-synonymous SNPs 79A $>$ C (Lys27Gln) and 208G $>$ A (Ala70Thr) in 200 healthy volunteers of Koreans, along with 206 Japanese, 200 Chinese-Americans, 150 Caucasian-Americans and 150 African-Americans in order to demonstrate ethnic differences. Our results suggest that the MAF of 208G $>$ A in Japanese is likely somewhat higher than in Koreans and Chinese-
Americans. These data provide fundamental and useful information for pharmacogenetic studies on cytidine deaminase-catalyzing drugs.

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References


