Examination of CYP2C8 rs1934951 polymorphism in Hungarian patients suffering from bisphosphonate-induced osteonecrosis

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Abstract

Osteonecrosis of the jaw (ONJ) is a major complication associated with long-term use of bisphosphonates which are often used in the treatment of osteoporosis and tumors with malignancy-related bone disease. Bisphosphonate-induced ONJ is an active area of investigation, however, its mechanism of action is still unclear. In this study, we aimed to investigate the effect of CYP2C8 rs1934951 SNP that was previously suggested to be associated with ONJ and its relationship to a number of clinical and biochemical factors in 46 Hungarian subjects with bisphosphonate-induced ONJ (35 with malignancy and 11 with osteoporosis). The polymorphism distribution was also determined in 223 healthy subjects.

All subjects underwent physical examination and completed a detailed questionnaire on family and medical histories and lifestyle habits. Blood samples were collected from each subject and genomic DNA was extracted. Genetic analysis of CYP2C8 rs1934951 polymorphism was carried out by pre-designed TaqMan primer/probe sets. The genetic data together with clinical and biochemical parameters were evaluated by Khi-square test, logistic regression analysis, Kruskal-Wallis nonparametric test.

There was no difference in CYP2C8 rs1934951 genotype or allele distribution between ONJ and healthy subjects. Significant correlation was seen between this polymorphism and the localization of ONJ among the affected patients. In the multiparametric logistic regression model, the risk of mandibular localization of ONJ was 19.2-fold higher in subjects with AG genotype than in normal GG genotype. ONJ presence in the mandibular region (76%) increased 3.3-fold compared to maxilla (23%) in case of AG carriers ($p \leq 0.041$). There was no significant variation of ONJ localization site in patients with GG genotype (mandible 58%: maxilla 42%, respectively).

In this study, we demonstrated a significant positive correlation between CYP2C8 rs1934951 polymorphism and the localization of ONJ among the affected patients.

Materials & Methods

Study population

The diagnosis of ONJ was based on the following criteria: exposed bone in the mandible, maxilla or both which persists for at least 8 weeks, in the absence of previous radiation and metastases in the jaws. Patients were classified in staging system for ONJ recommended by AAOMS.

The control group for the analysis of CYP2C8 *rs1934951* SNP included blood samples from healthy Hungarian unrelated patients. Exclusion criteria were history of bone, metabolic, any chronic illness; or any medication known to influence bone metabolism. The study was approved by the Regional Committee of Science and Research Ethics, Semmelweis University (ad.8-101/2009-1018EKU).

Genotyping of CYP2C8

Genomic DNA was extracted using High Pure PCR Template Purification kit (Roche Diagnostics, GmbH, Germany). DNA quality and quantity were determined with NanoDrop B-100 spectrophotometer (NanoDrop Technologies, USA).

The genetic analysis of this CYP2C8 (*rs1934951*) polymorphism was carried out by pre-designed TaqMan primer/probe sets (Applied Biosystems, USA): the PCR mixture contained 1 µL genomic DNA (50 ng/µL), 0.50 µL 40x TaqMan SNP Genotyping Assay (ID number: C_361409_1), 10 µL of TaqMan Universal PCR Assay Mix and 8.50 µL ultrapure PCR water. Cycling conditions comprised an initial cycle at 60°C for 1 min and 95°C for 10 min, followed by 55 cycles of 92°C for 15 s and 60°C for 1 min, and by a final step at 60°C for 1 min. The fluorescent intensity of the PCR products was measured using the ABI 7500 RT-PCR System.

Statistical analysis

The distribution of CYP2C8 *rs1934951* genotypes and alleles in healthy and ONJ populations was analyzed by Chi2-tests. We also tested the effect of CYP2C8 genotypes on the localization of ONJ applying univariate Chi2-tests and a multivariate logistic regression model using SPSS 15.0 software. P-value less than 0.05 was considered significant.

We used principal components analysis (PCA) to summarize multivariate genetic data structure in terms of a few important and uncorrelated dimensions, called the components. In the graphical biplot display for any two components, the observations (in this case, the patients) appear as points, whereas the variables are emphasized by lines pointing to their positions. This simultaneous representation, i.e. the biplot, allows for the evaluation of the grouping of patients and of the relative importance and correlations of variables in influencing this configuration. Computations were performed by the SYNTAX 2000 program package.

Results

Study population

The median age of ONJ patients was 69.5 (range: 60-81) years. The median age of control patients was 67 (range: 57-78) years. There was no significant difference between the ONJ and control groups in age, smoking habits, calcium intake, alcohol and caffeine consumption, and physical activity. The descriptive statistics of the examined ONJ patients are shown in Table 1.

Characteristics	ONJ patients *n = 46*
Sex	
Male	7
Female	39
Disorder	
Osteoporosis	11
Breast cancer	21
Prostate cancer	5
Multiple myeloma	5
Renal cancer	2
Cervix cancer	2
Bisphosphonate types	
Alendronate	6
Pamidronate	10
Zoledronate	23
Ibandronate	17
Risedronate	3
Clodronate	3
Bisphosphonate application	
Intravenous	33
Oral	22
Intravenous and oral[a]	*9*
Hormone deprivation therapy	
Yes	13
No	33
Recurrence	
Yes	14
No	32
ONJ stage	
0	5
1	6
2	28
3	7
ONJ localization	
Mandible	32
Maxilla	19
Mandible and Maxilla[b]	*5*
CYP2C8 genotype	
AA	1
AG	14
GG	31

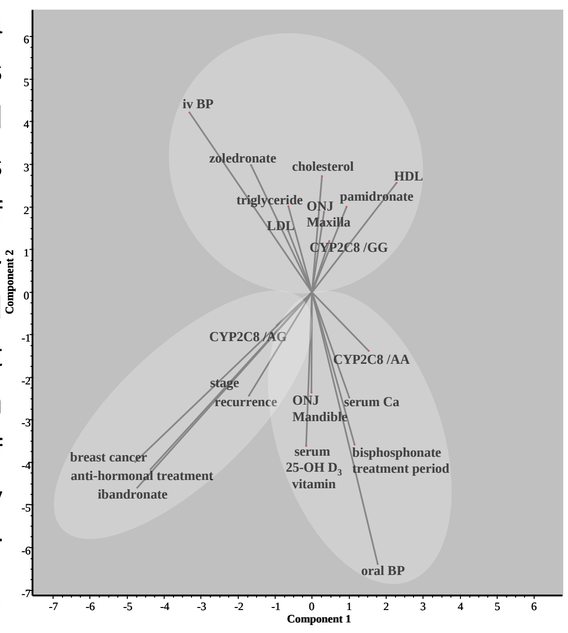
CYP2C8 genotyping

We found a significant effect of CYP2C8 genotype on the localization of ONJ among the affected patients. The occurrence of the mandibular ONJ was significantly higher in case of AG carriers than among subjects with GG homozygous genotype (Chi2=5.447, p=0.02). In the multivariate logistic regression model the AG genotype was associated with a 19.2-fold increased risk for mandibular ONJ compared to the GG genotype.

ONJ presence in the mandibular region (76%) increased 3.3-fold compared to maxilla (23%) in case of AG carriers ($p \leq 0.041$). There was no significant variation of ONJ localization site in patients with GG genotype (mandible 58%: maxilla 42%, respectively).

Multivariate data analysis

By Principal component analysisi (PCA) strong positive correlation was detected between maxillar localization of ONJ and a group of variables including intravenous bisphosphonate application and serum lipid markers. Mandibular localization of ONJ was correlated positively with another set of variables including serum calcium and 25-OH-vitamin D levels, oral BP application and the length of BP therapy. Also, different variable groups could be ordered to each CYP2C8 rs1934951 allele. The presence of A allele (AA and AG genotype) was positively associated not only with mandibular localization of ONJ but also with oral BP use, serum calcium and 25-OH-vitamin D levels. GG genotype was related to maxillar localization of ONJ, intravenous BP application and serum lipid markers.



Summary & Discussion

- **Significant correlation was detected between CYP2C8 *rs1934951* SNP and the localization of BP-induced osteonecrosis of the jaw.**
- **AG genotype is associated with a 19.2-fold increased risk for mandibular ONJ compared to the GG genotype.**
- **Applying PCA we could sharply separate different groups of variables significantly related to the pathological process.**
- **Mandibular appearance of ONJ was correlated positively with serum calcium, 25-OH-vitamin D levels and the length of BP therapy.**
- **The incidence of maxillar localization was elevated in ONJ patients with higher levels of serum lipids.**

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