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SHORT COMMUNICATION

A rare UGT2B7 variant creates a novel N-glycosylation site at codon 121 with impaired enzyme activity

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Running Title: A rare functional UGT2B7 $D^{121}N$ variant

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Non-standard abbreviations:

AcMPAG, mycophenolic acid acyl-glucuronide; AZT, zidovudine (3'-azido-3'-deoxythymidine); Endo H: Endoglycosidase H; HEK293, human embryo kidney 293 cells; MPA, mycophenolic acid; MPAG, mycophenolic acid phenolic-glucuronide; UDPGA, UDP-glucuronic acid; UGT, UDP-glucuronosyltransferase.

Abstract

UDP-glucuronosyltransferase (UGT) superfamily are glycoproteins resident of the endoplasmic reticulum membranes that undergo post-translational modifications (PTM). UGT2B7 is of particular interest because of its action on a wide variety of drugs. Most studies currently survey common variants and are only examining a small fraction of the genetic diversity. However, rare variants (frequency <1%) might have significant effect as they are predicted to greatly outnumber common variants in the human genome. Here, we discovered a rare single nucleotide *UGT2B7* variant of potential pharmacogenetic relevance that encodes a nonconservative amino acid substitution at codon 121. This low-frequency variation, found in two individuals of a population of 305 healthy volunteers, leads to the translation of an asparagine (Asn) instead of an aspartic acid (Asp) (UGT2B7 p.D^{121}N). This amino acid change was predicted to create a putative N-glycosylation motif NX(S/T) subsequently validated upon endoglycosidase H treatment of microsomal fractions and inhibition of N-glycosylation of endogenously produced UGT2B7 with tunicamycin from HEK293 cells. The presence of an additional N-linked glycan on the UGT2B7 enzyme, likely affecting proper protein folding, resulted in a significant decrease, respectively by 49 and 40%, in the formation of zidovudine and mycophenolic acid glucuronides. A systematic survey of the dbSNP database uncovered 32 rare and naturally occurring missense variations predicted to create or disrupt N-glycosylation sequence motifs in the other UGT2B enzymes. Collectively, these variants have the potential to increase the proportion of variance explained in the UGT pathway due to changes in PTM such as N-linked glycosylation with consequences on drug metabolism.

INTRODUCTION

Metabolic enzymes of the UDP-glucuronosyltransferase (UGT) superfamily catalyze glucuronidation reactions involved in the disposition of endogenous molecules, drugs and other xenobiotics. UGTs are glycoproteins resident of the endoplasmic reticulum (ER) membranes that undergo post-translational modifications (PTM) such as glycosylation and phosphorylation (Mackenzie 1990, Chakraborty et al. 2012, Riches and Collier 2015). This family of proteins are involved in drug metabolism, accounting for approximately 55% of the 200 most prescribed drugs (Guillemette et al. 2014). UGT2B7 is of particular interest as it is the most prevalent member of this enzyme family, conjugates almost one fifth of all drugs known to be conjugated to glucuronic acid (Stingl et al. 2014). For instance, UGT2B7 is involved in the inactivation and elimination of carboxylic acid-containing drugs including the nonsteroidal anti-inflammatory drugs (NSAIDs), and of a large variety of other drugs including opioids, the anticonvulsant valproic acid, morphine, codeine, efavirenz, fenofibric acid, mycophenolic acid (MPA) and zidovudine (AZT) (Stingl et al. 2014).

Glucuronide formation varies greatly between individuals and may be explained in part by the presence of single nucleotide polymorphisms (SNPs) (Guillemette et al. 2014). In the case of UGT2B7, there is a well-established connection to variation in drug efficacy and toxicity, especially for the most common polymorphism *UGT2B7*2* allele that encodes a nonconservative amino acid substitution $His^{268}Tyr$ in the substrate-binding domain linked to altered pharmacokinetics of several drugs but not all substrates examined (Bhasker et al. 2000, Stingl et al. 2014). Most pharmacogenomics studies currently survey common variants and thus are only observing a small fraction of the genetic diversity in any gene. Different forms of genetic variations within a *UGT* locus, including common and rare coding and regulatory variants, can

exist and have separate and yet cumulative effects. Rare variants (allele frequency <1%) may also cause interindividual differences in therapeutic effects and adverse reactions to drugs but have been much less studied (Nelson et al. 2012). This is highly relevant since rare variants are predicted to greatly outnumber common variants in the human genome (Marth et al. 2011) and they may be very important in the genomic contribution to treatment response and toxicity (Ramsey et al. 2012, Gillis et al. 2014). For example, it was recently established that as much as 92% of known variants in the cytochrome P450 superfamily have an allelic frequency below 1%, and 83% below 0.1% (Fujikura et al. 2015). Similar distribution of variants by allelic frequency might be observed in UGT2B7 and other UGTs, underscoring the importance of studying rare variants.

Here we report the identification of a novel rare missense *UGT2B7* variant (NM_001074.2:c.361G>A) in a Caucasian cohort of 305 healthy volunteers. This rare variation leads to the translation of an asparagine (Asn) instead of an aspartic acid (Asp) at position 121 of the UGT2B7 protein (NP_001065.2:p.Asp121Asn; $D^{121}N$). Investigation of its functional impact using bioinformatics tools predicted that this variation creates a putative N-glycosylation site, which was experimentally validated. We then studied whether this additional PTM site affects glucuronidation of AZT and MPA, demonstrating a drastic reduction in UGT2B7 catalytic activity. Lastly, we established a comprehensive dataset of 32 rare variants potentially affecting gain or loss of N-glycosylation sites in other family members, with some located in regions highly conserved across *UGT* genes, by integrating data from the dbSNP database along with their respective allelic frequency. Collectively, these low-frequency variants have the potential to increase the proportion of variance explained in the glucuronidation pathway.

MATERIALS AND METHODS

Genotyping of healthy Caucasian volunteers. The variation at position 361 in the *UGT2B7* gene causing amino acid change $Asp^{121}Asn$ was initially observed in one of 52 participants of a previous pharmacokinetic study (Levesque et al. 2007, Levesque et al. 2008). These healthy volunteers were selected in a population of 305 Caucasian subjects that were subsequently genotyped for the variation at codon 121 using specific primers, as described (Levesque et al. 2007, Levesque et al. 2008), and a second subject was found to carry this variation.

Heterologous expression of the variant UGT2B7 enzyme and enzymatic assays. A HEK293 cell line expressing the variant UGT2B7 protein at codon 121 ($UGT2B7Asn^{121}$) was established by mutagenesis using primers 5'-CCCAACAACTCATCCTCTCTTAAAATTGAAA-3' (forward) and 5'-TTTCAATTTTAAGAGAGGATGAGTTGTTGGG-3' (reverse) based on the reference UGT2B7 cDNA (Menard et al. 2011). Relative quantification of UGT protein content in microsomal proteins was performed by immunoblot analysis using an anti-UGT2B antibody EL-93 (dilution 1:2000) (Lepine et al. 2004) and with an anti-calnexin antibody (dilution 1:5000; Stressgen Biotechnologies, Victoria, Canada) to normalize for sample loading. Kinetic parameters were assessed for both cell lines in the presence of increasing concentrations of substrates, MPA (25 to 1500 µM; MP Biomedicals, Santa Ana, CA) or AZT (100 to 5000 µM; Sigma-Aldrich, St. Louis, MO) and fixed UDPGA (5 mM), or co-substrate UDPGA (from Sigma-Aldrich, St. Louis, MO) (50 to 5000 µM; fixed AZT (500 µM or 1 mM)), for 1-hour incubation at 37°C. Enzymatic assays and liquid chromatography-mass spectrometry were performed to assess glucuronide (G) formation as described (Benoit-Biancamano et al. 2007). Absolute velocities (V_{max}: pmol/min/mg protein) were normalized for UGT protein content assessed by western blotting and expressed as relative V_{max} (pmol/min/mg protein/UGT content).

Kinetic parameters, according to the Michaelis-Menten model, were calculated with Sigma Plot 11 using the Enzyme Kinetics 1.3 module (SYSTAT Software Inc., San Jose, CA). Data are derived from at least two independent experiments performed in triplicates. *P*-value calculations using Student's t-test were performed with Excel 2016 (Microsoft Corporation, Redmond, WA).

Experimental confirmation of N-glycosylation using endoglycosidase digestion. Enzymes were obtained from New England BioLabs Inc. (Ipswich, MA). Microsomes (20 µg) from HEK293-UGT2B7 cell lines were incubated in Glycoprotein Denaturing Buffer (0.5% SDS, 40 mM DTT) for 10 minutes at 100°C to ensure denaturation of the protein content. For endoglycosidase H (Endo H) treatment, GlycoBuffer 3 (50 mM Sodium Citrate (pH 6 @ 25°C) and Endo H (500 U) were added to microsomes whereas for O-glycosidase treatment, microsomes were supplemented with GlycoBuffer 2 (50 mM Sodium Phosphate (pH 7.5 @ 25°C), NP-40 (1 %), neuraminidase (100 U) and O-glycosidase (40 000 U), in a 20 µl final volume. Samples were incubated for 1h at 37°C and subsequently resolved by 10% SDS-polyacrylamide gel electrophoresis using standard procedure. Immunodetection of UGT2B7 was conducted with a polyclonal UGT2B7 antibody (1:5000; 16661-1-AP, ProteinTech Group, Rosemont, IL).

Inhibition of endogenous glycosylation of UGT2B7 was conducted as described with some modifications (Nakajuma et al. 2010). Briefly, HEK293 cells plated in 10 cdm-dishes were transiently transfected with 4 ug of pcDNA3 constructs driving expression of reference UGT2B7 or the c121 variant (Menard et al. 2011) using Lipofectamine 2000 (Invitrogen). Inhibition of glycosylation was achieved with tunicamycin (Sigma-Aldrich) added at time of transfection (final concentration 0.1 and 1 µg/ml). Controls consisted of vehicle only (0.1% ethanol). Cells were harvested 24h/40h post-transfection by washing twice in PBS then homogenates were

prepared by harvesting cells in phosphate-buffered saline containing 0.5 mM DTT. To control for ER stress, cells were transfected as above, thapsigargin (0.5 µM; Sigma-Aldrich) was added at time of transfection and cells were harvested 24 h post-treatment as above. Protein concentration in homogenates was determined by a bicinchoninic acid assay. Immunodetection of UGT2B7 was as described above.

Analysis of UGT2B genetic variations. Single nucleotide variations (SNPs) for UGT2B coding sequences were retrieved from the dbSNP database using the NCBI browser (U.S. National Library of Medecine, Bethesda, MD). Alignments of UGT2Bs were obtained using the Clustal O (1.2.1) multiple sequence alignment tool. Coding variations were analyzed to establish whether they affect the sequence context Asn-X-Ser/Thr (create or disrupt NX(S/T) motif), where X is any amino acid except proline.

RESULTS AND DISCUSSION

Our analysis of *UGT2B7* gene sequences in a population of 305 healthy volunteers uncovered a novel missense variation observed in two individuals and corresponding to a nonsynonymous coding variation at codon 121 ($Asp^{121}Asn$). A Sanger sequencing chromatogram is presented in **Fig.1A** designating a double peak of an adenine (A) and a guanine (G) at position 361 (allelic frequency of 0.328% for the variant A allele). This population was previously used to identify candidates carrying specific *UGT1A* and *UGT2B7* genetic variations for a pharmacokinetic study of mycophenolate mofetyl (Levesque et al. 2007, Levesque et al. 2008). An *in silico* analysis for prediction of PTM sites using the NetNGlyc 1.0 tool (http://www.cbs.dtu.dk/services/NetNGlyc/) also confirmed with the GlycoEP software (Chauhan et al. 2013), revealed that this amino acid change creates a putative asparagine-linked N-glycosylation motif NX(S/T) consisting of an asparagine followed 2 positions downstream by

a threonine as pictured in **Fig.1D**. Three such motifs are typically found in the UGT2B7 protein, of which glycosylation at positions 68 and 315 was experimentally validated (Nagaoka et al. 2012). The western blot analysis of HEK293 microsomal preparations showed that the variant UGT2B7Asn^{121} protein has a higher molecular weight than the UGT2B7Asp^{121} enzyme, which is consistent with the addition of an oligosaccharide by N-glycosylation. In contrast to O-glycosidase treatment, when treated with Endo H, oligosaccharides are removed leaving both UGT2B7 proteins at the same lower molecular weight, thus confirming the presence of an additional N-glycosylation on the variant UGT2B7Asn^{121} protein (**Fig.1B**). As further evidence of N-glycosylation causing the enhanced mobility shift of the UGT2B7Asn^{121} protein, N-glycosylation of endogenously produced UGT2B7 was inhibited with tunicamycin (Nakajima et al. 2010). Both the reference and variant UGT2B7 were detected as multiple protein bands with low drug concentration, suggesting a partially perturbed glycosylation, whereas the high drug concentration prevented the formation of slower migrating proteins, thus confirming that the mobility shift is caused by N-glycosylation (**Fig.1C**). Inhibition was not due to a general ER stress impairing glycosylation, given that thapsigargin did not perturb the mobility of either UGT2B7 (**Fig.1C**) We also used mass spectrometry analysis with the goal to detect the glycosylated peptide containing the sequence at codon 121. This approach, using cellular fractions from HEK293-UGT2B7 cell models enriched for UGT2B7 by affinity purification and treated or not with endoglycosidase PNGase F, was inconclusive. It permitted the detection of multiple UGT2B7 tryptic peptides (protein coverage up to 29%; data not shown) but not the codon 121-bearing peptide.

Previous data support that N-glycosylation plays a significant role in the enzymatic activity of UGT2B7 (Barbier et al. 2000, Nagaoka et al. 2012), suggesting that the novel UGT2B7Asn^{121}

variat may affect enzyme activity. A significant alteration in the conjugation of AZT and MPA was observed, with a decreased activity by 49% and 40%, respectively, associated with the variant Asn^{121} protein compared to the reference Asp^{121} enzyme, suggesting an altered protein folding. No significant differences were noted in the affinity (K_m) of the enzyme (**Table 1, Fig.1E**). Nagaoka and colleagues showed that disruption of N-glycosylation sites at position 68 and 315 by mutagenesis leads to significant changes in the activity of the UGT2B7 enzyme (Nagaoka et al. 2012). Similarly, abolition of the N-glycosylation of UGT2B15 resulted in decreased enzyme activity without changing K_m (Barbier et al. 2000). In line, a rare *UGT1A1* variant creating a glycosylation site ($K^{402}T$) was reported to cause a drastic decrease in UGT activity associated with severe hyperbilirubinemia (Crigler-Najjar type 1) (Sneitz et al. 2010). These observations underscore the need for further research on the impact of the N-glyco variants on UGT2B7 protein function as well as on other UGTs.

Our goal was next to build a framework for better understanding the effects of non-synonymous variations on the N-glycosylation of UGT2B enzymes. Through a systematic survey of publicly available data (dbSNP database), we revealed numerous naturally occurring missense variations, most with low allelic frequency (below 0.01%), predicted to affect N-glycosylation of UGT2B enzymes (**Table 2, Fig.1F**). Very little information is still available regarding the glycosylation profile of UGT enzymes, precluding us from establishing the potential functional relevance of these rare variants. Clustering of the variants according to their position in the UGT sequences helped visualize the fact that many of them localize in the same regions of UGT1, UGT2A or UGT2B enzymes. These potentially correspond to conserved and functional NX(S/T) motifs across several UGT proteins (**Fig.1F)**, hence making them interesting candidates for further *in vitro* validation. For example, the first cluster is noteworthy because N-glycosylation in this

region of UGT2B7 and UGT2B15, on residues 68 and 65 respectively, was experimentally validated in liver tissue by a method combining multiple digestion and hydrazide chemistry (Chen et al. 2009). Likewise, codon 69 of UGT2B4, UGT2B11 and UGT2B28 is affected by naturally occurring rare variants creating at this position the final serine or threonine of an NX(S/T) glycosylation motif, whereas a similar motif is present in this region for UGT1A and UGT2A enzymes. A comprehensive survey of the impact of these additional rare variants on the N-glycosylation of UGT enzymes will be required to evaluate if variations that lead to changes in the glycosylation pattern of a UGT protein can be damaging.

In conclusion, we discovered a rare UGT2B7 variant resulting in a NX(S/T) glycosylation gain that significantly affects rates of drug glucuronidation. A more thorough understanding of the significance of this variant in the context of drug treatment would require genotyping a larger population, initially to acquire a better appreciation of the allelic frequency across populations and to perform pharmacokinetic analyses in individuals carrying this variation. Likewise, our exhaustive analysis of variations data in additional drug conjugating UGT enzymes exposes numerous missense variations with low allelic frequency potentially creating or disrupting N-glycosylation sites, with some in regions conserved across all UGT enzymes. Accordingly, a large proportion of variability in the UGT pathway may be due to rare variants of significant effect size with a profound impact on their biological function due to changes in PTM such as N-linked glycosylation.

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Authorship Contributions

Participated in research design: Benoit-Biancamano, Guillemette

Conducted experiments: Girard-Bock, Benoit-Biancamano, Villeneuve, Desjardins

Performed data analysis: Girard-Bock, Benoit-Biancamano, Villeneuve, Desjardins, Guillemette

Wrote or contributed to the writing of the manuscript: Girard-Bock, Benoit-Biancamano, Guillemette

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Legends for Figures

Figure 1. A) Sanger sequencing chromatogram revealing an individual heterozygote for the novel nonsense variation at position 361 ((NC_000004.12:g.69096881G>A, NM_001074.2:c.361G>A, NP_001065.2:p.Asp121Asn)) of the *UGT2B7* gene. The arrow indicates the position of the nucleotide variation. **B)** *N*-glycosylation profile of the UGT2B7 enzyme (Asp^{121}) and variant protein (Asn^{121}) assessed by Western blot analysis. Microsomal preparations (20 ug) were untreated or treated with endoglycosidases to cleave attached oligosaccharides. **C)** Inhibition of endogenous N-glycosylation. HEK293 cells transiently transfected to express UGT2B7 (Asp^{121}) or variant UGT2B7 (Asn^{121}) were treated with either vehicle (CTR), tunicamycin (0.1 or 1.0 µg/ml) or thapsigargin (0.5 µM) at time of transfection. UGT2B7 were immunodetected in cell homogenates (10 µg). **D)** Graphical representation of the

UGT2B7 protein sequence with putative domains, N-glycosylation sites (blue), those experimentally validated (marked with a star, (Nagaoka et al. 2012)), and the new site created in the UGT2B7Asn^{121} variant protein (red) as predicted with the NetNGlyc 1.0 tool. **E)** Glucuronide formation by the UGT2B7Asp^{121} reference enzyme (blue) and the variant UGT2B7Asn^{121} (red) using varying concentrations of MPA (25 to 1500 µM), AZT (100 to 5000 µM) or UDPGA (50 to 5000 µM in the presence of 500 µM of AZT). **F)** Partial sequence alignment of the 19 UGT1 and UGT2 enzymes in which putative N-glycosylation motifs (grey) and positions of rare variants (listed in Table 1) either creating (green) or disrupting (red) such motifs are indicated. Amino acid positions given above sequence alignments correspond to the UGT2B7 protein and letters represent clusters of putative N-glycosylation sites among UGTs (see **Table 2**)

TABLE 1.

Kinetic parameters for UGT2B7 substrates (AZT and MPA) using microsomal protein preparations isolated from HEK293 cells stably expressing the UGT2B7 enzyme (Asp^{121}) and the novel variant UGT2B7 protein (Asn^{121})[1].

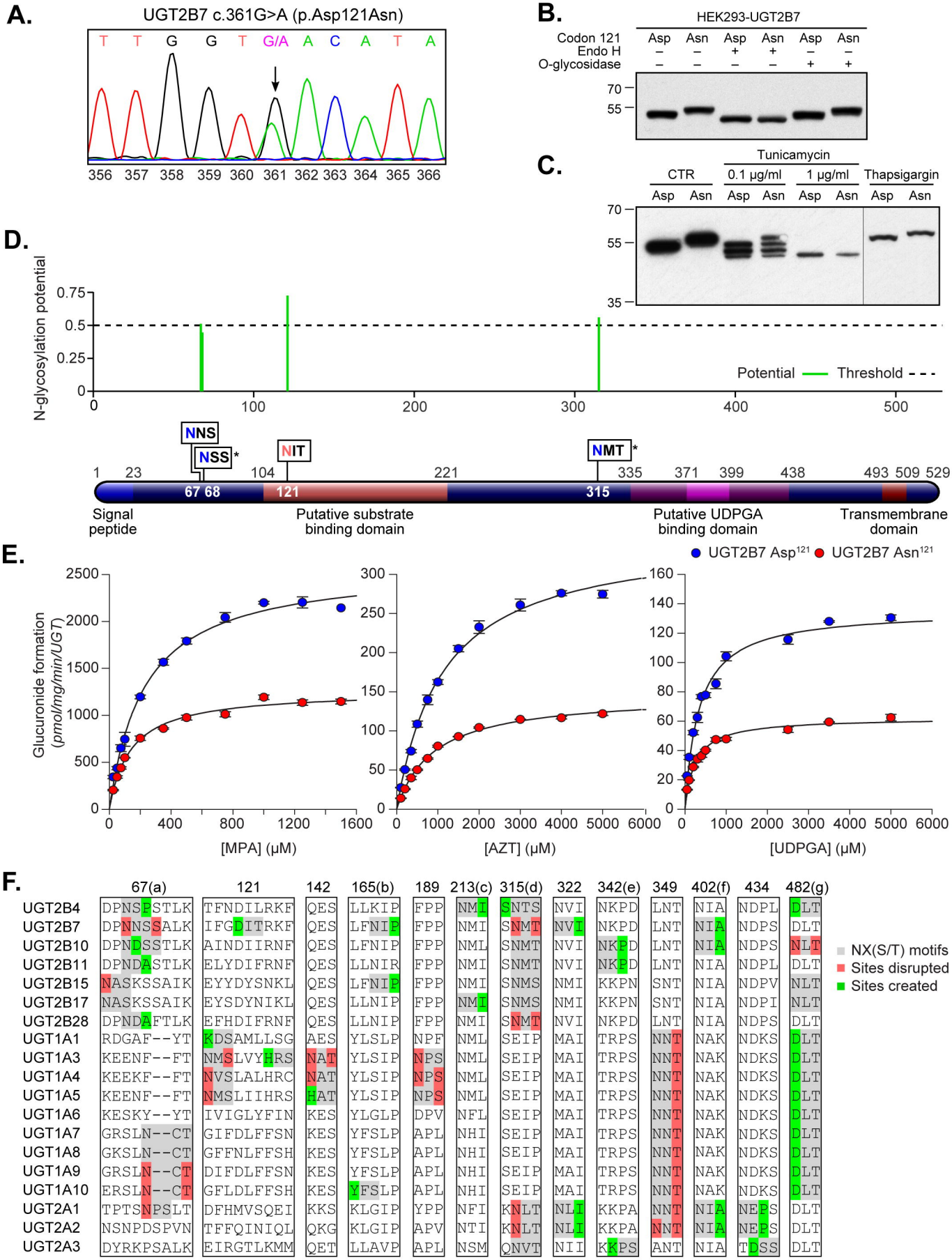
	Substrate MPA			**Substrate AZT**			**Co-substrate UDPGA**[2]		
	K_m	Relative V_{max}	CL_{int}	K_m	Relative V_{max}	CL_{int}	K_m	Relative V_{max}	CL_{int}
UGT2B7	*μM*	*pmol/min/mg/UGT content*	*μL/min/mg*	*μM*	*pmol/min/mg/UGT content*	*μL/min/mg*	*μM*	*pmol/min/mg/UGT content*	*μL/min/mg*
Asp^{121}	174 ± 46	2194 ± 65	13	1018 ± 152	267 ± 13	0.26	314 ± 37	139 ± 12	0.45
Asn^{121}	152 ± 29	1323 ± 121**	8	968 ± 100	137 ± 21*	0.14	231 ± 24	58 ± 6*	0.25

[1]Rates of glucuronide formation were adjusted relative to UGT2B7 protein content determined by Western blot. Values correspond to the mean of at least two independent experiments performed in triplicate. Relative Acyl-MPA-G, AZT-G formation rates and co-substrate utilization rates were significantly different between UGT2B7Asp^{121} and UGT2B7Asn^{121}; CL_{int} = clearance (V_{max}/K_m); **P* < 0.05 and ***P*< 0.01 vs. Asp^{121}. [2]In the presence of fixed concentration of AZT (500 μM). Similar results were observed using AZT at 1 mM (K_m= 400 ± 26 μM and V_{max}=156 ± 3 pmol/min/mg/UGT content for UGT2B7Asp^{121} and K_m = 240 ± 31 μM and V_{max} = 56 ± 2 pmol/min/mg/UGT content for UGT2B7Asn^{121}).

TABLE 2. *Naturally occurring rare variants in UGT2B family members predicted to create (gain) or disrupt (loss) N-glycosylation sites (NX(S/T) motif).*

Clusters	UGT	Variant	Position chr.4	Amino acid	NX(S/T) motif		MAF (%)
					sequence	effect	
a	UGT2B4	rs767028471	69495657	Pro69Ser	NSP	gain	< 0.01
	UGT2B7	rs748435925	69096719	Asn67His	NNS	loss	< 0.01
	UGT2B7	rs373587868	69096729	Ser70Phe	NSS	loss	< 0.01
	UGT2B10	rs61750900	68816218	Asp67Asn	DSS	gain	<0.01
	UGT2B11	rs756939149	69214518	Ala69Thr	NDA	gain	< 0.01
	UGT2B15	rs770648228	68670425	Asn65Ser	NAS	loss	< 0.01
	UGT2B28	rs150261084	69280705	Ala69Thr	NDA	gain	0.14
-	UGT2B15	rs368012995	68670362	Leu86Ser	NYL	gain	-
-	UGT2B7	This report	69096881	Asp121Asn	DIT	gain	0.33
b	UGT2B7	rs747704916	69097019	Pro167Ser	NIP	gain	< 0.01
	UGT2B15	rs747378153	68670117	Pro168Thr	NIP	gain	< 0.01
-	UGT2B10	rs764895973	68816534	Ser172Asn	SFS	gain	< 0.01
c	UGT2B4	rs760430619	69495218	Ile215Thr	NMI	gain	< 0.01
	UGT2B17	rs747935682	68567838	Ile216Thr	NMI	gain	< 0.01
-	UGT2B17	rs748669369	68560628	Ile305Thr	NGI	gain	< 0.01
d	UGT2B4	rs756195714	69489500	Ser314Asn	SNT	gain	-
	UGT2B7	rs755080081	69102879	Asn315Tyr	NMT	loss	< 0.01
	UGT2B7	rs752908839	69102885	Thr317Ala	NMT	loss	< 0.01
	UGT2B28	rs768879480	69286824	Asn315Asp	NMT	loss	< 0.01
	UGT2B28	rs144043239	69286831	Thr317Ile	NMT	loss	< 0.01
-	UGT2B7	rs745439209	69102907	Ile324Thr	NVI	gain	< 0.01
e	UGT2B10	rs767451406	68826437	Pro343Ser	NKP	gain	< 0.01
	UGT2B11	rs201637982	69205540	Pro344Thr	NKP	gain	< 0.01
f	UGT2B7	rs201964275	69108222	Ala404Thr	NIA	gain	< 0.01
	UGT2B10	rs750605168	68827448	Ala403Ser	NIA	gain	< 0.01
-	UGT2B10	rs545266191	68827497	Met419Thr	NTM	gain	0.02
-	UGT2B4	rs375220784	69480893	Met443Thr	NAM	gain	< 0.01
-	UGT2B28	rs781334372	69294551	Lys444Asn	KLS	gain	< 0.01
g	UGT2B4	rs767837637	69480777	Asp482Asn	DLT	gain	< 0.01
	UGT2B10	rs112561475	68830733	Asn481Asp	NLT	loss	1.30
	UGT2B10	rs567502684	68830734	Asn481Ser	NLT	loss	< 0.01
	UGT2B10	rs536035975	68830740	Thr483Asn	NLT	loss	0.10
-	UGT2B4	rs770843089	69480747	Asp492Asn	DVT	gain	< 0.01

Horizontal lines group variants according to their positions. MAF: Minor allele frequency.


A.
UGT2B7 c.361G>A (p.Asp121Asn)
T T G G T G/A A C A T A
356 357 358 359 360 361 362 363 364 365 366
B.
HEK293-UGT2B7
Codon 121 Asp Asn Asp Asn Asp Asn
Endo H – – + + – –
O-glycosidase – – – – + +
70
55
C.
Tunicamycin
CTR
0.1 µg/ml
1 µg/ml
Thapsigargin
Asp Asn Asp Asn Asp Asn Asp Asn
70
55
35
D.
N-glycosylation potential
0.75
0.5
0.25
0
0 100 200 300 400 500
Potential
Threshold
NNS
NSS*
NIT
NMT*
1 23 104 221 335 371 399 438 493 509 529
67 68
121
315
Signal peptide
Putative substrate binding domain
Putative UDPGA binding domain
Transmembrane domain
E.
UGT2B7 Asp121
UGT2B7 Asn121
Glucuronide formation (pmol/mg/min/UGT)
[MPA] (µM)
[AZT] (µM)
[UDPGA] (µM)
F.
67(a) 121 142 165(b) 189 213(c) 315(d) 322 342(e) 349 402(f) 434 482(g)
UGT2B4 DPNSPSTLK TFNDILRKF QES LLKIP FPP NMI SNTS NVI NKPD LNT NIA NDPL DLT
UGT2B7 DPNNSSALK IFGDITRKF QES LFNIP FPP NMI SNMT NVI NKPD LNT NIA NDPS DLT
UGT2B10 DPNDSSTLK AINDIIRNF QES LFNIP FPP NML SNMT NVI NKPD LNT NIA NDPS NLT
UGT2B11 DPNDASTLK ELYDIFRNF QES LLNIR FPP NMI SNMT NVI NKPD LNT NIA NDPL DLT
UGT2B15 NASKSSAIK EYYDYSNKL QES LFNIP FPP NMI SNMS NMI KKPN SNT NIA NDPV NLT
UGT2B17 NASKSSAIK EYSDYNIKL QES LLNIP FPP NMI SNMS NMI KKPN SNT NIA NDPI NLT
UGT2B28 DPNDAFTLK EFHDIFRNF QES LLNIP FPP NMI SNMT NVI NKPD LNT NIA NDPS DLT
UGT1A1 RDGAF--YT KDSAMLLSG AES YLSLP NPF NML SEIP MAI TRPS NNT NAK NDKS DLT
UGT1A3 KEENF--FT NMSLVYHRS NAT YLSIP NPS NML SEIP MAI TRPS NNT NAK NDKS DLT
UGT1A4 KEEKF--FT NVSLALHRC NAT YLSIP NPS NML SEIP MAI TRPS NNT NAK NDKS DLT
UGT1A5 KEENF--FT NMSLIIHRS HAT YLSIP NPS NML SEIP MAI TRPS NNT NAK NDKS DLT
UGT1A6 KESKY--YT IVIGLYFIN KES YLGLP DPV NFL SEIP MAI TRPS NNT NAK NDKS DLT
UGT1A7 GRSLN--CT GIFDLFFSN KES YFSLP APL NHI SEIP MAI TRPS NNT NAK NDKS DLT
UGT1A8 GKSLN--CT GFFNLFFSH KES YFSLP APL NHI SEIP MAI TRPS NNT NAK NDKS DLT
UGT1A9 GRSLN--CT DIFDLFFSN KES YFSLP APL NHI SEIP MAI TRPS NNT NAK NDKS DLT
UGT1A10 ERSLN--CT GFLDLFFSH KES YFSLP APL NHI SEIP MAI TRPS NNT NAK NDKS DLT
UGT2A1 TPTSNPSLT DFHMVSQEI KKS KLGIP YPP NFI KNLT NLI KKPA NNT NIA NEPS DLT
UGT2A2 NSNPDSPVN TFFQINIQL QKG KLGIP APV NTI KNLT NLI KKPA NNT NIA NEPS DLT
UGT2A3 DYRKPSALK EIRGTLKMM QET LLAVP APL NSM QNVT NII KKPS ANT NIA TDSS DLT
NX(S/T) motifs
Sites disrupted
Sites created