International Journal of Basic and Applied Biology
Print ISSN: 2349 – 5820; Online ISSN: 2349 – 5839 ; Volume. 2 No. 2, November 2014. pp. 63 – 68

http://www.krishisanskriti.org/ijbab.html

Aspirin Resistance: An Emerging Clinical Predicament is Associated with Single Nucleotide Polymorphisms

Shreya Majumdar[1], Sonakshi Rustagi[2], Sreshtha Mondal[3], Vani Rijhwani[4], Jasvinder Kaur[5], Poonam Sharma[6]

Gargi College
University of Delh

Abstract: **People use prescribed drugs all the time and there are different responses to a particular drug shown by different individuals. These responses largely have to do with single nucleotide polymorphisms. Aspirin is a salicylate drug often used as an analgesic, antipyretic, anti-inflammatory medication. It also has an anti-platelet effect by inhibiting the production of thromboxane. Approximately 40, 000 tones of this drug is being consumed every year. However, there are individual variations in response to aspirin, creating the term "aspirin non-responsiveness" or "aspirin resistance". Aspirin and its interaction with platelet aggregation inhibitor pathway genes (forty nine genes involved) were studied to hit upon the gene mutations, which gave rise to different responses to the same drug. Twelve of these exon mutations changed the originally coded amino acid with a new one (PTGDR, ITGB3, P2RY12, PLA2G4A, PLA2G4B, VWF, PTGS1, TBXAS1, TBX2R2, F2, F2RL3, and GNA13). These polymorphisms lie in the extracellular, cytoplasmic, signal sequence and transmembrane domains of the protein, α-helical regions and β-pleated sheets. These are significant pertaining to the transformation in polarity and charge of the amino acid side chains that in turn modify their interactions with other amino acid residues and aqueous surroundings. This alters the normal functioning of the genes and finally leads to differential responses to the drug aspirin.**

1. INTRODUCTION

The development and function of an organism is in large part controlled by genes. Mutations can lead to changes in the structure of an encoded protein or to a decrease or complete loss in its expression. Because a change in the DNA sequence affects all copies of the encoded protein, mutations can be particularly damaging to a cell or organism. A mutation in one DNA nucleotide (SNP, single-nucleotide polymorphism) can prevent a gene from turning into a protein. These seemingly small differences can have very large consequences, such as how a person responds to a prescription drug. Prescription drugs interact with signaling pathways in our bodies in very specific ways. If the protein that the drug interacts with has been mutated in such a way that the drug cannot interact with it or if the protein is not being made at all, the drug may not function, as it should. Aspirin, also known as acetylsalicylic acid, is a salicylate drug, often used as an analgesic to relieve minor aches and pains, as an antipyretic to reduce fever, and as an anti-inflammatory medication. Aspirin also has an antiplatelet effect by inhibiting the production of thromboxane, which under normal circumstances binds platelet molecules together to create a patch over damaged walls of blood vessels [1]. Because the platelet patch can become too large and also block blood flow, locally and downstream, aspirin is also used long-term, at low doses, to help prevent heart attacks, strokes, and blood clot formation in people at high risk of developing blood clots. Clinical studies have demonstrated that aspirin therapy is associated with a 12–25% reduction in non-fatal myocardial infarction (MI), non-fatal stroke or vascular death among high risk patients. However, there are individual variations in response to aspirin creating the term "aspirin non-responsiveness" or "aspirin resistance" [1,2] . Although aspirin resistance has been reported to occur in 5.5% to 60% of the population, the exact prevalence of aspirin resistance is still unclear due to lack of a standard definition of aspirin response. It has been shown that aspirin resistant patients have about a 4-fold risk of fatal and non-fatal cardiovascular, cerebrovascular, or vascular events compared with aspirin responsive patients [2].

There are 49 Genes involved in the Pathway

ADCY3	CD36	COL1A1	COL1A2	COL3A1	COL4A1	COL4A2
COL4	COL4A4	COL4A5	COL4A6	F2	F2R	F2RL3
FGA	FGB	FGG	GNA11	GNA12	GNA13	GNA15
GNAI1	GNAI2	GNAI3	GNAQ	GNAS	GNB3	GP1BB
GP5	GP6	GP9	ITGA2	ITGA2B	ITGB3	P2RX1

P2RY1	P2RY12	PLA2G2A	PLA2G4A	PLA2G4B	PLCB1	PLCG2
PTGDR	PTGER2	PTGIR	PTGS1	TBXA2R	TBXAS1	VWF

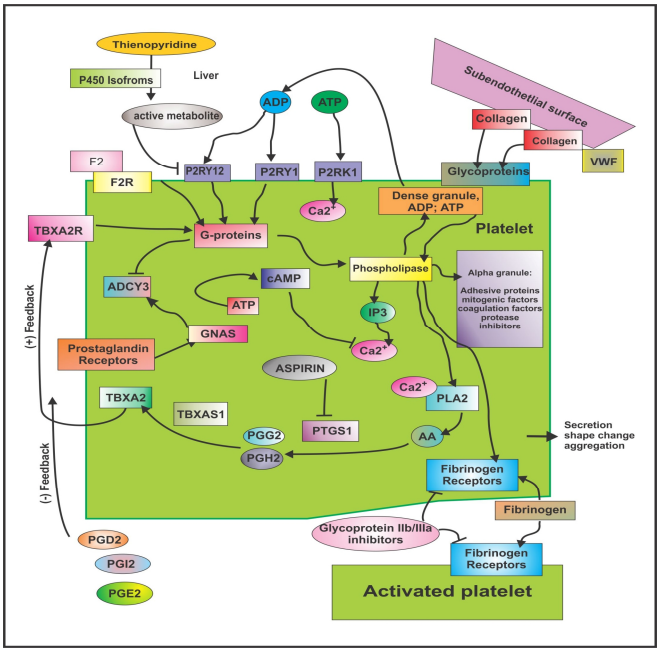

Figure 1: Platelet aggregation pathway [3]

2. METHODOLOGY

The Pharmacogenics Knowledgebase, a pharmacogenics resource was used for noting information about the drug aspirin including its generic or trade (brand) names, its chemical structure, the conditions in which it is used and its general effect on the human body [4]. Pharmacogenics of the drug was understood, which explains how the alleles (versions) of a gene (or genes) that a person has inherited changes its personal biology in a way that makes a drug more or less effective. The gene alleles which are involved in the pathway were noted and information about their variable response to the drug was gathered and recorded in the form of a table. SNP database of the NCBI website was used for this purpose [5]. rsID of the alleles of interest was searched for, that gave information about the allele of interest. It was further noted whether the allele is an intron or an exon. If the mutation was in an exon, then, recorded the corresponding 'allele change' for each gene. Alleles may also arise from mutations in parts of the gene other than exons i.e. 'NearGene' mutations were also noted. For each allele with a mutation in an exon, the "Amino Acid Sequence Change" was noted.

3. RESULTS AND DISCUSSION

Clinical manifestations associated with some of these genes are:

PTGDR: Receptor for prostaglandin D2 (PGD2). Coupled to the G(i)-protein. Amino acid polymorphism at position 190 from glutamic acid to lysine lies in the extracellular domain of the protein. Glutamate prefers sharply turning regions on the surface of the protein and is negatively charged whereas lysine is positively charged [6,7].

ITGB3: It encodes an integrin receptor found on the surface of platelets. It is involved in the cross-linking of platelets with fibrin. Integrin alpha-IIb/beta-3 is a receptor for fibronectin, fibrinogen, plasminogen, prothrombin and thrombospondin. Following activation integrin alpha-IIb/beta-3 brings about platelet/platelet interaction through binding of soluble fibrinogen. This step leads to rapid platelet aggregation which physically plugs ruptured endothelial surface [8]. Aspirin is known to reduce platelet aggregation. The presence of this mutation is associated with a 2.8-fold increase in the risk of first myocardial infarction. The mutation might affect the formation of the prothrombinase complex on platelets and, consequently, thrombin-mediated coagulation reactions, such as fibrinogen cleavage and factor XIII or factor V activation. Polymorphism at position 59 is seen where proline is found in place of leucine, this part lies in the extracellular domain of the protein [9]. Being hydrophobic, leucine prefers to be buried in protein hydrophobic cores. It also shows a preference for being within alpha helices more so than in beta strands. The side chain is very non-reactive and is thus rarely directly involved in protein functions like catalysis, although it can play a role in substrate recognition. In particular, hydrophobic amino acids can be involved in binding/recognition of hydrophobic ligands such as lipids [7]. Proline is unique in that it is the only amino acid where the side chain is connected to the protein backbone twice, forming a five-membered ring. It means that proline is unable to occupy many of the main-chain conformations easily adopted by all other amino acids. For this reason proline is often found in very tight turns in protein structures (i.e. where the polypeptide chain must change direction). It can also function to introduce kinks into α-helices, since it is unable to adopt a normal helical conformation [7]. Thus, due to the complete alteration in the structural property of the amino acid side

P2RY12: Receptor for ADP and ATP coupled to G-proteins that inhibit the adenylyl cyclase second messenger system. It negatively couples to adenylyl cyclase via $G\alpha_i$ resulting in

slow, irreversible platelet aggregation. Aspirin is an antagonist to its action [10]. Due to the polymorphism, arginine is substituted for glutamine at position 256, the residue resides in transmembrane helical domain [10]. Both of these are amphipathic, meaning that they contain hydrophobic and polar areas.. In some instances it is possible for such amino acids to play a dual role, with part of the side chain being buried in the protein and another being exposed to water [7]. Arginine is positively charged polar amino acid, whereas, glutamine is neutral. Arginines are also frequently involved in salt-bridges where they pair with a negatively charged aspartate or glutamate to create stabilizing hydrogen bonds that can be important for protein stability. Arginines are quite frequent in protein active or binding sites [7]. Position 256-259 forms ADP binding site, the polymorphism may decrease binding affinity for ADP. The positive charge means that they can interact with negatively-charged non-protein atoms. However, a neutral amino acid in its place can void off interactions that were otherwise important [10].

PLA2G4A: Cytosolic phospholipase A2 selectively hydrolyzes arachidonyl phospholipids releasing arachidonic acid. It hydrolyzes arachidonic acid from cellular membrane phospholipids, thereby providing enzymatic substrates for the synthesis of eicosanoids, such as prostaglandins and leukotrienes. It is implicated in the initiation of the inflammatory response also. Serine to proline polymorphism is situated at position 111 in an alpha helix, where proline might introduce a kink in the helix, thus, destabilizing the structure. However, proline is often mimicked by serine due to its smaller structure; it is often found within tight turns [11, 7].

PLA2G4B: Calcium-dependent phospholipase A2 that selectively hydrolyzes glycerophospholipids with a preference for arachidonoyl phospholipids. Has a much weaker activity than PLA2G4A. Glutamate is found in place of aspartate at position 297 in the protein that forms a part of the PLA2c domain. The PLA2c domain is the catalytic lipase domain in cytosolic phospholipase A2 (cPLA2) [7,8].

VWF: Von Willebrand factor. Important in the maintenance of hemostasis, it promotes adhesion of platelets to the sites of vascular injury by forming a molecular bridge between sub-endothelial collagen matrix and platelet-surface receptor complex GPIb-IX-V. Also acts as a chaperone for coagulation factor VIII, delivering it to the site of injury, stabilizing its heterodimeric structure and protecting it from premature clearance from plasma [8]. Arginine is changed to histidine at position 1374. Located in the VWFA 1 domain; binding site for platelet glycoprotein Ib [12].

PTGS1: Prostaglandin-endoperoxide synthase (fatty acid cyclooxygenase; PGH synthase) is the key enzyme in prostaglandin biosynthesis. The cyclooxygenase activity of the enzyme is inhibited by non-steroidal anti-inflammatory drugs (NSAID) such as aspirin. In platelets, it is involved in the generation of thromboxane A2 (TXA2), which promotes platelet activation and aggregation, vasoconstriction and proliferation of vascular smooth muscle cells [13]. Polymorphism at position 17 changes proline to leucine, this region forms a part of the signal sequence of the protein. Leucine likes to be buried in hydrophobic cores and likes to be placed in alpha helices, whereas, proline introduces a kink in alpha helices as already discussed. Single nucleotide polymorphisms (SNPs) give rise to non-conservative amino acid changes in the signal peptide (Pro(17) to Leu) of cyclooxygenase-1 [7].

TBXA2R: Receptor for thromboxane A2 (TXA2), a potent stimulator of platelet aggregation. The activity of this receptor is mediated by a G-protein that activates a phosphatidylinositol-calcium second messenger system and activates phospholipase C. Thromboxane A2 is an arachidonate metabolite that is a potent stimulator of platelet aggregation and a constrictor of vascular and respiratory smooth muscles. TXA2 has been implicated as a mediator in diseases such as myocardial infarction, stroke, and bronchial asthma. A mutation in this gene results in a bleeding disorder. Arginine to leucine polymorphism at position 60 is brought about a single change in the codon from G to T, this region lies in the cytoplasmic domain of the protein. The leucine side chain is very non-reactive and is thus rarely directly involved in protein functions like catalysis, whereas, arginine frequently pairs with a negatively charged aspartate or glutamate to create stabilizing hydrogen bonds that can be important for protein stability. This conversion thus may lead to destabilizing the protein structure, ultimately altering its function. TXA2 binding is not affected in this SNP but leads to defective interaction with G proteins and impairs phospholipase C and adenylyl cyclase activation.

TBXAS1: Thromboxane A synthase 1 is a endoplasmic reticulum membrane protein catalyzes the conversion of prostaglandin H2 to thromboxane A2, a potent vasoconstrictor and inducer of platelet aggregation. The enzyme plays a role in several pathophysiological processes including hemostasis, cardiovascular disease, and stroke. Alternatively, spliced transcript variants encoding different

isoforms have been found for this gene [8]. Genetic variation in TBXAS1 by mutation or polymorphism is associated with reduced platelet ASA responsiveness. The polymorphism is from leucine to proline at position 82 and 487 that lies in the transmembrane helical domain and cytoplasmic domains respectively [8].

F2: High affinity receptor for activated thrombin coupled to G proteins that stimulate phosphoinositide hydrolysis. It may be involved in platelet activation and in vascular development [14]. Polymorphism is at position 165 from threonine to methionine which is a part of extracellular helical domain of protein [15]. Threonine can reside both within the interior of a protein or on the protein surface. Threonines are quite common in protein functional centers. The hydroxyl group of threonine is fairly reactive, being able to form hydrogen bonds with a variety of polar substrates. Intracellular threonines can also be phosphorylated and in the extracellular environment and they can be O-glycosylated [7]. The methionine side chain is fairly non-reactive, and is thus rarely directly involved in protein function. Like other hydrophobic amino acids, it can play a role in binding/recognition of hydrophobic ligands such as lipids. However, unlike the proper aliphatic amino acids, methionine contains a sulphur atom, that can be involved in binding to atoms such as metals. Whereas, the sulphur atom in cysteine is connected to a hydrogen atom making it quite reactive; methionine's sulphur is connected to a methyl group. This means that the roles that methionine can play in protein function are much more limited [7].

F2RL3: F2RL3 (Coagulation factor II receptor-like 3), activated by cleavage of their N-terminal domains by serine proteases. Hydrolysis reveals a tethered peptide ligand, which interacts with the receptor within extracellular loop-2 to affect transmembrane signaling. Polymorphism at position 120 from alanine to threonine is a part of transmembrane helical domain of the protein [8]. Alanine's side chain, being very short, means that it is not particularly hydrophobic, rarely directly involved in protein function, but it can play a role in substrate recognition or specificity, particularly in interactions with other non-reactive atoms such as carbon. On the contrary, protein kinases frequently attach phosphates to these threonine residues as part of a signal transduction process [7].

GNA13: Guanine nucleotide-binding proteins (G proteins) alpha-13 are involved as modulators or transducers in various transmembrane signaling systems [8]**.** Polymorphism from glycine to arginine at position 40 is situated in an alpha helical portion of the protein [8]. Glycine contains hydrogen in its side chain. The uniqueness of glycine means that it can play a distinct functional role, such as using its backbone (without a side chain) to bind to phosphates. Arginine is a polar positively charged amino acid which frequently pairs with a negatively charged aspartate or glutamate to create stabilizing hydrogen bonds that can be important for protein stability [7].

4. CONCLUSION

The work focuses on the single nucleotide polymorphisms that cause different people to respond differently to aspirin. Forty nine genes are involved in the platelet aggregation pathway. Out of these, twelve genes show changes in amino acid sequences, which are PTGDR, ITGB3, P2RY12, PLA2G4A, PLA2G4B, VWF, PTGS1, TBXAS1, TBX2R2, F2, F2RL3, and GNA13. The mutations in the non-coding region of DNA (except the first) may not directly affect the functioning of gene per se, but it may be in the middle of instructions for how the gene should be properly turned into mRNA. In PTGDR, a natural variant changes a negatively charged amino acid (Glutamic acid) to positively charged (lysine). In P2RY12, SNP at position 256 changes arginine for glutamine which may decrease binding affinity for ADP. Serine to proline polymorphism in PLA2G4A is situated in an alpha helix, where proline might introduce a kink in the helix, thus, destabilizing the structure. In PTGS1, Single nucleotide polymorphisms (SNPs) from proline to leucine gives rise to non-conservative amino acid changes in the signal peptide (Pro(17) to Leu) of cyclooxygenase-1. In F2, polymorphism at position 165 changes threonine to methionine. The methionine side chain is fairly non-reactive, and is thus rarely directly involved in protein function. In F2RL3, polymorphism is from alanine to threonine at position 120 which is part of transmembrane helical domain. Polymorphism from glycine to arginine at position 40 in GNA13 is situated in an alpha helical portion of the protein; glycine is neutral whereas, arginine is positively charged.

In conclusion, three types of mutations were observed: exon mutations that resulted in amino acid change; exon mutations that change the codon sequence of DNA but did not result in amino acid change; intron mutations and near gene mutations. Out of these, exon mutations that changed the amino acid sequence were found to be of more importance than others as these changed the polarity and charge of the amino acid that causes change in its interaction with other amino acids and its aqueous environment, altering the normal functioning of the genes which may be the cause for different responses to the drug aspirin by different individuals.

Table 1: The gene alleles involved in platelet aggregation pathway and the SNP's that affect their DNA and RNA sequence, thereby altering the final product formed and the interaction with aspirin. Only exon mutations with reported sequence changes and/or reported effects are presented.

GENE	rsID	Exon/ intron	Codon seq. change (DNA)	Codon seq. change (RNA)	Amino acid change	Effect on amino acid	Location
PTGDR	rs139745811	Exon	GAC - AAG	GAC – AAG	Glu190lys	Negatively to positively charged	14q22.1
ITGB3	rs 5918	Exon	GTG - GCG	GUG – GCG	Leu59Pro	N/A	17q21.32
PLA2G2A	rs 4744	Exon	TAC - TAT	UAC -UAU	Tyr- Tyr	N/A	1p35
P2RY12	rs 121917885	Exon	CGA - CAA	CGA-CAA	Arg 256Gln	Positively Charged to Polar Uncharged	3q24-q25
PLA2G4A	rs 121434634	Exon	TCT - CCT	UCU – CCU	Ser111Pro	Polar to Nonpolar	1q25
PLA2G4B	rs267604194	Exon	GAC - GAA	GAC – GAA	Asp297Glu	N/A	15q11.2-q21.3
PLCG2	rs 267604657	Exon	TTC - TTT	UUC – UUU	Phe - Phe	N/A	16q24.1
PTGS1	rs3842787	Exon	CCC - CTC	CCC - CUC	Pro17Leu	Polar Uncharged to Nonpolar	9q32-q33.3
TBXA2R	rs 34377097	Exon	CGC - CTC	CGC - CUC	Arg60Leu	Positively Charged to Nonpolar	19p13.3
TBXAS1	rs13306050	Exon	CTT - CCT	CUU – CCU	Leu82,487 Pro	Nonpolar to Polar Uncharged	7q34-q35
VWF	rs 1800382	Exon	CGC - CAC	CGC - CAC	Arg1374His	N/A	12p13.3
F2	rs5896	Exon	ACG - ATG	ACG - AUG	Thr165Met	Polar Uncharged to Non-polar	11
F2RL3	rs773902	Exon	GCT - ACT	GCU- ACU	Ala120Thr	Non-polar to polar uncharged	19
GNA15	rs1637656	Exon	CTC -CTT	CUC- CUU	Leu - Leu	N/A	19p13.3
ITGA2	rs1062535	Exon	ACG - ACA	ACG - ACA	Thr - Thr	N/A	5q11.2
F2RL3	rs773902	Exon	GCT - ACT	GCU- ACU	Ala120Thr	Non-polar to polar uncharged	19
GNAI2	rs137853226	exon	CGC - GGC	CGC - GGC	Arg - Gly	Positively Charged to Nonpolar	3p21.31
GNAI3	rs387907178	exon	GGT - CGT	GGU - CGU	Gly40Arg	Non-polar to Positively Charged	1p13

5. ACKNOWLEDGEMENTS

The work was supported by Gargi College, University of Delhi and DBT STAR college grant

6. REFERENCES

[1] Hankey, G. J., Eikelboom, J. W., "Aspirin resistance", *The Lancet infectious diseases*, 367, 9510, February 2006, pp. 606-617.

[2] Berger, J. S., Brown, D. L., Becker, R. C., "Low-dose aspirin in patients with stable cardiovascular disease: a meta-analysis", *American Journal of Medicine*, 121, 1, January 2008, pp. 43-9.

[3] Sangkuhl, K., Shuldiner, A. R., Klein, T. E., Altman, R. B., "Platelet aggregation pathway", *Pharmacogenetics and Genomics*, 21,8, August 2011, pp. 516-521.

[4] Whirl-Carrillo, M., McDonagh, E. M., Hebert, J. M.., Gong, L., Sangkuhl, K., Thorn, C. F., Altman, R. B., Klein, T. E., "Pharmacogenomics Knowledge for Personalized Medicine", *Clinical Pharmacology and Therapeutics*, 92,4, October 2012, pp. 414-417

[5] Sherry, S. T., Ward, M. H., Kholodov, M., Baker, J., Phan, L., Smigielski, E. M., Sirotkin, K., "dbSNP: the NCBI database of genetic variation", *Nucleic Acids Research*, 29, 1, January 2001, pp. 308-311.

[6] Xue, L., Gyles, S. L., Barrow, A., Pettipher, R "Inhibition of PI3K and calcineurin suppresses chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2)-dependent responses of Th2 lymphocytes to prostaglandin D(2)", *Biochemical Pharmacology*, 73, 6, March 2007, pp. 843-853

[7] Betts, M. J., Russell, R. B., "Amino Acid Properties and Consequences of Substitutions" in *Bioinformatics for Geneticists*, (eds M. R. Barnes and I. C. Gray), John Wiley & Sons, Ltd, Chichester, UK, May 2003, pp. 290-316.

[8] "The UniProt Consortium Activities at the Universal Protein Resource (UniProt)", *Nucleic Acids Research*, 42, D1, January 2014, pp. D191-D198.

[9] Cargill, M., Altshuler, D., Ireland, J., Sklar, P., Ardlie, K., Patil, N., Shaw, N., Lane, C. R., Lim, E. P., Kalyanaraman, N., Nemesh, J., Ziaugra, L., Friedland, L., Rolfe, A., Warrington, J., Lipshutz, R., Daley, G. Q., Lander, E. S., "Characterization of single-nucleotide polymorphisms in coding regions of human genes", *Nature Genetics*, 22, July 1999, pp. 231-238

[10] Cattaneo, M., Zighetti, M. L., Lombardi, R., Martinez, C., Lecchi, A., Conley, P. B., Ware, J., Ruggeri, Z. M., "Molecular bases of defective signal transduction in the platelet P2Y12 receptor of a patient with congenital bleeding", *Proceedings of the National Academy of Sciences U.S.A.*, 100, 4, February 2003, pp. 1978-1983

[11] Adler, D. H., Cogan, J. D., Phillips, J. A., Schnetz-Boutaud, N., Milne, G. L., Iverson, T., Stein, J. A., Brenner, D. A., Morrow, J. D., Boutaud, O., Oates, J. A.,"Inherited human cPLA(2alpha) deficiency is associated with impaired eicosanoid biosynthesis, small intestinal ulceration, and platelet dysfunction", *Journal of Clinical Investigation*, 118, 6, June 2008, pp. 2121-2131

[12] Castaman, G., Eikenboom, C. J. C., Rodeghiero, F., Briet, K., Reitsma, P. H., "A novel candidate mutation (Arg611-->His) in type I 'platelet discordant' von Willebrand's disease with desmopressin-induced thrombocytopenia", *British Journal of Haematology*, 89, 3, March 1995, pp. 656-658

[13] Yagmur, E., Weiskirchen, R., Schedel, A., Bugert, P., "PTGS1 compound heterozygosity impairs gene expression and platelet aggregation and is associated with severe bleeding complications", *Thrombosis and Haemostasis*, 110, 5, November 2013, pp. 1083-105.

[14] Glenn, K. C., Frost, G. H., Bergmann, J. S., Carney, D. H., "Synthetic peptides bind to high-affinity thrombin receptors and modulate thrombin mitogenesis", *Peptide Research*, 1, 2, November 1988, pp. 65-73

[15] Cargill, M., Altshuler, D., Ireland, J., Sklar, P., Ardlie, K., Patil, N., Shaw, N., Lane, C. R., Lim, E. P., Kalyanaraman, N., Nemesh, J., Ziaugra, L., Friedland, L., Rolfe, A., Warrington, J., Lipshutz, R., Daley, G. Q., Lander, E. S., "Characterization of single-nucleotide polymorphisms in coding regions of human genes", *Nature Genetics*, 22, July 1999, pp. 231-238