Current Updates in the Genetic Polymorphisms of Human Organic Anion Transporters (OATs)

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Abstract

The Organic Anion Transporters (OATs) are a family of important membrane proteins responsible for the absorption, distribution and excretion of a wide range of substances, including many clinically important drugs. Therefore, the function of OATs is the key determinant of drug response in human. Human genetic polymorphisms often result in the functional alternation of OATs, which in consequence, leads to the interpatient variation of therapies. In this review, we summarize the current findings about OATs, with a focus on the genetic polymorphisms and their influence on transporter function and drug performance, the information of which is critical in elucidating the essential roles of OATs played in body, as well as forming the basis of future therapeutic optimization in individuals.

Keywords: Organic anion transporters; Genetic polymorphism; Single nucleotide polymorphism

Abbreviations: OAT: Organic Anion Transporters; SNPs: Single Nucleotide Polymorphisms; PAH: Para-Amino Hippurate; ES: Estrone-3-Sulfate; AF: Allele Frequency

Introduction

Interrapent variation of drug response becomes a critical issue in rational pharmacotherapy [1,2]. It is known that drug-metabolizing enzymes and influx/exflux transporters are responsible for the Absorption, Distribution, Metabolism and Elimination (ADME) of drugs in the body, therefore, the function of these genes are the key determinants of therapeutic outcomes in individuals [1-5].

The Solute Carrier Transporters (SLCs) are a superfamily of membrane proteins, responsible for the cellular influx of various substances, including many clinically important drugs. Among all the SLC members, the Organic Anion Transporters (OATs) are essential SLCs, playing critical roles in drug disposition and elimination. Intensive scientific and medical interests have been directed towards OATs, due to their involvement in excretion of various therapeutic agents [6-8]. Since, genetic variations often lead to functional alternation of these proteins; naturally occurred polymorphisms of OATs have been widely explored in the last decade, so as to elucidate the variable response of drugs in human.

Transporter Function and Tissue Distribution of OATs

Up to date, there are ten OAT isomembers, identified in humans. Among them, OAT1–4 have been extensively studied. OAT1 is mainly localized across the basolateral membrane of proximal tubules in the kidney, where it mediates the transport of various substances, by an outwardly directed dicarboxylate concentration gradient [9,10]. OAT2 is predominantly expressed in the liver, and weakly in the kidney [11]. As a sodium-independent multispecific organic anion/DimethylDicarboxylate exchanger, OAT2 is highly involved in the hepatic handling of organic anions [12]. OAT3 is also found at the basolateral membrane of proximal tubule cells, where it is responsible for transporting organic anions across the membrane, through a tertiary transport mechanism [13-17]. Dissimilar to OAT1 and 3, OAT4 is expressed at the apical membrane of renal proximal tubules and the basolateral membrane of syncytiotrophoblasts, in the placenta [18,19]. Therefore, it plays an important role in the reabsorption of organic anions from urine, as well as mediates the transfer of nutrients from the mother to the fetus [20]. OAT5 and OAT7 are expressed in human liver, while OAT10 were found in the apical membrane of proximal tubules cells [21-23]. Minimal information is available for these OATs, in particular, their genetic polymorphisms.

OATs are characterized by their multispecific substrate recognition. They are capable of dealing with small, amphiphilic organic anions with diverse structures, uncharged molecules, as well as some organic cations. Therefore, their substrate spectrum covers a broad range of common drugs, such as β-lactam antibiotics, anti-virals, angiotensin converting-enzyme inhibitors, non-steroidal anti-inflammatory drugs, diuretics and antineoplastics [7,24-26]. Due to their localizations at the boundary epithelia, they are also involved in the renal excretion and detoxification of a variety of endogenous compounds, including hormones and neurotransmitter metabolites [27]. In addition, the interactions of OATs with various natural compounds have also been elucidated [28-31]. Therefore, drug-drug/food interactions might occur, when drugs and other compounds are co-administered, and compete for the same transporter.

Molecular Regulation of OATs

It has been reported that OATs can be regulated through multiple signaling pathways. Previous studies have revealed that, Hepatocyte

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Nuclear Factor (HNF)-4α and other HNF isoforms, such as HNF-1α and/or β, could activate OAT genes at the transcriptional level [32-34]. Likewise, OATs were also subject to the regulation of HNF-1α homodimer, HNF-1β homodimer or HNF-1 α/β hereodimer [35]. Additionally, Ogasawara et al. [36] described that OAT promoter activity could be affected by the phosphorylation of CAMP-response element binding protein 1 and activating transcription factor 1. However, Kikuchi et al. [35] discovered that promoter methylation could down regulate OAT function. At post-translational level, Protein Kinase C (PKC) regulated OAT functions through carrier mediated trafficking [37-41]. In addition, OAT activities could also be manipulated by phosphorylation at serine residue, or by epidermal growth factor [42-45].

Knockout Mice Models of OATs

Knockout mouse model has been recognized as the clinical relevant tool, in characterizing specific gene. OAT1 and OAT3 knockout mice models manifested a profound loss of organic anion transport, although no obvious morphological or physiological abnormalities were observed [46-49]. In OAT1 knockout mice, the uptake of its model substrate, Para-Amino Hippurate (PAH) was significantly inhibited in isolated renal slices and urinary secretion, whereas the transport of taurocholate, Estrone-3-Sulfate (ES), PAH, and fluorescein were remarkably reduced in the OAT3-null mice [46,47]. Furthermore, OAT3-knockout mice appeared to have a lower renal Blood Pressure (BP) compared to the normal little mates [49], which observation suggested the potential treatment of high blood pressure, through regulating OAT3 function. More recently, Xue et al. [48] discovered that OAT1 and OAT3 are responsible for the cellular transport of Aristolochic Acid I (AAI), which agent causes severe kidney injury. Further study also revealed that lesions were significantly relieved in OAT1 and OAT3 knockout mice, therefore, these two transporters could become the novel targets in AAI treatment in the future.

Genetic Polymorphisms of OATs

As discussed formerly, naturally occurring genetic variations in OATs may account for various interpatient responses towards drug treatment. It is crucial to discover polymorphisms in these genes, and to investigate the pharmacological consequences of these mutations. Currently, relatively more research has been done regarding genetic polymorphisms in OAT1 – 4, whilst that of the other OAT isomers has not been explored.

OAT1

To thoroughly investigate individual differences in response to drugs, 96 healthy individuals representing the major ethnic geographic divisions were analyzed by Xu et al. [50]. Five SNPs were identified in the coding regions of hOAT1, three of which are synonymous (252G>T, 351A>G, and 1233C>T) and found in several ethnic groups (Indo-Pakistani, Japanese, Sub-Saharan African, Ashkenazi Jewish, North Saharan African and Northern European); whereas two nonsynonymous SNPs, 20T>C and 149G>A, were only discovered in the Southeast Asian and Sub-Saharan African population, respectively. Three SNPs, 149G>A, 252G>T, and 351A>G were also reported in the study of 92 individuals from African, Asian, and Caucasian origins by Blesby et al. [51]. In addition, seventeen SNPs were simultaneously identified in this study, among which, five were in the untranslated region, six were intrinsic and the other six were exonic. Two of the non-silent mutations in the translating region resulted in amino acid substitutions of R50H and K525I, respectively. R50H was found in both the African American and Pygmy ethnic groups, while K525I was represented by an individual of African American descent [51]. Furthermore, Fujita et al. [2] analyzed 276 DNA samples from US population and reported nine novel coding region mutations, including five non-synonymous SNPs coding the variants of P104L, I226T, A256V, R239W, and R454Q. Recently, eight SNPs including two novel SNPs (424G>T in intron 1 and 1377A>G in exon 9) were described by Shin et al. [52] with genotyping of the cohort of 50 Korean subjects; however, no amino acid alteration was observed in this study. A later study with 183 human subjects from five ethnic groups demonstrated the allelic frequencies of 505C>T and 149G>A (R50H) as 0.23 and 0.017, respectively [53], which was consistent with the earlier studies [2,51]. In the untranslated and intronic regions of SLC22A6, a total of five SNPs were identified by Iida et al. [54] from a cohort of 48 Japanese. Furthermore, one SNP (-3,655G>A) was also spotted in the evolutionarily conserved 5’ regulatory region of SLC22A6 [55]. This polymorphism was present in a Pacific Islander descent, and accounted for 0.5% in the sample population [55].

In order to investigate the potential impact of polymorphisms on OAT1 activity, functional analysis was conducted in various studies with a range of substrates. The R50H variant was reported to have an increased uptake of adefovir, cidofovir, and tenofovir, in contrast to an unchanged transport of PAH [51]. The function of variants, K525I, R50H, P104L, I1226T, A256V, R239W and R454Q was found to be identical to that of the reference transporter [2,51]. Interestingly, the function of R454Q variant was completely abolished in uptaking PAH, OTA and MTX; however, it has no influence on the renal clearance of adefovir [2]. Further kinetic studies revealed that both $V_{\text{max}}$ and $K_{\text{m}}$ of R50H and R293W variants, were not considerably distinct from the reference in the influx of PAH and OTA [2]. Likewise, Vormfelde et al. [56] indicated that four genetic variations with the highest allele frequency of 0.29 had no effect on the torsemide renal clearance. Up to now, the protein expressions of OAT1 genetic variants has not yet been investigated.

OAT2

Currently, there were relatively fewer natural occurring SNPs identified in OAT2 gene as compared to OAT1, OAT3 and OAT4. As shown in Table 1, Iida et al. [54] screened 48 Japanese candidates and detected one synonymous SNP in the coding region (1269C>T), four intronic SNPs and two SNPs in the 3' non-coding region Similarly, Xu et al. [50] analyzed 96 subjects with ethnically diverse background and reported the same SNP (1269C>T, as well as three additional non-synonymous SNPs (329C>T, 571G>A and 1520G>A), which coded the transporter variants T110I, V192I and G507D, respectively. All of the non-synonymous SNPs mentioned above, had low allele frequency (0.5%, n=192), with only one individual for each SNP identified in the entire cohort. The three human subjects were originated from Sub-Saharan Africa (329C>T), Indo-Pakistani (571G>A) and Japan (1520G>A), respectively [50]. However, the SNP 1269C>T appeared to be more common with a minor allele frequency of 40%, across a wide range of ethnic groups [50]. In the study of 50 Korean human subjects, an additional synonymous SNP (1539G>A) and three more intronic SNPs (14+269G>A, 17+154T>C and 19+206A>G), were recently reported [52]. However, Ogasawara et al. [57] observed no polymorphism in the promoter region of OAT2, with 63 kidney samples from Japanese.

The molecular consequences of these SNPs largely remain unclear. The only study ever conducted was by Shin et al. [52], in which the
| Gene        | dbSNP     | Genetic Polymorphism | Minor Allele Frequency (%) | Position | Functional Change | Protein Expression | Transporter Kinetic Characteristics |
|------------|-----------|----------------------|---------------------------|----------|-------------------|-------------------|-------------------------------------|
| SLC22A6/OAT1/NKT | rs4149171 | -3855G>A[55]         | 0.5[55]                   | 5' UTR   | N.D.              | N.D.              | N.D.                                |
|            | rs57677322 | -560G>G[51, 54]      | 29.5[51]                  | 5' UTR   | N.D.              | N.D.              | N.D.                                |
| rs95434    |           | G>A[51]              | N.D.                      | 5' SLC22A6| TOR CL no change  | N.D.              | N.D.                                |
|            | rs11566826 | 14G>A[2, 50, 51, 53] | 1.1[2], 0.5[50], 4.2[51], 1.7[53] | Arg50His | PAH[2.51]/OTA[2]/MTX[2] uptake no change | N.D.              | Kₐ and Vₘₐₓ of PAH / OTA / ADV / CDV no change [2] |
| rs11566830 |           | 18G>T[2]             | 0.4[2]                    | Lys66Lys | N.D.              | N.D.              | N.D.                                |
| rs11566830 |           | 18C>T[51]             | 0.6[51]                   | Asn60Asn | N.D.              | N.D.              | N.D.                                |
| rs11566825 |           | 18G>A[2]             | 0.2[2]                    | Gly62Gly | N.D.              | N.D.              | N.D.                                |
| rs11566828 |           | 25G>T[2]             | 3.6[2], 4[50], 2.7[51]    | Pro84Pro | N.D.              | N.D.              | N.D.                                |
| rs1156627  |           | 311C>T[2]            | 0.2[2]                    | Pro104Leu| PAH / OTA / MTX uptake no change[2] | N.D.              | N.D.                                |
| rs1156629  |           | 351A>G[2, 50, 51]    | 3.6[2], 4[50], 3.2[51]   | Pro117Pro| N.D.              | N.D.              | N.D.                                |
| rs1156623  |           | 67T>C[2]             | 0.2[2]                    | Ile226Thr| PAH / OTA / MTX uptake no change[2] | N.D.              | N.D.                                |
| rs1156624  |           | 767C>T[2]            | 0.2[2]                    | Ala256Val| PAH / OTA / MTX uptake no change[2] | N.D.              | N.D.                                |
| rs138717295|           | 873C>T[51]           | 0.6[51]                   | Gly279Gly| N.D.              | N.D.              | N.D.                                |
| rs45607933 |           | 877C>T[2]            | 0.6[2]                    | Arg293Trp| PAH / OTA / MTX uptake no change[2] | N.D.              | Kₐ and Vₘₐₓ of PAH / OTA / ADV / CDV no change[2] |
| rs11566819 |           | 1233C>T[2, 50]       | 0.2[2], 1[50]            | Leu411Leu| N.D.              | N.D.              | N.D.                                |
| rs1156634  |           | 1361G>A[2]           | 0.2[2]                    | Arg454Gln| PAH / OTA / MTX transport loss[2] | N.D.              | N.D.                                |
| rs1156635  |           | 1470C>G[2, 51]       | 0.4[2], 0.5[51]           | Tyr490Tyr| N.D.              | N.D.              | N.D.                                |
| rs10897312 |           | 1575A>T[51]          | 0.5[51]                   | Lys525Ile| PAH uptake no change[51]  | N.D.              | Kₐ and Vₘₐₓ of ADV / CDV / TNV no change[51] |
| rs4149174  |           | 168G>A[52]           | 8.3[51]                   | Lys66Lys | N.D.              | N.D.              | N.D.                                |
| rs3017670  |           | -447T>C[52]          | 5[52]                     | Intron8  | N.D.              | N.D.              | N.D.                                |
| rs2276300  |           | 33C>T[51, 52, 54]    | 5.6[51], 16[52]           | Intron5  | N.D.              | N.D.              | N.D.                                |
| rs4149174  |           | 168C>T[52, 54]       | 45[52]                    | Intron6  | N.D.              | N.D.              | N.D.                                |
| rs10897312 |           | C>T[56]              | N.D.                      | Intron  | TOR CL no change [56] | N.D.              | N.D.                                |
|            |           | -847T>C[51]          | 0.5[51]                   | 3' UTR   | N.D.              | N.D.              | N.D.                                |
| rs736342   | C>T [56] | N.D. | 3'SLC22A6 TOR CL no change [56] | N.D. | N.D. |
|------------|----------|------|---------------------------------|------|------|
| rs73625099 | 329C>T [50] | 0.5 [50] | Thr110Ile N.D. | N.D. | N.D. |
| 571G>A [50] | 0.5 [50] | Val192Ile N.D. | N.D. | N.D. |
| rs2270860  | 1269C>T [50, 54] | 40 [50, 14][52] | Ser423Ser N.D. | N.D. | N.D. |
| rs70953693 | 1520G>A [50] | 0.5 [50] | Gly507Asp N.D. | N.D. | N.D. |
| rs70953694 | 1539G>A [52] | 2.0 [52] | Thr513Thr N.D. | N.D. | N.D. |
| rs2814647  | 1644G>A [52, 54] | 22.0 [52] | N.D. | N.D. |
| 842G>A [54] | N.D. | intron 4 N.D. | N.D. | N.D. |
| rs1574430  | 33C>A [54] | 20.0 [52] | N.D. | N.D. |
| rs2841648  | 183A>C [52, 54] | 22.0 [52] | N.D. | N.D. |
| rs2841647  | 184C>T [54] | 0.5 [54] | N.D. | N.D. |
| rs11231306 | -1822G>C [55] | 46.9 [55] | N.D. | N.D. |
| rs11386069 | 183G>A [55] | 0.5 [55] | N.D. | N.D. |
| rs3840764  | -153G>A [50,54, 58] | 8[50], 7.5[58] | Pro51Pro N.D. | N.D. | N.D. |
| rs10792369 | T>C [56] | 29 [56] | 5' UTR N.D. | N.D. | N.D. |
| rs948980  | -578C>G [54,57] | 18.3 [57] | 5' FR N.D. | N.D. | N.D. |
| rs948979  | -515C>A [56,57] | 23 [56, 15.9][57] | 5' UTR N.D. | N.D. |
| rs3069069 | -461T>C [54,57] | 26.2[57] | N.D. | N.D. |
| rs94002361 | -19C>A [57] | 1.6[57] | N.D. | N.D. |
| rs11231306 | -1822G>C [55] | 46.9 [55] | N.D. | N.D. |
| rs11386069 | 183G>A [55] | 0.5 [55] | N.D. | N.D. |
| rs4149180  | 153G>A [50,54,58] | 8[50], 7.5[58] | Pro51Pro N.D. | N.D. | N.D. |
| rs11568479 | 387C>A [1] | 0.2[1] | Phe129Leu N.D. | N.D. | N.D. |
| rs45566039 | 445C>A [1] | 0.4[14, 15] | Arg149Ser N.D. | N.D. | N.D. |
| rs201866442 | 523A>G [50] | 0.5[50] | Ile175Val N.D. | N.D. | N.D. |
| rs11568496 | 715C>T [1] | 0.2[14, 15] | Gln239Ter N.D. | N.D. |
| rs2276299  | 723G>A [50, 54,56,58] | 24 [50, 13][56], 27.5 [58] | Thr241Thr TOR [56] / PRA CL no change[58] | N.D. | N.D. |
| rs11568493 | 779T>G [1] | 0.2 [14, 15] | Ile260Arg N.D. | N.D. |
| rs11568492 | 829C>T [1] | 0.2 [14, 15] | Arg277Trp N.D. | N.D. |
| rs45438191 | 842T>C [1] | 1.7 [1] | Val281Ala N.D. | N.D. |
| rs11568482 | 913A>T [1] | 0.9 [1, 59] | Ile305Phe N.D. | N.D. |
| rs11568481 | 929C>T [1] | 0.2[1] | Ala310Val N.D. | N.D. | N.D. |
| 1166C>T [58] | 0.8[58] | Ala389Val N.D. | N.D. |
| rs11568497 | 1195G>T [1] | 0.2[1] | Ala399Ser N.D. | N.D. |
| rs11568486 | 1342G>A [1] | 0.7[1] | Val448Ile N.D. | N.D. |
| 1777C>C [54] | N.D. | intron 2 N.D. | N.D. | N.D. |
| rs      | SNP     | Allele Change | Description | Genotype |Frequency | OR | Reference |
|---------|---------|---------------|-------------|----------|----------|----|-----------|
| 6201T>G | G       | N.D.          | intron 2    | N.D.     | N.D.     | N.D. |          |
| 79G>C   | C       | N.D.          | intron 3    | N.D.     | N.D.     | N.D. |          |
| 524T>C  | C       | N.D.          | intron 5    | N.D.     | N.D.     | N.D. |          |
| 386G>A  | A       | N.D.          | intron 7    | N.D.     | N.D.     | N.D. |          |
| rs953894| G       | 25[56]        | intron 7    | TOR CL no change[56] | N.D. | N.D. |          |
| rs2187383| A       | 36[56]        | intron 8    | N.D.     | N.D.     | N.D. |          |
| rs4963326| T       | 31[56]        | intron 9    | N.D.     | N.D.     | N.D. |          |
| SLC22A11/OAT4 | G>T | A>T[56]        | 3' SLC22A11 | TOR CL [56] | N.D. | N.D. |          |
| rs3759053| -18C>T  | 45[56], 3.2[57] | 5' PR       | TOR CL [56] | Promoter activity and mRNA expression no change[57] | N.D. |          |
| rs75976740| 37G>A   | 0.5[50]       | Val13Met    | ES uptake no change [62] | N.D. | N.D. |          |
| rs11231819| 86T>C   | N.D.          | Leu29Pro    | ES [81, 62] / OTA / UA [61] uptake loss | Minimal to no protein[61] ≤ 10% of WT[62] | N.D. |          |
| rs11231820| 91A>G   | N.D.          | Ile31Val    | ES [62] / OTA / UA [61] uptake no change | N.D. | N.D. |          |
| rs35008345| 142C>T  | 0.5[50], 0.78[61] | Arg48Ter    | ES [61, 62] OTA / UA [61] uptake loss | Minimal to no protein[61] | N.D. |          |
| rs200043601| 185G>A  | 0.5[50]       | Thr62Arg    | ES uptake no change | N.D. | N.D. |          |
| rs2277313 | 312C>T  | 1[50]         | Ser104Ser   | N.D.     | N.D.     | N.D. |          |
| PMT-2945 | 361C>T  | 0.5[61]       | Arg121Cys   | ES / OTA / UA uptake no change [61] | N.D. | K_s of ES no change V_{max} of ES [61] |          |
| rs61744144| 463G>A   | 0.5[50], 0.38[61] | Val155Met  | ES [61, 62] / OTA / UA [61] uptake no change | N.D. | K_s and V_{max} of ES no change[61] |          |
| rs11275832| 464T>G   | 0.18[61], 1[62] | Val155Gly   | ES uptake [62] / OTA / UA [61] uptake no change | [62] | K_{V_{max}} of ES no change[62] K_{V_{max}} of ES no change[61] |          |
| rs72559737| 483C>A   | 3[50]         | Gly161Gly   | N.D.     | N.D.     | N.D. |          |
| rs732C>T  | 50[62]   | 0.5[50]       | Ala244Val   | ES uptake no change | N.D. | N.D. |          |
| rs147522958| 832G>A   | 0.5[50]       | Glu278Lys   | ES / DHEA / OTA uptake no change [60] | N.D. | K_{V_{max}} of ES no change [60] |          |
| rs847C>T  | 80[60]   | 1[60]         | Leu283Leu   | N.D.     | N.D.     | N.D. |          |
| rs141760078| 1015G>A  | 1[50]         | Val339Met   | ES uptake no change | N.D. | N.D. |          |
| PMT-2917 | 1028G>T  | 0.18[61]      | Arg343Leu   | ES / OTA / UA uptake no change [61] | N.D. | N.D. |          |
| rs1175C>T  | 50[62]   | 0.5[50]       | Thr392Ile   | ES uptake [62] ≤ 10% of WT [62] | K of ES no change V_{max} of ES [62] |          |
| PMT-2910 | 1406A>G  | 0.2[61]       | His469Arg   | ES / OTA / UA uptake loss[61] | [61] | N.D. |          |
| PMT-2911 | 1471C>G  | 0.18[61]      | Leu491Val   | ES / OTA / UA uptake no change[61] | N.D. | N.D. |          |
| rs3782099| C>T[56] | 47[56]        | intron 7    | TOR CL [56] | N.D. | N.D. |          |
| rs1783811| G>A[56] | 31[56]        | intron 8    | TOR CL [56] | N.D. | N.D. |          |
| rs2076267| T>C[56] | 48[56]        | intron 9    | TOR CL [56] | N.D. | N.D. |          |
| rs528211| G>A[56] | 35[56]        | 3' SLC22A11 | TOR CL no change[56] | N.D. | N.D. |          |
protein expression of the genetic variations of SLC22A7 was explored in 34 Korean liver samples. No significant difference was observed between the homozygous wild-type and the heterozygous mutant groups, in this study. However, it is noticed that this study was quite limited by only examining the various haplotypes, but not individual variant transporter protein.

### OAT3

As a key transporter expressed in kidney and brain, the genetic polymorphisms of OAT3 has been widely examined in various ethnic populations. Two synonymous SNPs were firstly identified in polymorphisms of OAT3 has been widely examined in various ethnic group [1,59]. The rest of the genetic variants were presented in more than one African-American, and A310V was exclusive to European-American. A399S was particularly observed in Mexican-American, Q239X and I260R were particularly with different ethnicity in these studies, F129L and A310S were only observed in one Japanese [52A>G, I175V], which was only detected in one Japanese individual (AF=5%). Erdman et al. [1] and Urban et al. [59] discovered more novel non-silent variants, which attributed to the following amino acid substitutions: F129L, R149S, Q239X, I260R, R277W, V281A, I305F, A310V, A399S and V448I. Among the 540 individuals with different ethnicity in these studies, F129L and A399S were only observed in Mexican-American, Q239X and I260R were particularly found in Asian-American, R277W and V281A only occurred in African-American, and A310V was exclusive to European-American [1,59]. The rest of the genetic variants were presented in more than one ethnic group [1,59]. Functional analysis of the OAT3 variants have been conducted, however, little information is available as to their protein expression. Nishizato et al. [58] firstly researched the pharmacokinetic consequences of OAT3 polymorphisms in uptaking pravastatin, which study signified none of the polymorphisms altered the renal clearance of this drug. Later study indicated that Estrone-3-Sulfate (ES) and cimetidine uptake was essentially lost in the RI49S, G239X and I260R variants, while R277W and I305F only demonstrated a significantly lower ES uptake comparing to that of the reference transporter. All the other variants assessed in the same study did not show a considerable difference in the uptake of either substrate [1,59]. More recently, Vormfelde et al. [56] molecularly investigated two 5’ UTR and one non-synonymous SNPs of OAT3, and none of these SNPs was shown to affect the renal clearance of the loop diuretic, torsemide.

### OAT4

Due to the tissue localization of OAT4, it is responsible for the renal reabsorption of various substances, as well as maternal-fetus transfer of a wide range of molecules. Xu et al. [50] initially revealed three synonymous and eight non-synonymous genomic polymorphisms from 96 ethnically different human subjects. These SNPs coded for eight transporter variants, V13M, R48X, T62R, V155M, A244V, E278K, V339M and T392I. All of the non-synonymous polymorphisms in this study, appeared to have low allele frequency (AF: 0.5-1%) with only one individual from the cohort [50]. All of the three identified silent SNPs were observed in one or more ethnic groups, with allele frequency of 0.5-3% [50]. Compared to the other OATs, more information is available regarding the molecular consequences of OAT4 genetic variants. The studies of us and others have also reported the functional analysis of several OAT4 genetic variants. In the study of 95 individuals, Vormfelde et al. [56] observed 10 polymorphisms in the 5’ or the 3’ UTRs, or the introns. Human subject carrying the SNP (rs11231809), showed the most pronounced decrease in torsemide clearance. Four SNPs (-18C>T, rs3782099, rs1783811 and rs2078267) resulted in moderate decrease of torsemide renal clearance, while the SNP (rs4930423) led to an increased clearance of such drug. Ogasawara et al. [57] also discovered one same mutation (-18C>T) from the Japanese cohort, which study concluded that it did not affect the promoter activity or the mRNA expression of this gene. Meanwhile, Lee et al. [60] explored the functional consequence of E278K, which mutant possessed a reduced uptake activity of ES, Dehydroepiandrosterone sulfate (DHEA-s) and ochratoxin A (OTA). This effect was associated with the decreased transporter-substrate affinity (an increased $K_m$) and reduced maximum transporting rate (a diminished $V_{max}$) [60]. The study of us and others concluded that variant transporter L29P, R48X and H469R, was functional deficient in uptaking ES, OTA and Uric Acid (UA) [61,62], whereas V155G and T392I prominently diminished ES uptake [62]. Our early study revealed that the functional reduction of T392I was mainly due to the impaired transporter membrane expression and transporter-substrate turnover rate [62]. Interestingly, L29P also led to less than 10% of transporter protein expressed on cell surface, while compared to that of reference transporter [62], which is consistent with the later observation of Shima et al. [61]. In our study, we also indicated the functional loss of V155G variant is mainly due to the reduced transporter-substrate affinity, while the transporter protein expression is not affected on the surface, as well as in the whole cell [62]. However, the kinetics analysis from Shima et al. [61] suggested that the functional decline of the same variant could be due to a decreased maximum transport rate. Due to the fact that the
HEK293 cells stably expressing V155G was used in the study of Shima et al. [61], and the protein expression of this variant was not able to be assessed, further analysis is expected to consolidate the conclusion. As to the variant R121C, it has been shown to have a considerable decrease in the V_max of ES, which resulted in the modest change in the cellular flux of ES, OTA and UA [61]. The more devastative variant reported by Shima et al. [61], R48X caused a termination in the protein sequence; therefore, as expected, it displayed a minimal to no protein expression from immunoblotting analysis. It was found that the variant H469R did not completely eradicate plasma membrane protein even though it terminated the transporter function totally [61].

Conclusion

Organic Anion Transporters (OATs) are a group of important transporter proteins, responsible for the cellular influx of a wide range of substances, including many clinical important drugs. The function of OATs is confirmed to largely impact on the absorption, distribution and elimination of drugs in body. Genetic mutations have been widely recognized in OATs, which could result in altering the protein sequence, or regulating the transcription of these genes. Functional analysis of the OAT polymorphisms revealed that the natural mutations may dampen or even eliminate the transporter activity in total. The underlying molecular mechanisms included, affecting the transporter-substrate affinity and/or the transporter turnover rate. Moreover, various OAT genetic variants have been found to have diminished protein expression on the cell surface, which is the only location for the protein to be active. Therefore, these mutational outcomes can have critical clinical consequences in particular, when OATs play important roles in the disposition, and/or clearance of specific drugs. Up to date, relatively more genetic polymorphisms are known for OAT1, OAT2, OAT3 and OAT4; whereas such information about the other OAT isoforms is yet to be explored. And the functional consequences, protein expression and transporter kinetics has been investigated in a number of reported OAT polymorphisms, including the polymorphisms in the intrinsic and untranslated regions. Similar work will then be requested to be conducted in the other OAT polymorphisms. In summary, the gained knowledge about OAT natural polymorphisms will largely contribute to establish a more comprehensive evaluation of drug performance, which will then form the basis of designing individualized dosage regimen in the future.

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