

Von Willebrand Disease: Mutations, Von Willebrand Factor Variance and Genetic Drift

Student: Cecilia Carlsson Pecharromán

Supervisor: Torbjörn Säll

Institution: Department of Biology, Lund University

E-mail: nat12cca@student.lu.se

Abstract

The most commonly inherited bleeding disorder in humans is the von Willebrand disease (VWD), which is categorized into Type 1, Type 2 (with subtypes 2A, 2B, 2M and 2N) and Type 3 (Chen *et al.*, 2016). In order to acquire a better method for its diagnosis, first the mutations causing the different types of the disorder were recorded. Secondly, the variance in the concentration of the von Willebrand factor (VWF) in the plasma was analysed since defects in the VWF gene cause the disorder (Goodeve *et al.*, 2007). The concentration of the VWF in the plasma was compared between seemingly healthy individuals with different gender, age and blood type. Finally, the genetic diversity within different populations was determined so as to discover if there was genetic drift between the populations.

1. Introduction

von Willebrand disease (VWD) is the most commonly inherited bleeding disorder in humans (Chen *et al.*, 2016). It is caused by genetic defects in the von Willebrand factor (VWF) gene (Goodeve *et al.*, 2007), which is located on the short arm of chromosome 12 (Swami and Kaur, 2016). The VWF is a large plasma glycoprotein synthesized by endothelial cells and megakaryocytes (Chen *et al.*, 2016), which binds to collagen at sites of vascular injury, mediates platelet adhesion and aggregation, and operates as a carrier protein for coagulation factor VIII (Leebeek and Eikenboom, 2016).

According to Chen *et al.* (2016) the VWD is categorized into three different types depending on the quantitative and qualitative defects of the VWF. Types 1 and 3 are caused by the presence of a lower amount of VWF, while type 2 is due to defects in VWF, such as abnormal

homodimerization and posttranslational modifications taking place during synthesis. The most common subtype is type 1 VWD, which is caused by a decrease in the concentration of VWF either because of a decrease in synthesis or due to an increase in clearance. Type 2 VWD is further categorized into four subtypes depending on the kind of mutation that produces the defective VWF. Type 2A is due to a decreased effectiveness in platelet clot formation caused by a missense mutation that leads to fewer binding sites for the platelet glycoprotein Ib (GpIb) on VWF. Type 2B is due to a gain of function mutation that results in an increased affinity of GpIb binding site on VWF for the platelet GpIb receptor, which leads to spontaneous binding in circulation and expulsion from plasma. Type 2M VWD is caused by mutations that impair VWF-platelet and VWF-collagen interactions. Type 2N VWD is caused by mutations that result in a reduced affinity of VWF to coagulation factor VIII. The least frequent subtype is type 3 VWD, which is caused by the absence of VWF.

In order to acquire a better method for the diagnosis of the VWD, first, the information from the VWD database concerning the type of VWD and the nucleotide changes that occur was compiled. Secondly, since genetic defects in the VWF gene cause the VWD (Goodeve *et al.*, 2007) the concentration of VWF in the plasma of seemingly healthy individuals, both family members that are not classified as diseased and control individuals, was analysed. The concentration of VWF in the plasma was observed in order to detect if its concentration varied with gender, age and blood type. The concentration of VWF in the plasma of healthy family members and control individuals were then compared to each other so as to detect if there was any variation between these two groups. Finally, the single-nucleotide polymorphisms (SNPs) within these two groups were analysed to establish the expected heterozygosity for each of the 110 SNPs, each of the 12 centers included in the data, and for the total population. The determined genetic diversity within different populations was used to find out the fixation index between centers in both family members and control individuals so as to discover if there was genetic drift between the different centers.

2. Material and Method

2.1. Nucleotide Changes

The data utilized in the project was provided by the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis, ISTH-SSC (2016). The VWD classification, nucleotide change and the references from this data were analysed to find out if

the same mutation causing the same type of VWD had been detected in separate studies, if different types of VWD were caused by the same mutation, and to find out if different mutations were located on the same base pair position.

2.2. Von Willebrand Factor Variance

Data about the VWF was then analysed by using the software SPSS. First, the mean concentration of VWF in healthy family members was calculated. The mean concentration of VWF for female and male healthy family members were also calculated, and then in order to find out if the concentration of VWF in healthy family members varies with gender the results obtained were compared to each other. Thereafter, a correlation test and a simple scatter graph were done to determine if the concentration of VWF in healthy family members varies with age. Then, in order to find out if the concentration of VWF in healthy family members varies depending on the blood type the mean concentration of VWF was calculated for each blood type and then compared to the other blood types. Boxplots were made so as to have a graphic representation of the variation of the concentration of VWF depending on gender and blood type. Afterwards, an analysis of variance (ANOVA) was made comparing all the variables with the concentration of VWF. These tests were also carried out for control individuals, except for the ones concerning the blood type since the data provided did not contain this information.

2.3. Heterozygosity and Fixation Index

Thereafter, the allele frequency for each of the 110 SNPs, for each of the 12 centers and for the total population of healthy individuals was calculated. This was done by using *Equation 1* and *Equation 2*, in which p represents the frequency of one of the alleles assigned the letter A , q represents the frequency of the other allele assigned the letter B , $f(AA)$ represents the frequency of genotype AA , $f(AB)$ represents the frequency of genotype AB , and N represents the genotyped population. Subsequently, the expected heterozygosity (H_{exp}) for each of the 110 SNPs, each of the 12 centers and for the total population of healthy individuals was calculated by using *Equation 3*.

$$p = \frac{2 \times f(AA) + f(AB)}{2 \times N} \quad \text{Equation 1}$$

$$q = 1 - p \quad \text{Equation 2}$$

$$H_{exp} = 1 - \Sigma(p^2 + q^2) \quad \text{Equation 3}$$

Next, the expected heterozygosity between centers (H_s) was calculated by using *Equation 4*, in which H_{EC} represents the expected heterozygosity for each center, N_C represents the number of individuals in each center, and N_T represents the number of individuals in the whole population. Finally, the fixation index between centers (F_{st}) was calculated according to *Equation 5*, in which H_T represents expected heterozygosity of the total population. These calculations were carried out for control individuals as well.

$$H_s = \frac{\Sigma(H_{EC} \times N_C)}{N_T} \quad \text{Equation 4}$$

$$F_{ST} = \frac{H_T - H_s}{H_T} \quad \text{Equation 6}$$

3. Results

3.1. Nucleotide Changes

Within the data from ISTH-SSC (2016) there are 58 cases in which the same mutation causing the same type of VWD is found in separate studies, 36 cases in which the same mutation causes different types of VWD, and 32 cases in which there are different mutations causing the same VWD type at the same position. These nucleotide changes and their position are presented in the Appendix, Table 4.

Most of the mutations are due to the replacement of the base located on a specific position with another base, but there are a few mutations that are due to deletions. In the position -1522 there is a deletion of CATTGTTTCCTTT which has been found in separate studies. The deletion of exon 18 has also been found in different studies. In the position 2435 there is a deletion of the base C which causes different types of VWD, and at the same position a change from base C to T is found as well. In between the positions 4222 and 4224 there is a deletion of AAG which causes different types of VWD, and in position 4453 there is a deletion of the base G which has been found in separate studies.

3.2. Von Willebrand Factor Variance

In healthy family members the mean concentration of VWF in the plasma is 96.40 IU/dl. The concentration of VWF in healthy family members is not significantly lower for males (94.24 IU/dl) than for females (98.65 IU/dl), as shown in Table 1 and in the Appendix, Figure 3.

The mean concentration of VWF in the plasma is 99.56 IU/dl for control individuals. The concentration of VWF in control individuals is not significantly lower for males (99.30 IU/dl) than for females (99.74 IU/dl), as shown in Table 1 and in the Appendix, Figure 4.

Table 1. The mean concentration of VWF in the plasma and its concentration in females and males for both healthy family members and control individuals.

Gender	Mean VWF (IU/dl) for Family	Mean VWF (IU/dl) for Control
Female	98.65	99.74
Male	94.24	99.30
Total	96.40	99.56

The concentration of VWF in healthy family members increases significantly at the 0.01 level with age, as shown in Figure 1, and the concentration of VWF in control individuals increases significantly at the 0.01 level with age as well, as shown in Figure 2.

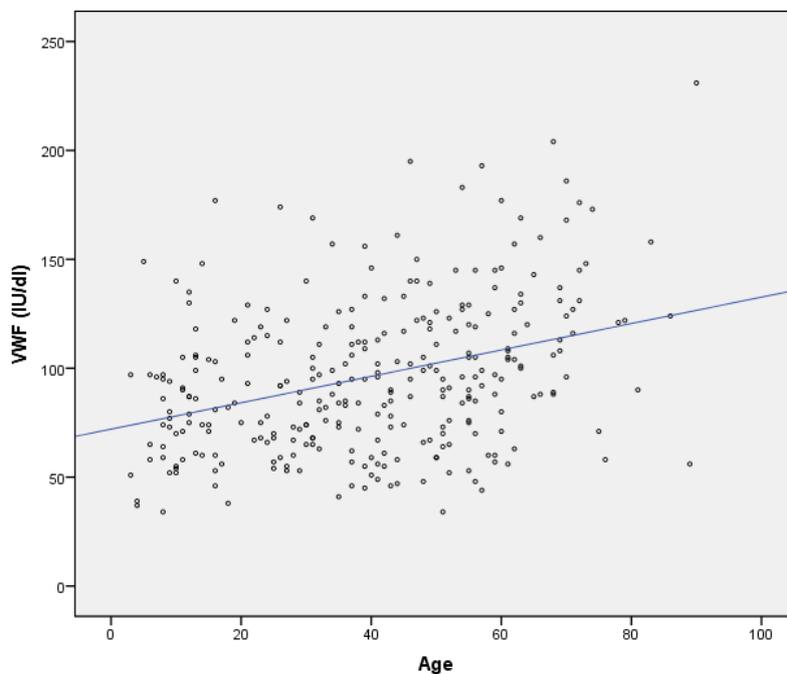


Figure 1. Simple scatter graph representing the increase in concentration of VWF in the plasma of healthy family members with age.

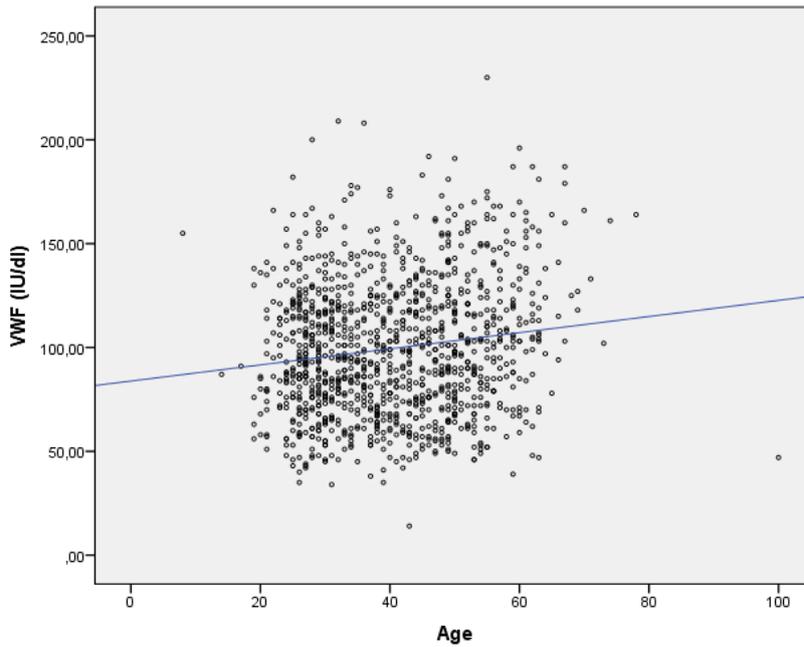


Figure 2. Simple scatter graph representing the increase in concentration of VWF in the plasma of control individuals with age.

In healthy family members concentration of VWF depends on the blood type, as shown in Table 2 and in the Appendix, Figure 5. The difference between the highest and lowest concentration is significant at the 0.01 level. The concentration of VWF is highest for the blood type A/A, 141.80 IU/dl, and lowest for the blood type O/O, 84.12 IU/dl.

Table 2. The mean concentration of VWF in the plasma of healthy family members for each different blood type (ABO).

ABO	Mean VWF (IU/dl)
A/A	141.80
A/A2	109.20
A/B	115.50
A2/A2	95.00
A2/B	93.50
B/B	120.50
O/A	107.12
O/A2	98.26
O/B	102.46
O/O	84.12

According to the ANOVA there does not exist any significant variation in the concentration of the VWF in the plasma of healthy family members that depends on neither gender nor age nor blood type, as shown in Table 3. The concentration of the VWF in the plasma of control

individuals does significantly vary at the 0.05 level with gender, as shown in Table 3 and at the 0.01 level with age.

Table 3. The significance value calculated by ANOVA of the variation in concentration of the VWF in the plasma depending on gender, age and blood type (ABO) of healthy family members and control individuals.

	Significance Value for Family	Significance Value for Control
Gender	0.500	0.032
Age	0.213	0.005
ABO	0.114	-

3.3. Heterozygosity and Fixation Index

The expected heterozygosity for the total population and for the population of each of center was 0.50 both in healthy family members. This was also the case for control individuals. The expected heterozygosity for the population of each of the SNP was more varied and included values ranging from 0 to 0.50 in both healthy family members and control individuals. These values are presented in the Appendix, Table 5. In healthy family members the fixation index between centers is 0.00038, and in control individual the fixation index is 0.00001.

4. Discussion

4.1. Nucleotide Changes

There are 58 cases in which the same mutation causing the same type of VWD has been in separate studies as presented in the Appendix, Table 4. Most of these nucleotide changes are due to the replacement of the base located on a specific position with another base. However, there is also a deletion of a few bases at the position -1522 and a deletion of exon 18.

Identical mutations have caused different types of VWD in 36 cases as presented in the Appendix, Table 4. Most of these nucleotide changes are due to the replacement of the base located on a specific position with another base. Nonetheless, there are deletions at the positions 2435 and from 4222 to 4224.

There are 32 cases in which different mutations causing the same VWD type are found on the same location as presented in the Appendix, Table 4. Most of these nucleotide changes are

due to the replacement of the base located on a specific position with another base, but on the position 2435 one of the two different mutations found is a deletion.

4.2. Von Willebrand Factor Variance

According to the values presented in Table 1 although the concentration of VWF in the plasma is higher in females there is not a significant difference between genders both in healthy family members and control individuals. Moreover, there is not a significant difference between the mean concentration of VWF in the plasma of healthy family members and control individuals. The results acquired from the ANOVA presented in Table 3 confirm that there is not a significant difference in the concentration of VWF in the plasma between genders in healthy family members. However, the results from the ANOVA in control individuals imply that the concentration of the VWF in the plasma does significantly vary at the 0.05 level with gender. This last result is compatible with the findings in Zhou *et al.* (2014), which show that there are differences between genders and that females have a significantly higher concentration of VWF in the plasma than men.

The concentration of the VWF in the plasma increases significantly at the 0.01 level with age in both healthy family members and control individuals, as shown in Figure 1 and Figure 2 respectively. This is confirmed for control individuals by the results acquired from the ANOVA presented in Table 3. In contrast for healthy family members the results acquired from the ANOVA do not show a significant increase in the concentration of the VWF in the plasma with age. Apart from this last result, the values obtained are in accordance with the findings in Ryds *et al.* (2015) and Sanders *et al.* (2014), which show there is a significant increase in the concentration of the VWF in the plasma.

On the one hand, the values presented in Table 2 imply that there is a significant variance in the concentration of the VWF in the plasma of healthy family members with different blood types. On the other hand, the results acquired from the ANOVA presented in Table 3 do not show a significant increase in concentration of the VWF in the plasma of healthy family members with different blood types. According to Franchini *et al.* (2007) and Haley *et al.* (2002) there is a significant difference in the concentration of the VWF in the plasma of individuals with different blood types and individuals with blood type O have a significantly lower concentration of the VWF in the plasma, which also is shown in Table 2.

Although according to one part of the tests the concentration of VWF in the plasma does not significantly depend on gender, age or blood type, another part of the results obtained agrees

with the information found in other studies. Whether or not the concentration of the VWF in the plasma significantly varies with age cannot be determined. However, assuming that the findings in other studies are true the concentration of the VWF in the plasma would be lowest for young males with blood type O and they would be more likely to have the disease.

4.3. Heterozygosity and Fixation Index

Both healthy family members and control individuals have a total population which is 50% heterozygous. The population of each center of both healthy family members and control individuals is 50% heterozygous as well. However, the heterozygosity in the populations of each SNP varies as shown in the Appendix, Table 5. Approximately half of the SNPs in healthy family members and control individuals are 50% heterozygous, whereas some SNPs have 0% heterozygous individuals because there only is one type of allele present, and other SNPs have a value somewhere in between these two because there is a somewhat higher number of homozygous individuals. The fact that completely homozygous individuals present in the population do not change the heterozygosity of the total population and the population of each center, might be because some individuals are completely homozygous for one of the alleles while other individuals are completely homozygous for the other allele thus cancelling each other out.

In healthy family members the fixation index between centers is 0.00038 and thus the difference between centers only accounts for 0.038% of the total genetic variation. The fixation index in control individuals is lower than for healthy family members, it is 0.00001 and thus the difference between centers only account for 0.001% of the total genetic variation. This suggests that there is little genetic drift between the populations of different centers in both healthy family members and control individuals.

4.4. Conclusion

Firstly, the VWD is caused in most cases by mutations that are due to the replacement of the base located on a specific position with another base. There are 58 cases of identical mutations found in separate studies that cause the same type of VWD, 36 cases of identical mutations causing different types of VWD, and 32 cases in which different mutations causing VWD have been found on the same location. Secondly, the variation in concentration of the VWF in the plasma with gender, age and blood type cannot be determined since the results obtained are contradictory. According to other studies there is a significant variation in concentration of the VWF in the plasma with gender (Zhou *et al.*, 2014), age (Ryds *et al.*,

2015) (Sanders *et al.*, 2014) and blood type (Franchini *et al.*, 2007) (Haley *et al.*, 2002). Finally, even though the heterozygosity in the populations of each SNP varies, the population of each center and the total population are 50% heterozygous, and there is little genetic drift between the populations of different centers in both healthy family members and control individuals.

5. References

Chen, J., Hinckley, J.D., Haberichter, S., Jacobi, P., Montgomery, R., Flood, V.H., Wong, R., Interlandi, G., Chung, D.W., López, J.A. and Di Paola, J. (2016) 'Variable content of von Willebrand factor mutant monomer drives the phenotypic variability in a family with von Willebrand disease', *Blood*, 126, pp. 262-269.

Franchini, M., Capra, F., Targher, G., Montagnana, M. and Lippi, G. (2007) 'Relationship between ABO blood group and von Willebrand factor levels: from biology to clinical implications', *Thrombosis Journal*, 5, pp. 1-5.

Goodeve, A., Eikenboom, J., Castaman, G., Rodeghiero, F., Federici, A.B., Batlle, J., Meyer, D., Mazurier, C., Goudemand, J., Schneppenheim, R., Budde, U., Ingerslev, J., Habart, D., Vorlova, Z., Holmberg, L., Lethagen, S., Pasi, J., Hill, F., Hashemi-Soteh, M., Baronciani, L., Halldén, C., Guilliat, A., Lester, W. and Peake, I. (2007) 'Phenotype and genotype of a cohort of families historically diagnosed with type 1 von Willebrand disease in the European study, Molecular and Clinical Markers for the Diagnosis and Management of Type 1 von Willebrand Disease (MCMDM-1VWD)', *Blood*, 109, pp. 112-121.

Haley, E., Babar, N., Ritter, C., Downes, K.A., Green, D., Shurin, S. and Sarode, R. (2002) 'Effect of ABO Blood Group on the Collagen-Binding Assay for von Willebrand Factor', *Am. J. Hematol.*, 71, pp. 229-231.

ISTH-SSC (2016) *VWF Online Database*. Available at: <http://www.ragtimedesign.com/vwf/mutation/mutationtableresults.php> (Accessed: 10 October 2016).

Leebeek, F.W.G. and Eikenboom, J.C.J. (2016) 'Von Willebrand's Disease', *N. Engl. J. Med.*, 375, pp. 2067-2080.

Ryds, N., Grabell, J., Lillicrap, D. and James, P.D. (2015) 'Changes in von Willebrand factor level and von Willebrand activity with age in type 1 von Willebrand disease'. *Haemophilia*, 21, pp. 636-641.

Sanders, Y.V., Giezenaar, M.A., Laros-van Gorkom, B.A.P., Meijer, K., van der Bom, J.G., Cnossen, M.H., Nijziel, M.R., Ypma, P.F., Fijnvandraat, K., Eikenboom, J., Mauser-Bunschoten, E.P. and Leebeek, F.W.G. (2014) 'von Willebrand disease and aging: an evolving phenotype'. *J. Thromb. Haemost.*, 12, pp. 1066–1075.

Swami, A. and Kaur, V. (2016) 'von Willebrand Disease: A Concise Review and Update for the Practicing Physician', *Clin. Appl. Thromb. Hemost.*, 0, pp. 1-11.

Zhou, Z., Yu, F., Buchanan, A., Fu, Y., Campos, M., Wu, K.K., Chambless, L.E., Folsom, A.R., Boerwinkle, E. and Dong, J. (2014) 'Possible Race and Gender Divergence in Association of Genetic Variations with Plasma von Willebrand Factor: A Study of ARIC and 1000 Genome Cohorts'. *PLoS ONE*, 9, pp. 1-6.

6. Appendix

6.1. Nucleotide Changes

Table 4. The position of nucleotide changes found in separate studies, the position of nucleotide changes causing different types of VWD, and the position of different nucleotide changes found on the same position causing the same type of VWD.

Nucleotide changes found in separate studies	Nucleotide changes causing different types of VWD	Different nucleotide changes found on the same position causing the same VWD
-2731C>T	100C>G	100C>G and 100C>T
-2615A>G	823T>C	421G>A and 421G>T
-2487G>A	1534-3C>A	449T>A and 449T>C
-1886A>C	1926G>A	823T>A and 823T>C
-1873A>G	2220G>A	2365A>C and 2365G>C
-1665G>C	2435delC	2435C>T and 2435delC
-1522delCATTGTTTCCTTT	2446C>T	2686-2A>G and 2686-1G>C
Exon 18 deletion	2561G>A	2820+1G>A and 2820+1G>C
1093C>T	2771 G>A	3388T>C and 3388T>G

1534-3C>A	3108+5G>A	3538G>A and 3538+1G>A
2344C>T	3179G>A	3797C>A and 3797C>T
2362T>C	3379+1G>A	3802C>A and 3802C>G
2372 C>T	3388T>G	3814T>C and 3814T>G
2384 A>G	3389G>T	3815G>C and 3815G>T
2435delC	3430T>G	3835G>A, 3835G>T and 3835T>G
2446C>T	3437A>G	3917G>A and 3917G>T
2451T>A	3445T>C	3923G>A and 3923G>C
2561G>A	3467C>T	3940G>C and 3940G>T
2771G>A	3586T>C	3944G>A and 3944G>T
3379+1G>A	3614G>A	4022G>A, 4022G>C and 4022G>T
3389G>T	3797C>T	4120C>A and 4120C>T
3430T>G	3835G>A	4121G>A and 4121G>T
3437A>G	3943C>T	4148T>C and 4148T>G
3445T>C	3944G>T	4368C>A and 4368C>G
3467C>T	4010C>T	4382C>A and 4382C>T
3614G>A	4079T>C	4508T>C and 4508T>G
3686T>G	4105T>A	4789C>G and 4789C>T
3692A>C	4120C>T	4790G>A and 4790G>T
3797C>T	4121G>A	5053+1G>A and 5053+1G>T
3835G>A	4222_4224delAAG	7430G>A and 7430G>C
3916C>T	4508T>C	7437G>A and 7437+1G>A
3922C>T	4517C>T	8155+3G>C and 8155+3G>T
3929C>T	4747C>T	
3931C>T	6187C>T	
3943C>T	7085G>T	
3946G>A	7630C>T	
4010C>T		
4022G>A		
4120C>T		
4121G>A		
4135C>T		
4238C>T		
4273A>T		
4453delG		

4514G>A		
4517C>T		
4628C>T		
4751A>G		
4789C>T		
4825G>A		
4883T>C		
4975C>T		
6620T>C		
7390 C>T		
7603C>T		

6.2. Von Willebrand Factor Variance

Figure 3. Boxplot comparing the concentration of VWF in the plasma of female and male healthy family members.

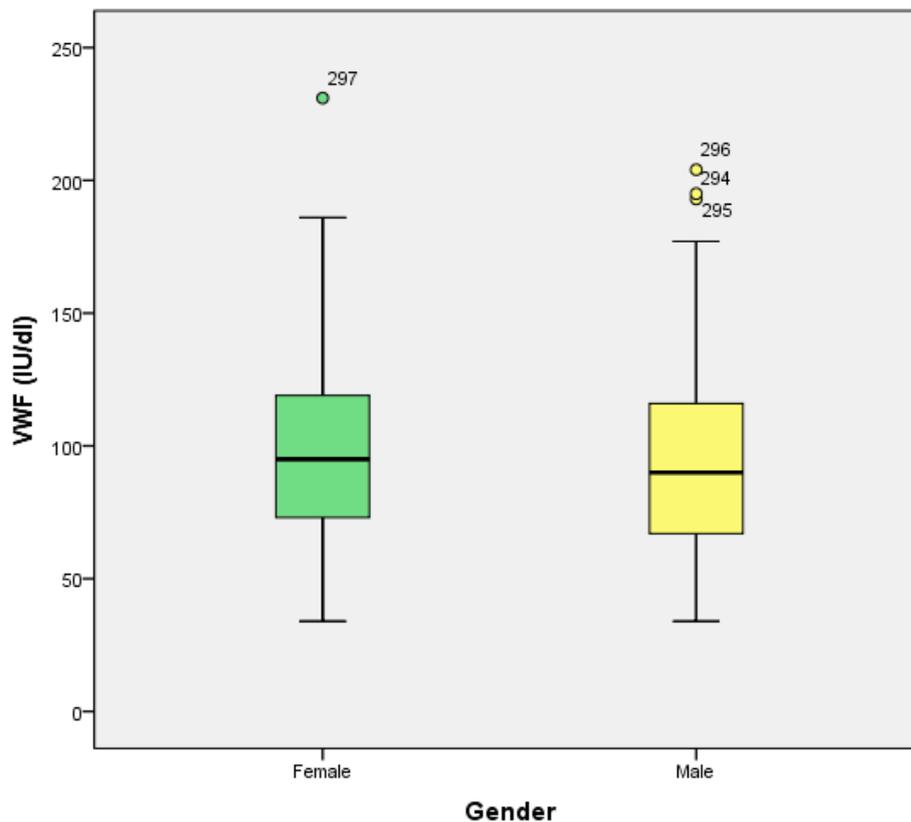


Figure 4. Boxplot comparing the concentration of VWF in the plasma of female and male control individuals.

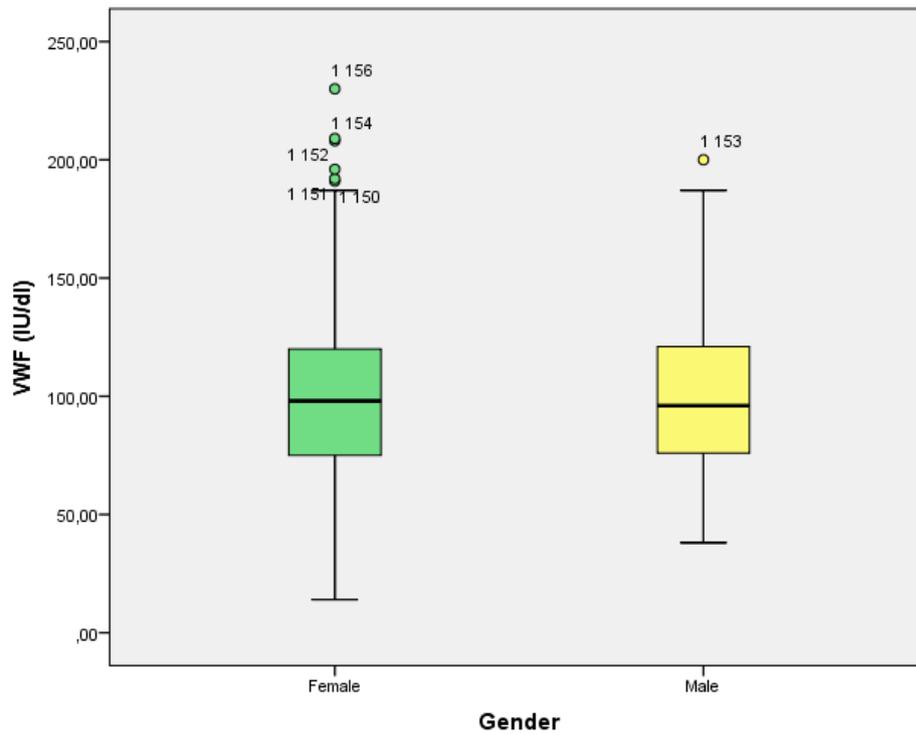
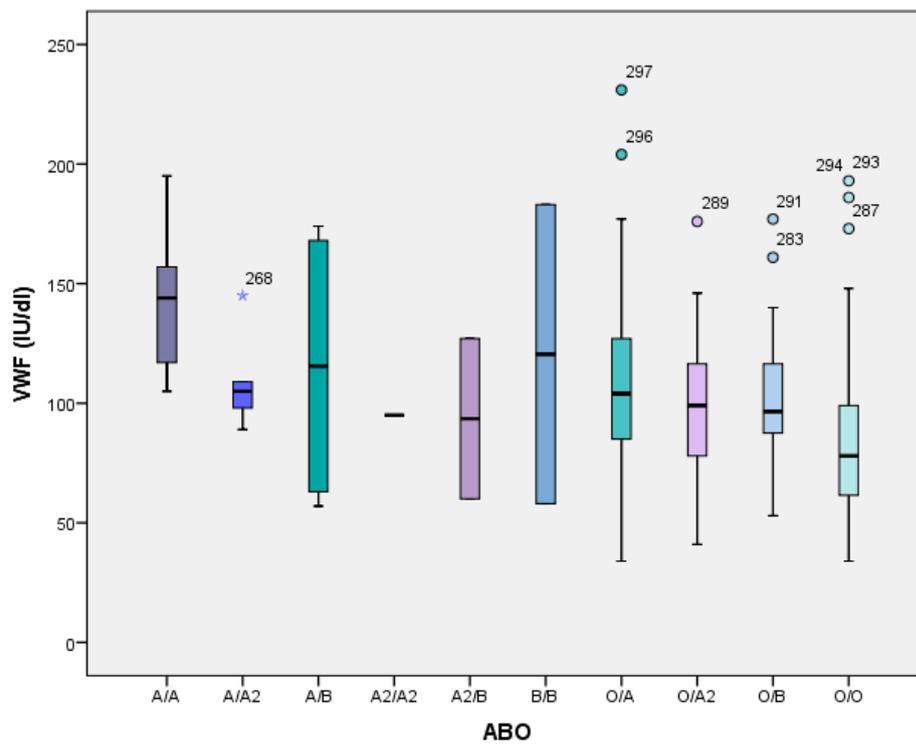


Figure 5. Boxplot comparing the concentration of VWF in the plasma of healthy family members with different blood types (ABO).



6.3. Heterozygosity and Fixation Index

Table 5. Expected heterozygosity (H_{exp}) for each SNP in both healthy family members and control individuals.

H_{exp} per SNP Family	H_{exp} per SNP Control
0.49	0.49
0.48	0.47
0.48	0.47
0.50	0.50
0.41	0.41
0.48	0.46
0.25	0.29
0.32	0.33
0.07	0.10
0.26	0.29
0.41	0.41
0.48	0.49
0.49	0.50
0.49	0.50
0.47	0.47
0.29	0.34
0.37	0.34
0.10	0.09
0.44	0.46
0.44	0.44
0.49	0.50
0.44	0.44
0.31	0.30
0.01	0.00
0.40	0.38
0.47	0.45
0.46	0.45
0.16	0.21
0.44	0.46
0.01	0.00
0.35	0.36
0.49	0.50
0.49	0.50
0.44	0.46
0.00	0.01
0.04	0.03
0.01	0.02
0.47	0.47
0.49	0.50
0.48	0.47
0.36	0.35

0.48	0.47
0.48	0.47
0.44	0.42
0.44	0.42
0.50	0.49
0.13	0.16
0.00	0.00
0.00	0.00
0.01	0.00
0.03	0.01
0.00	0.00
0.49	0.49
0.02	0.00
0.01	0.00
0.34	0.36
0.34	0.36
0.34	0.36
0.34	0.35
0.14	0.16
0.04	0.03
0.04	0.01
0.44	0.44
0.48	0.48
0.45	0.44
0.44	0.44
0.34	0.36
0.47	0.46
0.39	0.39
0.21	0.16
0.38	0.40
0.00	0.00
0.43	0.44
0.43	0.44
0.49	0.49
0.01	0.01
0.41	0.40
0.45	0.45
0.47	0.48
0.50	0.50
0.38	0.44
0.21	0.23
0.21	0.23
0.50	0.50
0.50	0.49
0.41	0.41
0.45	0.46
0.41	0.41

0.42	0.42
0.50	0.50
0.47	0.46
0.46	0.47
0.48	0.49
0.46	0.47
0.46	0.47
0.46	0.47
0.45	0.46
0.45	0.47
0.45	0.48
0.45	0.48
0.46	0.48
0.47	0.43
0.50	0.49
0.50	0.48
0.49	0.48
0.50	0.50
0.50	0.50
0.48	0.48
0.49	0.49
0.50	0.50