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SHORT COMMUNICATION

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Determination of −3858G → A and −164C → A genetic polymorphisms of CYP1A2 in blood and saliva by rapid allelic discrimination: large difference in the prevalence of the −3858G → A mutation between Caucasians and Asians

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Abstract *Introduction*: Two mutations in CYP1A2, −164C → A (allele CYP1A2*F) and −3858G → A (allele CYP1A2*C), affecting the inducibility of the enzyme, have been published. The aim of this study was to develop a high throughput allelic discrimination assay for these mutations in both saliva and blood and to determine their frequency in Caucasians.
Methods: An allelic discrimination assay, based on the fluorogenic 5′-nuclease activity (TaqMan), was developed for the two mutations. Genomic DNA extracted from 17 saliva and 100 blood samples from Caucasians was analysed.
Results and conclusions: For the −164C → A mutation, we found an allelic frequency of 68% in the Caucasian population, comparable with data published for Asians and Caucasians. For the −3858G → A mutation, the allele frequency was only 2% in Caucasians, a much lower value than the ˜25% reported in Asians ($P < 0.001$). The presented allelic discrimination allows fast and accurate detection of these two mutations. Genotype calls were 100% identical for DNA from saliva and blood. Saliva is easily accessible and represents an excellent alternative to the traditionally used venous blood for genotyping.

Keywords CYP1A2 · Inducibility · Genotyping

Introduction

To improve drug effectiveness or reduce toxicity, increasing importance is attributed to genotyping of drug metabolising enzymes [1]. As a member of the cytochrome P_{450} superfamily, CYP1A2 plays an important role in the activation of carcinogens and in the metabolism of many drugs [2, 3, 4, 5, 6, 7, 8, 9]. No functional genetic polymorphism explaining the high inter-individual variability of the enzyme activity has been reported until now [10, 11, 12]. However, two mutations associated with inducibility of CYP1A2 gene expression have been described, namely the −164C → A mutation in intron 1 (allele CYP1A2*F) and the −3858G → A mutation in the precoding region (allele CYP1A2*C) [13, 14, 15, 16, 17, 18]. It was suggested that the −164C → A mutation in the CYP1A2 gene might be in linkage with the −3858G → A mutation [13]. The published methods to identify these mutations are based on polymerase chain reaction—restriction fragment length polymorphism (PCR-RFLP). The aim of the present study was the development of a rapid allelic discrimination technique for the identification of these mutations, to determine their prevalence in the Caucasian population, and—because saliva is the preferred matrix for non-invasive CYP1A2 phenotyping [19, 20]—to investigate the use of saliva as a source of genomic DNA. This allows to combine CYP1A2 phenotyping and genotyping in the same matrix.

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Methods

Subjects

Genomic DNA samples were from 100 Caucasians from Switzerland. Saliva samples were collected from Caucasian and Chinese staff members and students. Participants were investigated after giving signed informed consent. This work was part of a larger study on the inducibility of CYP1A2 activity, approved by the ethics committee of the University Hospital Basel.

DNA isolation and mutation analysis

Genomic DNA was isolated from blood or saliva with the QIAamp DNA Blood Kit (Qiagen, Basel, Switzerland) according to the manufacturer's recommendations for blood, and as described by van Schie and Wilson for saliva [21].

Allelic discrimination using the 5′-nuclease activity assay was adapted to detect the −164C → A and −3858G → A mutations. Primers and probes, designed using the Primer Express 1.5 software

Table 1 Sequences and positions of primers, wild-type (WT) and mutant (MT) TaqMan probes and MGB probes are listed for mutations −164C → A and −3858G → A. The polymorphic nucleotides in the probes are underlined. Positions are according to accession number AF253322

Mutation	Position		Sequence
−164C → A	34725	Forward primer	5′-GGCTCCTTTCCAGCTCTCAGA
−164C → A	34859	Reverse primer	5′-TGATAAACACTGATGCGTGTTCTG
−164C → A	34770	WT probe (anti-sense)	5′FAM-ATGCGTCCTG**G**GCCCACAGA-TAMRA
−164C → A	34769	MT probe (anti-sense)	5′VIC-CATGCGTCCTG**T**GCCCACAGAG-TAMRA
−3858G → A	31034	Forward primer	5′-GGAGTGCAGTGGTGCGATCT
−3858G → A	31133	Reverse primer	5′-CACACCTGTAATTCCAGCTACTCG
−3858G → A	31073	WT probe (anti-sense)	5′FAM-CCTCTC**G**GATTCAA-MGB
−3858G → A	31073	MT probe (anti-sense)	5′VIC-CCTCTC**A**GATTCAAG-MGB

(Applied Biosystems, Rotkreuz, Switzerland), are shown in Table 1. Probes for −164C → A are antisense, probes for the −3858G → A mutation are minor groove binder (MGB) probes. Probes were labelled with the fluorophores FAM or VIC. Primers were from Microsynth (Balgach, Switzerland), TaqMan probes and Universal PCR Master Mix were from Applied Biosystems. A 25-μl total reaction volume was used and, post-PCR, the allelic specific fluorescence was measured on the ABI prism 7700 (Applied Biosystems) using the Sequence Detection Systems 1.7 software for allelic discrimination. The genotype calls were attributed automatically.

PCRs with restriction analysis for the two mutations were performed, using the published primers and restriction enzymes [14, 15].

To validate the use of genomic DNA from saliva, DNA was extracted from both blood and saliva of ten volunteers and the presence of the two CYP1A2 mutations was compared.

Sequence analysis for verifying the −3858G → A mutation was performed on 17 DNA samples by Microsynth (Balgach, Switzerland). In parallel to the 5′-allelic discrimination assay, RFLP-PCR was performed on ten saliva samples to validate the results of the 5′-nuclease assay.

Statistical analysis

The allele frequency in our subjects is given together with the 95% confidence interval. Differences in allele frequencies between the two populations were analysed with the χ^2 test. A P value < 0.05 was considered statistically significant. Statistical analysis was performed with Statview.

Results

The 5′-allelic discrimination method was adapted to detect the −164C → A or −3858G → A mutations in the CYP1A2 gene and over 100 DNA samples from Caucasians were analysed.

Regarding the −3858G → A mutation, PCR-RFLP on 20 Caucasian DNA samples did not reveal a homozygote sample needed to establish the TaqMan allelic discrimination method for this mutation. Sequencing confirmed the absence of the mutation in all these samples. As the mutation was published to be present in the Japanese and Chinese populations, we screened 14 Chinese volunteers and found four heterozygotes and three homozygotes for the mutation. After establishing the Taqman allelic discrimination method, four −3858G → A mutations were detected in 100 Caucasian DNA samples screened.

Considering the −164C → A mutation, nine subjects with the −164C/C genotype were detected. Since, as discussed above, the homozygous −3858A/A form was not found, the combination with the −164C/C genotype was also absent in our population.

Table 2 shows the allele frequencies for the two mutations from our data and that published previously [14, 15, 16, 17, 18, 22, 23]. The results show no difference between Caucasian and Asian populations for the −164C → A mutation, but a significantly low frequency of the −3858G → A mutation in Caucasians ($P < 0.001$).

The yield of DNA from 1 ml saliva varied from 5.5 μg to 46.2 μg (20.5 ± 11.4 μg, mean ± SD). PCR runs revealed no disturbing interference when DNA originating from saliva was used. The allelic discrimination method gave a 100% agreement of the results between saliva and blood.

Table 2 Allele frequencies of CYP1A2 mutations in different ethnic groups

Source	Number of alleles	Allele frequency −164C → A (95% CI*)	Allele frequency −3858G → A (95% CI*)
Nakajima et al. 1999 [14]	232	–	0.23 (0.17–0.28)
Sachse et al. 1999 [15]	472	0.68 (0.63–0.71)	–
Mihara et al. 2000 [22]	202	–	0.22 (0.16–0.27)
Han et al. 2001 [16]	278	0.67 (0.62–0.72)	0.25 (0.20–0.30)
Shimoda et al. 2002 [23]	80	0.70 (0.60–0.80)	0.24 (0.15–0.33)
Sachse et al. 2003 [18]	130	0.66 (0.58–0.74)	0.008 (0.00–0.02)
Hamdy et al. 2003 [17]	424	0.68 (0.64–0.72)	0.07 (0.04–0.09)
Present study	200	0.68 (0.62–0.74)	0.02 (0.01–0.03)

*95% CI, 95% confidence interval

Discussion

The methods published so far for the detection of the −164C → A and −3858G → A mutations are PCR-RFLP based [14, 15].

Allelic discrimination described here allows a high throughput, a general need in genetic studies [24], and reveals a high accuracy. As shown in the current and previous investigations [25, 26], the method allows an automated assignment of the genotype. The differentiation of the genotypes can be optimised by the use of MGB probes, resulting in a better clustering of the fluorescence signals. Frequencies of the C and A allele for the −164C → A mutation in our population were 32% and 68%, respectively. These results are in good agreement with frequencies found in Caucasians and in the studies in Asian populations [13, 15, 16, 17, 18, 23]. For the −3858G → A mutation, we found a frequency for the G allele of 98%, whereas the mutant A allele represented only 2%. This is confirmed by a recent publication, whereas in Asian populations frequencies are reported in a narrow range between 22% and 25% for the A allele [13, 14, 16, 18, 23]. Han et al. reported that with the −3858A/A variant (−2964A/A in the original publication) present, the −164C/A or −164C/C variants (734C/A in the original publication) are absent. The −3858A/A variant in combination with −164C alleles would result in reduced inducibility of CYP1A2 and a linkage of the −3858A/A with the −164A/A variant would restore inducibility of CYP1A2. We could not confirm this hypothesis because of the absence of the −3858A/A variant in our population of 100 Caucasians analysed. The very low incidence of the −3858G → A mutation in Caucasians suggests that this mutation has no significant influence on CYP1A2 activity, since the frequency of slow CYP1A2 metabolisers is not different between Caucasians and Asians [27, 28, 29]. Thus, the contribution of other genetic or environmental factors seems to be more important for CYP1A2 activity. Since the contributions of these two mutations to CYP1A2 activity were not very important in previous studies [14, 15, 16], further prospective studies in larger populations are needed, analysing the relationship between inducibility of CYP1A2 and the presence of the −164C → A and −3858G → A mutations.

Although several publications report the use of saliva, hair and fingernails for genotyping [21, 30, 31, 32, 33], in the hospital setting genotyping is usually based on invasive blood sampling. Since saliva is the preferred matrix for non-invasive determination of the CYP1A2 phenotype by caffeine as a probe drug [19], we validated the use of saliva as a source of genomic DNA instead of blood. This allows CYP1A2 phenotyping and genotyping in one sample, obtained non-invasively. The amount of DNA we have isolated from 1 ml of saliva was sufficient for several PCR-based tests. Similar to blood, DNA isolated from saliva gave no interference in the consecutive PCR assays, indicating that saliva could replace blood as a DNA source for genotyping.

Conclusions

While the frequency of the −164C → A mutation in our study is in agreement with published data for Caucasians and Asians, the frequency of the −3858G → A mutation is significantly lower in Caucasians than Asians. The use of 5′-nuclease activity combined with fluorogenic probes allows fast and high throughput allelic discrimination of these two mutations and gives 100% agreement with the PCR-RFLP method. Saliva is a reliable and easily available source of genomic DNA for genotyping.

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References

1. Ingelman-Sundberg M (2001) Genetic susceptibility to adverse effects of drugs and environmental toxicants. The role of the CYP family of enzymes. Mutat Res 482:11–19
2. Boobis AR, Lynch AM, Murray S, de la Torre R, Solans A, Farre M, Segura J, Gooderham NJ, Davies DS (1994) CYP1A2-catalyzed conversion of dietary heterocyclic amines to their proximate carcinogens is their major route of metabolism in humans. Cancer Res 54:89–94
3. Butler MA, Guengerich FP, Kadlubar FF (1989) Metabolic oxidation of the carcinogens 4-aminobiphenyl and 4,4′ -methylene-bis(2-chloroaniline) by human hepatic microsomes and by purified rat hepatic cytochrome P-450 monooxygenases. Cancer Res 49:25–31
4. Gu L, Gonzalez FJ, Kalow W, Tang BK (1992) Biotransformation of caffeine, paraxanthine, theobromine and theophylline by cDNA-expressed human CYP1A2 and CYP2E1. Pharmacogenetics 2:73–77
5. Lemoine A, Gautier JC, Azoulay D, Kiffel L, Belloc C, Guengerich FP, Maurel P, Beaune P, Leroux JP (1993) Major pathway of imipramine metabolism is catalyzed by cytochromes P-450 1A2 and P-450 3A4 in human liver. Mol Pharmacol 43:827–832
6. McManus ME, Burgess WM, Veronese ME, Huggett A, Quattrochi LC, Tukey RH (1990) Metabolism of 2-acetylaminofluorene and benzo(a)pyrene and activation of food-derived heterocyclic amine mutagens by human cytochromes P-450. Cancer Res 50:3367–3376
7. Nelson DR, Koymans L, Kamataki T, Stegeman JJ, Feyereisen R, Waxman DJ, Waterman MR, Gotoh O, Coon MJ, Estabrook RW, Gunsalus IC, Nebert DW (1996) P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. Pharmacogenetics 6:1–42
8. Minchin RF, McManus ME, Boobis AR, Davies DS, Thorgeirsson SS (1985) Polymorphic metabolism of the carcinogen 2-acetylaminofluorene in human liver microsomes. Carcinogenesis 6:1721–1724
9. Shader RI, Granda BW, von Moltke LL, Giancarlo GM, Greenblatt DJ (1999) Inhibition of human cytochrome P450 isoforms in vitro by zafirlukast. Biopharm Drug Dispos 20:385–388
10. Nakajima M, Yokoi T, Mizutani M, Shin S, Kadlubar FF, Kamataki T (1994) Phenotyping of CYP1A2 in Japanese population by analysis of caffeine urinary metabolites: absence

of mutation prescribing the phenotype in the CYP1A2 gene. Cancer Epidemiol Biomarkers Prev 3:413–421
11. Tantcheva-Poor I, Zaigler M, Rietbrock S, Fuhr U (1999) Estimation of cytochrome P-450 CYP1A2 activity in 863 healthy Caucasians using a saliva-based caffeine test. Pharmacogenetics 9:131–144
12. Welfare MR, Aitkin M, Bassendine MF, Daly AK (1999) Detailed modelling of caffeine metabolism and examination of the CYP1A2 gene: lack of a polymorphism in CYP1A2 in Caucasians. Pharmacogenetics 9:367–375
13. Han XM, Chen XP, Wu QN, Jiang CH, Zhou HH (2000) G-2964A and C734A genetic polymorphisms of CYP1A2 in Chinese population. Acta Pharmacol Sin 21:1031–1034
14. Nakajima M, Yokoi T, Mizutani M, Kinoshita M, Funayama M, Kamataki T (1999) Genetic polymorphism in the 5′ -flanking region of human CYP1A2 gene: effect on the CYP1A2 inducibility in humans. J Biochem 125:803–808
15. Sachse C, Brockmoller J, Bauer S, Roots I (1999) Functional significance of a C → A polymorphism in intron 1 of the cytochrome P450 CYP1A2 gene tested with caffeine. Br J Clin Pharmacol 47:445–449
16. Han XM, Ou-Yang DS, Lu PX, Jiang CH, Shu Y, Chen XP, Tan ZR, Zhou HH (2001) Plasma caffeine metabolite ratio (17X/137X) in vivo associated with G-2964A and C734A polymorphisms of human CYP1A2. Pharmacogenetics 11:429–435
17. Hamdy SI, Hiratsuka M, Narahara K, Endo N, El-Enany M, Moursi N, Ahmed MS, Mizugaki M (2003) Genotyping of four genetic polymorphisms in the CYP1A2 gene in the Egyptian population. Br J Clin Pharmacol 55:321–4
18. Sachse C, Bhambra U, Smith G, Lightfoot TJ, Barrett JH, Scollay J, Garner RC, Boobis AR, Wolf CR, Gooderham NJ (2003) Polymorphisms in the cytochrome P450 CYP1A2 gene (CYP1A2) in colorectal cancer patients and controls: allele frequencies, linkage disequilibrium and influence on caffeine metabolism. Br J Clin Pharmacol 55:68–76
19. Fuhr U, Rost KL (1994) Simple and reliable CYP1A2 phenotyping by the paraxanthine/caffeine ratio in plasma and in saliva. Pharmacogenetics 4:109–116
20. Tang BK, Zhou Y, Kadar D, Kalow W (1994) Caffeine as a probe for CYP1A2 activity: potential influence of renal factors on urinary phenotypic trait measurements. Pharmacogenetics 4:117–124
21. van Schie RC, Wilson ME (1997) Saliva: a convenient source of DNA for analysis of bi-allelic polymorphisms of Fc gamma receptor IIA (CD32) and Fc gamma receptor IIIB (CD16). J Immunol Methods 208:91–101
22. Mihara K, Suzuki A, Kondo T, Yasui N, Furukori H, Nagashima U, Ono S, Kaneko S, Otani K, Inoue Y (2000) Effect of a genetic polymorphism of CYP1A2 inducibility on the steady state plasma concentrations of haloperidol and reduced haloperidol in Japanese patients with schizophrenia. Ther Drug Monit 22:245–249
23. Shimoda K, Someya T, Morita S, Hirokane G, Yokono A, Takahashi S, Okawa M (2002) Lack of impact of CYP1A2 genetic polymorphism (C/A polymorphism at position 734 in intron 1 and G/A polymorphism at position −2964 in the 5′-flanking region of CYP1A2) on the plasma concentration of haloperidol in smoking male Japanese with schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 26:261–265
24. Caraco Y (1998) Genetic determinants of drug responsiveness and drug interactions. Ther Drug Monit 20:517–524
25. Happich D, Schwaab R, Hanfland P, Hoernschemeyer D (1999) Allelic discrimination of factor V Leiden using a 5′-nuclease assay. Thromb Haemost 82:1294–1296
26. Yuan CC, Peterson RJ, Wang CD, Goodsaid F, Waters DJ (2000) 5′ Nuclease assays for the loci CCR5-+/Delta32, CCR2-V64I, and SDF1-G801A related to pathogenesis of AIDS. Clin Chem 46:24–30
27. Bartoli A, Xiaodong S, Gatti G, Cipolla G, Marchiselli R, Perucca E (1996) The influence of ethnic factors and gender on CYP1A2-mediated drug disposition: a comparative study in Caucasian and Chinese subjects using phenacetin as a marker substrate. Ther Drug Monit 18:586–591
28. Campbell ME, Spielberg SP, Kalow W (1987) A urinary metabolite ratio that reflects systemic caffeine clearance. Clin Pharmacol Ther 42:157–165
29. Kalow W, Tang BK (1991) Use of caffeine metabolite ratios to explore CYP1A2 and xanthine oxidase activities. Clin Pharmacol Ther 50:508–519
30. Tanigawara Y, Kita T, Hirono M, Sakaeda T, Komada F, Okumura K (2001) Identification of N-acetyltransferase 2 and CYP2C19 genotypes for hair, buccal cell swabs, or fingernails compared with blood. Ther Drug Monit 23:341–346
31. van Schie RC, Wilson ME (2000) Evaluation of human FcgammaRIIA (CD32) and FcgammaRIIIB (CD16) polymorphisms in Caucasians and African-Americans using salivary DNA. Clin Diagn Lab Immunol 7:676–681
32. Anderson TD, Ross JP, Roby RK, Lee DA, Holland MM (1999) A validation study for the extraction and analysis of DNA from human nail material and its application to forensic casework. J Forensic Sci 44:1053–1056
33. Lijnen I, Willems G (2001) DNA research in forensic dentistry. Methods Find Exp Clin Pharmacol 23:511–517