Table 3. Case reports concerning hepatotoxicity in fiscal year 2008

Molecular entities	Hepatic function abnormal	Liver disorder	Jaundice	Hepatitis fulminant	Hepatitis acute	Hepatitis	Cholestasis
Terbinafine HCl	52	47	3	0	5	11	2
Fluvastatin Na	33	21	0	0	0	1	0
Itraconazole	17	10	0	0	0	0	0
Loxoprofen Na	12	15	0	0	0	2	0
Carbamazepine	11	13	0	1	1	2	0
Rosuvastatin Ca	15	7	0	1	0	3	0
Tegafur/Uracil	12	10	1	3	1	0	0
Ticlopidine HCl	9	12	4	0	0	1	15
Gefitinib	16	2	0	1	0	0	0
Cyclosporine	11	3	3	0	0	0	1
Atorvastatin Ca	7	8	0	0	0	0	1
Fenofibrate	7	5	0	0	0	4	0
Acarbose	2	8	0	0	0	0	0
Tranilast	6	4	1	0	0	1	1
Aspirin	1	8	1	0	0	1	0
Non-pyrines (4)	3	5	0	0	0	0	0
Voglibose	3	4	1	0	1	3	0
Temozolomide	6	1	0	0	0	0	0
Cefcapene pivoxil HCl	3	3	2	2	0	0	0
Total	1,063	771	90	82	68	58	55

Based on the open data source PMDA drug safety information website: http://www.info.pmda.go.jp/fukusayou/menu_fukusayou_attention.html

is associated with significant mortality.[10] We counted the event number of major suspected drugs in domestic cases concerning various hepatotoxicities based on the open data source from PMDA from April 2008 to March 2009, and the results are shown in **Table 3**. Highly reported hepatotoxicities were abnormal hepatic function, liver disorder, jaundice, fulminant hepatitis, acute hepatitis, hepatitis, and cholestasis (**Table 3**). Although the difference between abnormal hepatic function and hepatic disorder is unclear, these MedDRA-PTs are exclusively used. The total number of case reports for hepatotoxicity was 2,509. Other hepatotoxicity related reports (more than 10 cases) not in cluded in **Table 3** were hepatic failure (44), hepatocellular injury (41), cholelithiasis (21), acute hepatic failure (19), autoimmune hepatitis (19), hyperbilirubinemia (17), veno-occlusive liver disease (15), cholecystitis (14), mixed liver injury (12), cholecystitis acute (11), jaundice cholestatic (10), and cholangitis (10). There were 89 other hepatotoxicity-related reports (less than 9 cases) not included in **Table 3**. The estimated frequency of reported hepatotoxicity ranges from 1 per 10,000 to 1 per 10 million patient-years of exposure[11] (**Table 3**).

Table 4. Case reports concerning rhabdomyolysis for fiscal years 2004 to 2008

Molecular entities	Fiscal year				
	2004	2005	2006	2007	2008
Atorvastatin	51	41	48	31	28
Bezafibrate	16	22	17	16	11
Pravastatin	21	24	19	9	11
Simvastatin	21	15	5	8	2
Levofloxacin	9	10	10	9	4
Fluvastatin	13	8	7	4	6
Omeprazole	10	8	10	3	4
Propofol	8	14	2	5	8
Rosuvastatin	—	0	4	15	14
Risperidone	7	5	5	5	5
Fenofibrate	10	9	3	1	5
Pitavastatin	4	3	10	3	9
Total	389	351	359	291	332

Based on the open data source PMDA drug safety information website: http://www.info.pmda.go.jp/fukusayou/menu_fukusayou_attention.html

Rhabdomyolysis, one of the most serious myopathies, is characterized by the leakage of muscle cell content, including electrolytes, myoglobin, and other sarcoplasmic proteins [*e.g.*, creatine kinase, aldolase, lactate dehydrogenase, alanine aminotransferase (AST), and aspartate aminotransferase (AST)] into the circulation.[12] Lipid-lowering drugs (*e.g.*, statins and fibrates) are well known causes of rhabdomyolysis, and reports produced by the US Food and Drug Administration (FDA) showed that the rate of fatal rhabdomyolysis was 0.15 per 1 million statin

prescriptions dispensed.[13,14] **Table 4** shows suspicious drugs in Japan, which are similar to US data, and the reported range of case numbers is 300 to 400 per year for the last 5 years (April 2004–March 2009).

Genomic analysis of severe adverse drug reactions

Severe ADRs affect only a minority of patients taking drugs. However, hereditary forms of severe ADRs and cases occurring in identical twins have been reported, implying involvement of certain genetic factors in predisposing individuals to such severe ADRs.[15,16] The genetic basis of ADRs can be categorized into two broad groups. The first group involves genes that drive pharmacological mechanisms (drug targets, drug metabolizing enzymes, and drug transporters).[17] Common mechanisms underlying these severe ADRs are unusual drug accumulation in the target organ due to polymorphisms in drug metabolizing enzyme and drug transporter genes, and unusual sensitivity in the target organ due to changes in drug target genes.[18] The second category involves the immune system in a drug-induced allergic reaction. One important molecule for ADRs associated with immune reactions is the human lymphocyte antigen (HLA), which plays a key role in initiation of immune responses and killing target cells by presenting antigens to the T-cell receptor.[19] The *HLA* gene region codes for three classical class I (HLA-A, HLA-B, and HLA-C) and three class II (HLA-DR, HLA-DP, and HLA-DQ) antigens. Class I antigens are recognized by cytotoxic $CD8^+$ T cells and class IIs by $CD4^+$ T cells. Of the highly polymorphic *HLA* genes, *HLA-B* is the most polymorphic with over 800 variants reported in the human genome.[20] *HLA* genes within each class encode structurally similar but distinct HLA proteins that bind and present HLA-type-specific antigenic peptides to T-cell receptors.[19,20] HLA disease associations that are related to genes with immunological and inflammatory functions have been identified in many autoimmune and inflammatory conditions.

In addition to these susceptible genes, recent advances in molecular biology have led to analysis of the association of whole genome polymorphisms with ADRs.[21,22] For example, recent high-density DNA microarrays can analyze more than one million genomic biomarkers at the same time. Therefore, association analysis has been conducted by both candidate gene and genome-wide analysis.

Severe cutaneous adverse drug reactions

Carbamazepine (CBZ) is one of the most widely used aromatic anticonvulsants and is often used as a pain-relief drug for prosopalgia. CBZ is metabolized by mainly hepatic CYP3A4, CYP2B6, and CYP2C8, which generate various potentially reactive metabolites, such as CBZ-10,11-epoxide; 3-hydroxy-CBZ; 2-hydroxy-CBZ; and CBZ-2,3-epoxide.[23,24] CBZ is generally well tolerated but also is associated with idiosyncratic adverse reactions such as SJS/TEN. A high frequency of CBZ-induced SJS/TEN was reported in Han Chinese (0.25% in new exposures to CBZ) compared to Caucasians (0.014% in new exposures to CBZ).[25–29] Furthermore, CBZ-induced SJS/TEN has been reported in identical twins.[15] These studies suggest that susceptibility to such reactions may be genetically determined. Since most reactive CBZ metabolites are detoxified to non-toxic dihydrodiols by liver microsomal epoxide hydrolase 1 (EPHX1) or to glutathione conjugates by glutathione S-transferase μ 1 (GSTM1),[30,31] some researchers have attempted to find the defective alleles of *EPHX* and *GST* genes in patients with SJS/TEN; however, these attempts have failed to find associations, indicating that reactive metabolite generation from CBZ is not sufficient to cause SJS/TEN.[32,33] Recently, Chung *et al.* reported a tight association between CBZ-induced SJS/TEN and *HLA-B*1502* allele in Han Chinese[25] (**Table 5**). They showed that all 44 Han Chinese patients with CBZ-induced SJS/TEN carried the *HLA-B*1502* allele and its odds ratio was 2,505 (95% confidence interval, 195 to 27,483, $Pc = 2.02 \times 10^{-32}$). The finding was further confirmed by the same group in

Table 5. Association between severe cutaneous adverse drug reaction and *HLA* type

Drug	HLA	Population	OR or (N)[a]	Pc[b]	Ref
Carbamazepine	B*1502	Han Chinese	2505	2.0×10^{-32}	25)
Carbamazepine	B*1502	Asian ancestry	(4/4)	–	37)
Carbamazepine	B*1502	European	(0/8)	–	37)
Carbamazepine	B*1502	Thai	25.5	0.0005	106)
Carbanazepine	B*1502	Japanese	(0/7)	–	36)
Carbamazepine	B*1502	Han Chinese	1357	1.6×10^{-41}	34)
Phenytoin	B*1502	Thai	18.5	0.005	106)
Antiepileptic	B*1502	Han Chinese	17.6	0.001	35)
Allopurinol	B*5801	Han Chinese	580	4.7×10^{-24}	44)
Allopurinol	B*5801	European	80	$< 10^{-6}$	5)
Allopurinol	B*5801	Japanese	40.8	$< 10^{-4}$	36)
Abacavir	B*5701	Australian	117	$< 10^{-4}$	48)
Abacavir	B*5701	British	24	$< 10^{-4}$	47)
Abacavir	B*5701	Australian	960	$< 10^{-4}$	50)
Abacavir	B*5701	White	(36/65)	–	49)
		Black	(0/9)	–	
		Other	(1/10)	–	
Abacavir	B*5701	Japanese	(0/7)	–	40)
Nevirapine	DRB1*0101 and high CD4	Caucasian Australian	18	0.0006	59)
Nevirapine	Cw*0802-B*1402	Sardinian	15	0.05	60)
Nevirapine	B*3505	Thai	18.96	4.6×10^{-6}	62)
Nevirapine	Cw8	Japanese	6.2	0.03	61)

[a] OR is odds ratio and (N) is sensitivity (carrier cases/all cases).
[b] Pc indicates corrected P value.

an additional study that included patients who were Han Chinese or Chinese descendants from Taiwan, Hong Kong, China, and the USA[34] (**Table 5**). The involvement of *HLA-B*1502* was also detected in SJS/TEN caused by other aromatic anti-epileptic drugs, such as phenytoin in Han Chinese[35] (**Table 5**). However, such strong association between *HLA-B*1502* and CBZ-induced SJS/TEN has not been detected in Caucasian and Japanese SJS/TEN patients[36,37] (**Table 5**). *HLA-B*1502* is present at a higher allele frequency in South-east Asian populations than in Caucasian and Japanese populations.[38] *HLA-B*1502* was not detected in 486 healthy Japanese subjects[39] and in 935 USA Caucasians (http://www.allelefrequencies.net/), while the allele frequency in Han Chinese is 8.6%.[25] The low frequency of *HLA-B*1502* in Caucasian and Japanese populations may account for the fact that no association between *HLA-B*1502* and CBZ-induced SJS/TEN was observed in Caucasians and Japanese.[36,40] Alternatively, these results suggest that *HLA-B*1502* is involved in the mechanism, but is not sufficient for CBZ-induced SJS/TEN. There could be other co-factors, such as virus infection or other variants of genes, for example, *CYP3A4*, *CYP2B6*, *CYP2C8*, *EPHX1*, and *GSTM1*; T cell receptors; genes related to apoptosis; or genes for co-stimulatory molecules involved in the interaction between antigen-presenting cells and T cells.

Allopurinol is a xanthine oxidase inhibitor that prevents the production of uric acid and is commonly used for hyperuricemia and gout.[41] Allopurinol is metabolized by xanthine oxidase to oxipurinol, which forms ribonucleotide adduct and ribonucleoside adduct.[42] Allopurinol has been reported to be a causative drug of a variety of delayed cutaneous adverse reactions, such as SJS/TEN.[43] Recently, a strong association of *HLA-B*5801* with allopurinol-induced severe cutaneous adverse reactions (drug-induced hypersensitivity syndrome and SJS/TEN) was found in Han Chinese in Taiwan[44] (**Table 5**). They showed that the *HLA-B*5801* allele was present in all patients (51/51) with allopurinol-induced severe cutaneous adverse reactions, but only in 15% of tolerant patients (20/135). The odds ratio was 580 (95% confidence interval, 34 to 9781, $Pc = 4.7 \times 10^{-24}$). Although the association was confirmed in Caucasians[5] and Japanese,[36] the odds ratio in Han Chinese (580) was much higher than that in Caucasians (80) and Japanese (40) (**Table 5**). Approximately 9 to 11% of Han Chinese are carriers of the allele, and its prevalence is generally lower in Caucasian (1 to 6%), Japanese (0.68%), and African (2 to 4%) populations.[43,44] These reports suggest that *HLA-B*5801* might be a genetic biomarker for allopurinol-induced severe cutaneous adverse reactions; however, the extent of the association showed ethnic differences.

Abacavir is a potent nucleoside analog reverse transcriptase inhibitor that is used in combination with other drugs to treat human immunodeficiency virus infection. The most serious adverse reaction of abacavir that limits its use in therapy is a hypersensitivity reaction which includes the combination of fever, skin rash, constitutional symptoms, gastrointestinal tract symptoms, and respiratory symptoms.[45] Hypersensitivity to abacavir occurs in approximately 5 to 8% of patients treated with abacavir, typically within 1 to 6 weeks of the initial dose.[45] Abacavir is metabolized by type 1 alcohol dehydrogenase to an aldehyde-reactive metabolite.[46] The initial association between *HLA-B*5701* and abacavir-induced hypersensitivity reaction was elucidated by two independent research groups in 2002[47,48] (**Table 5**). The association was reported only in Caucasians and not in Africans or Japanese[40,49] (**Table 5**) because the allelic frequency of *HLA-B*5701* in Caucasians is approximately 8%, but low in individuals of African or Asian descent.[47,48] Fine recombinant genetic mapping has identified a significant linkage disequilibrium of the haplotypic M493T polymorphism of heat shock protein-Hom (Hsp70-Hom M493T) and *HLA-B*5701* in abacavir-induced hypersensitivity reaction cases in Western Australians, which enhanced the discrimination of hypersensitive subjects from the tolerant control (odds ratio, 3,893; $Pc < 0.00001$) when compared to *HLA-B*5701* only (odds ratio, 960; $Pc < 0.00001$).[50] The Hsp70-Hom M493T polymorphism may facilitate the loading of abacavir- or its metabolite-hapten endogenous peptide onto *HLA-B*5701*.[51] A large randomized, controlled clinical trial assessing the clinical effectiveness of *HLA-B*5701* screening in Caucasians (PREDICT-1 study),[52] and a case-control study of *HLA-B*5701* in both Caucasians and African Americans (SHAPE study),[53] were highly supportive of the use of *HLA-B*5701* screening in clinical practice to exclude *HLA-B*5701* carriers from patients treated with abacavir. However, 7 Japanese patients, who were all *HLA-B*5701*-negative, had abacavir-induced hypersensitivity reactions.[40] Thus, the genetic screening of *HLA-B*5701* is unlikely to be effective for the Japanese.

Nevirapine is a non-nucleoside reverse transcriptase inhibitor that is used in combination with antiretroviral therapy.[54] The major treatment-limiting toxicity associated with nevirapine use is skin rash and hypersensitivity, which emerge in 5% of patients who have initiated nevirapine therapy.[55] Nevirapine is metabolized by CYP3A4 predominantly, and to a lesser degree by CYP2B6 and other CYP isoforms, to several hydroxylated metabolites: 12-hydroxynevirapine has been implicated as a putative nevirapine metabolite causing hypersensitivity reactions.[56] $CD4^+$ T cells have been shown to be involved in the nevirapine-induced hypersensitivity reaction.[57,58] Thus, a high level of $CD4^+$ T cells (more than 25% above normal) is one risk factor for nevirapine-induced hypersensitivity reaction.[55] In addition to $CD4^+$ T cell levels, associations between several types of HLA and

nevirapine-induced hypersensitivity reactions have been reported in different countries or populations. For example, *HLA-DRB1*0101* (Western Australian),[59] *HLA-Cw* 0802-B*1402* haplotype (Sardinian patients, people in Italian autonomous regions),[60] *HLA-Cw8* (Japanese),[61] and *HLA-B*3505* (Thai)[62] have been reported (**Table 5**). These results imply that the primary determining *HLA* allele may be different among populations in the nevirapine-induced hypersensitivity reaction.

Drug-induced liver injury

Several patterns of DILI exist, with the most useful classification being hepatocellular, cholestatic, or a combination of both.[63] Hepatocellular injury involves marked elevation of serum ALT and AST levels, usually preceding an increase in total bilirubin and no increase or only modest increases in alkaline phosphatase (ALP) levels.[9] In cholestatic injury, increases in ALP levels predominate and precede increases in ALT and AST.[9] The patterns in which a drug causes liver injury are regarded as being either predictable (dose-dependent) or unpredictable (idiosyncratic).[64] Acetaminophen-induced hepatotoxicity has been considered the classic example of a dose-related hepatotoxin,[65] although few other drugs fit this pattern.[64] Rather, the majority of drugs that are capable of producing liver injury do so in an unpredictable fashion with variable latency periods.[9,66]

Single nucleotide polymorphisms (SNPs) in drug metabolizing enzymes and drug transporters, which regulate the metabolism and disposition of drugs, represent the best studied set of pharmaceutically important genetic markers of DILI. *N*-Acetyltransferase (NAT) functions by acetylating drugs, therefore causing active drug metabolites to be detoxified. Deficient alleles of *NAT* (such as *NAT1*14* and **15* and *NAT2*5*, **6*, and **7*), which reduce detoxification activity, increase the toxicity of drugs including isoniazid, sulfonamides, and procainamides.[67,68] The allele distribution of the Caucasian population differs from that reported in the Japanese population.[67,69] Another enzyme pathway of importance is that of glutathione in the detoxification of reactive metabolites. Genetically determined deficiencies in glutathione synthetase and GST have been associated with increased hepatotoxicity of certain drugs, including acetaminophen, metronidazole, and nitrofurantoin.[70,71] Frequencies of GST-deficient alleles show ethnic differences. For example, the homozygous deletion genotype frequency in *GSTM1* ranges from 0.38 to 0.67 in Caucasians, from 0.33 to 0.63 in East Asians, and from 0.22 to 0.35 in Africans and African-Americans.[72] Pacific Islanders have the highest reported frequency of homozygous deletion genotypes (0.64–1.0) of any group studied.[73,74] Troglitazone is a 2,4-thiazolidinedione anti-diabetic drug with insulin-sensitizing activities.[75,76] Troglitazone-associated idiosyncratic hepatic dysfunction and hepatic failure were reported after introduction of the drug into the market.[77–79] Yamamoto *et al.* reported that CYP3A4 catalyzed troglitazone into an epoxide of a quinone metabolite which may be eliminated by GSTs and EPHX.[80] To address the susceptible genetic factors responsible for the hepatotoxicity associated with the drug, Watanabe *et al.* performed a genetic polymorphic analysis by a target gene approach in troglitazone-treated Japanese patients with type 2 diabetes mellitus[81] (**Table 6**). They observed a correlation between hepatic failure and both *GSTT1* and *GSTM1* null genotypes. They reported that the odds ratio was 3.692 and its 95% confidence interval was 1.354 to 10.066 (Pc = 0.008). A more recent example of drug hepatotoxicity resulting from genetic polymorphisms of drug metabolizing enzymes and drug transporters is that of diclofenac, a non-steroidal anti-inflammatory drug that is among the most common drugs to cause idiosyncratic hepatotoxicity. Diclofenac-induced hepatotoxicity occurs at a rate of 6 per 100,000 users, and 8 to 20% of the patients who develop jaundice die of liver failure. It has been concluded that diclofenac hepatotoxicity is associated with the possession of variant UDP-glucuronosyltransferase 2B7 (*UGT2B7*2*), ATP-binding cassette transporter C2 (*ABCC2*, $-24C>T$), and *CYP2C8* haplotypes[82] (**Table 6**).

Table 6. Association between drug-induced liver injury and genetic polymorphisms

Drug	Gene variant	Population	OR[a]	Pc[b]	Ref
Troglitazone	GSTM1/T1	Japanese	3.69	0.008	81)
Ticlopidine	HLA-A*3303	Japanese	36.5	7.32×10^{-7}	86)
Diclofenac	UGT2B7*2	Unknown	8.5	0.03	82)
	ABCC2(C-24T)	Unknown	5.0	0.005	
	CYP2C8 haplotypes	Unknown	–	0.04	
Flucloxacillin	HLA-B*5701	European	45.0	8.7×10^{-33}	21)

[a] OR is the odds ratio.
[b] Pc indicates corrected P value.

Immune-mediated mechanisms via the reactive metabolite binding to macromolecules are believed to be associated with idiosyncratic DILI. *HLA* has been considered to be involved in T-cell mediated cytotoxic reactions and drug-induced allergic reactions. Therefore, *HLA* might be another type of candidate genetic biomarker of DILI.[64,66] Ticlopidine, an anti-platelet agent, which has been widely used for the secondary prevention of atherothrombosis,[83] has shown severe hepatotoxicity, mainly of the cholestatic type,[84] and there appears to be an increased rate of hepatic adverse reactions in Japanese compared with Caucasian patients.[85] Hirata *et al.* explored genetic risk factors for ticlopidine-induced hepatotoxicity using 22 Japanese patients with ticlopidine-induced hepatotoxicity and 85 Japanese patients who tolerated ticlopidine therapy without ex-

periencing adverse reactions and they found a significant correlation between ticlopidine-induced hepatotoxicity and *HLA-A*3303*[86] (**Table 6**). Allelic frequency of *HLA-A*3303* is 7.54% in Japanese, 0.6% in Caucasians, and 4.5% in African-Americans (http://www.allelefrequencies.net/). They reported that 12 patients (86%) among 14 patients who showed ticlopidine-induced cholestatic hepatotoxicity had *HLA-A*3303* and the odds ratio was 36.50 (95% confidence interval, 7.25 to 183.82). Another example of the correlation between *HLA* and DILI is that of flucloxacillin, which is widely used in many European countries and Australia for treatment of staphylococcal infection. Its use has been associated with a characteristic cholestatic hepatitis that is more common in females, the elderly, and patients with prolonged treatment courses.[87-89] In the United Kingdom, the incidence of flucloxacillin-related DILI has been estimated at 8.5 in every 100,000 new users in days 1 to 45 after starting treatment.[89] Daly *et al.* conducted a genome-wide association study using 51 cases (white European ancestry) of flucloxacillin-related DILI and 282 controls matched for sex and ancestry and found the strongest correlation between flucloxacillin-related DILI and a genetic marker (rs2395029) in complete linkage disequilibrium with *HLA-B*5701*[21] (**Table 6**). They reported that the odds ratio was 45 (95% confidence interval, 19.4 to 105). Among 51 cases, 43 patients (84%) carried the risk allele (G), which has a frequency of approximately 5% in the population controls and in European populations generally.

Statin-induced myopathy

In rare cases, statins can cause muscle pain or weakness in association with elevated creatine kinase levels (*i.e.*, myopathy), and occasionally this leads to muscle breakdown and myoglobin release (*i.e.*, rhabdomyolysis), with a risk of kidney failure and death.[90] The mechanisms by which statins cause myopathy remain unknown but appear to be related to statin concentrations in blood and muscle.[90]

Morimoto *et al.* studied genetic factors contributing to the risk of stain-induced myopathy and showed that the frequencies of *OATP-C*15* [tagged by 388A>G (N130D) and 521T>C, V174A], a mutant allele of *OATP-C* (OATP1B1, *SLC21A6/SLCO1B1*) was significantly higher in Japanese patients with myopathy who were receiving pravastatin or atorvastatin than in patients without myopathy[91] (**Table 7**). They also found another *OATP-C* mutant allele, 1628T>G (L543W), which is located in exon 12 of *SLC21A6/SLCO1B1* in a Japanese patient with pravastatin-induced myopathy[92] (**Table 7**). They examined the transporting activity for pravastatin and other substrates and found that the activity decreased significantly in HEK293 cells expressing mutant proteins with V174A and L543W compared to those in cells expressing *OATP-C*1a*, the reference allele of *OATC-C*.[93,94] OATP-C has been shown to mediate the hepatic uptake of statins.[95] From these results, they speculated that patients who are carrying these defective *OATP-C* mutant alleles have increased plasma concentrations of these statins and are thus more susceptible to the myotoxic effects of these statins compared to non-carrier patients treated with pravastatin and atorvastatin.[92] In fact, Ide *et al.* recently reported that *OATP-C*15* significantly influenced the relative bioavailability [F(rel)] of pravastatin; F(rel) was increased 1.50- and 1.95-fold in heterozygous and homozygous participants, respectively, for the *OATP-C*15* allele in comparison with participants without the allele from a covariate analysis of population pharmacokinetic analysis.[96]

Table 7. Candidates of *OATP-C* genomic biomarkers for statin-induced myopathy

Variant	*In vitro* activity	Statin	Ref
521T>C	Decrease	Pravastatin or atorvastatin	91)
521T>C	Decrease	Simvastatin	22)
1628T>G	Decrease	Pravastatin	91, 94)

The SEARCH Collaborative Group, which aims to determine whether a daily dose of 80 mg of simvastatin safely produces greater benefit than a daily dose of 20 mg, found 98 definite or incipient cases of myopathy among 6,031 participants who were assigned to receive 80 mg of simvastatin.[22] All participants were from the United Kingdom, but their ethnicity was not specified. They performed a genome-wide association study using approximately 300,000 genomic markers in 85 subjects with definite or incipient myopathy and 90 controls, and found a single strong association of myopathy with the rs4149056 (521T>C) SNP located within *SLCO1B1* (**Table 7**). They reported that the odds ratio for myopathy was 4.5 (95% confidence interval, 2.6 to 7.7) per copy of the C allele, and 16.9 (95% confidence interval, 4.7 to 61.1) in CC as compared with TT homozygotes. They concluded that this variant of *SLCO1B1* is strongly associated with an increased risk of statin-induced myopathy.

These studies suggest that variant *OATP-C* decreased the hepatic uptake of statin and increased blood and muscle concentrations of statin. The increase of the blood and muscle concentrations of statin may cause myopathy or rhabdomyolysis. Genotyping these *SLCO1B1* variants might help to achieve the benefits of statin therapy more safely.

Use of pharmacogenetic biomarkers in clinical practice

In principle, identifying genetic risk factors for severe ADRs, particularly type B reactions, could significantly

decrease the incidence rate of ADRs and improve the process of drug development.[97] Among these type B ADRs which we consider in this review, the usefulness of abacavir *HLA*-genetic biomarker (*HLA-B*5701*) has been confirmed in Caucasians from several prospective studies, such as the PREDICT-1 study.[52] The association of CBZ-induced SJS/TEN and an *HLA*-genetic biomarker (*HLA-B*1502*) in Han Chinese is extremely high compared with other drugs.[25] Therefore, *HLA-B*1502* screening is recommended for CBZ in clinical practice by the US FDA, and *HLA-B*5701* screening is recommended for abacavir by the US FDA and European Medical Agency. Before treatment with CBZ or abacavir, *HLA* analysis should be performed to exclude *HLA-B*1502* or *HLA-B*5701* unless the patient is from a population who shows extremely low frequency of these *HLA* types. Such exclusion of patients from treatment with causative drugs would markedly reduce the possibility of severe ADRs and prevent overestimation of severe cutaneous ADRs that could otherwise result in excessive discontinuation of treatment.[34,98,99] In Japan, package inserts of CBZ and abacavir describe these research results.

Perspective on pharmacogenetic biomarkers

An unresolved issue for genetic biomarkers is ethnic differences, since these *HLA* markers show ethnic specificity. For example, an *HLA* marker of abacavir (*B*5701*) or CBZ (*B*1502*) is present only in Caucasians or Han Chinese (and South East Asians), respectively, and its usefulness has not been shown in other populations such as Japanese.[37,40] On the other hand, the association between allopurinol treatment and *HLA-B*5801* was observed not only in Han Chinese but also in Caucasians and Japanese, although the odds ratios were lower than that of CBZ.[5,36,44] Therefore, it is absolutely necessary to explore the *HLA* marker for each population against each drug and also to find the universal genetic biomarker (if one exists) of severe ADRs for clinical practice. As shown for nevirapine, the association between the rash with hepatitis and *HLA-DRB1*0101* was observed in Western Australian patients with $CD4^+$ T-cell levels greater than 25% above normal levels.[59] This case suggests that the combination of *HLA* genetic biomarkers and other biomarkers might be useful to predict ADRs for some drugs. A prospective study or comparative study with other populations is necessary for *HLA* biomarkers of ticlopidine- and flucloxacillin-related DILI and *SLCO1B1* biomarkers of statin-induced myopathy.

In most cases of allergic reactions, such as SJS/TEN and DILI, *HLA*-drug toxicity associations are thought to arise as a result of the interaction of a specific *HLA* allele with the drug or its metabolite, causing an immune reaction to be triggered.[38,100,101] As shown in the abacavir-induced hypersensitivity reaction, the drug metabolite may play an important role in the allergic reaction process,[102] suggesting that sequential reactions from drug metabolism to the immune mechanism can exist in the allergic process. Thus, drug toxicities that are driven primarily by the immune response may require bioactivation of the drug to a specific metabolite to evoke the specific immune response that will lead to the generation of an adverse reaction.[38,100,101] These complex mechanisms may be involved in most cases of allergic reactions because reactive metabolites have been detected not only in abacavir but also in nevirapine, CBZ, and allopurinol.

Most of the currently available genetic biomarkers are limited in relation to *HLA*, drug metabolizing enzymes, and drug transporters.[17] Considering that the technology to identify genetic variants across the whole genome is advancing rapidly, many more significant genetic factors for ADRs are likely to be identified in the future. In such whole-genome case-control analysis, there might be critical points to resolve. The first problem is the size of case and control groups. Accrual of large numbers of cases is necessary for genome-wide association study of genetic factors underlying severe ADRs, even though the number of patients with specific types of ADRs is small.[103] Control subjects for such studies should be matched for drug exposure, concomitant use of other drugs that could affect the pharmacokinetics and pharmacodynamics of the drug in question, and subject background such as age, gender, and ethnicity.[103] The second problem is objective diagnosis of ADRs. Because one drug could induce many ADR phenotypes in which the mechanism may be different, standardization of diagnosis is necessary.[40,103] These critical points affect the sufficient statistical power to detect the genetic biomarker. In order to resolve these problems, several regional networks to study severe ADRs have been established, including our research group in Japan (for SJS/TEN, DILI, and myopathy in Japanese),[36] European collaboration for studying the genetic basis of adverse drug reactions (EUDRAGENE for six severe ADRs in multiple European populations),[104] the United States Drug Induced Liver Injury Network (DILIN, for DILI),[105] and the International Serious Adverse Event Consortium (SAEC, for SJS/TEN and DILI in global populations).[21] These networks involve scientists in regulatory agencies, healthcare systems, and pharmaceutical industries as well as academia. Moreover, for the goal of standardizing phenotypes and comparing ethnicity regarding genetic risk factors for severe ADRs, these networks may form a global consortium together with new networks from other communities in the future.

Conclusions

Specific types of *HLA* which showed strong association with severe cutaneous ADRs and DILI have been found as candidate pharmacogenetic biomarkers for each ADR. The *HLA* type was different for different causative drugs,

and the allelic frequency of *HLA* genetic polymorphisms showed ethnic differences. The genetic polymorphism of drug transporter gene *SLCO1B1* has been shown to be associated with statin-induced myopathy. It is necessary to conduct prospective studies to establish valid pharmacogenetic biomarkers for severe ADRs. A large research network for the collection of DNA samples from patients with ADRs is also necessary to explore a variety of pharmacogenetic biomarkers for ADRs.

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Short Communication

*CYP3A4*16* and *CYP3A4*18* Alleles Found in East Asians Exhibit Differential Catalytic Activities for Seven CYP3A4 Substrate Drugs[S]

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ABSTRACT:

CYP3A4, the major form of cytochrome P450 (P450) expressed in the adult human liver, is involved in the metabolism of approximately 50% of commonly prescribed drugs. Several genetic polymorphisms in *CYP3A4* are known to affect its catalytic activity and to contribute in part to interindividual differences in the pharmacokinetics and pharmacodynamics of CYP3A4 substrate drugs. In this study, catalytic activities of the two alleles found in East Asians, *CYP3A4*16* (T185S) and *CYP3A4*18* (L293P), were assessed using the following seven substrates: midazolam, carbamazepine, atorvastatin, paclitaxel, docetaxel, irinotecan, and terfenadine. The holoprotein levels of CYP3A4.16 and CYP3A4.18 were significantly higher and lower, respectively, than that of CYP3A4.1 when expressed in Sf21 insect cell microsomes together with human NADPH-P450 reductase. CYP3A4.16 exhibited intrinsic clearances (V_{max}/K_m) that were lowered considerably (by 84–60%) for metabolism of midazolam, carbamazepine, atorvastatin, paclitaxel, and irinotecan compared with CYP3A4.1 due to increased K_m with or without decreased V_{max} values, whereas no apparent decrease in intrinsic clearance was observed for docetaxel. On the other hand, K_m values for CYP3A4.18 were comparable to those for CYP3A4.1 for all substrates except terfenadine; but V_{max} values were lower for midazolam, paclitaxel, docetaxel, and irinotecan, resulting in partially reduced intrinsic clearance values (by 34–52%). These results demonstrated that the impacts of both alleles on CYP3A4 catalytic activities depend on the substrates used. Thus, to evaluate the influences of both alleles on the pharmacokinetics of CYP3A4-metabolized drugs and their drug-drug interactions, substrate drug-dependent characteristics should be considered for each drug.

Introduction

CYP3A4, the major form of cytochrome P450 (P450) expressed in the adult human liver, is involved in the metabolism of approximately 50% of commonly prescribed drugs (Guengerich, 1999). CYP3A4 is capable of oxidizing a wide range of structurally diverse drugs as well as endogenous compounds. For example, many anticancer drugs, such as docetaxel, paclitaxel, etoposide, tamoxifen, irinotecan, vinblastine, and cyclophosphamide, are known to be metabolized by CYP3A4.

The expression and catalytic activity of CYP3A are highly variable among individuals, and this variability is partially attributable to genetic factors (Ozdemir et al., 2000). Several *CYP3A4* genetic polymorphisms are known to affect the metabolism of CYP3A4 substrate drugs (www.cypalleles.ki.se/cyp3a4.htm). In addition, *CYP3A4* alleles were reported to exhibit large ethnic differences in their distribution. In the Japanese, four alleles with amino acid alterations, *CYP3A4*6* (D277EfsX8), *CYP3A4*11* (T363M), *CYP3A4*16* (T185S), and *CYP3A4*18* (L293P), are found at frequencies of <0.001, 0.002, 0.014 to 0.05, and 0.013 to 0.028, respectively (Lamba et al., 2002; Yamamoto et al., 2003; Fukushima-Uesaka et al., 2004). Of these alleles, *CYP3A4*16* has also been detected in Korean (allele frequency, 0.002) and Mexican populations (allele frequency, 0.05) and *CYP3A4*18* is distributed commonly among East Asians such as Chinese (allele frequency, 0.008–0.01), Koreans (allele frequency, 0.012–0.017), and Malaysians (allele frequency, 0.021) (Wen et al., 2004; Hu et al., 2005; Lee et al., 2007; Ruzilawati et al., 2007; Kang et al., 2009).

*CYP3A4*16* and *CYP3A4*18* are reported to affect both in vitro and in vivo catalytic activities toward several substrates and to be involved in the interindividual differences in the pharmacokinetics and pharmacodynamics of CYP3A4 substrate drugs. CYP3A4.16 exhibited an approximately 50% reduction in intrinsic clearance (V_{max}/K_m) for testosterone (TST) 6β-hydroxylation activity in vitro (Murayama et al., 2002). We recently demonstrated the substrate-dependent altered kinetics of CYP3A4.16 for midazolam (MDZ) and carbamazepine (CBZ) (Maekawa et al., 2009). The intrinsic clearance for 1′-hydroxymidazolam (1′-OH-MDZ), 4-hydroxymidazolam (4-

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ABBREVIATIONS: P450, cytochrome P450; APC, 7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino] carbonyloxycamptothecin; TST, testosterone; MDZ, midazolam; CBZ, carbamazepine; 1′-OH-MDZ, 1′-hydroxymidazolam; ATV, atorvastatin; PTX, paclitaxel; DTX, docetaxel; IRN, irinotecan; TFN, terfenadine; 4-OH-MDZ, 4-hydroxymidazolam; 3′-*p*-OH-PTX, 3′-*p*-hydroxypaclitaxel; 2-OH-ATV, 2-hydroxyatorvastatin; 4-OH-ATV, 4-hydroxyatorvastatin; NPC, 7-ethyl-10-(4-amino-1-piperidino) carbonyloxycamptothecin; OR, NADPH P450 reductase.

OH-MDZ), and CBZ 10,11-epoxide formation decreased by 50, 30, and 74%, respectively, compared with the wild type. In vivo, heterozygous *CYP3A4*16* patients administered paclitaxel (PTX) showed significantly reduced 3′-*p*-hydroxypaclitaxel (3′-*p*-OH-PTX)/PTX area under the plasma concentration-time curve ratios (Nakajima et al., 2006). In addition, decreased metabolism of irinotecan (IRN) to the inactive metabolite 7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino] carbonyloxycamptothecin (APC) was observed with *CYP3A4*16* (Sai et al., 2008).

In contrast to *CYP3A4*16*, *CYP3A4*18* seems to be bidirectional in terms of its catalytic activity toward different substrates, although different evaluation systems were used for each study. For example, the CYP3A4.18 protein exhibited increased activity for TST and chlorpyrifos (Dai et al., 2001), but not for nifedipine (Lee et al., 2005) in vitro. On the other hand, for the conventional probe drug MDZ, CYP3A4.18 showed decreased metabolism in vitro but not in vivo (Lee et al., 2007). Kang et al. (2009) demonstrated that *CYP3A4*18* is the gain-of-function allele for metabolism of several CYP3A4 substrates, including sex steroids like estrogens, leading to a relative sex-hormone deficiency that may predispose older women to osteoporosis.

In this study, to evaluate the effects of *CYP3A4*16* and *CYP3A4*18* on the catalytic activity toward structurally diverse substrates, recombinant wild-type (CYP3A4.1) and variant CYP3A4 enzymes (CYP3A4.16 and CYP3A4.18) were expressed using baculovirus-insect cell systems. The seven substrates used in the investigation were MDZ, CBZ, atorvastatin (ATV), PTX, docetaxel (DTX), IRN, and terfenadine (TFN) (Supplemental Fig. S1).

Materials and Methods

Materials. Purified human cytochrome b_5 was purchased from either Invitrogen (Carlsbad, CA) or Oxford Biomedical Research (Rochester, MI). MDZ and PTX were obtained from Wako Pure Chemicals (Osaka, Japan). 1′-OH-MDZ and 4-OH-MDZ were obtained from BD Gentest (Woburn, MA). CBZ, CBZ 10,11-epoxide, 3′-*p*-OH-PTX, and TFN and its metabolite *t*-butylhydroxyterfenadine were purchased from Sigma-Aldrich (St. Louis, MO). A second TFN metabolite, α,α-diphenyl-4-piperidinomethanol, was obtained from Fine & Performance Chemicals Ltd (Middlesbrough, UK). ATV, its metabolites 2-hydroxyatorvastatin (2-OH-ATV) and 4-hydroxyatorvastatin (4-OH-ATV), and DTX and its metabolite, DTX hydroxy *tert*-butyl carbamate (M2), were obtained from Toronto Research Chemicals Inc. (North York, ON, Canada). IRN and its CYP3A4 metabolites, APC, and 7-ethyl-10-(4-amino-1-piperidino) carbonyloxycamptothecin (NPC), were kindly supplied by Yakult (Tokyo, Japan). All other chemicals and solvents used were of the highest commercially available grade or analytical grade.

Expression of Recombinant Wild-Type and Mutant CYP3A4 Proteins. Insect cell microsomes coexpressing CYP3A4 (wild type or variants) and NADPH P450 reductase (OR) were prepared according to methods described previously (Maekawa et al., 2009). The cytochrome P450 content and OR activity in microsomes were measured (Phillips and Langdon, 1962; Omura and Sato, 1964), and Western blotting of CYP3A4 and OR was performed as described previously (Maekawa et al., 2009).

Assay for CYP3A4 Activity. To compare alterations in kinetic parameters among substrates, three batches of wild-type and two variant enzyme preparations were used for all kinetic studies. Kinetic analysis on all seven CYP3A4 substrates was performed under proper conditions for the incubation time and P450 concentrations such that linear relationships for metabolite formation were obtained.

Catalytic activities for MDZ 1′- and 4-hydroxylations and CBZ 10,11-epoxide formation were measured as described previously (Maekawa et al., 2009), with slight modifications. For other substrates (ATV, PTX, DTX, IRN, and TFN), the incubation conditions were similar to those used for MDZ and CBZ. For all substrates, CYP3A4s from insect microsomes and purified cytochrome b_5 were mixed together (CYP3A4/b_5 ratio, 1:4), and protein concentrations and the OR/P450 ratio in the CYP3A4 wild-type and variant reaction mixtures were adjusted to be equivalent by adding both control (uninfected) microsomes and microsomes expressing solely OR. MDZ (0.2–200 μM), CBZ (10–500 μM), ATV (5–120 μM), PTX (1–50 μM), DTX (0.25–64 μM), IRN (5–400 μM), or TFN (0.0125–160 μM) was added into aliquots of the above-mentioned enzyme preparations. The reaction was started by adding NADPH generation system and terminated by adding appropriate stop solutions containing suitable internal standard for the measurement of each metabolite. Samples were mixed well and then spun at 13,000*g* for 3 to 5 min.

Metabolite analyses for MDZ, CBZ, ATV, and PTX were carried out on a tandem quadrupole mass spectrometer (Micromass Quattro Premier XE; Waters, Milford, MA) interfaced with an Acquity UPLC System (Waters) equipped with an Acquity BEH C18 column (1.7 μm, 2.1 × 30 mm; Waters) kept at 50°C. Two solutions (solution A, 10 mM ammonium acetate; solution B, 90% acetonitrile containing 10 mM ammonium acetate) were used as the mobile phase. Metabolites were eluted by linear gradient, increasing solution B. Detections were performed by monitoring the transitions of *m/z* 342 to 203 (1′-OH-MDZ), *m/z* 342 to 234 (4-OH-MDZ), *m/z* 253 to 180 (CBZ 10,11-epoxide), *m/z* 575 to 440 (2-OH-ATV and 4-OH-ATV), and *m/z* 870 to 122 (3′-*p*-OH-PTX).

For IRN, TFN, and DTX, a time-of-flight mass spectrometer (Micromass LCT Premier XE; Waters) interfaced with an Acquity UPLC System, equipped with Acquity BEH C18 column (1.7 μm, 2.1 × 100 mm; Waters), and kept at 40°C was used for metabolite analyses. The mobile phase consisted of a mixture of acetonitrile/methanol/distilled water containing 0.1% (v/v) formic acid (14:14:72 for IRN, 21:21:58 for TFN, and 15:45:40 for DTX) delivered isocratically at a flow rate of 0.3 ml/min. Detections were performed by monitoring the $M+H^+$ ions, *m/z* 824.3493 ± 0.02 (*t*-butyl hydroxyl DTX), 519.2243 ± 0.02 (NPC), 619.2768 ± 0.02 (APC), 488.3165 ± 0.02 (*t*-butyl hydroxyl TFN), and 268.1701 ± 0.02 (α-α-diphenyl-4-piperidinomethanol).

Kinetic parameters were calculated using the computer program designed for nonlinear regression analysis (MULTI program) (Yamaoka et al., 1986). Kinetic parameters for MDZ 4-hydroxylation, ATV 2- and 4-hydroxylation, PTX 3′-*p*-hydroxylation, IRN oxidation to NPC, and DTX *t*-butyl hydroxylation were determined by the hyperbolic Michaelis-Menten model (eq. 1). The substrate inhibition model (eq. 2) was used for MDZ 1′-hydroxylation, TFN *C*-hydroxylation, and TFN *N*-demethylation, where K_s is the substrate inhibition constant. In the case of the 10,11-epoxidation of CBZ, kinetic parameters were determined by the modified two-site equation ($V_{max1} = 0$) (Korzekwa et al., 1998) (eq. 3).

$$V = V_{max}S/(K_m + S) \quad (1)$$

$$V = V_{max}S/(K_m + S + S^2/K_s) \quad (2)$$

$$V = (V_{max2}S^2/K_{m1}K_{m2})/(1 + S/K_{m1} + S^2/K_{m1}K_{m2}) \quad (3)$$

Kinetic data were determined as the mean ± S.D. for three microsomal preparations derived from separate baculovirus infections, and statistical analysis was conducted by Dunnett's multiple comparison test in SAS (SAS Institute, Cary, NC). A p value of <0.05 was set as a statistically significant difference.

Results and Discussion

Expression of Wild-Type and Variant CYP3A4s in Insect Cells. Wild-type (CYP3A4.1) and variant proteins (CYP3A4.16 and CYP3A4.18) were coexpressed with human OR in Sf21 insect cells. Typical CO difference spectra with a maximum absorbance at 450 nm were obtained for all microsomal fraction preparations (Supplemental Fig. S2). CYP3A4.18 exhibited a larger peak at 420 nm than either CYP3A4.1 or CYP3A4.16. In three independent expression experiments, holoenzyme contents in the variant CYP3A4.16 (230.8 ± 25.2 pmol/mg microsomal protein) and CYP3A4.18 microsomes (51.3 ± 3.2 pmol/mg microsomal protein) were significantly higher and lower ($p < 0.05$), respectively, than that in the wild-type

TABLE 1

Kinetic parameters for 10 catalytic reactions using seven substrates by CYP3A4.1, CYP3A4.16, and CYP3A4.18

Data are represented by mean ± S.D. of three different expression experiments.

	K_m	V_{max}	Intrinsic Clearance (V_{max}/K_m)	K_s
	μM	*pmol/min/pmol P450*	*μl/min/pmol P450*	*μM*
MDZ 1′-hydroxylation				
CYP3A4.1	1.9 ± 0.1	28.1 ± 2.8	14.8 ± 1.5	407 ± 32
CYP3A4.16	2.6 ± 0.1***	15.0 ± 3.5**	5.8 ± 1.7***	986 ± 302*
CYP3A4.18	2.0 ± 0.1	17.5 ± 3.2*	8.8 ± 1.6**	713 ± 168
MDZ 4-hydroxylation				
CYP3A4.1	23.1 ± 5.2	12.9 ± 0.1	0.58 ± 0.14	
CYP3A4.16	51.5 ± 3.5***	11.7 ± 1.4	0.23 ± 0.04*	
CYP3A4.18	22.3 ± 3.5	9.2 ± 2.0*	0.42 ± 0.11	
CBZ epoxidation[a]				
CYP3A4.1	21.9 ± 5.2 (K_{m1}) 165 ± 15 (K_{m2})	15.5 ± 2.5	0.095 ± 0.018	
CYP3A4.16	48.0 ± 3.7***(K_{m1}) 603 ± 204** (K_{m2})	11.0 ± 1.0	0.020 ± 0.008***	
CYP3A4.18	20.0 ± 2.7 (K_{m1}) 172 ± 32 (K_{m2})	15.7 ± 4.5	0.090 ± 0.011	
ATV 2-hydroxylation				
CYP3A4.1	24.2 ± 7.6	6.6 ± 1.1	0.29 ± 0.08	
CYP3A4.16	87.4 ± 22.6**	8.2 ± 1.9	0.10 ± 0.02*	
CYP3A4.18	20.2 ± 6.8	3.8 ± 1.3	0.20 ± 0.11	
ATV 4-hydroxylation				
CYP3A4.1	19.6 ± 4.0	16.1 ± 5.0	0.84 ± 0.29	
CYP3A4.16	65.4 ± 19.3**	8.4 ± 1.4	0.14 ± 0.05*	
CYP3A4.18	16.1 ± 3.4	11.1 ± 4.2	0.71 ± 0.31	
PTX 3′-*p*-hydroxylation				
CYP3A4.1	2.9 ± 0.1	0.55 ± 0.06	0.19 ± 0.03	
CYP3A4.16	12.9 ± 2.4***	0.55 ± 0.13	0.04 ± 0.02***	
CYP3A4.18	2.7 ± 0.1	0.24 ± 0.07**	0.09 ± 0.03**	
DTX *t*-butyl hydroxylation				
CYP3A4.1	2.5 ± 0.1	0.38 ± 0.01	0.16 ± 0.01	
CYP3A4.16	1.8 ± 0.2**	0.26 ± 0.03**	0.14 ± 0.01	
CYP3A4.18	2.3 ± 0.2	0.24 ± 0.04**	0.10 ± 0.03*	
IRN oxidation to NPC				
CYP3A4.1	19.3 ± 2.7	1.4 ± 0.2	0.07 ± 0.02	
CYP3A4.16	34.0 ± 2.9**	0.9 ± 0.4	0.03 ± 0.01*	
CYP3A4.18	19.7 ± 2.8	0.7 ± 0.1*	0.04 ± 0.01*	
TFN *t*-butyl hydroxylation				
CYP3A4.1	3.4 ± 0.3	3.4 ± 0.6	1.0 ± 0.2	218 ± 5
CYP3A4.16	3.5 ± 0.5	2.1 ± 0.1*	0.6 ± 0.1*	51 ± 8
CYP3A4.18	6.0 ± 1.2*	3.1 ± 0.5	0.5 ± 0.1*	311 ± 131
TFN *N* -demethylation[b]				
CYP3A4.1	2.4 ± 0.5	2.2 ± 0.4	0.95 ± 0.25	629 ± 244
CYP3A4.16	2.1 ± 0.2	1.5 ± 0.3	0.72 ± 0.12	92 ± 8
CYP3A4.18	3.4 ± 0.3*	1.9 ± 0.2	0.57 ± 0.10	

* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ versus the wild-type (Dunnett's multiple comparison test).
[a] For CBZ epoxidation, K_{m1} and K_{m2}, V_{max2}, and intrinsic clearance (V_{max2}/K_{m2}) values are indicated in each column.
[b] For TFN *N*-demethylation, kinetic profile of CYP3A4.18 was better fitted to the Michaelis-Menten model than to the substrate inhibition model.

CYP3A4.1 microsomes (104.4 ± 23.9 pmol/mg microsomal protein). OR activity varied among the preparations but was not significantly different ($p > 0.05$) among CYP3A4.1 (1032.3 ± 88.2 nmol cytochrome *c* reduced/min/mg protein), CYP3A4.16 (659.4 ± 254.6 cytochrome *c* reduced/min/mg protein), and CYP3A4.18 (1019.1 ± 260.1 cytochrome *c* reduced/min/mg protein). On the other hand, total (apoenzyme and holoenzyme) CYP3A4 protein expression levels in insect cell microsomes were not significantly different ($p > 0.05$) between the wild type and variants by immunoblot analysis (data not shown).

Catalytic Activities of Wild-Type and Variant CYP3A4s. To characterize the substrate-dependent functional alterations of *CYP3A4*16* and *CYP3A4*18*, CYP3A4 catalytic activities of wild type and variants toward the seven substrates (MDZ, CBZ, ATV, PTX, DTX, IRN, and TFN) were measured. For four of the substrates, two different metabolites were detected: 1′- and 4-OH-MDZ from MDZ, 2- and 4-OH-ATV from ATV, APC and NPC from IRN, and *t*-butylhydroxy-TFN and α-α diphenyl-4-piperidinomethanol (azacyclonol) from TFN. Because the level of APC formed from IRN was too low to quantify precisely under our experimental conditions, kinetic analysis for IRN was performed only for NPC formation. The kinetic profiles are shown in Supplemental Fig. S3, and kinetic parameters are summarized in Table 1. The variant-to-wild-type ratios (percent) of intrinsic clearance values (V_{max}/K_m) are compared among substrates used (Fig. 1).

CYP3A4.16 showed significantly higher K_m values than CYP3A4.1 for seven catalytic reactions: MDZ 1′- and 4-hydroxylations ($p < 0.001$), CBZ 10,11-epoxidation ($p < 0.01$), ATV 2- and 4-hydroxylations ($p < 0.01$), PTX 3′-*p*-hydroxylation ($p < 0.001$), and IRN oxidation to NPC ($p < 0.01$). The V_{max} value of CYP3A4.16 was significantly lower (by 47%) ($p < 0.01$) for MDZ 1′-hydroxylation, but not for MDZ 4-hydroxylation (91% of the wild type), suggesting that catalytic site-dependent changes in V_{max} values occurred. The intrinsic clearance (V_{max}/K_m) of CYP3A4.16 was significantly reduced compared to that of CYP3A4.1 for the following catalytic reactions: MDZ 1′- and 4-hydroxylations (by 61 and 60%,

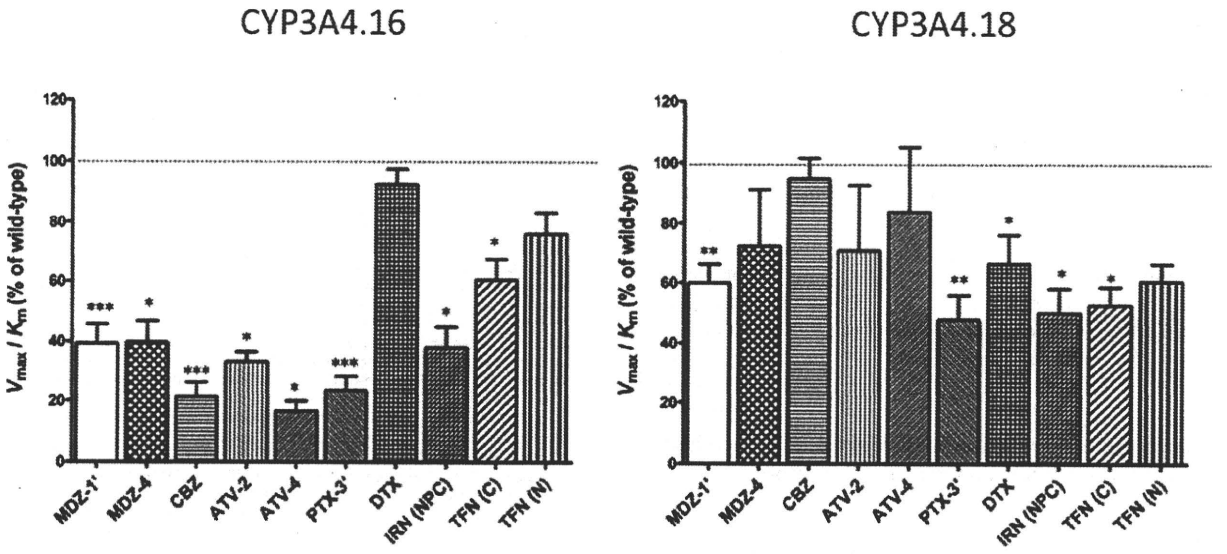

FIG. 1. The percent ratios of intrinsic clearance of variants to that of the wild type. Data are represented by mean ± S.D. of three different expression experiments. MDZ-1′, MDZ 1′-hydroxylation; MDZ-4, MDZ 4-hydroxylation; CBZ, CBZ 10,11-epoxidation; ATV-2, ATV 2-hydroxylation; ATV-4, ATV 4-hydroxylation; PTX-3′, PTX 3′-*p*-hydroxylation; DTX, DTX *t*-butyl hydroxylation; IRN (NPC), IRN oxidation to NPC; TFN (C), TFN *t*-butyl hydroxylation; TFN (N), TFN *N*-demethylation. * $p <$ 0.05, ** $p < 0.01$, and *** $p < 0.001$ versus the wild-type (Dunnett's multiple comparison test).

$p < 0.001$ and $p < 0.05$, respectively), CBZ 10,11-epoxidation (by 79%, $p < 0.001$), ATV 2- and 4-hydroxylation (by 67 and 84%, respectively, $p < 0.05$), PTX 3′-*p*-hydroxylation (by 77%, $p <$ 0.001), IRN oxidation to NPC (by 62%, $p < 0.05$), and TFN *t*-butyl hydroxylation (by 40%, $p < 0.05$). In contrast, for DTX hydroxylation and TFN *N*-demethylation, no significant differences ($p > 0.05$) in the intrinsic clearance values were observed between CYP3A4.1 and CYP3A4.16.

Our results were consistent with those by Miyazaki et al. (2008), who found that recombinant CYP3A4.16 expressed in *Escherichia coli* is markedly deficient in MDZ, TST, and nifedipine metabolisms with lower V_{max} and increased K_m relative to CYP3A4.1. Thr185 in the E helix is far away from the active site and is not located in the substrate recognition site. Further studies are necessary to elucidate the role of this residue in the binding of structurally diverse CYP3A4 substrates to the substrate recognition site.

In agreement with the lower in vitro catalytic activity of CYP3A4.16 toward PTX and IRN, *CYP3A4*16* heterozygous patients administered PTX or IRN were reported to show significantly reduced metabolite-to-substrate area under the plasma concentration-time curve ratios, which are parameters for in vivo CYP3A4 activity (Nakajima et al., 2006; Sai et al., 2008). As for substrates for which the clinical significance of *CYP3A4*16* has not been evaluated, this study demonstrated that ATV metabolism was markedly affected by CYP3A4.16. Because CYP3A4 (but not CYP3A5) is the major enzyme involved in the formation of the two ATV metabolites: 2- and 4-OH-ATV (Park et al., 2008), the clinical relevance of *CYP3A4*16* for efficacy and/or adverse reactions of ATV should be further investigated. In contrast, CYP3A4.16 retained its catalytic activity toward DTX, and thus it is predicted that this allele does not substantially influence the metabolism of DTX in vivo.

For CYP3A4.18, the reduced intrinsic clearances were observed for MDZ 1′-hydroxylation (by 40%, $p < 0.01$), PTX 3′-*p*-hydroxylation (by 52%, $p < 0.01$), DTX *t*-butyl hydroxylation (by 32%, $p < 0.05$), IRN oxidation to NPC (by 50%, $p < 0.05$), and TFN *t*-butyl hydroxylation (by 48%, $p < 0.05$) compared with CYP3A4.1. Except for TFN, the lowered V_{max} values for CYP3A4.18 resulted in lower activity in contrast to those for CYP3A4.16, which exhibited increased K_m values for most substrates. On the other hand, CYP3A4.18 had similar kinetic profiles to CYP3A4.1 in their values for K_m, V_{max}, and intrinsic clearance for oxidation of CBZ (Table 1; Supplemental Fig. S3), which has the lowest molecular weight among the seven substrates (Supplemental Fig. S1).

For the substrates MDZ, PTX, and IRN, it was reported that heterozygous *CYP3A4*1/CYP3A4*18* did not affect their pharmacokinetics (Nakajima et al., 2006; Lee et al., 2007; Sai et al., 2008). Because our in vitro results with CYP3A4.18 showed a partial decrease in V_{max} values for these drugs, an in vivo-in vitro correlation was not observed, at least for heterozygotes. Further studies are necessary to evaluate the clinical relevance of homozygous *CYP3A4*18*.

By molecular modeling studies, Kang et al. (2009) demonstrated that the L293P substitution at the beginning of the I helix caused significant secondary structural changes in the I helix and reduced protein stability. Our spectral analysis that CYP3A4.18 preparations contained more P420 than CYP3A4.1 might also be in agreement with their modeling. These possible conformational changes in CYP3A4.18 may affect substrate access depending on the substrate structure.

In conclusion, the substrate-dependent functional alterations of CYP3A4.16 and CYP3A4.18 were assessed toward seven structurally diverse substrates, MDZ, CBZ, ATV, PTX, DTX, IRN, and TFN. Compared to the wild type, CYP3A4.16 exhibited more than 60% reduced activity toward MDZ, CBZ, ATV, PTX, and IRN due to increased K_m values. In contrast, CYP3A4.18 showed a moderate reduction in its catalytic activity (by 34–52%) for MDZ, PTX, DTX, and IRN due to decreased V_{max} values. Thus, to evaluate the influences of both alleles on the pharmacokinetics of other CYP3A4-metabolized drugs and their drug-drug interactions, substrate drug-dependent characteristics should be elucidated for each drug.

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KEIKO MAEKAWA
NORIKO HARAKAWA
TAKUYA YOSHIMURA
SU-RYANG KIM
YOSHIYUKI FUJIMURA
FUMIKA AOHARA
KIMIE SAI
NORIKO KATORI
MASAHIRO TOHKIN
MIKIHIKO NAITO
RYUICHI HASEGAWA
HARUHIRO OKUDA
JUN-ICHI SAWADA[1]
TAKURO NIWA
YOSHIRO SAITO

[1] Current affiliation: Office of Biologics I, Pharmaceuticals and Medical Devices Agency, Chiyoda-ku, Tokyo, Japan.

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Address correspondence to: Dr. Keiko Maekawa, Division of Medicinal Safety Science, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan. E-mail: maekawa@nihs.go.jp

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REVIEW

Recommended nomenclature for five mammalian carboxylesterase gene families: human, mouse, and rat genes and proteins

Roger S. Holmes · Matthew W. Wright · Stanley J. F. Laulederkind · Laura A. Cox · Masakiyo Hosokawa · Teruko Imai · Shun Ishibashi · Richard Lehner · Masao Miyazaki · Everett J. Perkins · Phillip M. Potter · Matthew R. Redinbo · Jacques Robert · Tetsuo Satoh · Tetsuro Yamashita · Bingfan Yan · Tsuyoshi Yokoi · Rudolf Zechner · Lois J. Maltais

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Abstract Mammalian carboxylesterase (*CES* or *Ces*) genes encode enzymes that participate in xenobiotic, drug, and lipid metabolism in the body and are members of at least five gene families. Tandem duplications have added more genes for some families, particularly for mouse and rat genomes, which has caused confusion in naming rodent *Ces* genes. This article describes a new nomenclature system for human, mouse, and rat carboxylesterase genes that identifies homolog gene families and allocates a unique name for each gene. The guidelines of human, mouse, and rat gene nomenclature committees were followed and "*CES*" (human) and "*Ces*" (mouse and rat) root

R. S. Holmes (✉) · L. A. Cox
Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX 78227-5301, USA
e-mail: rholmes@sfbrgenetics.org

R. S. Holmes · L. A. Cox
Southwest National Primate Research Center, Southwest Foundation for Biomedical Research, San Antonio, TX, USA

R. S. Holmes
School of Biomolecular and Physical Sciences, Griffith University, Brisbane, QLD, Australia

M. W. Wright
European Bioinformatics Institute, Wellcome Trust Genome Campus, Cambridge, UK

S. J. F. Laulederkind
Rat Genome Database, Human Molecular Genetics Center, Medical College of Wisconsin, Milwaukee, WI, USA

M. Hosokawa
Laboratory of Drug Metabolism and Biopharmaceutics, Chiba Institute of Science, Choshi, Chiba, Japan

T. Imai
Graduate School of Pharmaceutical Sciences, Kumamoto University, Kumamoto, Japan

S. Ishibashi
Department of Medicine, Jichi Medical University, Shimotsuke, Tochigi, Japan

R. Lehner
CIHR Group on Molecular and Cell Biology of Lipids, University of Alberta, Edmonton, AB, Canada

M. Miyazaki
The Institute of Glycoscience, Tokai University, Kanagawa, Japan

E. J. Perkins
Department of Drug Disposition, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN, USA

P. M. Potter
Department of Molecular Pharmacology, St. Jude Children's Research Hospital, Memphis, TN, USA

M. R. Redinbo
Department of Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

J. Robert
Laboratoire de Pharmacologie, Institut Bergonié, Bordeaux Cedex, France

T. Satoh
Graduate School of Pharmaceutical Sciences, Chiba University, Chiba, Japan

T. Yamashita
Department of Agro-bioscience, Iwate University, Morioka, Japan

symbols were used followed by the family number (e.g., human *CES1*). Where multiple genes were identified for a family or where a clash occurred with an existing gene name, a letter was added (e.g., human *CES4A*; mouse and rat *Ces1a*) that reflected gene relatedness among rodent species (e.g., mouse and rat *Ces1a*). Pseudogenes were named by adding "*P*" and a number to the human gene name (e.g., human *CES1P1*) or by using a new letter followed by *ps* for mouse and rat *Ces* pseudogenes (e.g., *Ces2d-ps*). Gene transcript isoforms were named by adding the GenBank accession ID to the gene symbol (e.g., human *CES1_AB119995* or mouse *Ces1e_BC019208*). This nomenclature improves our understanding of human, mouse, and rat *CES/Ces* gene families and facilitates research into the structure, function, and evolution of these gene families. It also serves as a model for naming *CES* genes from other mammalian species.

Introduction

Five families of mammalian carboxylesterases (CES; E.C.3.1.1.1) have been described, including CES1, the major liver enzyme (Ghosh 2000; Holmes et al. 2009a; Munger et al. 1991; Shibita et al. 1993); CES2, the major intestinal enzyme (Holmes et al. 2009a; Langmann et al. 1997; Schewer et al. 1997); CES3, expressed in brain, liver, and colon (Holmes et al. 2010; Sanghani et al. 2004); CES5 (also called CES7 or cauxin), a major urinary protein of the domestic cat also present in human tissues (Holmes et al. 2008a; Miyazaki et al. 2003, 2006; Zhang et al. 2009); and CES6, a predicted CES-like enzyme in brain (Clark et al. 2003; Holmes et al. 2009a; reviewed by Williams et al. 2010). These enzymes catalyze hydrolytic and transesterification reactions with xenobiotics, anticancer prodrugs, and narcotics (Ohtsuka et al. 2003; Redinbo and Potter 2005; Satoh and Hosokawa 1998, 2006; Satoh et al. 2002), the conversion of lung alveolar surfactant (Ruppert et al. 2006), and several lipid metabolic reactions (Becker et al. 1994; Diczfalusy et al. 2001; Ghosh 2000; Hosokawa et al. 2007; Tsujita and Okuda 1993); they may also assist with the assembly of low-density lipoprotein particles in liver (Wang et al. 2007).

Structures for human and animal *CES* genes have been reported, including rodent *CES1*- and *CES2*-"like" genes (Dolinsky et al. 2001; Ghosh et al. 1995; Hosokawa et al. 2007) and human *CES1* and *CES2* genes (Becker et al. 1994; Ghosh 2000; Langmann et al. 1997; Marsh et al. 2004). Predicted gene structures have been also described for the human *CES3*, *CES5*, and *CES6* genes, which are localized with *CES1* and *CES2* in two contiguous *CES* gene clusters on human chromosome 16 (Holmes et al. 2008a, 2009a, b, 2010). In addition, a *CES1*-like pseudogene (currently designated *CES4*) is located with the *CES1–CES5* gene cluster (Yan et al. 1999). Mammalian *CES* genes usually contain 12–14 exons of DNA encoding CES enzyme sequences which may be shuffled during mRNA synthesis, generating several *CES* transcripts and enzymes encoded by each of the *CES* genes (see Thierry-Mieg and Thierry-Mieg 2006). There are significant sequence similarities for the five *CES* families, especially for key regions previously identified for human liver CES1 (Bencharit et al. 2003, 2006; Fleming et al. 2005). Three-dimensional structural analyses of human CES1 have identified three major ligand binding sites, including the broad-specificity active site, the "side door," and the "Z-site," where substrates, fatty acids, and cholesterol analogs, respectively, are bound; and an active site 'gate', which may facilitate product release following catalysis (Bencharit et al. 2003, 2006; Fleming et al. 2005).

Because of the confusion associated with the current nomenclature for mammalian *CES* genes, particularly for mouse and rat *Ces* genes where significant gene duplication events have generated a large number of *Ces1*-like and *Ces2*-like genes (Berning et al. 1985; Dolinsky et al. 2001; Ghosh et al. 1995; Hosokawa et al. 2007; Satoh and Hosokawa 1995), this article proposes a new nomenclature system that enables easy identification of *CES* family members for this enzyme. The nomenclature follows the guidelines of the human, mouse, and rat gene nomenclature committees and allocates a new name for each human (*CES*) or mouse and rat (*Ces*) gene. It also names and identifies the gene family origin for identified *CES* pseudogenes and provides a system for naming transcript isoforms derived from each of the *CES* genes. The nomenclature has the flexibility to accommodate new human, mouse, and rat *CES* genes and will assist further research into the structure, function, and evolution of these gene families as well as serve as a model for naming *CES* genes from other mammalian species.

B. Yan
Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI, USA

T. Yokoi
Division of Pharmaceutical Sciences, Graduate School of Medical Science, Kanazawa University, Kanazawa, Japan

R. Zechner
Institute of Molecular Biosciences, University of Graz, Graz, Austria

L. J. Maltais
The Jackson Laboratory, Bar Harbor, ME, USA

Guiding principles for the new *CES* nomenclature

The new nomenclature system for human, mouse, and rat *CES* genes and enzymes is based on the identification of homolog gene families and a subsequent allocation of a unique gene name for each of the genes observed from genome databases or reported from previous studies. It follows the guidelines of the human, mouse, and rat gene nomenclature committees and recommends the naming of homolog *CES* or *Ces* genes among species. The italicized root symbol "*CES*" for human and "*Ces*" for mouse and rat genes were used, followed by an number describing the gene family (examples include *CES1* for human *CES* family 1 or *Ces1* for mouse and rat *Ces* family 1 genes) (Tables 1, 2, 3). For mammalian genomes in which multiple genes were identified or a gene required a name that clashed with an existing name, a capital letter (for human genes) (e.g., *CES4A*) or a lower-case letter (for mouse and rat genes) (e.g., *Ces1a*, *Ces1b* for multiple mouse *Ces1*-like genes) was added after the number. The letter used for multiple genes reflected the relatedness of the genes across species (e.g., reflecting higher degrees of identity for mouse and rat *Ces1a* genes). When a human *CES* pseudogene was identified, a capital "P" and a number were added to the gene name (e.g., *CES1P1*), whereas for mouse and rat *Ces* pseudogenes, a unique lower-case letter was used followed by "-ps" (e.g., *Ces2d-ps*). Transcript isoforms of human (*CES*) and mouse and rat (*Ces*) gene transcripts were designated by following the gene name with the GenBank transcript ID, such as human *CES1_AB119997* and *CES1_AB187225*, which differs from the current nomenclature used for human *CES1* isoforms (*CES1A1* and *CES1A2*, respectively) (see Table 1).

Human *CES* genes

Table 1 summarizes the locations and exonic structures for human *CES* genes based upon previous reports for human *CES1* and *CES2* (Becker et al. 1994; Ghosh 2000; Langmann et al. 1997; Marsh et al. 2004) and predictions for human *CES3* (Holmes et al. 2010), *CES4A* (Holmes et al. 2009a), and *CES5A* (Holmes et al. 2008a) [the February 2009 human reference sequence (GRCh37) was used in this study (Rhead et al. 2010)]. Human *CES1P1* (a *CES1*-like pseudogene), *CES1*, and *CES5A* were located in a cluster (cluster 1) on chromosome 16, while *CES2*, *CES3*, and *CES4A* were in a separate cluster (cluster 2) on the same chromosome. Cluster 1 *CES* genes (*CES1* and *CES5A*) were transcribed on the negative strand, whereas cluster 2 genes (*CES2*, *CES3*, and *CES4A*) were transcribed on the positive strand. Figure 1 summarizes the predicted exonic start sites for human *CES* genes, with *CES1* and *CES4A* containing 14 exons, *CES3* and *CES5A* 13 exons, and *CES2* with 12 exons. These exon start sites were in identical or similar positions to those reported for *CES1* (Ghosh 2000; March et al. 2004). Figure 2 shows the comparative structures for human *CES* reference sequences and transcripts described on the AceView website (http://www.ncbi.nlm.nih.gov/IEB/Research/Acembly/) (Thierry-Mieg and Thierry-Mieg 2006). The *CES* gene and transcript sequences varied in size from 11 kb for *CES2* to 79 kb for *CES5A* and exhibited distinct structures in each case. Moreover, several isoforms were generated in vivo for each of the human *CES* genes and have different structures as a result of transcriptional events, including truncation of the 5′ ends, differential presence or absence of exons, alternative splicing or retention of introns, or overlapping exons with different boundaries. In addition, the isoforms are differentially expressed in tissues of the body and may perform distinctive metabolic roles. *CES* isoforms were named by using the gene name followed by the GenBank ID for the specific transcript. Recent studies of human *CES1* have described at least two major isoform transcripts, designated as *CES1A1* (*AB119997*) and *CES1A2* (*AB119996*) (Tanimoto et al. 2007). These isoforms have been redesignated as *CES1_AB119997* and *CES1_AB119997*, respectively (see Table 1) and encode sequences that differ by only four amino acid residues within the N-terminal region (exon 1) (Tanimoto et al. 2007). Distinct 5′-untranslated consensus sequences for binding transcription factors were reported. They suggested differences in transcriptional regulation and functional roles in contributing to CPT-11 chemosensitivity for these isoforms (Hosokawa et al. 2008; Tanimoto et al. 2007; Yoshimura et al. 2008). Fukami et al. (2008) have also examined human *CES* isoform structure and proposed that *CES1P1*, a *CES1*-like pseudogene on chromosome 16 (designated as *CES1A3*), was derived from the *CES1_AB119997* isoform.

Human CES amino acid sequences and structures

An alignment of the amino acid sequences for human CES-like protein subunits is shown in Fig. 1, together with a description of several features for these enzymes. The sequences have been derived from previously reported sequences for CES1 (Munger et al. 1991; Shibata et al. 1993), CES2 (Langmann et al. 1997; Schewer et al. 1997), CES3 (Sanghani et al. 2004), CES4A (previously CES6 or CES8) (Holmes et al. 2009a); and CES5A (previously CES7) (Holmes et al. 2008a) (Table 1). Alignments of the human CES subunits showed between 39 and 46% sequence identities, which suggests that these are products of separate but related gene families, whereas sequence alignments of human CES1 and CES2 with mouse

Table 1 Human *CES1*, *CES1P1*, *CES2*, *CES3*, *CES4A*, and *CES5A* genes and subunits

Human *CES* gene	Chromosome 16 coordinates	Gene size (bp)	Exons strand	Subunit MW	Amino acids	GenBank ID	Other gene names	Expression tissues (relative level of gene expression)	NCBI RefSeq transcript	UNIPROT ID
CES1	54,394,465–54,424,468	30,004	14 −ve	62,521	567	L07765	*hCE-1, CES1A1, HU1, EST1*	liver, lung, others [×3.8]	NM_001025195	P23141
CES1P1	55,794,511–55,808,824	14,314	6 +ve	ps	ps	AF106005	*CES4*	pseudogene	NR_003276	
CES2	65,527,040–65,535,426	8,387	12 +ve	61,807	559	BC032095	*CE-2, HU2, hCE-2*	brain, kidney, intestine [×4.5]	NM_003869	O00748
CES3	65,552,712–65,564,450	11,739	13 +ve	62,282	571	BC053670	*ES31, CE3*	colon, brain, others [×0.5]	NM_024922	Q9H6X7
CES4A	65,580,177–65,600,543	20,367	14 +ve	60,366	561	BC166638	*ESTHL, CES8, CE5*	brain, lung, kidney [×0.7]	NM_173815	Q5XG92
CES5A	54,437,867–54,466,634	28,768	13 −ve	63,936	575	BC039073	*CES7, CE4*	brain, lung, testis [×0.1]	NM_001143685	Q6NT32

Human *CES* gene	Human *CES* transcript isoform names	Gene size (bp)	Exons strand	Subunit MW	Amino acids	GenBank ID	Other names for human CES isoforms	AceView[a] human CES isoform name	NCBI RefSeq transcript	Transcript length (bp)
CES1	*CES1_AB119997*	30,380	14 −ve	62,592	568	AB119997	*CES1A1*	CES1, variant aApr07	NM_001025195	2,084
	CES1_AB119996	30,380	14 −ve	62,521	**567**	AB119996	*CES1A2*	CES1, variant bApr07	NM_001025194	2,081
	CES1_AK290623	30,310	14 −ve	62,393	566	AK290623	*CES1A3*	CES1, variant cApr07	NM_001266	2,007
CES2[d]	*CES2_BC032095*	10,890	12 +ve	68,899	**559**	BC032095	*CES2A1*	CES2, variant aApr07	NM_003869	4,177
	CES2_AL713761	10,660	12 +ve	67,051	607	AL713761	*CES2A2*	CES2, variant bApr07	NM_198061	3,901
	CES2_AK095522	10,590	12 +ve	61,566	560	AK095522	*CES2A3*	CES2, variant cApr07	NM_003869	4,140
CES3	*CES3_AY358609*	13,920	13 +ve	62,282	**571**	AY358609	*CES3A1*	COesterase.1, variant aApr07	NM_024922	3,894
	CES3_BC053670	12,160	13 +ve	61,967	568	BC053670	*CES3A2*	COesterase.1, variant bApr07	BC053670[b]	2,123
CES4A	*CES4A_BC166638*	20,367	14 +ve	60,366	**561**	BC166638	*CES4A1*	c	NM_173815	2,135
CES5A	*CES5A_BC069501*	29,217	13 −ve	63,926	**575**	BC069501	*CES5A1*	CES7, variant aApr07	NM_001143685.1	2,285
	CES5A_BC069548	29,217	12 −ve	58,201	525	BC069548	*CES5A2*	CES7, variant bApr07	NM_145024	2,135

RefSeq, GenBank, and UNIPROT IDs provide the sources for the gene and protein sequences; the relative gene expression level for human CES genes in comparison with the expression of an average human gene is given in brackets. Gene sizes are given as base pairs of nucleotides. CES isoform sequences aligned in Fig. 1 are bold

ps pseudogene (*CES1P1*), *+ve and –ve* transcription strand direction

[a] http://www.ncbi.nlm.nih.gov/IEB/Research/Acembly/

[b] GenBank ID number

[c] No current AceView isoform name available

[d] The human CES2_BC032095 isoform transcript contains multiple transcription start sites with the shorter CES2 sequence (559 residues) previously reported (Pindel et al. 1997; Schewer et al. 1997)

Table 2 Mouse *Ces* genes and subunits

Mouse CES gene (proposed)	Chr 8 coordinates	Gene size (bp)	Exons Strand[a]	Subunit MW	Amino acids	GenBank ID	MGI ID_YZ	Current MGI symbol_YZ	Current gene symbols	NCBI transcript	Vega ID	Ensembl ID	UNIPROT ID	Tissue expression (relative)[b]
Ces1a	95,544,116–95,572,091	27,979	14 – ve	61,744	563	BC089371	MGI:3648919	Gm4976	*EG244595*	NM_001013764	None	ENSMUSG00000071047	Q5FWH4	Fetal liver [0.08]
Ces1b	95,580,789–95,603,815	23,027	13 – ve	62,197	567	*NM_001081372	MGI:3779470	Gm5158	*CesN*	NM_001081372	None	ENSMUSG00000078964		Liver [×2.0]
Ces1c	95,622,914–95,655,182	32,268	13 – ve	61,172	554	BC028907	MGI:95420	Es1	*Es1, Ces-N*	NM_007954	ENSMUSG00000024453	ENSMUSG00000057400	P23953	Liver [×2.0]
Ces1d	95,690,157–95,721,618	31,462	14 – ve	61,788	565	BC019198	MGI:2148202	Ces3	*Ces3*	NM_053200	ENSMUSG00000024539	ENSMUSG00000056973	Q8VCT4	Tongue, liver [×2.2]
Ces1e	95,725,306–95,753,320	28,015	14 – ve	61,582	562	BC019208	MGI:95432	Es22	*Es22*	NM_133660	ENSMUSG00000024532	ENSMUSG00000061959	Q64176	Liver, kidney [0.4]
Ces1f	95,780,331–95,803,599	23,269	14 – ve	61,698	561	BC013479	MGI:234564	AU018778	*CesML1, TGH-2*	NM_144930	ENSMUSG00000024519	ENSMUSG00000031725	Q91WU0	Tongue, kidney [2.6]
Ces1g	95,826,807–95,861,053	34,247	14 – ve	62,680	565	BC021150	MGI:88378	Ces1	*Ces1*	NM_021456	ENSMUSG00000024535	ENSMUSG00000057074	Q3UW56	Tongue, kidney [2.6]
Ces1h	95,875,926–95,903,624	27,699	14 – ve	62,087	562	AK009689	MGI:75704	2310039D24Rik	*AK009689*	XM_134476	ENSMUSG00000033579	ENSMUSG00000074156		Tongue, kidney [2.6]
Ces2a	107,257,972–107,265,313	7,342	12 +ve	61,940	558	BC024491	MGI:2142491	Ces6	*Ces6*	NM_133960	OTTMUSG00000027410	ENSMUSG00000055730	Q8QZR3	Liver, colon [×1.0]
Ces2b	107,355,572–107,362,353	6,782	12 +ve	61,927	556	BC015286	MGI:2448547	BC015286	*BC015286*	NM_198172	OTTMUSG00000027467	ENSMUSG00000050097	Q6PDB7	Kidney, colon [0.1]
Ces2c	107,371,033–107,378,161	7,129	12 +ve	62,470	561	BC031170	MGI:2389505	Ces2	*Ces2*	NM_145603	OTTMUSG00000027466	ENSMUSG00000061825	Q91WG0	Kidney, colon [1.2]
Ces2d-ps	107,391,388–107,397,764	3,762	6 +ve			BC034182	MGI:3704319	Gm9756		XR_002069	None	ENSMUSG00000031884		Pseudogene
Ces2e	107,450,221–107,457,611	7,391	12 +ve	62,735	560	BC055062	MGI:2443170	Ces5	*Ces5*	NM_172759	None	ENSMUSG00000031886	Q8BK48	Liver, intestine [0.6]
Ces2f	107,471,256–107,479,862	7,335	12 +ve	62,707	561	BC117742	MGI:1919153	2310038E17Rik		NM_001079865	None	ENSMUSG00000062826	Q08ED5	Tongue, thymus [0.2]
Ces2g	107,485,688–107,492,328	6,771	10 +ve	52,731	478	BC027185	MGI:1919611	2210023G06Rik		NM_197999	None	ENSMUSG00000031877		Kidney, stomach [0.7]
Ces2h	107,524,753–107,544,307	19,554					MGI:3648740	Gm5744		XM_488149	None	None		Not available
Ces3a	107,572,572–107,582,000	21,512	13 +ve	61,510	554	AK138932	MGI:102773	Es31	*Es31*	NM_198672	None	ENSMUSG00000069922	Q63880	Liver, aorta [1.1]
Ces3b	107,607,670–107,617,468	9,799	14 +ve	63,007	568	BC019047	Gm4738	Es31L	*Es31L*	NM_144511	None	ENSMUSG00000062181		Liver [0.5]
Ces4a	107,655,852–107,673,417	17,566	14 +ve	62,123	563	BC026374	BC026374	Ces8	*Ces8*	NM_146213	OTTMUSG00000027469	ENSMUSG00000060560		Skin [0.1]