UMYU Scientifica, Vol. 2 NO. 4, December 2023, Pp 023 – 030
https://doi.org/10.56919/usci.2324.003
ISSN: 2955 – 1145 (print); 2955 – 1153 (online)
A periodical of the Faculty of Natural and Applied Sciences, UMYU, Katsina

ORIGINAL RESEARCH ARTICLE

Genotyping of Uridine-Diphosphate Glucuronosyltransferases-1A1 (UGT1A1) Enzyme and Its Genetic Variant Allele Determination Using Polymerase Chain Reaction and Gel Electrophoresis.

Midia Adugo[1], Samuel J. Bunu[2], Robinson T. Dodoru[3], Edebi N. Vaikosen[2] and Benjamin U. Ebeshi[2].

[1]Pharmacy Department, Niger Delta University Teaching Hospital, Okolobiri, Yenagoa, Bayelsa, Nigeria
[2]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa, Nigeria
[3]Institute for Biomedical Research and Innovations, College of Health Sciences, University of Uyo, Akwa Ibom State, Nigeria.

ABSTRACT

Dolutegravir is an integrase inhibitor that prevents the integration of the viral genome into the host cell's DNA, thus halting HIV replication. The study aimed to conduct genotyping of immunocompromised patients in some Southern States of Nigeria on dolutegravir-based highly active antiretroviral therapy for the *UGT1A1*6* and *UGT1A1*28* variant alleles using gel electrophoresis and polymerase chain reaction. 52 HIV/AIDS patients participated in the study. Specific primers for *UGT1A1*6* and *UGT1A1*28*: U1F1 forward primer: 5 – AGATACTGTTGATCCCAGTG - 3 and U211R reverse primer: 5 - CTTCAAGGTGTAAAATGGTC-3, was used for the gene amplification, followed by restriction digestion with *Ava II*. DNA concentrations were quantified with a NanoDrop-1000 spectrophotometer. Restriction fragment length polymorphism (RFLP) techniques were used for genotyping and Gel electrophoresis to determine the heterozygosity and homozygosity of UGT1A1 alleles. After the polymerase chain reaction (PCR), all DNA samples appeared at 280 base pairs on a 1% agarose gel electrophoretic medium. RFLP analysis confirmed the PCR results; thus, no mutations were observed in all the samples. There were no *UGT1A1* genetic polymorphisms among the ethnic groups studied, although there was a mild significant link between dolutegravir and neuropsychiatric side effects in the patients (at p-value = 0.08).

ARTICLE HISTORY

Received July 15, 2013.
Accepted November 17, 2023.
Published December 13, 2023.

KEYWORDS

UGT1A1, Polymorphism, Doluegravir, HIV/AIDS, NanoDrop-1000.

INTRODUCTION

Over the last 30 years, human-immune-deficiency Virus (HIV) infection has skyrocketed, affecting all social and racial groups worldwide and having a considerable impact on health and many other sectors (Janeway et al., 2001; WHO, 2023). The relationship between the plasma levels of dolutegravir (DTG), an integrase inhibitor used in the management of HIV/AID, and Uridine-Diphosphate Glucuronosyltransferases-1A1 enzyme (UGT1A1) gene polymorphisms have been reported (Yagura et al., 2017). The researchers discovered that the median DTG concentration was 1.06g/mL among 107 individuals. The mean DTG plasma levels of individuals with both normal alleles were significantly lower than the *UGT1A1*6* homozygous individuals who had 1.43g/mL (Huik et al., 2022; Yagura et al., 2017). Pharmacogenetics refers to how individual variances in drug response result from point mutations, insertions, duplications, multiplications, and deletions in the genes that code for the enzymes (Mroziewicz & Tyndale, 2010). Pharmacogenetic polymorphism occurs when a gene that sensitizes an enzyme that metabolizes a drug has more than one different allele (greater than 1%) at the same locus and impacts how a medication interacts with the body (Oates & Lopez, 2018; Surendiran et al., 2008). Gene polymorphism in individual groups or populations can result in DNA sequence variety. Genetic polymorphisms include single nucleotide polymorphisms (SNPs), sequence duplications, deletions, recombination, and repetitions (Ismail, 2012; Rozas, 2009). When a gene's polymer sequence changes permanently and deviates from the sequence present in most people, it is referred to as a genetic mutation or genetic polymorphism (Chauhan,

Correspondence: Midia Adugo. Pharmacy Department, Niger Delta University Teaching Hospital, Okolobiri, Yenagoa, Bayelsa, Nigeria. ✉ adugomidia@gmail.com. Phone Number: +234 706 513 2894.
How to cite: Adugo, M., Bunu, S. J., Dodoru, R. T., Vaikosen, E. N., & Ebeshi, B. U. (2023). Genotyping of Uridine-Diphosphate Glucuronosyltransferases-1A1 (UGT1A1) Enzyme and Its Genetic Variant Allele Determination Using Polymerase Chain Reaction and Gel Electrophoresis. *UMYU Scientifica*, 2(4), 23 – 30. https://doi.org/10.56919/usci.2324.003

2018). From a single DNA base pair (bp) to a chromosome's vast phase, which houses several genes, mutations can affect anything (Mahdieh & Rabbani, 2013; NIH, 2017).

Dolutegravir inhibits retroviral DNA strand transfer and host cell integration by binding to the active site of the HIV integrase (Ribera & Podzamczer, 2015). The strand transfer phase is required for HIV replication, yet it also inhibits viral activity (Mirambeau, 2008). This drug specifically affects human host cells, resulting in high tolerance and low toxicity (Fantauzzi & Mezzaroma, 2014; Rhee et al., 2019). Dolutegravir's prolonged impact in clinical investigations demonstrates that it has a major impediment to resistance due to its tight binding and lengthy dissociative half-life (McCormack, 2014). The UGT3 UDP-sugar transferase family has been linked to xenobiotic conjugation. The human UGTs are located in the endoplasmic reticulum (ER), exhibiting tissue-specific expression (Yang et al., 2017). The human intestine had around 6 UGTs (including UGT1A1, UGT1A10, UGT2B7, and UGT2B17), whereas the human kidney included only UGT1A9, UGT2B7, and UGT1A64, (Meech et al., 2019; Zhou et al., 2019). Because of its unique role in conjugative bilirubin detoxification, UGT1A1 is clinically important (Zhu et al., 2020). Previous research has shown that genetics and environmental factors can both alter UGT1A1 expression and function, resulting in lower protein levels or activity (Gervai, 2009; Tiffon, 2018). UGT1A1 is a highly polymorphic enzyme. Polymorphisms in UGT1A1 frequently result in lower expression, lower enzyme activity, or even complete activity loss (Gil & Sasiadek, 2012; Teh et al., 2012). Hyperbilirubinemia caused by decreased UGT1A1 expression or function can range in severity from mild Gilbert's syndrome to severe kernicterus and Crigler-Najjar syndrome (Mi et al., 2019). Polymorphic UGT1A1 mutations have also been associated with drug-induced liver damage (Yang et al., 2018). Small-molecule UGT1A1 inhibitors may majorly impact its catalytic activity, resulting in undesirable side effects such as drug/herb-drug interactions (D/HDI) interactions (Goon et al., 2016). Drugs such as nilotinib, indinavir, and sorafenib, as well as herbal remedies such as milk thistle, green tea, and emodin, have been shown to inhibit UGT1A1 (Archibald et al., 2016; Fasinu & Rapp, 2019).

Some studies on UGT1A1-ligand interactions have revealed that UGT1A1 contains a large number of ligand-binding sites (Lv et al., 2019; Marques & Ikediobi, 2010). Because UGT1A1 is required for bilirubin metabolism, xenobiotics, food/herbal components, and environmental pollutants that inhibit this conjugative enzyme may result in hyperbilirubinemia. UGT1A1 blockage may also result in clinically severe DDI/HDI due to UGT1A1's critical functions in the detoxification of numerous medications with tight therapeutic windows (Chang et al., 2013; Dean, 2012). Reports have shown that *UGT1A1* gene mutations substantially impair bilirubin metabolism, and this gene is a key sensitizer of the dolutegravir metabolizing enzyme (UGT1A1). It has been demonstrated that younger age, carrying UGT1A1*6 and/or UGT1A1*28 alleles, is associated with high plasma DTG concentrations, posing risk factors for neuropsychiatric adverse events (Yagura et al., 2017). There are few or no reports on dolutegravir polymorphism or adverse events in Africa, including Nigeria; hence, this study aimed to conduct genotyping of Uridine-Diphosphate Glucuronosyltransferases-1A1 enzyme and determining genetic variant allele using the gel electrophoresis and polymerase chain reaction. This will provide significant information on how individual of this regimen handles the drug, the effectiveness, tolerability, and possible adverse events among the study population.

MATERIALS AND METHOD

Study Population

The study population consisted of Southern Nigeria HIV/AIDS-positive patients aged 18 and above who agreed to participate in the study. Fifty-two (52) HIV/AIDS positive but unrelated patients, aged 18 years and above, who were receiving dolutegravir (50 mg daily) in combination with either lamivudine or tenofovir and met the study inclusion criteria were chosen at random from various States of Southern Nigeria. Potential subjects were explained the study procedures, after which they were free to choose whether or not to participate in the study.

Sample Collection

A study laboratory scientist at the Medical Laboratory Department of the FMC Yenagoa was involved in blood sample collection. From each participant, 5.0ml blood samples were collected by veno-puncture into EDTA tubes from the HIV patients who have received 50mg of Dolutegravir for at least 3 weeks. All the samples were stored at (-20 °C) until further use.

Genotyping for UGT1A1

DNA samples were prepared using the QIAamp DNA Blood Mini Kit, purchased from Germany. After preparation, DNA sample purity was determined by measuring their concentrations using a NanoDrop-1000 spectrophotometer following the technique described by Bunu et al. (2020). The purity of the DNA Samples was obtained by calculating the absorbance ratio of 260 nm and 280 nm (A260/A280), respectively. UGT1A1 genotyping was carried out using real-time PCR and restriction fragment length polymorphism (PCR-RFLP) techniques, as described by Ebeshi et al. (2011). Specific primers for UGT1A1*6 and UGT1A1*28 with the following sequences: U1F1 forward: 5 – AGATACTGTTGATCCCAGTG - 3 and U211R reverse: 5 - CTTCAAGGTGTAAAATGGTC-3, was used for the gene amplification, followed by restriction digestion with Ava II (Huang et al., 2004). The PCR was performed in a total of 25 µL reaction mixture containing 5ng genomic DNA, 200 µM of deoxynucleoside

triphosphate mixture (i.e. dNTPs: dATP, dCTP, dGTP, dTTP) (Roche Biochemicals), 0.2µM of each primer, 2.0 mM of $MgCl_2$, 1xPCR buffer, 1U of Taq polymerase (Roche Biochemicals). A programmable thermal cycler was employed in all PCR reactions. The UGT1A1*6 and UGT1A1*28 PCR cycles start with a 3-minute denaturation of the DNA, followed by 35 cycles of denaturation at 94oC for 30 seconds, primer annealing at 52 ℃ for 30 seconds, primer extension at 72 ℃ for 1 minute, and a final extension at 72 ℃ for 6 minutes. The RFLP reaction was carried out for 15 minutes at 65 ℃ in a 20 µl reaction mixture containing 1 µl of the restriction enzyme (Ava II), 5 µl of 10x NE Buffer, 8 µl of PCR Amplicon, and 6 µl of nuclease-free water.

DNA Quantification

The Nanodrop-1000 spectrophotometer was used to quantify DNA. A software installation system was linked to the spectrophotometer. To blank the machine, two microliters of nuclease-free water were utilized. Two microliters of extracted DNA products were placed on the pedestal, and the quantity and purity of the extracted DNA were determined at 260 nm and 280 nm (A260/A280) wavelength, as displayed on the monitor by the program.

Gel Electrophoresis

The digested PCR-RFLP products (amplicons) were visualized on 1 % agarose gel electrophoresis. 1.5 g of agarose was weighed into a conical flask, and 100 ml of 1xTBE gel buffer solution was added to make the agarose gel. By heating the agarose in the microwave for 2 minutes, allowing it to cool (55 ℃), and then adding 1.5 µl of EZ vision dye to the gel and pouring it onto the plate, the agarose was completely dissolved. 5 µl of PCR product and 20 µl of digestion product were loaded onto the gel for analysis, along with 10 µl of the diluted molecular weight maker. The gel was electrophoretically run at 130V for 2.5 to 3.0cm migration. The Gel Photosystem P1-1002 was used to view the gel's results.

Data Analysis and Ethical Approval

Statistical data were analyzed using ANOVA with SPSS software, version 23.0, Excel spreadsheet, and GraphPad Inc USA software. The study protocol was approved by the Federal Medical Centre, Yenagoa, Bayelsa State, Nigeria.

RESULTS

The information obtained from participants was coded with A01 – A52, with their demographic data, such as age, marital status, and state of origin, as well as the patient weight at the time of data collection, are presented in Table 1

The Agarose gel electrophoresis of UGT1A1 gene of DNA sample from HIV-positive patients on dolutegravir-based combination therapy. Lane 1 - 10 represents the positive UGT1A1 gene bands (280bp). Lane M represents the 100bp DNA Ladder of 1000bp. This is presented in plate 1.

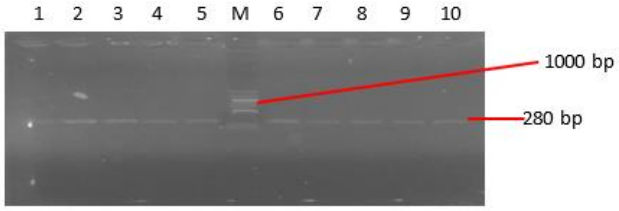

PLATE 1: Agarose gel electrophoresis of UGT1A1 gene DNA samples 1 - 10.

The Agarose gel electrophoresis of UGT1A1 gene of DNA sample from HIV-positive patients on dolutegravir-based combination therapy. Lane 11 - 21 represents the positive UGT1A1 gene bands (280bp). Lane M represents the 100bp DNA Ladder of 1000bp. This is presented in plate 2.

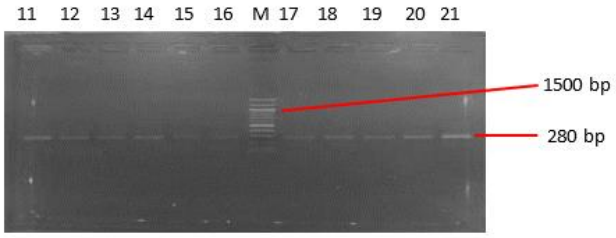

PLATE 2: Agarose gel electrophoresis of UGT1A1 gene of DNA samples 11 – 21.

The agarose gel electrophoresis of *UGT1A1 gene* of DNA sample from HIV-positive patients on dolutegravir-based combination therapy. Lane 22 - 32 represents the positive UGT1A1 gene bands (280bp). Lane M represents the 100bp DNA Ladder of 1000bp. This is presented in plate 3.

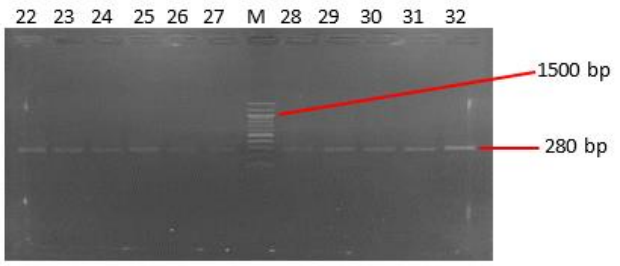

PLATE 3: Agarose gel electrophoresis of *UGT1A1 gene* of DNA samples 22 – 32.

The agarose gel electrophoresis of UGT1A1 gene of DNA sample from HIV-positive patients on dolutegravir-based combination therapy. Lane 33 - 43 represents the positive UGT1A1 gene bands (280bp). Lane M represents the 100bp DNA Ladder of 1000bp. This is presented in plate 4.

Table 1. Demographic Data of study participants

S/N	Lab Code	Gender	Age (Years)	Marital Status	State of Origin	Weight (Kg)
1.	A01	F	44	M	BY	87
2.	A02	F	29	S	AN	58
3.	A03	F	37	M	AB	81
4.	A04	F	40	M	RIV	56
5.	A05	M	53	M	DE	62
6.	A06	F	34	M	AK	65
7.	A07	M	37	M	BY	60
8.	A08	F	35	M	BY	65
9.	A09	M	40	M	AN	51
10.	A10	M	57	M	RIV	54
11.	A11	F	33	M	BY	56
12.	A12	F	47	D/S	BY	55
13.	A13	F	27	M	EN	71
14.	A14	F	34	S	BY	71
15.	A15	M	48	M	RIV	75
16.	A16	F	31	S	BY	75
17.	A17	F	28	M	BY	60
18.	A18	F	38	M	AN	92
19.	A19	F	45	M	BY	89
20.	A20	M	38	M	BY	62
21.	A21	F	34	M	AB	65
22.	A22	F	53	D/S	IM	103
23.	A23	F	44	M	IM	60
24.	A24	F	27	S	CR	50
25.	A25	F	42	W	IM	57
26.	A26	F	37	M	AN	80
27.	A27	F	32	S	AK	48
28.	A28	F	36	M	AK	68
29.	A29	F	34	M	AB	65
30.	A30	F	53	D/S	AK	60
31.	A31	M	42	M	EN	86
32.	A32	F	51	M	RIV	54
33.	A33	F	41	M	AK	65
34.	A34	M	59	WR	ED	67
35.	A35	F	43	S	DE	67
36.	A36	F	40	S	CR	64
37.	A37	M	45	M	AK	55
38.	A38	F	51	W	IM	80
39.	A39	F	37	M	AK	65
40.	A40	M	58	M	IM	58
41.	A41	F	30	M	RIV	82
42.	A42	M	50	M	AK	65
43.	A43	F	38	M	RIV	55
44.	A44	F	58	D/S	RIV	68
45.	A45	F	31	M	RIV	59
46.	A46	F	63	W	DE	57
47.	A47	M	41	M	ED	74
48.	A48	F	42	M	CR	75
49.	A49	M	38	M	EB	70
50.	A50	F	27	M	AB	58
51.	A51	F	48	M	BY	57
52.	A52	F	38	M	BY	65

***Key**: BY – Bayelsa, AN – Anambra, AB – Abia, RIV – Rivers, DE – Delta, EB – Ebonyi, EN – Enugu, IM – Imo, CR – Cross river, AK – Akwa Ibom, ED – Edo. All Participants were on TLD – Tenofovir, Lamivudine, and Dolutegravir combination therapy.*

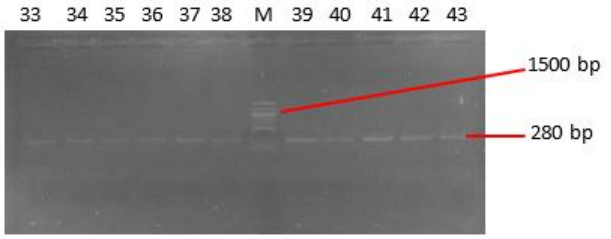

PLATE 4: Agarose gel electrophoresis of UGT1A1 gene of DNA samples 33 - 43.

The agarose gel electrophoresis of UGT1A1 gene of DNA sample from HIV-positive patients on dolutegravir-based combination therapy. Lane 44 - 50 and 52 represent the positive UGT1A1 gene bands (280bp). Lane 51 represents a failed amplification. Lane M represents the 100bp DNA Ladder of 1000bp. This is presented in plate 5.

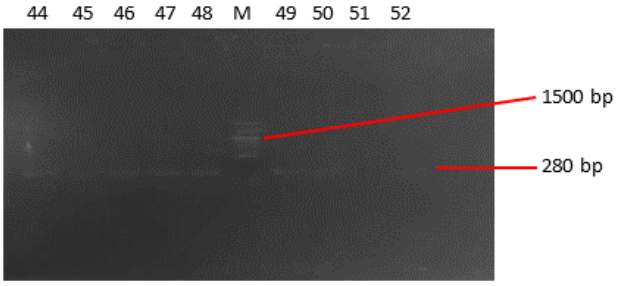

PLATE 5: Agarose gel electrophoresis of UGT1A1 gene of DNA samples 44 – 52.

The RFLP Agarose gel electrophoresis of UGT1A1 gene of DNA sample from HIV-positive patients on dolutegravir-based combination therapy. Lane 1 - 12 represents the positive UGT1A1 gene bands (280bp) and a negative result of the RFLP due to the absence of polymorphic bands on the gel at the end of the analysis. Lane M represents the 100bp DNA Ladder of 1000bp. This is shown in Plate 6.

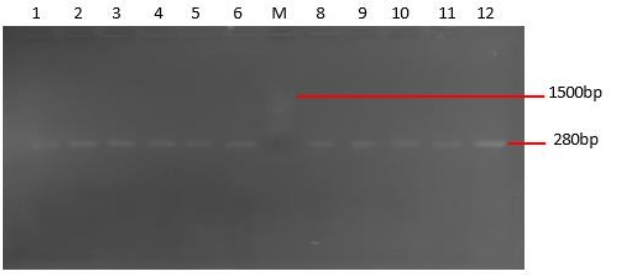

PLATE 6: RFLP Agarose gel electrophoresis of UGT1A1 gene of DNA representative samples 1 -12.

DISCUSSION

Genetic polymorphisms are the occurrence of many alleles at the same locus with a frequency greater than 1% in the DNA. The six blood groups are classic examples of genetic polymorphism, with more than two morphs like A, B, AB, and O being the most common variants in the entire human population, albeit in varying proportions (Daniels, 2005). A polymorphism is any difference in DNA sequence that exists in the genome, such as single nucleotide polymorphisms (SNPs). This is the most common type of genetic variation. Simple insertions/deletions (Indels) are DNA nucleotide insertions or deletions (Bessenyei et al., 2004; Rozas, 2009). Polymorphic repetitive elements - an active transposable component can cause polymorphism in the human genome by inserting itself in various locations, such as repetitive elements of the short DNA stretch discovered by Arthrobacter luteus (Alu) and the long interspersed nuclear elements-1 (LINE1) families (Liehr, 2021; Rishishwar et al., 2015). Polymorphism has become important in modern health, paving the path for personalized care.

After the DNA quantification with NanoDrop-1000, the PCR amplification efficiency was close to 100% in all samples at 280 molecular base pairs (bp), a favourable parameter assay in real-time PCR analysis. In the presence of an inhibitor or an external contaminant, such as phenolic compounds, PCR efficiency can be increased, whereas high amounts and purity of DNA can increase inhibitor levels (Bunu et al., 2020). All DNA samples from HIV-positive patients enrolled for the study appeared at 280bp following amplification using a 100bp DNA molecular ladder, as seen on a 1% agarose gel electrophoretic medium. This implies that all subjects possessed the right gene (UGT1A1) required to code for the UGT1A1 enzyme, which is responsible for dolutegravir metabolism (Plate 1 - 5).

Finally, the Restriction Fragment Length Polymorphism (RFLP) study yielded nearly identical results to the PCR analysis. Using a 100bp DNA molecular ladder, all patient samples remained at 280bp after amplification, restriction enzyme digestion (Ava II), and visualization on a 1% agarose gel electrophoretic medium. This merely demonstrated that there are no evident genetic variations of the gene (UGT1A1) coding for the dolutegravir metabolizing enzyme (UGT1A1) among the ethnic groups studied (Plate 6). This also implies that all participants carry the UGT1A1*1 wild-type allele, which is linked to normal enzymatic function. Compared to previous studies on genetic polymorphism of some highly active antiretroviral therapeutic agents like efavirenz, where genetic polymorphism incidence (CYP2B6) was significantly high, there was no observed genetic polymorphism of dolutegravir among the study population (Bunu et al., 2022). Also, age has been identified as one of the contributing factors associated with DTG neuropsychiatric adverse events (Yagura et al., 2017). This suggests that DTG is not the appropriate antiretroviral regimen for pregnant women or children who are HIV-positive. The participants in this current study showed no DTG polymorphism or serious adverse events. Pregnant women and individuals under 18 were excluded from the study.

CONCLUSION

DNA concentrations were obtained with sufficient yields. Polymerase Chain Reaction (PCR) samples demonstrated at 280 molecular base pairs (bp) on 1% agarose gel

electrophoretic medium that all individuals possessed the required gene to code for the UGT1A1 enzyme, which is responsible for dolutegravir metabolism. RFLP analysis yielded nearly identical results to the PCR study after digestion with *Ava II*. This simply demonstrated that there is no prevalence of genetic polymorphisms of *UGT1A1* gene coding for dolutegravir metabolizing enzyme (UGT1A1) among the selected states in Southern Nigeria, chosen for the study, but a mild significant correlation between dolutegravir and neuropsychiatric side effects among the patients (p-value = 0.08).

REFERENCES

Archibald, M., Pritchard, T., Nehoff, H., Rosengren, R. J., Greish, K., & Taurin, S. (2016). A combination of sorafenib and nilotinib reduces the growth of castrate-resistant prostate cancer. *Int J Nanomedicine*, *11*, 179-200. **[Crossref]**

Bessenyei, B., Marka, M., Urban, L., Zeher, M., & Semsei, I. (2004). Single nucleotide polymorphisms: aging and diseases. *Biogerontology*, *5*(5), 291-303. **[Crossref]**

Bunu, S. J., Denmo Otele, Tolulope Alade, & Dodoru, R. T. (2020). Determination of Serum DNA Purity among Patients Undergoing Antiretroviral Therapy using NanoDrop-1000 Spectrophotometer and Polymerase Chain Reaction. *Biomedical and Biotechnology Research Journal*, *4*, 214-219. **[Crossref]**

Bunu, S. J., Owaba, A. D. C., Vaikosen, E. N., & Ebeshi, B. U. (2022). The Cyp2b6 Gene Polymorphism and Phenotypic Correlation of Efavirenz-Based Combination Therapy Among the Niger Delta Ethnic Population: Implications in Modern Pharmacogenomics. *Pharmgenomics Pers Med*, *15*, 45-54. **[Crossref]**

Chang, J. H., Plise, E., Cheong, J., Ho, Q., & Lin, M. (2013). Evaluating the in vitro inhibition of UGT1A1, OATP1B1, OATP1B3, MRP2, and BSEP in predicting drug-induced hyperbilirubinemia. *Mol Pharm*, *10*(8), 3067-3075. **[Crossref]**

Chauhan, T. (2018). Genetic Mutations- Definition, Types, Causes and Examples. *Genetic Education, Mutation* Retrieved 01 August 2023, from https://geneticeducation.co.in/genetic-mutations-definition-types-causes-and-examples/

Daniels, G. (2005). The molecular genetics of blood group polymorphism. *Transpl Immunol*, *14*(3-4), 143-153. **[Crossref]**

Dean, L. (2012). Irinotecan Therapy and UGT1A1 Genotype. In V. M. Pratt, S. A. Scott, M. Pirmohamed, B. Esquivel, B. L. Kattman, & A. J. Malheiro (Eds.), *Medical Genetics Summaries*. https://www.ncbi.nlm.nih.gov/pubmed/28520360

Ebeshi, U. B., Oluseye, O. B., & Collen, M. M. (2011). Cytochrome P450 2D6 (CYP2D6) Genotype and Phenotype Determination in Nigeria Population. *Asian Journal of Pharmaceutical and Health Sciences*, *1*(2), 47-54. https://ajphs.com/sites/default/files/AsianJPharmHealthSci-1-2-47.pdf

Fantauzzi, A., & Mezzaroma, I. (2014). Dolutegravir: clinical efficacy and role in HIV therapy. *Ther Adv Chronic Dis*, *5*(4), 164-177. **[Crossref]**

Fasinu, P. S., & Rapp, G. K. (2019). Herbal Interaction With Chemotherapeutic Drugs - A Focus on Clinically Significant Findings. *Front Oncol*, *9*, 1356. **[Crossref]**

Gervai, J. (2009). Environmental and genetic influences on early attachment. *Child Adolesc Psychiatry Ment Health*, *3*(1), 25. **[Crossref]**

Gil, J., & Sasiadek, M. M. (2012). Gilbert syndrome: the UGT1A1*28 promoter polymorphism as a biomarker of multifactorial diseases and drug metabolism. *Biomark Med*, *6*(2), 223-230. **[Crossref]**

Goon, C. P., Wang, L. Z., Wong, F. C., Thuya, W. L., Ho, P. C., & Goh, B. C. (2016). UGT1A1 Mediated Drug Interactions and its Clinical Relevance. *Curr Drug Metab*, *17*(2), 100-106. **[Crossref]**

Huang, W., Zhang, J., & Moore, D. D. (2004). A traditional herbal medicine enhances bilirubin clearance by activating the nuclear receptor CAR. *J Clin Invest*, *113*(1), 137-143. **[Crossref]**

Huik, K., Hill, S., George, J., Pau, A., Kuriakose, S., Lange, C. M., Dee, N., Stoll, P., Khan, M., Rehman, T., Rehm, C. A., Dewar, R., Grossman, Z., & Maldarelli, F. (2022). High-level dolutegravir resistance can emerge rapidly from few variants and spread by recombination: implications for integrase strand transfer inhibitor salvage therapy. *AIDS*, *36*(13), 1835-1840. **[Crossref]**

Ismail, S. E., Mona. (2012). Genetic polymorphism studies in humans. *Middle East Journal of Medical Genetics*, *1*(2), 57-63,. **[Crossref]**

Janeway, C. J., Travers, P., & Walport, M. (2001). Immunobiology: The Immune System in Health and Disease. In *Inherited immunodeficiency diseases* (Vol. 5th edition). Garland Science.

https://www.ncbi.nlm.nih.gov/books/NBK27109/

Liehr, T. (2021). Repetitive Elements in Humans. *Int J Mol Sci*, *22*(4). **[Crossref]**

Lv, X., Xia, Y., Finel, M., Wu, J., Ge, G., & Yang, L. (2019). Recent progress and challenges in screening and characterization of UGT1A1 inhibitors. *Acta Pharm Sin B*, *9*(2), 258-278. **[Crossref]**

Mahdieh, N., & Rabbani, B. (2013). An overview of mutation detection methods in genetic disorders. *Iran J Pediatr*, *23*(4), 375-388. https://www.ncbi.nlm.nih.gov/pubmed/24427490

Marques, S. C., & Ikediobi, O. N. (2010). The clinical application of UGT1A1 pharmacogenetic testing: gene-environment interactions. *Hum Genomics*, *4*(4), 238-249. **[Crossref]**

McCormack, P. L. (2014). Dolutegravir: a review of its use in the management of HIV-1 infection in adolescents and adults. *Drugs*, *74*(11), 1241-1252. **[Crossref]**

Meech, R., Hu, D. G., McKinnon, R. A., Mubarokah, S. N., Haines, A. Z., Nair, P. C., Rowland, A., & Mackenzie, P. I. (2019). The UDP-Glycosyltransferase (UGT) Superfamily: New Members, New Functions, and Novel Paradigms. *Physiol Rev*, *99*(2), 1153-1222. **[Crossref]**

Mi, X. X., Yan, J., Ma, X. J., Zhu, G. L., Gao, Y. D., Yang, W. J., Kong, X. W., Chen, G. Y., Shi, J. P., & Gong, L. (2019). Analysis of the UGT1A1 Genotype in Hyperbilirubinemia Patients: Differences in Allele Frequency and Distribution. *Biomed Res Int*, *2019*, 6272174. **[Crossref]**

Mirambeau, G. (2008). [How proviral DNA is integrated into the host cell DNA and how this process can be inhibited]. *Enferm Infecc Microbiol Clin*, *26 Suppl 12*, 11-16. **[Crossref]** (Como se integra el ADN proviral en el ADN de la celula del huesped y como se puede inhibir el proceso.)

Mroziewicz, M., & Tyndale, R. F. (2010). Pharmacogenetics: a tool for identifying genetic factors in drug dependence and response to treatment. *Addict Sci Clin Pract*, *5*(2), 17-29. https://www.ncbi.nlm.nih.gov/pubmed/22002450

NIH. (2017). Understanding Genetic Variance and Phenotype Expression. In E. National Academies of Sciences, and Medicine; Health and Medicine Division; Board on Health Care Services; Board on the Health of Select Populations; Committee on the Evidence Base for Genetic Testing. (Ed.), *An Evidence Framework for Genetic Testing*. National Academies Press (US). https://www.ncbi.nlm.nih.gov/books/NBK425811/

Oates, J. T., & Lopez, D. (2018). Pharmacogenetics: An Important Part of Drug Development with A Focus on Its Application. *Int J Biomed Investig*, *1*(2). **[Crossref]**

Rhee, S. Y., Grant, P. M., Tzou, P. L., Barrow, G., Harrigan, P. R., Ioannidis, J. P. A., & Shafer, R. W. (2019). A systematic review of the genetic mechanisms of dolutegravir resistance. *J Antimicrob Chemother*, *74*(11), 3135-3149. **[Crossref]**

Ribera, E., & Podzamczer, D. (2015). Mechanisms of action, pharmacology, and interactions of dolutegravir. *Enferm Infecc Microbiol Clin*, *33 Suppl 1*, 2-8. **[Crossref]** (Mecanismo de accion, farmacologia e interacciones de dolutegravir.)

Rishishwar, L., Tellez Villa, C. E., & Jordan, I. K. (2015). Transposable element polymorphisms recapitulate human evolution. *Mob DNA*, *6*, 21. **[Crossref]**

Rozas, J. (2009). DNA sequence polymorphism analysis using DnaSP. *Methods Mol Biol*, *537*, 337-350. **[Crossref]**

Surendiran, A., Pradhan, S. C., & Adithan, C. (2008). Role of pharmacogenomics in drug discovery and development. *Indian J Pharmacol*, *40*(4), 137-143. **[Crossref]**

Teh, L. K., Hashim, H., Zakaria, Z. A., & Salleh, M. Z. (2012). Polymorphisms of UGT1A1*6, UGT1A1*27, and UGT1A1*28 in three major ethnic groups from Malaysia. *Indian J Med Res*, *136*(2), 249-259. https://www.ncbi.nlm.nih.gov/pubmed/22960892

Tiffon, C. (2018). The Impact of Nutrition and Environmental Epigenetics on Human Health and Disease. *Int J Mol Sci*, *19*(11). **[Crossref]**

WHO. (2023). *HIV and AIDS* https://www.who.int/news-room/fact-sheets/detail/hiv-aids

Yagura, H., Watanabe, D., Kushida, H., Tomishima, K., Togami, H., Hirano, A., Takahashi, M., Hirota, K., Ikuma, M., Kasai, D., Nishida, Y., Yoshino, M., Yamazaki, K., Uehira, T., & Shirasaka, T. (2017). Impact of UGT1A1 gene polymorphisms on plasma dolutegravir trough concentrations and neuropsychiatric adverse events in Japanese individuals infected with HIV-1. *BMC Infect Dis*, *17*(1), 622. **[Crossref]**

Yang, N., Sun, R., Liao, X., Aa, J., & Wang, G. (2017). UDP-glucuronosyltransferases (UGTs) and their related metabolic cross-talk with internal homeostasis: A systematic review of UGT isoforms for precision medicine. *Pharmacol Res*, *121*, 169-183. **[Crossref]**

Yang, Y., Zhou, M., Hu, M., Cui, Y., Zhong, Q., Liang, L., & Huang, F. (2018). UGT1A1*6 and UGT1A1*28 polymorphisms are correlated with irinotecan-induced toxicity: A meta-analysis. *Asia Pac J Clin Oncol*, *14*(5), e479-e489. **[Crossref]**

Zhou, Y., Fu, W. B., Si, F. L., Yan, Z. T., Zhang, Y. J., He, Q. Y., & Chen, B. (2019). UDP-glycosyltransferase genes and their association and mutations associated with pyrethroid resistance in Anopheles sinensis (Diptera: Culicidae). *Malar J*, *18*(1), 62. **[Crossref]**

Zhu, Y. D., Guan, X. Q., Chen, J., Peng, S., Finel, M., Zhao, Y. Y., Wang, R. M., Bi, H. C., Lei, M., Wang, D. D., & Ge, G. B. (2020). Neobavaisoflavone Induces Bilirubin Metabolizing Enzyme UGT1A1 via PPARalpha and PPARgamma. *Front Pharmacol*, *11*, 628314. **[Crossref]**