PHARMACOGENOMIC OF MATAMORPHINE IN TYPE -2 DIABETES

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ABSTRACT

One of the main causes of death with a fast-rising prevalence is type 2 diabetes. After lifestyle changes fail, pharmacological treatment is the first recommended course of action. However, a sizable portion of patients exhibit or develop treatment resistance over time and as their diseases worsen. Additionally, not every type 2 diabetic patient responds the same way to medication. Although nongenetic factors (hepatic, renal, and intestinal) are present, genetic explanations account for the majority of this heterogeneity. Despite the fact that there are still some unresolved issues, pharmacogenomics studies have described a link between single nucleotide polymorphisms and medication resistance. The most trustworthy method for examining allelic variants to far is next-generation sequencing, which enables the simultaneous investigation of nucleotide variants and gene expression on a genome-wide scale. Here, we evaluate. the association between insulin signaling and CYP2C9 gene polymorphisms, a gene involved in drug metabolism (ABCC8, KCNJ11, and PPARG). We also point out how recent developments in sequencing technology have made it possible for researchers to conduct extensive pharmacogenomics studies. The discovery of allelic variations linked to drug resistance will provide a strong foundation for developing individualized therapeutic strategies for the management of type 2 diabetes.

***Keywords**: Type 2 diabetes, Pharmacogenomics, Pharmacological treatment, Metabolism, Insulin.*

INTRODUCTION

Pharmacogenomics is the study of how genetics affect drug response, which can vary from losing the desired therapeutic benefit at one end of the spectrum to having an unfavourable drug reaction at the other [1,2]. The most often prescribed medication for the treatment of type 2 diabetes, metformin, was recently the subject of a workshop sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). Metformin's effectiveness varies widely, and it occasionally causes major side effects [3]. This issue includes a report on that workshop. In addition to providing information on the current status of metformin pharmacogenomics [4] also give an overview of the field of pharmacogenomics as a whole. The data from pharmacogenomics is the current state of the field of pharmacogenomics. Pharmacogenomic data is increasingly being used in clinical settings to modify drug dosage or prevent negative drug reactions [5]. Contrary to most medications, metformin does not go through biotransformation. It is not broken down [6]. On the other hand, it is moved into and out of cells and organs [7–9]. Metformin transporter genes such as OCT1 (SLC22A1), MATE1 (SLC47A1), and MATE2 (SLC47A2) have been the subject of successful pharmacogenomic investigations [7–9].

Diabetes is a chronic disease, which over time leads to cardiovascular and blood vessels damage and neuro, and

retinopathy, with a dramatic impact on health and high costs for all National Health Systems. Intensive programs that consider lifestyle changes to reduce T2D risk have revealed a moderate efficacy in reducing diabetes incidence in at-risk individuals [15]. When lifestyle changes are not sufficient to ameliorate the clinical features of T2D patients, it is necessary to design an appropriate pharmacological approach. In this scenario, the pharmacogenomics is a discipline that studies the importance of optimal treatment to patients, starting from the knowledge about the genetic and molecular etiology of the disease. Several studies have shown a widespread variability in glycemic response tolerability, and a plethora of variable effects in patients treated with similar antidiabetic drug [16, 17]. The foundation of pharmacogenomics is represented by these lines of evidence [14]. SNPs typically have a major role in determining interindividual variability (SNPs). Particularly, genes directly (or indirectly) linked to the activity (or metabolism) of oral antidiabetic medications have been identified to account for a significant portion of the genetic variability seen in T2D patients (OAD). Following the failure of lifestyle adjustments, the assumption of these medications is the initial intervention step in the therapy of T2D. Therefore, finding genetic variations linked to altered medication responsiveness is crucial for diabetes research since it will allow for a more individualised therapy strategy.

However, other biological, non-genetic variables, such as hepatic, renal, and intestinal processes, can have an impact on the pharmacodynamics of OADs. These Considerations underline the significance of taking into account both the genetics and the phenotype of T2D patients in order to select the most effective therapy strategy [15]. The association between treatment responsiveness in T2D patients and SNPs is described in this review, along with the organs that are crucial for drug metabolism or action. We also go through recent developments in sequencing technology, emphasising how they can significantly boost pharmacogenomics research. The development of individualised therapeutic strategies for the treatment of type 2 diabetes will undoubtedly be supported by the recent technological advances in the identification of allelic variations linked to altered medication response.

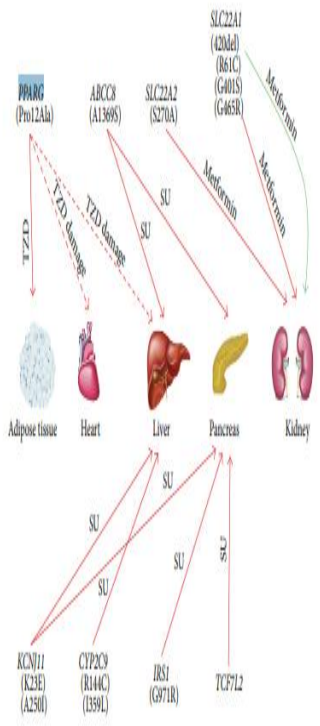

FIGURE 1: Interactions between gene products and OADs on target organs. Genes and the related "at-risk" SNPs (in brackets) are shown in the upper part. Arrows indicate if a SNP has a negative impact on the responsiveness to a given drug in a specific organ. Red arrows indicate increased drug resistance (or altered drug metabolism), whereas green arrows indicate a beneficial effect of such a SNP. Dashed lines indicate side effects of a given drug. TZD = thiazolidinedione; SU = sulphonylureas; MET = metformin.

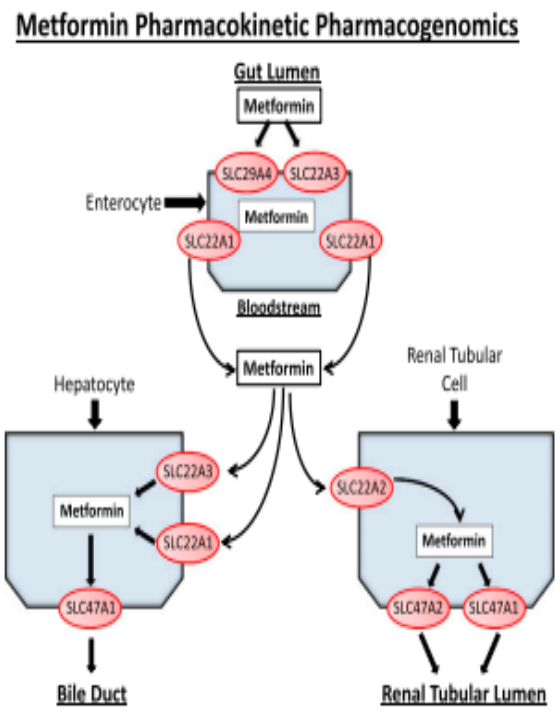

Figure 1—Schematic representation of the cellular locations of SLC transporters that contribute to metformin pharmacokinetics. It has been reported that the genes encoding SLC22A1, MATE1, and MATE2 are genetically polymorphic and that these polymorphisms contribute to individual variation in metformin pharmacokinetics (7–9). SLC22A1 = OCT1, SLC22A2 = OCT2, SLC22A3 = OCT3, SLC29A4 = ENT4, SLC47A1 = MATE1, and SLC47A2 = MATE2.

Even though the MATE1 and MATE2 genes have common genetic polymorphisms that have been linked to altered metformin-related glucose-lowering effects, the clinical utility of these transporter gene polymorphisms has yet to be proven, despite the fact that OCT1 encodes the main metformin transporter in the liver and is functionally genetically polymorphic 40 genes were subjected to a candidate gene research that discovered polymorphisms in these possible metformin target pathways, including the AMP-activated protein kinase pathway (10) . One of the biggest causes of death in modern civilization is diabetes [11]. According to the International Diabetes Federation's most recent report from 2013, there were 382 million cases of diabetes globally with an adult-onset rate of roughly 8.4%. The World Health Organization (WHO) has classified this problem as a "global outbreak" because it is predicted that by 2035, there would be 592 million cases worldwide [11]. Type 1 diabetes mellitus (T1D), also known as "insulin-dependent diabetes" or "juvenile diabetes," [12] and type diabetes mellitus (T2D), sometimes known as "noninsulin-dependent diabetes," are the two more prevalent types of diabetes that are both caused by abnormalities in insulin function. The first kind has an early onset and results from an utter lack of insulin [13], whereas the second type—the most common type—has an older onset and results from an impaired function of the hormone [14]. More than 5% of people in affluent countries have T2D, and its prevalence is rising globally. Diabetes is a chronic condition that over time damages the heart, blood vessels, nerves, kidneys, and eyes (causing retinopathy). Diabetes is a chronic disease having a significant negative impact on health and substantial expenses for all National Health Systems [12]. Over time, diabetes causes damage to the heart, blood vessels, nerves, kidneys, and retina. Intensive programmes that take into account lifestyle modifications to lower T2D risk have shown a moderate effectiveness in lowering diabetes incidence in at-risk patients [15]. Designing an adequate pharmaceutical strategy is important when lifestyle adjustments are insufficient to improve the clinical aspects of T2D patients. For all National Health Systems, there is a tic influence on health and high cost [12]. Intensive programmes that take lifestyle changes into account to lower the risk of T2D have shown a modest reduction in the incidence of diabetes in those at risk [15]. To improve the clinical aspects of T2D

patients when lifestyle changes are insufficient, an effective pharmaceutical strategy must be developed. In this case, the study of pharmacogenomics, which begins with knowledge of the genetic and molecular causes of the disease, examines the significance of providing patients with the best possible care. In patients receiving comparable anti-diabetic medications, numerous studies have revealed a wide range of variability in glycaemic response tolerance and a variety of varied effects [16, 17]. The foundation of pharmacogenomics is these lines of evidence [14].

Pharmaceutical Genomics of Diabetes Drugs

The sulphonylureas, metformin, and thiazolidinediones are now the medicines used in T2D treatment that are the most popular (troglitazone, pioglitazone, and rosiglitazone). The major proteins that are either activated by the administration of oral antidiabetic medications or involved in their absorption and metabolism are schematized in Figure 2.

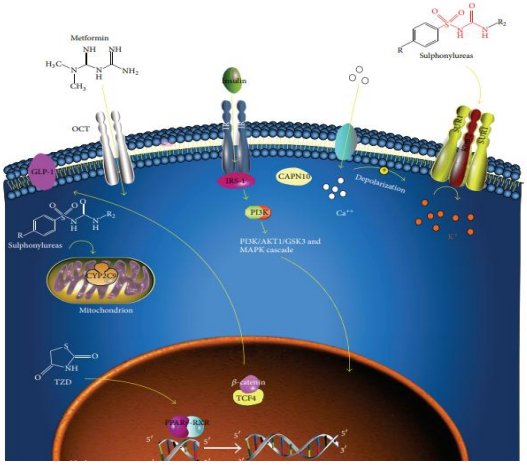

Figure 3: Main proteins involved in uptake and metabolism of OADs. IR = insulin receptor; GLP-1 = glucagon-like peptide-1; SUR1 = sulphonylureas receptor 1; Kir6.2 = potassium inward rectifier 6.2 subunit; PI3K = phosphoinositide 3-kinase; TCF4 = transcription factor 4; RXR = retinoid X receptor; PI3K/AKT1/GSK3 = phosphoinositide 3-kinase/RAC-alpha serine/threonine-protein kinase/glycogen synthase kinase 3; MAPK = mitogen-activated protein kinases Pharmacogenetics studies often take a few clinical outcomes into account when assessing medication response. The guidelines' definition of HbA1c levels of 7% and an overall decrease in HbA1c stand out as the most relevant parameters to take into account in T2D pharmacogenetics investigations [18]. The medicine of interest's use during early or late stages of the disease, where there is a very limited chance of achieving a meaningful therapeutic benefit, is another essential factor. The medicine of interest's use during early or late stages of the disease, where there is a very limited chance of achieving a meaningful therapeutic benefit, is another essential factor. We have compiled a list of SNPs in Table 1 that, based on GWASs, are frequently linked to altered medication responsiveness in T2D. These investigations have frequently shown a lack of a statistically meaningful relationship between SNPs and the closest gene's expression levels, demonstrating a wide range of variability. These association studies have also highlighted the ethnic differences in the expression patterns of SNPs in tissues that are essential for maintaining glucose homeostasis [19].

Effects of Sulphonylureas and Polymorphisms in the ABCC8, KCNJ11, TCF7L2, CYP2C9, IRS1, and CAPN10 Genes.

Sulphonyl urea effects are affected by polymorphisms in the genes ABCC8,

KCNJ11, TCF7L2, CYP2C9, IRS1, and CAPN10. Sulphonylureas (SUs) are often used medications for the treatment of T2D. Various side effects, such as weight gain and an increased risk of hypoglycaemia, have been reported often despite the widespread use of these medications in clinical practise [20, 21]. The four primary SUs that are now used to treat T2D are glibenclamide, gliclazide, glipizide, and glimepiride [22]. All SUs promotes glucose-stimulated insulin release from the pancreatic -cells via binding to the sulphonyl urea receptor 1 (SUR1). As a result, SUs work by interacting with the proteins that make up the ATP sensitive potassium (KATP) channel and causing it to close. In contrast to the outer pore of the KATP channel, which is made up of four K+ ions, Four molecules of SUR1 [23]. The ATP generated by the mitochondrial oxidation of glucose results in the closing of the KATP channel, which depolarizes the -cell membrane and increases the entrance of Ca2+ ions. This is followed by the release of pre synthesized insulin from the -cells. In the end, sulphonylureas bind to a particular receptor outside the KATP channel, causing the closing of these channels and the release of insulin.

The Effects of SLC22A1 Gene Polymorphisms on Metformin

In addition to SU, metformin is a medication that is widely used to treat T2D. Since it becomes positively charged at physiological pH, its pharmacokinetic characteristics are altered [24]. Metformin is eliminated in the urine rather than being processed in the liver like sulphonylureas are. Therefore, genetic variations in the genes encoding metabolising enzymes had no effect on the glucose-lowering action of metformin. Even in this instance, pharmacogenetics has benefited from GWASs to comprehend how SNPs in the genes that encode metformin transporters affect the medication's clinical effects conducted a GWAS on 1024 individuals on metformin treatment, examining 700 K polymorphisms [25, 26]. For genotyping purposes, researchers employed the Go DARTS and United Kingdom Prospective Diabetes Study (UKPDS) populations and got the same outcomes [22].

Pro12Ala 5. PPARG Polymorphism and Thiazolidinedione Responsiveness

Peroxisome Proliferator-Activated Receptor (PPARG), a nuclear receptor, is an important transcription factor in the metabolism of lipids and glucose. Numerous metabolic target genes, including lipoprotein lipase, fatty-acid transcript protein, and aquaporin, which control triglyceride hydrolysis, fatty acid absorption, and glycerol uptake, can be activated by it [27, 28]. Due to the large number of target genes, ligands, and coregulators (coactivators or corepressors) as well as the existence of many isoforms, some of which even have opposite or dominantly negative activity, PPARG is a master gene of adipogenesis and its roles are extremely complex [28, 29]. Indeed, numerous investigations have found PPARG transcripts to exist, strongly indicating that alternative splicing plays a significant role in how such a nuclear receptor functions [30, 31]. PPARG polymorphisms have been extensive analysis over the past ten years, both in the coding and regulatory domains, to determine whether they may be linked to pathologic phenotypes like T2D. [32,33].

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Diabetes and Next-Generation Sequencing

Studies based on genotyping and transcriptomics have increasingly moved away from hybridization-based methods and toward sequencing-based methods during the past few years. Indeed, an increasing number of studies have demonstrated how polymorphisms might alter gene expression variance among populations as a result of the development of the Next-Generation Sequencing (NGS) technique and new improved sequencing platforms [34, 35].

These results demonstrate that the complexity of T2D and other multifactorial disorders cannot be fully captured by GWAS alone. In fact, the possible causal involvement of SNPs in the susceptibility to complex diseases is only foreseeable in the absence of functional investigations. It is obvious that combining various NGS applications (such RNA-, ChIP-, and DNASeq) may aid doctors in analysing the genetic and epigenetic complexity that underlies complex traits/diseases, including cancer [36]. The most popular and effective method for genome sequencing, studying gene expression, and examining epigenetic markers is currently next-generation sequencing (NGS). Recent years have seen a huge decrease in the cost of experiments because to this sequencing technology, which has also resulted in a significant increase in data production.

Among its uses, RNA-Seq has significantly enhanced transcriptome analysis because of its capacity to identify and quantify low expressed genes, alternative splicing events, posttranscriptional RNA editing, and SNP expression [35, 37]. This is made possible by the type of sequence (read length), the sequence quality, the high throughput, and the low cost of RNA-Seq [38].

Conclusions

Since more research is demonstrating how ncRNAs play a role in human diseases, NGS has the potential to dramatically

advance our understanding of SNPs, medication response, and the non-coding portion of the human genome.

To the best of our knowledge, no comprehensive examination of SNPs in regulatory regions that might modify nucleotide methylation, alter chromatin structure, or effect novel binding sites for transcription factors and microRNAs (miRNAs) has yet been described. The use of NGS to investigate this new possible avenue seems vital in light of this concern

All things considered, we anticipate that NGS will considerably enhance the identification of genetic variations linked to altered medication responsiveness in T2D and that a thorough investigation of better drug use in clinic is anticipated to be guided by how these variations alter gene expression and epigenetic mechanisms.

References

[1] Weinshilboum RM, Wang L. Pharmacogenetics and pharmacogenomics: development, science, and translation. Annu Rev Genomics Hum Genet 2006;7: 223–245

[2] Wang L, McLeod HL, Weinshilboum RM. Genomics and drug response. N Engl J Med 2011;364:1144–1153

[3] Bailey CJ, Turner RC. Metformin. N Engl J Med 1996;334:574–579

[4]Pawlyk AC, Giacomini KM, McKeon C, Shuldiner AR, Florez JC. Metformin pharmacogenomics: current status and future directions. Diabetes 2014;63: 2590–2599

[5] Bielinski SJ, Olson JE, Pathak J, et al. Preemptive genotyping for personalized medicine: design of the right drug, right dose, right time-using genomic data to individualize treatment protocol. Mayo Clin Proc 2014;89: 25–33

[6] Gong L, Goswami S, Giacomini KM, Altman RB, Klein TE. Metformin pathways: pharmacokinetics and pharmacodynamics. Pharmacogenet Genomics 2012;22:820–827

[7] Shu Y, Sheardown SA, Brown C, et al. Effect of genetic variation in the organic cation transporter 1 (OCT1) on metformin action. J Clin Invest 2007;117: 1422–1431

[8] Shu Y, Brown C, Castro RA, et al. Effect of genetic variation in the organic cation transporter 1, OCT1, on metformin pharmacokinetics. Clin Pharmacol Ther 2008;83:273–280

[9] Stocker SL, Morrissey KM, Yee SW, et al. The effect of novel promoter variants in MATE1 and MATE2 on the pharmacokinetics and pharmacodynamics of metformin. Clin Pharmacol Ther 2013;93:186–194

[10] Jablonski KA, McAteer JB, de Bakker PIW, et al.; Diabetes Prevention Program Research Group. Common variants in 40 genes assessed for diabetes incidence and response to metformin and lifestyle intervention in the diabetes prevention program. Diabetes 2010;59:2672–2681

[11] J. E. Shaw, R. A. Sicree, and P. Z. Zimmet, "Global estimates of the prevalence of diabetes for 2010 and 2030," Diabetes Research and Clinical Practice, vol. 87, no. 1, pp. 4–14, 2010.

[12] World Health Organization, Diabetes, World Health Organization, Geneva, Switzerland, 2015.

[13] A. Hattersley, J. Bruining, J. Shield, P. Njolstad, and K. C. Donaghue, "The diagnosis and management of monogenic diabetes in children and adolescents," Pediatric Diabetes, vol. 10, no. 12, pp. 33–42, 2009.

[14] G. C. Mannino and G. Sesti, "Individualized therapy for type 2 diabetes: clinical implications of pharmacogenetic data," Molecular Diagnosis & Therapy, vol. 16, no. 5, pp. 285–302, 2012

[15] M. L. Becker, E. R. Pearson, and I. Tka´c, "Pharmacogenetics of ˇ oral antidiabetic drugs," International Journal of Endocrinology, vol. 2013, Article ID 686315, 10 pages, 2013.

[16] J. K. Di Stefano and R. M.Watanabe, "Pharmacogenetics of antidiabetes drugs," Pharmaceuticals, vol. 3, no. 8, pp. 2610–2646, 2010.

[17] L. K. Billings and J. C. Florez, "The genetics of type 2 diabetes: what have we learned from GWAS?" Annals of the New York Academy of Sciences, vol. 1212, pp. 59–77, 2010.

[18] A. Vella, "Pharmacogenetics for type 2 diabetes: practical considerations for study design," Journal of Diabetes Science and Technology, vol. 3, no. 4, pp. 705–709, 2009.

[19] H. P. Kang, X. Yang, R. Chen et al., "Integration of diseasespecific single nucleotide polymorphisms, expression quantitative trait loci and co-expression networks reveal novel candidate genes for type 2 diabetes," Diabetologia, vol. 55, no. 8, pp. 2205– 2213, 2012.

[20] J. Belsey and G. Krishnarajah, "Glycaemic control and adverse events in patients with type 2 diabetes treated with metformin + sulphonylurea: a meta-analysis," Diabetes, Obesity and Metabolism, vol. 10, no. 1, pp. 1–7, 2008.

[21] P. L. Drury and T. Cundy, "Glycemic management of type 2 diabetes mellitus," The New England Journal of Medicine, vol. 367, no. 2, pp. 182–183, 2012.

[22] M. Rendell, "The role of sulphonylureas in the management of type 2 diabetes mellitus," Drugs, vol. 64, no. 12, pp. 1339–1358, 2004.

[23] S.-L. Shyng and C. G. Nichols, "Octameric stoichiometry of the KATP channel complex," Journal of General Physiology, vol. 110, no. 6, pp. 655–664, 1997.

[24] G. G. Graham, J. Punt, M. Arora et al., "Clinical pharmacokinetics of metformin," Clinical Pharmacokinetics, vol. 50, no. 2, pp. 81–98, 2011.

[25] K. Zhou, C. Bellenguez, C. C. A. Spencer et al., "Common variants near ATM are associated with glycemic response to metformin in type 2 diabetes," Nature Genetics, vol. 43, no. 2, pp. 117–120, 2011.

[26] N. van Leeuwen, G. Nijpels, M. L. Becker et al., "A gene variant near ATM is significantly associated with metformin treatment response in type 2 diabetes: a replication and meta-analysis of five cohorts," Diabetologia, vol. 55, no. 7, pp. 1971–1977, 2012.

[27] M. Agostini, E. Schoenmakers, C. Mitchell et al., "Non-DNA binding, dominant-negative, human PPARγ mutations cause lipodystrophic insulin resistance," Cell Metabolism, vol. 4, no. 4, pp. 303–311, 2006.

[28] V. Costa, M. A. Gallo, F. Letizia, M. Aprile, A. Casamassimi, and A. Ciccodicola, "PPARG: gene expression regulation and nextgeneration sequencing for unsolved issues," PPAR Research, vol. 2010, Article ID 409168, 17 pages, 2010.

[29] M. Aprile, M. R. Ambrosio, V. D'Esposito et al., "PPARG in human adipogenesis: differential contribution of canonical transcripts and dominant negative isoforms," PPAR Research, vol. 2014, Article ID 537865, 11 pages, 2014.

[30] C. Knouff and J. Auwerx, "Peroxisome proliferator-activated receptor-γ calls for activation in moderation: lessons from genetics and pharmacology," Endocrine Reviews, vol. 25, no. 6, pp. 899–918, 2004.

[31] S. Heikkinen, J. Auwerx, and C. A. Argmann, "PPARγ in human and mouse physiology," Biochimica et Biophysica Acta, vol. 1771, no. 8, pp. 999–1013, 2007.

[32] W. He, "PPARγ2Pro12Ala polymorphism and human health," PPAR Research, vol. 2009, Article ID 849538, 15 pages, 2009.

[33] A. Ciccodicola, V. Costa, A. Casamassimi et al., "Characterization of a novel polymorphism in PPARG regulatory region associated with type 2 diabetes and diabetic retinopathy in italy," Journal of Biomedicine and Biotechnology, vol. 2009, Article ID 126917, 7 pages, 2009.

[34] A. Battle, S. Mostafavi, X. Zhu et al., "Characterizing the genetic basis of transcriptome diversity through RNA-sequencing of 922 individuals,"Genome Research, vol. 24, no. 1, pp. 14–24, 2014.

[35] S. B. Montgomery and E. T. Dermitzakis, "From expression QTLs to personalized transcriptomics," Nature Reviews Genetics, vol. 12, no. 4, pp. 277–282, 2011

[36] V. Costa, M. Aprile, R. Esposito, and A. Ciccodicola, "RNASeq and human complex diseases: recent accomplishments and future perspectives," European Journal of Human Genetics, vol. 21, no. 2, pp. 134–142, 2013.

[37] N. K. Yadav, P. Shukla, A. Omer, S. Pareek, and R. K. Singh, "Next generation sequencing: potential and application in drug discovery," The Scientific World Journal, vol. 2014, Article ID 802437, 7 pages, 2014.

[38] M. L. Metzker, "Emerging technologies in DNA sequencing," Genome Research, vol. 15, no. 12, pp. 1767–1776, 2005.

[39] Marguerat, B. T. Wilhelm, and J. Bahler, "Next-generation ¨ sequencing: applications beyond Genomes," Biochemical Society Transactions, vol. 36, no. 5, pp. 1091–1096, 2008.

[40] M. Sultan, M. H. Schulz, H. Richard et al., "A global view of gene activity and alternative splicing by deep sequencing of the human transcriptome," Science, vol. 321, no. 5891, pp. 956–960, 200

[41]. World Health Organization, Diabetes, World Health Organization, Geneva, Switzerland, 2015.

[42]. ***Sharma Shailesh****, Mithesh phale et al. "Exploration and formulation of oral selective polysaccharide based, Budesonide-B-cyclodextrin for colon specific drug" international Research journal of current trends in Pharmaceutical Research, IJCTPR, Dec2016, Vol 4(6), PP262-270.*

[43]. Bishnoi, et al. "A Pharmacovigilance Study of Antihypertensive Medicines" World Journal OF Pharmacy & Pharmaceutical Sciences, Jan.2021 Volume 10, Issue 3, 1081-1089.