アレル）遺伝子多型診断系においても、すべて12merのプローブの組合せが最も感度良く、正確に遺伝子診断できた。

イムノクロマトグラフィーの際に用いられる展開バッファーによって、DNA検出試験紙上の検出ラインの濃淡が変化することが判明した。リン酸バッファーとトリスバッファーを比較した結果、リン酸バッファーを用いた方が非特異的な反応を抑制することができた。また、55mMリン酸ナトリウム、150mM NaCl（pH7.2）の時に最も感度良く、正確に遺伝子診断することができた。

D. 考察

従来用いていたロシュ製の DNA 検出試験紙は、現在発売中止となり、入手することは困難である。そこで、第一化学薬品製の DNA 検出試験紙に変更したが、これまでのプローブ条件では最高のパフォーマンスが得られているとは言えず、診断系のブラッシュアップが必要であった。今回、ミトコンドリア DNA（1555A>G 多型）及び NAT2（*5, *6, *7 アレル）の遺伝子多型診断において、プローブ鎖長を 12mer にすることで、検出感度と正確性が向上した。このことは、従来のロシュ製の試験紙と第一化学薬品製の試験紙では製品特性がことなることを示唆している。また、イムノクロマトグラフィー時の展開バッファーの組成も各社の試験紙に最も適した条件を見いだす必要があると考えられた。

現在、CYP2C19（*2, *3 アレル）及び TPMT（*3C アレル）検出のための条件も検討中であり、来年度に報告する予定である。

E. 結論

これまでに我々が構築してきた薬剤反応性に関与する遺伝子多型診断法である CASSOH法のブラッシュアップを行い、対象としたミトコンドリアDNA（1555A>G多型）及び NAT2（*5, *6, *7アレル）の検出では、プローブ鎖長を12merにすることで最も高い検出感度と正確性が得られた。また、第一化学薬品製の試験紙に適した展開バッファーの組成を決定できた。

F. 健康危険情報

G. 研究発表

1. 論文発表

なし

2. 学会発表

アミノグリコシド系抗生剤による副作用の回避を目的としたミトコンドリア DNA1555A>G 多型遺伝子診断と遺伝カウンセリング、作山佳奈子、平塚真弘、呉繁夫、松原洋一、金野由美子、佐々木崇光、水柿道直、第 44 回日本薬学会東北支部大会、仙台、2005 年 10 月

H. 知的財産権の出願・登録状況

特になし

厚生労働科学研究費補助金(萌芽的先端医療技術推進研究事業)

分担研究報告書

臨床的に有用な薬理遺伝的遺伝子多型情報の収集と評価

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研究要旨

近年、ヒトゲノム解析の進展により薬剤反応性の一部が薬物代謝酵素や薬物トランスポーター、薬物受容体遺伝子などのSNPの影響を大きく受けることが明らかにされている。将来的には薬物投与前に薬物動態や薬効発現に関連する遺伝子のSNP診断を行い、患者個々に最適な投与薬剤の選択及び投与量の調整を行うことが可能になる。このような個別化薬物療法オーダーメイド医療の展開には、簡易迅速なSNP検出法の開発が必須である。今回、日本人集団において臨床的に有用な薬剤反応性遺伝子のSNP検出法を開発すべく、薬剤反応性に影響を及ぼすと考えられる遺伝子を文献情報よりリストアップした。また、それらの遺伝子多型の人種差の有無、遺伝子診断法の種類を調査した。その結果、ミトコンドリアDNA(1555A>G多型)、CYP2C19(*2, *3アレル)、NAT2(*5, *6, *7アレル)及びTPMT(*3Cアレル)のSNPが、日本人で比較的頻度が高く、遺伝子型－表現型の関連性が明確であり、臨床的に遺伝子多型情報として有用であると判断した。

A. 研究目的

近年、薬剤反応性遺伝子における一塩基多型(SNP)解析に基づき、医薬品に対するレスポンダー・ノンレスポンダーの識別や副作用予測を行うことで、患者個々に適した投与薬剤の用法・用量の選択が可能になると考えられている。現在国内外において、種々の薬剤反応性遺伝子のSNPと投与薬物の血中濃度・治療効果、副作用発現等の相関性に関する情報が積み上げられている。しかし、薬物療法に影響を与えるSNPを有する患者に対して、臨床レベルでその診断を行い、薬物の適正使用を行っている施設は非常に少ないと言える。この原因として、簡易迅速な遺伝子診断法がスタンダード化されていないことが理由の一つとして考えられる。今回、「オーダーメイド薬物療法のための革新的なベッドサイド遺伝子診断法の開発と応用」の中で、臨床的に有用な薬剤反応性遺伝子のSNP検出法を開発すべく、その候補遺伝子を選択した。

B. 研究方法

薬剤反応性に影響を及ぼすと考えられる遺伝子を文献情報よりリストアップした。また、それらの遺伝子多型の人種差の有無、遺伝子診断法の種類を調査した。また、日本人にお

いて遺伝子多型の頻度分布が明らかでないものに対しては、約200人の日本人ボランティア検体を用いて、SNPスクリーニングを行った。

（倫理面への配慮）

今回の研究プロトコールは本邦における「ヒトゲノム・遺伝子解析研究に関する倫理指針」を遵守し、東北薬科大学・大学院倫理委員会および東北大学医学部倫理委員会に申請・承認された同名課題「薬剤反応性遺伝子の多型性が薬効及び薬物動態に与える影響に関する研究」に従って実施された。

C. 研究結果

様々な薬剤反応性に影響を及ぼすと考えられる遺伝子を文献情報よりリストアップし、その中で日本人種と白人種でSNPの頻度分布やハプロタイプに差があるものをピックアップできた。また、それらのSNPを検出するための遺伝子診断法を整理し、総説（Clin. Chim. Acta, 363, 177-186 (2006)）としてまとめた。次に、文献情報からリストアップした薬剤反応性遺伝子の中から、特に遺伝子型-表現型の関連性が明確であるもの及び日本人集団で比較的頻度が高い遺伝子を今回の遺伝子診断法で検出するための薬剤反応性遺伝子として選択した。その結果、ミトコンドリアDNA（1555A>G多型）、CYP2C19（*2, *3アレル）、NAT2（*5, *6, *7アレル）及びTPMT（*3Cアレル）のSNPが、日本人で比較的頻度が高く、遺伝子型－表現型の関連性が明確であり、臨床的に遺伝子多型情報として有用であると判断した。

また、CYP2D6及びMTHFRに関しては、現在本邦で繁用されている多くの医薬品の代謝に関与し、遺伝子多型の存在も報告されている。今回、日本人集団におけるSNPスクリーニングの結果、これまでに報告のないアミノ酸置換を起こす新規SNPが同定された。今後、これらのSNPにより酵素タンパク質の機能低下が起こるか否かを検証する必要がある。

D. 考察

今回の文献調査により、日本人集団において遺伝子多型情報を利用したオーダーメイド薬物療法を推進する場合、遺伝子型－表現型の関連が明確であり、比較的遺伝子多型頻度が高く、臨床的に有用である遺伝子は、ミトコンドリア DNA（1555A>G 多型）、CYP2C19（*2, *3 アレル）、NAT2（*5, *6, *7 アレル）及び TPMT（*3C アレル）と判断した。ミトコンドリア DNA（1555A>G 多型）は、アミノグリコシド系抗生物質の投与による不可逆的感音性難聴発症の危険因子である。CYP2C19 は消化性潰瘍治療薬オメプラゾール、ランソプラゾールの代謝酵素であり、この遺伝子に SNP を有するヒトでは代謝が遅延し、薬効が現れやすい。NAT2 は抗結核薬イソニアジドの代謝酵素であり、この遺伝子にSNPを有するヒトはイソニアジド投与により副作用が発現しやすい。TPMT は抗がん剤 6-メルカプトプリンの代謝酵素であり、この遺伝子に SNP を有するヒトは 6-メルカプトプリン投与により骨髄抑制などの重篤な副作用が起こりやすい。これらの遺伝子多型情報は薬物療法を行う前に有益な情報となる可能性が高い。

また、日本人集団でSNPスクリーニングしたCYP2D6及びMTHFRにおいて、新規SNPが同定されたが、それらの頻度は低く、遺伝子型－表現型の関連性も現在検討中であるため、今回の対象遺伝子からは除外した。

E. 結論

現時点で、遺伝子多型と薬剤反応性の関連性が明らかであり、臨床的に遺伝子多型診断が有用であると判断された遺伝子は、ミトコンドリアDNA(1555A>G多型)、CYP2C19(*2, *3アレル)、NAT2(*5, *6, *7アレル)及びTPMT(*3Cアレル)であった。

F. 健康危険情報

G. 研究発表

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H. 知的財産権の出願・登録状況

特になし

Ⅲ. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Hiratsuka M, Matsubara Y, Kure S, Mizugaki M, et al.	Competitive allele-specific short oligonucleotide hybridization (CASSOH) with enzyme-linked immunosorbent assay (ELISA) for the detection of pharmacogenetic single nucleotide polymorphisms (SNPs)	J Biochem Biophys Methods			in press
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Ⅳ. 研究成果の刊行物・別刷

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Competitive allele-specific short oligonucleotide hybridization (CASSOH) with enzyme-linked immunosorbent assay (ELISA) for the detection of pharmacogenetic single nucleotide polymorphisms (SNPs)

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Abstract

Individualization of drug therapy through genetic testing would maximize the effectiveness of medication and minimize its risks. Recent progress in genetic testing technologies has been remarkable, and they have been applied for the analysis of genetic polymorphisms that regulate drug responses. Clinical application of genetic information to individual health care requires simple and rapid identification of nucleotide changes in clinical settings. We previously reported a novel DNA diagnostic method for detecting single nucleotide polymorphisms (SNPs) using competitive allele-specific short oligonucleotide hybridization (CASSOH) with an immunochromatographic strip. We have developed the method further in order to incorporate an enzyme-linked immunosorbent assay (ELISA) into the final detection step; this

Abbreviations: CASSOH, competitive allele-specific short oligonucleotide hybridization; ELISA, enzyme-linked immunosorbent assay; SNP, single nucleotide polymorphism; mtDNA, mitochondrial DNA; NAT2, *N*-acetyltransferase 2; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; PCR-SSCP, polymerase chain reaction-single strand conformational polymorphism; FITC, fluorescein isothiocianate; HRP, horseradish peroxidase; TMB, 3,3′,5,5′-tetramethylbenzidine.

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enables multiple SNP detection. Special ELISA chips have been fabricated so that disposal of buffer waste is not required and handling procedures are minimized. This method (CASSOH-ELISA) has been successfully applied for the detection of clinically important SNPs in drug metabolism, such as *N*-acetyltransferase 2, *NAT2*6* (590G>A) and *NAT*7* (857G>A), and mitochondrial DNA (1555A>G). It would also facilitate point-of-care genetic testing for potentially diverse clinical applications.

Keywords: Genotyping; Single nucleotide polymorphism; Pharmacogenetics

1. Introduction

Single nucleotide polymorphisms (SNPs) are the most common type of genetic variations found in individuals. Clinical application of pharmacogenetic SNPs information is important in customizing the species of the drug and providing optimal dosage and schedule for individual patients. Pharmacogenetics involves determination of genetic polymorphisms that influence drug exposure levels. Specifically, increased toxicity or altered efficacy can result from variations in a gene that encodes an important drug-metabolizing enzyme. Alterations in genes that are known to influence the drug response and/or toxicity include SNPs at the loci for *N*-acetyltransferase 2 (*NAT2*) [1–3] and mitochondrial DNA (mtDNA) [4].

N-Acetyltransferase 2 (NAT2) exhibits hereditary determined polymorphism, and the individual phenotypes can be classified as rapid, intermediate, or slow acetylators according to their acetylation activity [1]. NAT2 metabolizes many drugs, including isoniazid, procainamide, and polycyclic amines such as several sulfonamides and hydralazines [5]. The relationship between polymorphic acetylation of isoniazid and procainamide by NAT2 and idiosyncratic drug toxicity has been well-documented [6]. This polymorphism shows racial differences; in the Caucasian population, nearly 50% are slow acetylators, whereas this frequency is only 10% in the Japanese population [7–9]. Among all the NAT2 allelic variants that have been identified [10], 3 variants (*NAT2*5* (341T>C), *NAT2*6* (590G>A), and *NAT2*7* (857G>A)) were shown to account for the majority of the slow acetylator genotype in Japanese subjects [9,11].

It has been reported that the administration of aminoglycoside antibiotics is likely to cause irreversible perceptive deafness in humans with an SNP at the 1555th base (A to G) in the 12S rRNA gene in mtDNA [4]. Aminoglycosides basically inhibit the synthesis of bacterial protein: however, in cases where the 1555th base is altered from A to G in the mtDNA in normal humans, the sequence shares a similar three-dimensional structure with bacterial 16S rRNA. Further, the site that originally does not have affinity becomes a target for aminoglycosides and protein synthesis involved in the mitochondrial electron transfer system, and oxidative phosphorylation is suppressed. As a result, ATP production decreases and hair cells in the internal ear show dysfunction [12]. Thus, it is necessary to perform routine genetic testing prior to aminoglycoside administration and promote counseling for mtDNA (A1555G) subjects to avoid side effects.

Prior to drug treatment, it is extremely desirable to use bedside genotyping methods to identify drug responders or nonresponders as well as patients with increased risk of toxicity. Numerous SNP detection methods have been developed, including PCR-RFLP, allele-specific PCR [13], PCR-SSCP [14], oligonucleotide ligation assay [15], TaqMan PCR [16–18], Invader assay [19,20], pyrosequencing [21], microarrays [22], and matrix-assisted laser desorption/ ionization-time of flight mass spectrometry [23]. However, these methods require either

cumbersome laboratory procedures or high-tech instrumentation for high-throughput analysis. None of these procedures are readily performed in local clinical laboratories in which molecular biology expertise is unavailable.

Matsubara and Kure [24] and our group [25] have recently developed a novel DNA diagnostic method for detecting SNPs using competitive allele-specific short oligonucleotide hybridization (CASSOH) with an immunochromatographic strip. The discrimination of a one-base mismatch is achieved by employing unusually short oligonucleotide probes (11–17 mer), which would reduce the reassociation of mismatched hybrids by a factor of two, thereby providing unsurpassed reliability and reproducibility. However, at present, the production of immunochromatographic strips (DNA detection test strips™ (Roche)) has been discontinued. In this study, we have devised a method for incorporating an enzyme-linked immunosorbent assay (ELISA) into the final detection step; this enables multiple SNP detection.

2. Materials and methods

2.1. Isolation of DNA from human blood

The local ethics committee of Tohoku Pharmaceutical University and Tohoku University Hospital approved the study, and all blood donors provided written, informed consent. DNA was isolated from K_2EDTA-treated anticoagulated peripheral blood using a DNA Extractor WB-Rapid kit (Wako Pure Chemical Industries, Osaka, Japan) or a QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions.

2.2. Oligonucleotides

The nonlabeled primers were synthesized by Fasmac, Inc. (Atsugi, Japan). The fluorescein isothiocyanate (FITC)-labeled primer and biotin-labeled probe were synthesized by Nihon Gene Research Laboratories, Inc. (Sendai, Japan).

2.3. CASSOH assay

The CASSOH assay for the detection of polymorphisms of *NAT2* (*NAT2*6* (590G>A) and *NAT2*7* (857G>A)) and mitochondrial DNA (1555A>G) was carried out according to the method described by Matsubara and Kure [24] and our group [25] with minor modifications. The principle of the method is illustrated in Fig. 1. A target sequence containing an SNP site was amplified by PCR using a pair of PCR primers, one of which was labeled with FITC at its 5′-end. The PCR reaction mixture also contained two sets of hybridization probes. One set was used for the detection of the variant type nucleotide sequence and consisted of an oligonucleotide containing the variant type sequence labeled with biotin at its 3′-end and an unlabeled oligonucleotide containing the wild sequence (reaction 1). The second set consisted of an oligonucleotide containing the wild sequence labeled with biotin at its 3′-end and an unlabeled oligonucleotide containing the variant type sequence (reaction 2). All hybridization probes were designed on the strand opposite to the FITC-labeled PCR primer. Table 1 presents a summary of the primers and probes used.

The PCR reaction was carried out in a mixture containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 250 μM each dNTP, 1 μM PCR forward primer, 1 μM PCR reverse primer, 600 nM biotinylated probe, 3 μM unlabeled competing probe, 1.25 U Ex Taq DNA polymerase

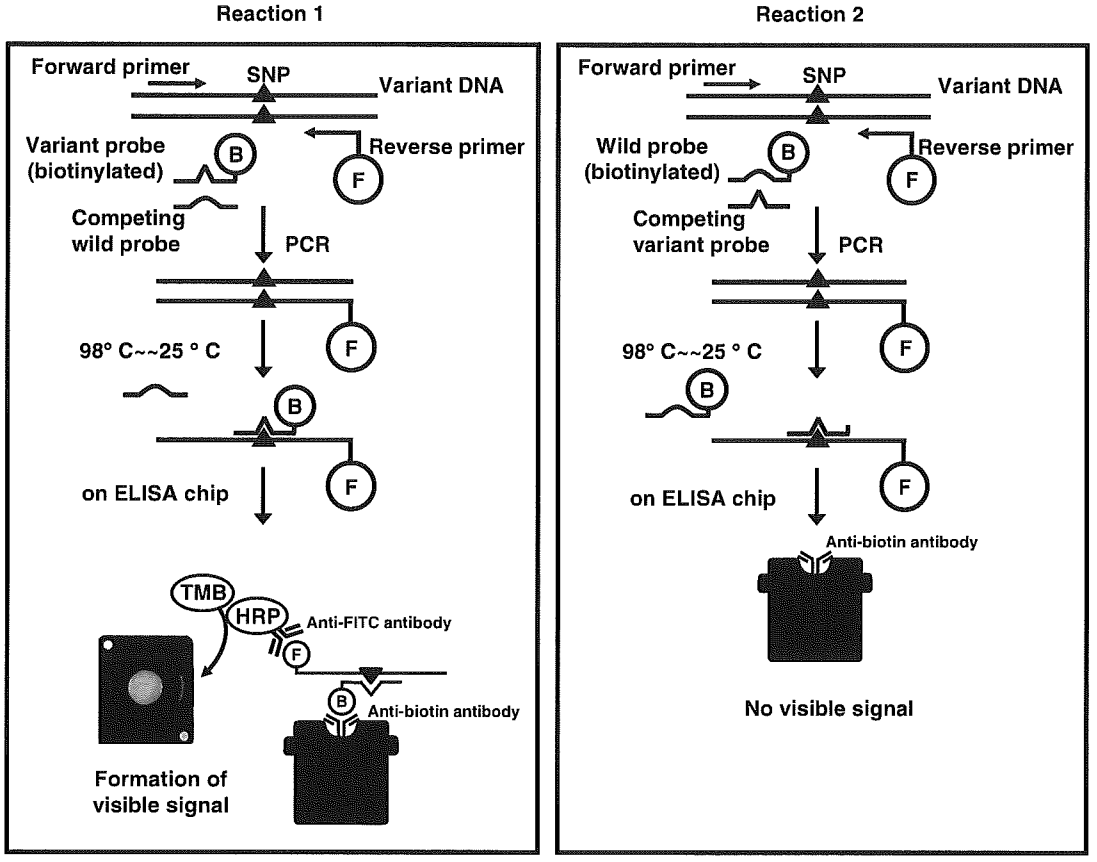

Fig. 1. CASSOH assay with the ELISA chip. Analysis of variant DNA using reaction 1 (left) for the detection of a variant sequence or reaction 2 (right) for the detection of a wild-type sequence. Closed triangles indicate an SNP site. F, fluorescein isothiocyanate (FITC) labeling; B, biotin labeling; HRP, horseradish peroxidase; TMB, 3,3′,5,5′-tetramethylbenzidine.

(Takara, Otsu, Japan), and 0.04–100 ng of genomic DNA in a total volume of 25 μL. The PCR reactions were performed in a BIO-RAD iCycler (Hercules, CA, USA) under the following cycling conditions: denaturation at 94 °C for 3 min followed by 35 cycles of denaturation at 98 °C for 10 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s, and, finally, successive incubations at 72 °C for 3 min, 98 °C for 3 min, 65 °C for 1 min, 55 °C for 1 min, 45 °C for 1 min, 35 °C for 1 min, and 25 °C for 1 min.

2.4. ELISA chip detection

As shown in Fig. 2, 50 μL of the block solution (1% casein in phosphate buffer (pH 7.2)) was pipetted onto the ELISA chip (Toyobo Co., Fukui, Japan). The ELISA chip uses a porous membrane filter (glass-fiber, 8 mm in diameter) immobilized anti-biotin antibody (IgG) on the top of the chip. The inside of the chip is filled with the absorbent pad which can hold a solution to approximately 400 μL. A mixture of 20 μL of the PCR reaction mixture plus 20 μL of the horseradish peroxidase (HRP)-labeled anti-FITC antibody solutions (DAKO, Glostrup, Denmark) was pipetted onto the ELISA chip. Following incubation for 4 min, 80 μL of the wash solution (10 mM phosphate buffer, 0.2% Tween 20) was pipetted onto the chip. After incubation for 1 min, 80 μL of the wash solution was again pipetted onto the chip. Incubation for 1 min was

Table 1
Sequences of PCR primers and hybridization probes

Gene (allele)		Forward primer		Reverse primer	
PCR primers					
*NAT2 (*6)*		5′-ttggaaacattaactgacattcttgag-3′		5′-FITC-tgtggttataaatgaagatgttggagac-3′	
*NAT2 (*7)*		5′-agggtatttttacatccctccagtt-3′		5′-FITC-ggtagagaggatatctgatagcacataagt-3′	
mtDNA		5′-cccaaactgggattagataccc-3′		5′-FITC-ttagctcagagcggtcaagttaag-3′	
Gene (allele)	Substitution	Detection of wild-type sequence		Detection of variant sequence	
		Biotinylated wild probe	Competing variant probe	Biotinylated variant probe	Competing wild probe
Hybridization probes					
*NAT2 (*6)*	590G>A	5′-aacctcGaacaa-Bio-3′	5′-gaacctcAaacaa-3′	5′-gaacctcAaacaa-Bio-3′	5′-aacctcGaacaa-3′
*NAT2 (*7)*	857G>A	5′-tgatgGatccct-Bio-3′	5′-gtgatgAatccct-3′	5′-gtgatgAatccct-Bio-3′	5′-tgatgGatccct-3′
mtDNA	1555A>G	5′-aggagAcaagtcg-Bio-3′	5′-aggagGcaagtc-3′	5′-aggagGcaagtc-Bio-3′	5′-aggagAcaagtcg-3′

Underlined nucleotides indicate targeted substitutions.
FITC, fluorescein isothiocyanate; Bio, biotin.

followed by pipetting 40 μL of a substrate solution containing 3,3′,5,5′-tetramethylbenzidine (TMB) onto the chip. The HRP-labeled anti-FITC antibody, FITC-labeled PCR products, and biotin-labeled probe complexes were further trapped by the anti-biotin antibody on the ELISA chip, resulting in a visible blue signal after 10 min. The genotype of the specimen was determined by the presence or absence of the blue signal in reactions 1 and 2.

3. Results

Fig. 3 shows representative results for *NAT2*6* (590G>A), *NAT2*7* (857G>A), and mtDNA (1555A>G) using the CASSOH-ELISA. False positive or false negative signals were not

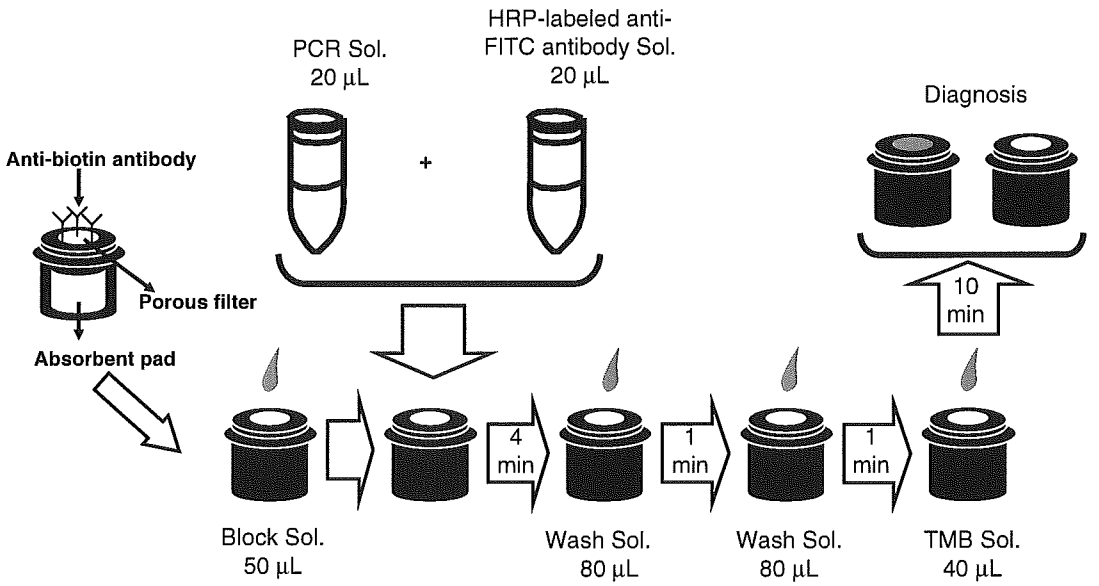

Fig. 2. Procedure for CASSOH with ELISA chips.

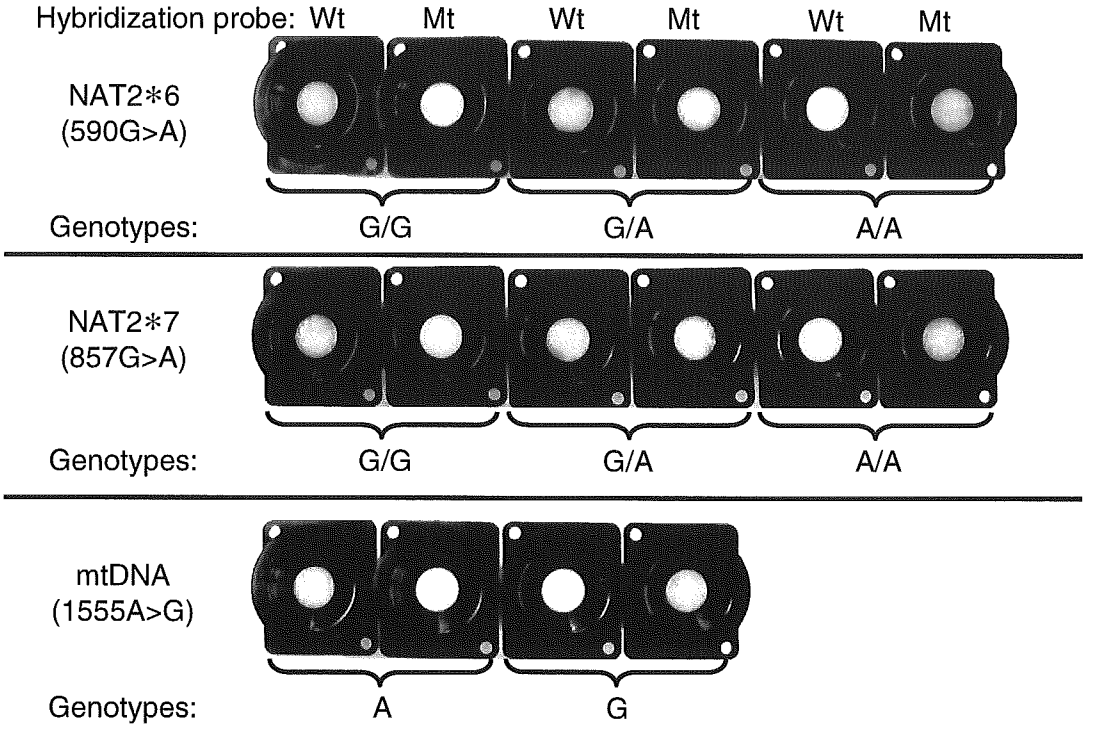

Fig. 3. Genotyping by CASSOH assay with ELISA chips. Detection of the *NAT2*6* (590G>A), *NAT2*7* (857G>A), and mtDNA (1555A>G) polymorphisms. Wt, detection probe for wild type; Mt, detection probe for variant type.

observed. A 100% match was observed in the genotyping results of the 590G-homozygotes, heterozygotes, and 590A-homozygotes of *NAT2*; the 857G-homozygotes, heterozygotes, and 857A-homozygotes of *NAT2*; and the 1555A-homoplasmy and 1555G-homoplasmy of mtDNA. Although signal intensities of the wild-type probes and variant probes in a heterozygous sample of *NAT2*6* were slightly different, no false negative signals were observed under the conditions. To estimate the precision and reproducibility of the assay, selected samples (n=3–17) of a known genotype were analyzed in duplicate. The genotype of all samples was tested by sequencing and CASSOH-ELISA, and identical results were obtained by the two methods (data not shown). Test results on the ELISA chip were stable for at least 60 min at room temperature (data not shown).

4. Discussion

The employment of the ELISA chip enabled low-tech detection of SNP, which is suitable for point-of-care DNA diagnosis in clinical settings. Advantages of the CASSOH-ELISA assay are that electrophoresis and disposal of buffer waste are not required and handling procedures are minimized. Although the ELISA chip cannot be purchased from other company except for the Toyobo Co., the ELISA part of this method can be carried out using a conventional ELISA plate. If the ELISA chip or palate is applied with samples, washed and detected blue signals automatically, SNP detection should be easier from the present way. One disadvantage of the ELISA chips in comparison with the immunochromatographic strip [24,25] is the number of steps required to complete the assay, which could increase the likelihood of carryover contamination. Sensitivity of the ELISA chip method was as high as that of the immunochromatographic strip method.

Primer design and reaction conditions were remarkably similar for the three different diagnostic tests. Therefore, the method may be readily applied to the detection of any SNP of interest, although empirical optimization of probe is required for each application. Especially, the length of an optimal wild-type probe may be different from that of an optimal mutant probe due to different base compositions.

The CASSOH-ELISA assay may be used for point-of-care genetic diagnosis for potentially diverse clinical application. For example, there is increasing evidence for the role of genetic polymorphisms in determining drug disposition and drug response and, thus, susceptibility to adverse drug reactions. Several SNPs that alter the rate of drug metabolism have been described in the genes of various drug-metabolizing enzymes, including CYP2C9, CYP2C19, CYP2D6, *N*-acetyltransferase 2, dihydropyrimidine dehydrogenase, and thiopurine methyltransferase [26]. Prospective bedside genotyping to identify poor drug metabolizers would allow individualization of drug therapy and thereby maximize efficacy and minimize toxicity.

5. Simplified description of the method and its future applications

In this research, we have developed a CASSOH-ELISA genotyping method for SNPs that are particularly important for the identification of drug responders or nonresponders and patients with increased risk of drug toxicity. This method is rapid and simple and should be suitable for routine clinical genotyping.

Acknowledgements

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Review

Genetic testing for pharmacogenetics and its clinical application in drug therapy

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Abstract

There is wide individual variation in drug responses and adverse effects. As the main causes of the variation in drug responses, attention has focused on the genetic polymorphisms that encode metabolic enzymes regulating pharmacodynamics and receptors modulating the affinity with the responsive sites. Tailor-made drug therapy analyzes genetic polymorphisms involved in drug responses before drug administration and selects drugs and doses suitable for the individual genetic background. Establishment of tailor-made drug therapy is expected to contribute to medical economy by avoiding wasteful drug administration. To promote such medical practice, it is necessary to use simple genetic testing that is clinically convenient. Currently, genetic testing using real-time PCR has been frequently employed at laboratories with its clinical application anticipated. As to the many genes involved in drug responses, to date, the application of patient genetic information to tailor-made drug therapy has been achieved at the practical level. Information on pharmacogenetics will be a critical factor in medical practice in the near future.

Keywords: Genetic testing; Genetic polymorphism; Pharmacogenetics; Real-time PCR; Single nucleotide polymorphism; Tailor-made drug therapy

Contents

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1. Introduction

There is wide individual variation in the pharmacodynamics of the administered drugs, effectiveness, and the appearance rates of adverse effects, and they are regulated by functions of the liver, kidneys, and heart, age, gender, circadian rhythm, diet, concomitantly administered drugs, and healthy foods. Recently, genetic polymorphism has drawn attention as one of such factors [1]. In particular, a single nucleotide polymorphism (SNP) of DNA is thought to produce a variation in drug responses and has become a representative research target in pharmacogenetics. In some cases, a difference in only one nucleotide results in a several- to ten-fold increase in maximum drug concentrations in the blood and frequent incidences of unexpected critical adverse effects. Today, it is anticipated that such genome information will be used to provide safer and more efficient tailor-made medicine for patients. If patient genome information is investigated before drug administration and appropriate drugs, doses, and administration timing can be predicted, a minimum drug dose will yield the maximum effect with the minimum side effects. If such medical practice is available, low efficiency in selecting drugs on a trial and error basis and drug administration as symptomatic therapy in cases of the emergence of side effects are expected to greatly change, which will contribute to medical economy. To date, polymorphisms of genes encoding drug metabolism enzymes, drug transporters, and drug receptors, which are involved in drug responses, have been reported and in some of them the association between pharmacodynamics and drug efficacy has been clarified [1,2].

In the future the expansion of tailor-made medicine using genome information, the development of simple and rapid genetic testing will be critical. Especially in the case of outpatients, unless results are returned within some tens of minutes to a few hours after the order of genetic testing, genome information cannot be used for prescription on the same day. Recently, new systems have been developed to detect genetic polymorphisms involved in drug responses in about 30 min to 2 hours by using real-time PCR.

In this review, we summarize the recent information on genetic polymorphisms involved in drug responses and demonstrate recent genetic testing including real-time PCR, the clinical application of tailor-made drug therapy, and the impact on medical economy.

2. Target genes of pharmacogenetics

2.1. P450 enzymes

A number of enzymes are involved in drug metabolism, and cytochrome P450 (CYP, P450) is regarded as the most important enzyme involved in drug metabolism, because many drugs are the substrate for the enzyme. P450 forms a superfamily consisting of a variety of molecules, and almost 20 kinds of enzymes involved in drug metabolism have been confirmed in humans (http://drnelson.utmem.edu/CytochromeP450.html). Of them, CYP3A4, CYP2D6, CYP2C9, CYP2C19, CYP2B6, and CYP1A2 play the most critical role in drug metabolism, and account for more than 90% of drugs metabolized by P450 [1,3]. P450 enzymes involved in the metabolism of the drugs currently available in medical practice are shown in Table 1. These molecules have proven genetic polymorphisms (Table 2), and a number of reports have shown the association between drug responses and genetic polymorphisms, in particular, as to CYP2D6, CYP2C9, and CYP2C19. Updated information can be found on the Human CYPallele Nomenclature Website (http://www.imm.ki.se/cypalleles). Phenotypes of P450 are divided into the extensive metabolizer (EM) that shows regular metabolic capacity and the poor metabolizer (PM) that shows low metabolic capacity. The PM phenotype carrying gene alterations on both alleles is inherited in an autosomal recessive manner. In addition, the categorization includes the intermediate metabolizer (IM) that shows the metabolic capacity between the PM and the EM, and the ultra-rapid metabolizer (UM) that shows higher metabolic capacity than the EM.

2.1.1. CYP2D6

In cases with CYP2D6, 5–10% of Caucasians are the PMs that have little enzymatic activity [4]. The frequency of the PM has racial diversity and the frequency of the CYP2D6 PM is less than 1% in Mongolian-origin races including Japanese [5,6]. Metabolism is delayed in a variety of drugs in the PM, and even the same drug dose is more likely to cause side effects, because plasma concentrations of the drug last longer at higher than normal levels. For example, in cases with the CYP2D6 PM, tricyclic antidepressants cause arrhythmia and thirstiness at higher rates [7]. To date, almost 50 genetic polymorphisms for CYP2D6

Table 1
Substrates of drug-metabolizing enzymes

Enzymes		Substrates
CYP1A2	Antidepressants	amitriptyline, clomipramine, fluvoxamine, imipramine
	Miscellaneous	clozapine, cyclobenzaprine, estradiol, haloperidol, mexiletine, naproxen, olanzapine, ondansetron, propranololriluzole, ropivacaine, tacrine, theophylline, verapamil, *R*-warfarin, zileuton, zolmitriptan
CYP2B6	Chemotherapeutic agents	cyclophosphamide, ifosfamide
	Miscellaneous	alfentanil, bupropion, efavirenz, ketamine, methadone, nevirapine, propofol, tamoxifen,
CYP2C9	Anticoagulants	*S*-warfarin
	Sulfonylureas	glibenclamide, glimepiride, glipizide, tolbutamide,
	NSAIDs	celecoxib, diclofenac, ibuprofen, mefenamic acid, meloxicam, naproxen, piroxicam, suprofen
	Antidepressants	amitriptyline, fluoxetine, imipramine
	Antihypertensives	irbesartan, losartan
	Miscellaneous	fluvastatin, nateglinide, phenytoin, rosiglitazone, tamoxifen, torsemide
CYP2C19	Benzodiazepines	diazepam
	Antidepressants	amitriptyline, clomipramine, imipramine
	Proton pump inhibitors	lansoprazole, omeprazole, pantoprazole, rabeprazole
	Miscellaneous	carisoprodol, citalopram, cyclophosphamide, hexobarbital, indomethacin, *S*-mephenytoin, mephobarbital, nelfinavir, nilutamide, phenobarbitone, phenytoin, primidone, progesterone, proguanil, propranolol, teniposide
CYP2D6	Antiarrhythmics	amiodarone, aprindine, encainide, fmexiletine, idocaine, lecainide, lsparteine, propafenone, *N*-propylajmaline
	Opiates	codeine, dextromethorphan, dihydrocodeine, ethlmorphine, hydrocodone, norcodeine, oxycodone, tramadol
	Antihypertensives	alprenolol, carvedilol, bufuralol, bunitrolol, bupranolol, debrisoquine, guanoxan, indoramin, *S*-metoprolol, propranolol, timolol
	Antidepressants	amiflamine, amitriptyline, brofaromine, citalopram, clomipramine, desipramine, desmethylcitalopram, fluoxetine, fluvoxamine, imipramine, maprotiline, minaprine, moclobemide, nortriptyline, paroxetine, tomoxetine, trimipramine, venlafaxine
	Antipsychotics	chlorpromazine, clozapine, haloperidol, perphenazine, risperidone, sertindole, thioridazine, zuclopentixol
	Miscellaneous	cinnarizine, dolansetron, methoxyamphetamine, methoxyphenamine, metoclopramide, nicergoline, ondansetron, phenformin, promethazine, tamoxifen, tolterodine, tropisetron
CYP3A4	Immune modulators	cyclosporine, tacrolimus
	Benzodiazepines	alprazolam, diazepam, midazolam, triazolam
	Ca channel blockers	amlodipine, diltiazem, felodipine, lercanidipine, nefedipine, nisoldipine, nitrendipine, verapamil
	Chemotherapeutic agents	busulfan, docetaxel, etoposide, irinotecan, paclitaxel, tamoxifen, vinblastine, vincristine
	HMG-CoA reductase inhibitors	atorvastatin, cerivastatin, lovastatin, simvastatin
	Estrogens, corticosteroids	estradiol, hydrocortisone, progesterone, testosterone
	Macrolide antibiotics	clarithromycin, erythromycin
	Protease inhibitors	indinavir, nelfinavir, ritonavir, saquinavir
	Antihistamines	astemizole, chlorpheniramin, terfenadine
	Miscellaneous	buspirone, cisapride, cilostazol, cocaine, dapsone, dextromethorphan, domperidone, fentanyl, finasteride, imatinib, lidocaine, methadone, nateglinide, pimozide, ondansetron, quinine, salmeterol, sidenafil, sirolimus, trazodone, zaleplon, zolpidem
TPMT	Chemotherapeutic agents	mercaptopurine, thioguanine
	Immune modulators	azathiprine
DPD	Chemotherapeutic agents	carmofur, doxifluridine, 5-fluorourasil, tegafur
NAT2		isoniazid, hydralazine, procainamide, sulfamethoxazole
UGT1A1		bilirubin, irinotecan
COMT		levodopa

have been reported (http://www.imm.ki.se/cypalleles). Of them, CYP2D6*3, CYP2D6*4, and CYP2D6*5 are the major genetic polymorphisms involved in the PM of Caucasians [8]. More than 90% of the PMs in Caucasians are ascribable to these three genetic polymorphisms. Moreover, the existence of CYP2D6*10, a genetic polymorphism of the IM that shows lower CYP2D6 metabolic capacity but not as low as the PM, has been identified [9]. This genetic polymorphism is markedly prevalent in Japanese and Chinese.

2.1.2. CYP2C9

CYP2C9 is involved in the metabolism of an antiepileptic agent phenytoin and an anticoagulant warfarin. To date, 12 CYP2C9 variant alleles have been reported (http://www.imm.ki.se/cypalleles). Of these, a decrease in activity was confirmed in cases with CYP2C9*3 by the expression system using COS cells and yeast and in vivo tests on the normal volunteers and patients whose genetic polymorphisms were known [10,11]. For example, in cases with phenytoin, oral clearance decreased to one quarter in the

Table 2
Pharmacogenetics of drug-metabolizing enzymes (P450)

Gene	Genotypes	Major allelic variants	Phenotypes: Frequency	
			Caucasian	Asian (Japanese)
CYP1A1	*1–*11			
CYP1A2	*1–*14	*1F, *2, *3, *4, *5, *6		
CYP1B1	*1–*26			
CYP2A6	*1–*17	*2, *4, *7, *9, *10		
CYP2A13	*1–*9			
CYP2B6	*1–*15			
CYP2C8	*1–*5			
CYP2C9	*1–*13	*3	EM: 97% PM: 3%	
CYP2C19	*1–*16	*2, *3	EM: 94–98% PM: 2–6%	EM: 80% PM: 20%
CYP2D6	*1–*51	*2×N, *3, *4, *5, *6, *10, *17	EM: 90–97% PM: 3–10%	EM: 99% PM: 1%
CYP2E1	*1–*7	*2, *3, *4		
CYP2J2	*1–*7			
CYP2R1	*1–*2			
CYP2S1	*1–*3			
CYP3A4	*1–*19	*4, *5, *6		
CYP3A5	*1–*10	*3, *6		
CYP3A7	*1			
CYP3A43	*1–*3			
CYP4B1	*1–*7			

A description of the alleles can be found on the human cytochrome P450 allele nomenclature committee home page (http://www.imm.ki.se/CYPalleles/).
EM=extensive metabolizer; IM=intermediate metabolizer; PM=poor metabolizer.

subjects with homozygous polymorphism for CYP2C9*3 [12]. Oral clearance for (S)-warfarin decreased to less than half in the subjects with heterozygous polymorphism for CYP2C9*3 (CYP2C9*1/*3) and to less than 10% in the patients with homozygous polymorphism for CYP2C9*3 [13].

2.1.3. CYP2C19

CYP2C19 is an enzyme involved in the hydroxylation of the S form of an anti-epileptic agent mephenytoin, and also in the metabolism of a series of proton pump inhibitors such as omeprazole. The PM for CYP2C19 is found in about 20% of Japanese and about 3% of Caucasians [14]. To date, 15 variant alleles responsible for the PM for CYP2C19 have been identified (http://www.imm.ki.se/cypalleles), and almost all PMs in Japanese are ascribable to the two genetic polymorphisms CYP2C19*2 and CYP2C19*3. Omeprazole concentrations in the blood after oral intake of omeprazole were significantly different in each genetic polymorphism for CYP2C19, and the concentration in the blood 10 h after the intake in the PM was comparable to the peak concentration in the EM [15]. When the area under the plasma concentration–time curve was compared, the PM was reported to be different from the EM by about 13 times [16].

2.2. Non-P450 enzymes

2.2.1. Dihydropyrimidine dehydrogenase (DPYD)

Dihydropyrimidine dehydrogenase (DPD) is a rate-limiting enzyme for the metabolism of an anticancer drug 5-FU, and more than 85% of administered 5-FU is metabolized by DPD. The gene encoding DPD is called DPYD, and 13 genetic polymorphisms have been reported [17,18]. In particular, the genetic polymorphism that decreases DPD activity has been reported to be DPYD*2 with a polymorphism at the splicing recognition site [19]. Administration of 5-FU to the patients with decreased DPD activity increases the adverse events such as leukocytopenia, stomatitis, diarrhea, nausea and vomiting, and cerebellum disorder [20].

2.2.2. Thiopurine S-methyltransferase (TPMT)

Thiopurine *S*-methyltransferase (TPMT) is involved in the detoxification and metabolism of an anti-leukemia drug 6-mercaptoprine (6-MP) and an immunosuppressant azathioprine. In Caucasian infant patients with acute myeloid leukemia, those who carried TPMT*2, TPMT*3A, TPMT*3B, or TPMT*3C showed significantly higher concentrations of the 6-MP metabolite in the red blood cells. In addition, a dose reduction or termination of the administration was reported to be necessary in all patients due to the adverse effects such as myelosuppression [21].

2.2.3. N-acetyltransferase 2 (NAT2)

An individual variation in *N*-acetylation activity of an anti-tuberculosis drug isoniazid has been reported to be ascribable to the genetic polymorphism for *N*-acetyltransferase 2 (NAT2). NAT2 is categorized into the rapid acetylator (RA), the intermediate acetylator (IA), and the slow acetylator (SA) according to the acetylation activity. The frequency of the SA is about 50% in Caucasians and about 10% in Japanese [22,23]. To date, more than 10 variant alleles have been identified (http://www.louisville.edu/medschool/pharmacology/NAT.html), and three genetic polymorphisms NAT2*5, NAT2*6, and NAT2*7, but not NAT2*4 (wild type alleles), are responsible for almost all SAs in Japanese [23]. Drug-induced hepatitis by isoniazid occurs more than twice in the SA for NAT2 than in the RA [24,25].

2.2.4. Other non-P450 enzymes

Other than the above-mentioned enzymes for drug metabolism, genetic polymorphisms for UDP-glucuronic acid transferase (UGT) 1A1 [26], glutathione-*S*-transferase (GST) [27], and thymidylic acid synthase (TS) [28] are thought to be responsible for the individual variation in drug responses. The relation between these genes and drug responses is shown in Table 3.