CHAPTER V

RESULT AND DISCUSSION

5.1 Result

All subjects in this study had answered the risk factor questionnaire which contained 23 questions. Five main risk factors in this study showed in category: sex and family history of myopia (table 4), near work activities (table 5) and outdoor activity and lighting (table 6).

Table 4. Distribution of sex and family history in myopia

Question	Criteria	Frequency	Percentage
Sex	Male	33	33%
	Female	67	67%
Eyes status	Both eyes using minus lens	50	50%
	Both eyes normal	50	50%
	One normal, one using minus lens	0	0%
Parental history of myopia	None	52	52%
	One parent	32	32%
	Both parents	16	16%
Paternal grandparent history of myopia	None	69	69%
	One grandparent	22	22%
	Both grandparent	9	9%
Maternal grandparent history of myopia	None	75	75%
	One grandparent	13	13%
	Both grandparent	12	12%
Sibling history of myopia	None	57	57%
	1	27	27%
	2	12	12%
	3	2	2%
	4	2	2%

Table 5. Distribution of near work activities as risk factor for myopia

Question	Criteria	Frequency	Percentage
Frequency of reading per week	Rare (1-2 times)	41	41%
	More frequent (3-5 times)	33	33%
	Frequent (>6 times)	25	25%
Duration of reading per day	<30 minutes	36	36%
	30 minutes-2 hours	44	44%
	> 2 hours	20	20%
Frequency of watching television per week	Rare (1-2 times)	55	55%
	More frequent (3-6 times)	45	45%
	Frequent (>7 times)	0	0%
Duration of watching television per day	< 1 hour	12	12%
	1-4 hours	53	53%
	> 4 hours	35	35%
Television viewing distance	Near (< 30 cm)	6	6%
	Middle (30-60 cm)	33	33%
	Far (> 60 cm)	61	61%
Frequency of using computer/gadget for academic activity per week	Never	20	20%
	Rare (1-3 times)	35	35%
	Frequent (> 4 times)	45	45%
Duration of using computer/gadget for academic activity per day	< 1 hour	31	31%
	1-4 hours	51	51%
	> 4 hours	18	18%
Frequency of using computer/gadget for pleasure per week	Never	17	17%
	Rare (1-3 times)	35	35%
	Frequent (> 4 times)	48	48%
Duration of using computer/gadget for pleasure per day	< 1 hour	22	22%
	1-4 hours	44	44%
	> 4 hours	34	34%

Table 6. Distribution of outdoor activity and lighting as a risk factor for myopia

Question	Criteria	Frequency	Percentage
Outdoor activity frequency per week	Never	5	5%
	Rare (1-2 times)	45	45%
	Frequent (≥ 3 times)	50	50%
Outdoor activity duration per day	< 30 minutes	16	16%
	30 minutes- 2 hours	45	45%
	> 2 hours	39	39%
Lighting while reading	Almost dark	0	0%
	Dim	19	19%
	Bright	81	81%
Television viewing lighting	Dim	26	26%
	Bright	74	74%

There are two questions, especially designed for myopic subject only, which are age when first time using minus lens and whether they use the spectacle while watching television or not.

Table 7. Frequency table for specific question for myopic subject

Question	Criteria	Frequency	Percentage
Age of using spectacle with minus lens	6	1	2%
	7	2	4%
	8	1	2%
	9	1	2%
	10	2	4%
	11	3	6%
	12	6	12%
	13	3	6%
	14	10	20%
	15	7	14%
	16	5	10%
	17	6	12%
	18	2	4%
	19	1	2%
Using spectacle while watching television	Yes	25	50%
	No	25	50%

Based on pedigree collected from 50 myopic subjects, subject who had myopia history in ≥2 generation was classifed as multigeneration group and 1 generation only as single generation group.

Table 8. Distribution of pedigree in case group

Classification	Frequency	Percentage
Multigeneration	38 subjects	76%
Single generation	12 subjects	24%
Total	50 subjects	100%

Myopic subjects were asked about their spherical lens size and the classified into three categories, which are low myop (≤3 dioptri), medium myop (3-6 dioptri), and high myop (>6dioptri). There are 82% subjects of low myop, 12% of medium myop, and 6% of high myop.

Table 9. Distribution of myopia based on refractive power

Classification	Frequency	Percentage
Low myop	41 subjects	82%
Medium myop	6 subjects	12%
High myop	3 subjects	6%
Total	50 subjects	100%

Myopia onset in high myopia group were 6, 7 and 10 years old. Subject with the biggest refractive error (-12 D) was subject with the youngest age of onset (6 years old). All myopic subjects were asked about their frequency of changing lens because of changing in refractive power in a last year. There were 17 people (34%) that never changed their lens because there was no subjective complaint, 25 people (50%) that changed their lens 1 time in the last year, 7 people (14%) that changed their lens 2

times in the last year, and 1 people (2%) that changed their lens 5 times in the last year.

5.1.1 Questionnaire Analysis

5.1.1.1 Sex

The *p* value for this criterion in this study was <0.05. This means there was a correlation between sex and myopic status in subjects, so the null hypothesis is rejected.

Table 10. Prevalence risk for sex as a risk factor for myopia

Risk Factor	Myopia	Normal	Total
Female	41	26	67
Male	9	24	33
Total	50	50	100

Prevalence risk = a/(a+b) : c/(c+d) = 41/67 : 9/33 = 2.3

In this study, prevalence risk for female for having myopia was >1. It means that female sex increases the risk for having myopia.

5.1.1.2 Family History of Myopia

History of myopia in the family also determined to be a risk factor for having myopia. In this study, the parental and sibling history were investigated. There was a correlation between parental history of myopia and myopic status in subjects because *p* value for parental history was <0.05. So, the null hypothesis was rejected.

Table 11. Prevalence risk for parental history of myopia as a risk factor for myopia

Parental History	Myopia	Normal	Total
Positive	35	13	48
Negative	15	37	52
Total	50	50	100

Prevalence risk = a/(a+b) : c/(c+d) = 35/48 : 15/52 = 2.5

Prevalence risk for parental history of myopia as a risk factor for having myopia was > 1. It means that having parents with history of myopia will increases the risk for having myopia in children.

The *p* value for paternal grandparent history of myopia and the correlation for early-onset myopia was <0.05 but not for the maternal grandparent. It means that there was a correlation between paternal grandparents history of myopia and myopic status in subjects, but not with maternal grandparents.

Table 12. Prevalence risk for paternal grandparent history of myopia as a risk factor for myopia

Grandparent History	Myopia	Normal	Total
Positive	22	9	31
Negative	28	41	69
Total	50	50	100

Prevalence risk = a/(a+b) : c/(c+d) = 22/31 : 28/69 = 1.7

Prevalence risk for paternal grandparent history of myopia as a risk factor for having myopia was >1. It showed that having grandparent from the father side with history of myopia will increases the risk for having myopia.

The *p* value of myopia history in siblings was <0.05 which showed a correlation between sibling history of myopia and myopic status in subjects.

Table 13. Prevalence risk for sibling history of myopia as a risk factor for myopia

Sibling History	Myopia	Normal	Total
Positive	29	14	43
Negative	21	36	57
Total	50	50	100

Prevalence risk = a/(a+b) : c/(c+d) = 29/43 : 21/57 = 1.8

Prevalence risk for sibling history of myopia as a risk factor for having myopia was >1. It means that having sibling with history of myopia will increases the risk for having myopia.

5.1.1.3 Outdoor Activity

Frequency and duration of doing outdoor activities were investigated in this study. The *p* value for both criteria was >0.05, showed no correlation between outdoor activities and myopic status in subjects, so the null hypothesis is accepted.

5.1.1.4 Near Work

Near work as a risk factor for having myopia in this study was described as reading, watching television, and using gadget/computer. Frequency of reading for pleasure, duration of reading for pleasure, and television viewing distance gave *p* value <0.05, showed a correlation between those 3 criteria and myopic status in subjects. So, the null hypothesis was rejected.

Table 14. Prevalence risk for reading and television viewing distance as a risk factor for myopia

Reading frequency	**Myopia**	**Normal**	**Total**
Frequent (≥6times per week)	18	8	26
Rare (0-5 times per week)	32	42	74
Total	50	50	100
Reading duration			
> 2 hours per day	14	6	20
< 2 hours per day	36	44	80
Total	50	50	100
Television viewing distance			
≤60 cm	34	5	39
>60 cm	16	45	61
Total			100

Prevalence risk for reading frequency

= a/(a+b) : c/(c+d) = 18/26 : 32/74 = 1.6

Prevalence risk for reading duration:

= a/(a+b) : c/(c+d) = 14/20 : 36/80 = 1.6

Prevalence risk for television viewing distance:

= a/(a+b) : c/(c+d) = 34/39 : 16/61 = 3.3

Prevalence risk >1 for television viewing distance and reading for pleasure as risk factors for having myopia showed a greater risk for having myopia.

Frequency and duration of watching television and frequency and duration of using computer/gadget gave *p* value >0.05. This also showed no correlation between those criteria and myopic status in subjects. So, the null hypothesis was accepted.

5.1.1.5 Lighting

Lighting quality while reading or watching television was determined to be one risk factor for having myopia. In this study, both criteria got *p* value >0.05, which showed no correlation between lighting and myopic status. So, the null hypothesis was accepted.

Table 15. *p* value for risk factor in questionnaire

Criteria	*p* value	Prevalence Risk	Confidence Interval 95%
Female sex	0.001	4.21	1.69-10.45
Positive parental history of myopia	0.0001	6.64	2.77-15.93
Positive paternal grandparent history of myopia	0.005	3.58	1.44-8.91
Positive maternal grandparent history of myopia	0.106	2.14	0.84-5.46
Positive sibling history of myopia	0.002	3.55	1.54-8.18
Low outdoor activity frequency	0.110	1.91	0.86-4.22
Short outdoor activity duration	0.151	1.81	0.80-4.09
High frequency of reading	0.023	2.95	1.14-7.65
Long duration of reading	0.046	2.85	0.99-8.17
Dim lighting while reading	0.799	1.14	0.42-3.10
High frequency of watching television	0.546	0.78	0.36-1.73
Long duration of watching television	0.834	1.09	0.48-2.48
Short television viewing distance	0.0001	19.13	6.38-57.37
Dim television viewing lighting	0.171	1.88	0.76-4.69
High frequency of using computer/gadget for academic activity	0.315	1.50	0.68-3.31
Long duration of using computer/gadget for academic activity	1.000	1.00	0.36-2.78
High frequency of using computer/gadget for pleasure	0.230	1.62	0.74-3.57
Long duration of using computer/gadget for pleasure	0.673	1.20	0.52-2.74

5.1.2 Corneal Curvature Analysis

Corneal curvature was measured by manual keratometer Takagi Japan model MT 377. Before measurement, the eyes were inspected for any condition that can alter corneal examination. After data collection, the corneal curvature radius was determined for both eyes. Corneal curvature prevalence in myopic and normal subject displayed in table 16 below.

Table 16. Corneal curvature prevalence

Eye	Corneal Curvature Radius	Frequency	Percentage
Right Eye	Steeper cornea (≤ 7.8 mm)	64	64%
	Flatter cornea (> 7.8 mm)	36	36%
Left Eye	Steeper cornea (≤ 7.8 mm)	64	64%
	Flatter cornea (> 7.8 mm)	36	36%

Mean value of corneal curvature radius in this study for right eye was 7.75 mm and for the left eye was 7.76.

Calculation for corneal curvature analysis were using unpaired t-test. This test gave *p* value >0.05, which showed that there was no difference between corneal curvature in myopic subjects and normal subjects (table 18). So, the null hypothesis is accepted.

Table 17. Corneal curvature in myopic and normal subjects

Eye	Status	n	Mean ± Standard Deviation (mm)	*p* value	CI 95%
Right	Myopic	50	7.72 ± 0.26	0.35	0.46 (0.05-0.14)
	Normal	50	7.77 ± 0.23		
Left	Myopic	50	7.73 ± 0.27	0.29	0.53 (0.05-0.15)
	Normal	50	7.78 ± 0.23		

Association between corneal curvature and myopia can be analysed if the numeric data of corneal curvature transformed into categorical data: steeper cornea (≤7.8 mm) and flatter cormea (>7.8 mm). The result came as an insignificant result because *p* value was >0.05 (table 18). This showed that corneal curvature was not associated with myopia.

Table 18. *p* value for association between corneal curvature and myopia

Corneal Curvature	*p* for Myopia	Prevalence Risk	CI 95%	*p* for Dioptri
Right eye	0.21	1.69	0.74-3.86	1.00
Left eye	0.41	1.42	0.62-3.22	1.00

Unpaired t-test to find a difference of corneal curvature in sex gave *p* value >0.05 which means there was no corneal curvature difference between all female and male (table 19).

Table 19. Corneal curvature in male and female subjects

Eye	Status	n	Mean ± Standard Deviation (mm)	*p* value	CI 95%
Right	Female	67	7.72 ± 0.23	0.16	0.07 (0.02-0.17)
	Male	33	7.79 ± 0.26		
Left	Female	67	7.72 ± 0.23	0.06	0.09 (0.04-0.20)
	Male	33	7.82 ± 0.26		

There were 70% of right eye and 68% of left eye that had steeper cornea (≤7.8 mm) in myopic group (table 20).

Table 20. Distribution of corneal curvature in myopic subjects

Sex	Right eye		Total	Left eye		Total
	≤7.8 mm	>7.8 mm		≤7.8 mm	>7.8 mm	
Myopic female	28 (68.3%)	13 (31.7%)	41 (82.0%)	27 (65.9%)	14 (34.1%)	41 (82.0%)
Myopic male	7 (77.8%)	2 (22.2%)	9 (18.0%)	7 (77.8%)	2 (22.2%)	9 (18.0%)
Total	35 (70.0%)	15 (30.0%)	50 (100.0%)	34 (68.0%)	16 (32.0%)	50 (100.0%)

Table 21. Corneal curvature in myopic subjects

Eye	Status	n	Median (minimum-maximum) (mm)	*p* value
Right	Myopic female	41	7.69 (7.07-8.63)	0.74
	Myopic male	9	7.72 (7.04-8.69)	
Eye	**Status**	**n**	**Mean ± Standard Deviation (mm)**	***p* value**
Left	Myopic female	41	7.71 ± 0.25	0.25
	Myopic male	9	7.83 ± 0.35	

Table 21 showed the analysis result to find the difference between corneal curvature in myopic female and myopic male. The *p* value was >0.05 which means there was no corneal curvature difference between myopic male and myopic female.

The frequency of changing lens showed myopia progressivity. There were no correlation between corneal curvature radius in both eyes and myopia progressivity.

5.1.3 *PDGFRA* Gene Polymorphisms

There were 7 SNPs in *PDGFRA* gene investigated in this study. Those SNPs are rs7676985, rs17084051, rs7677751, rs2307049, rs7682912, rs7660560, rs2114039. At first, these SNPs were investigated using High Resolution Melting (HRM) curve analysis. But this method gave poor result in genotyping the SNP because there were many other polymorphisms around those SNPs.

Molecular investigation method was turned into Amplification Refractory Mutation System (ARMS) PCR. This method were using 3 primers in a tube: 1 forward universal primer, 1 reverse universal primer, and 1 forward allele specific primer. PCR product will be shown as 2 band: outer band as a control band and inner band as an allele specific band. Every allele in every SNPs were investigated in different reactions.

Visualization of PCR optimization result was using 1% agarose gel, ran over 35 minutes with electrical voltage 120 V (Bio Rad). Agarose gel was added 3 µl ethidium bromide 10 µg/ µl (Invitrogen). The gel then visualized under UV light in Gel Doc (Bio Rad).

5.1.3.1 SNP rs7676985 G>A

PCR reaction for rs7676985 using ARMS PCR technique: hold 1 95°C 3', 40 times (95°C 10", 62°C 30", 72°C 30"), and hold 2 72°C 2'. PCR reagents was 5 µl KAPA SYBR Fast Universal qPCR kit, 1 µl for each 5 pmol/ µl primer, 1 µl DNA sample 10 µg/ µl, and 1 µl H_2O PCR grade.

Primer set A that used to find wildtype allele in rs7676985 was forward outer 5'-GCATTTTTATCCATGCCTGACAAGC-3', reverse outer 5'-CTAGGAACACCTTCCTCACCCCTG-3', and a forward inner 5'-GCCTAGGTCCTTCTTATAATTTCACG-3'. Primer set A produced control band at 522 bp and inner band at 204 bp. But, only few DNA samples that could be amplified succesfully with this primer set. The other DNA samples were then amplified with primer set B to find wildtype allele which was forward outer 5'-GTCTGGATACCATTCCTGGGTGGT-3', reverse outer 5'-CTAGGAACACCTTCCTCACCCCTG-3', and forward inner 5'-GCCTAGGTCCTTCTTATAATTTCACG-3'. Primer set B produced control band at 452 bp and inner band 204 bp.

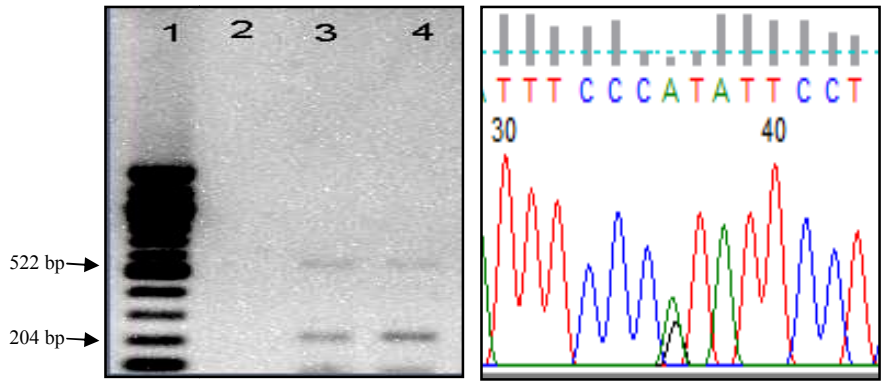

Figure 7. Electrophoresis result of rs7676985 ARMS PCR using primer set A. **Left:** Wildtype allele G shown as control band at 522 bp and inner band at 204 bp. (lane 1: marker ladder 100bp , 2: blank, 3: positive control, 4: wildtype allele positive) **Right:** positive control was having allele heterozygote G and A shown in sequencing result.

Subject who did not have that inner band product classified as had no wildtype allele for this SNP.

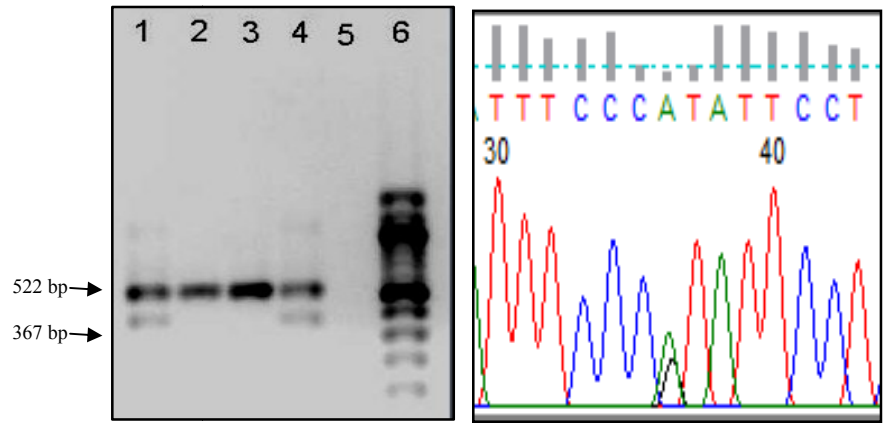

Figure 8. Electrophoresis result of rs7676985 ARMS PCR using primer set C. **Left:** Mutant allele A shown as control band at 522 bp and inner band at 367 bp. (Lane 1: mutant allele positive, 2-3: mutant allele negative, 4: positive control, 5: blank, 6: marker ladder 100 bp). **Right:** positive control was having allele heterozygote A and G shown in sequencing result.

The reaction for mutant allele was using primer set C (forward outer: 5'-GCATTTTTATCCATGCCTGACAAGC-3', reverse outer: 5'-CTAGGAACACCTTCCTCACCCCTG-3', reverse inner: 5'-ACAACCCGGGCAGGGAGGAAGAT-3') that produced control band at 522 bp. Mutant allele was recognized by inner band product at 367 bp. Subject who did not have that inner band product classified as had no mutant allele for this SNP.

Table 22. Allele distribution of rs7676985 G>A

Allele	Eye Status		Total
	Myop	Normal	
G/G	37 (55.2%)	30 (44.8%)	67 (67%)
A/A	1 (50%)	1 (50%)	2 (2%)
G/A	12 (38.7%)	19 (61.3%)	31 (31%)
Total	50 (50%)	50 (50%)	100 (100%)

There were 86% of wildtype allele G and 14% of mutant type allele A of rs7676985 *PDGFRA* gene polymorphism in South Sumatera tribes with

early-onset myopia. There were 79% of wildtype allele G and 21% of mutant type allele A of rs7676985 *PDGFRA* gene polymorphism in South Sumatera tribes with normal eyes.

Tabel 23. Allele frequency of rs7676985 G>A

Allele	Frequency (n)	Percentage (%)
G	165	82.5
A	35	17.5
Total	200	100

Wildtype allele G was distributed in most of the subjects (82.5%), and only 17.5% subjects were having mutant allele A. There was no correlation between mutant allele in rs7676985 and corneal curvature because the *p* value was >0.05 for both eyes (*p* value=0.958). There was no correlation between mutant allele in rs7676985 and myopic status because the *p* value was >0.05 (*p* value=0.137).

5.1.3.2 SNP rs17084051 C>A

PCR reaction for rs17084051 using ARMS PCR technique: hold 1 95°C 3', 40 times (95°C 10", 60°C 30", 72°C 30"), and hold 2 72°C 2'. PCR reagents mix was 5 µl KAPA SYBR Fast Universal qPCR kit, 1 µl for each 5 pmol/ µl primer, 1 µl DNA sample 10 µg/ µl, and 1 µl H_2O PCR grade.

Primer set A that used to find wildtype allele in rs17084051 was forward outer 5'-AAGAGAGACAATTTTCTCTCTGCTGG-3', reverse outer 5'-AAGTAGAAGATCCCTCACCCTTAAGC-3', and reverse inner

5’-CTAATCTGGAAGTAACAAAGTTTGTGAG-3’ that produced control band at 602 bp and inner band for wildtype allele C at 357 bp (figure 9).

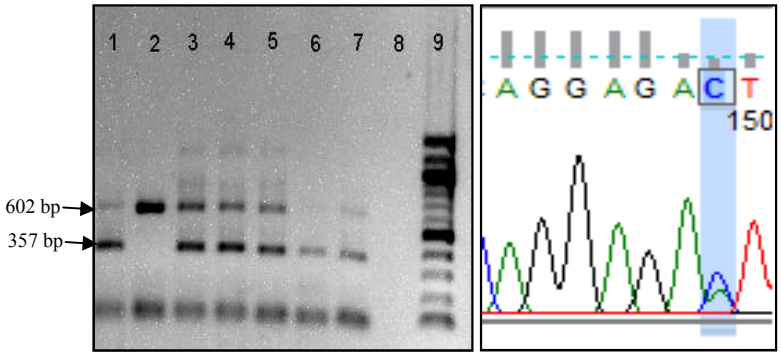

Figure 9. Electrophoresis result of rs17084051 ARMS PCR using primer set A. **Left:** Wildtype allele C shown as control band at 602 bp and inner band at 357 bp. (Lane 1: wildtype allele positive, 2: wildtype allele negative, 3-6: wildtype allele positive, 7: positive control, 8: blank, 9: marker ladder 100 bp). Right**:** sequencing result for positive control . **Right:** positive control was having allele heterozygote C and A shown in sequencing result.

There were some DNA samples that could not be amplified with primer set A, but could be amplified with primer set B. Primer set B was design to identify wildtype allele C in rs17084051, which were forward outer 5’-TTCTACGGGAGAAGGCTGG-3’, reverse outer: 5’-CCTAGTCCTCCGACTCTGCT-3’ and reverse inner: 5’-CTAATCTGGAAGTAACAAAGTTTGTGAG-3’. Primer set B produced control band at 239 bp and inner band at 207 bp (figure 12).

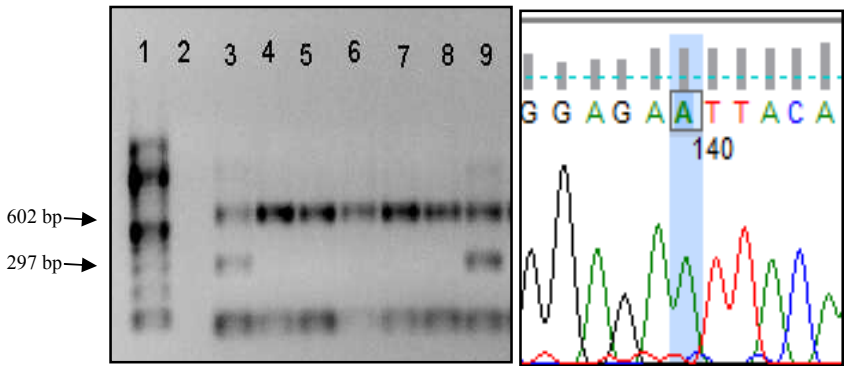

Figure 10. Electrophoresis result of rs17084051 ARMS PCR using primer set C. **Left:** Mutant allele A shown as control band at 602 bp and inner band at 297 bp. (Lane 1: marker ladder 100bp, 2: blank, 3: positive control, 4-8: mutant allele negative, 9: mutant allele positive). **Right:** positive control was having allele A shown in sequencing result.

Mutant allele was recognized by control band at 602 bp and inner band product at 297 bp using primer set C (forward outer: 5'-AAGAGAGACAATTTTCTCTCTGCTGG-3', reverse outer: 5'-AAGTAGAAGATCCCTCACCCTTAAGC-3', forward inner: 5'-GAGTGGTTGCATCTGACAGGAAAA-3'). Subject who did not have that inner band product classified as had no mutant allele for this SNP.

There were some DNA samples that could not be amplified with primer set C, but could be amplified with primer set D to identify mutant allele A in rs17084051 (forward outer: 5'-TTCTACGGGAGAAGGCTGG-3', reverse outer: 5'- CCTAGTCCTCCGACTCTGCT-3', forward inner: 5'-GAGTGGTTGCATCTGACAGGAAAA-3'). This primer produced control band at 239 bp and inner band for mutant allele A at 83 bp.

Table 24. Allele distribution of rs 17084051 C>A

Allele	Eye Status		Total
	Myop	Normal	
C/C	16 (35.6%)	29 (64.4%)	45 (45.0%)
A/A	0 (0.0%)	2 (100.0%)	2 (2.0%)
C/A	34 (64.2%)	19 (35.8%)	53 (53.0%)
Total	50 (50.0%)	50 (50.0%)	100 (100.0%)

There were 66% of wildtype allele C and 34% of mutant type allele A of rs17084051 *PDGFRA* gene polymorphism in South Sumatera tribes with early-onset myopia. There were 77% of wildtype allele C and 23% of mutant type allele A of rs17084051 *PDGFRA* gene polymorphism in South Sumatera tribes with normal eyes.

Table 25. Allele Frequency of rs17084051 C>A

Allele	Frequency (n)	Percentage (%)
C	143	71.5
A	57	28.5
Total	200	100

Allele frequency of rs17084051 was dominated by wildtype allele (71.5%). Minor allele frequency (MAF) was 0.29. There was no correlation between allele mutant in rs17084051 and corneal curvature because the *p* value was >0.05 for both eyes. There was a correlation between myopic status and the mutant allele in rs17084051 (*p* value 0.009). Prevalence risk for subject who had mutant allele in rs17084051 was 1.7 greater chance for having myopia.

5.1.3.3 SNP rs7677751 C>T

PCR reaction for rs7677751 using ARMS PCR technique: hold 1 95°C 3', 40 times (95°C 10", 55°C 30", 72°C 30"), and hold 2 72°C 2'. PCR reagents mix was 5 µl KAPA SYBR Fast Universal qPCR kit, 1 µl for each 5 pmol/ µl primer, 1 µl DNA sample 10 µg/ µl, and 1 µl H_2O PCR grade.

Primer set A (forward outer: 5'-ATCTCTCCCACTTTCTCTCCCTTCTT-3', reverse outer: 5'-TTTCTCAGCTTCCAAACTAGAGCTGA-3', reverse inner: 5'-CCTAGAATTTCTGAAACTTCCAACAATTTG-3') produced control band at 352 bp and inner band for wildtype allele C at 227 bp. But only few DNA samples that could be amplified using primer set A, so the others were amplified using primer set B. Primer set B was forward outer: 5'-TCTCCCTTCTTCACTTTCTCTATTG-3', reverse outer: 5'-TTTCAATGGTCCTTTAAACCATTT-3', reverse inner: 5'-CCTAGAATTTCTGAAACTTCCAACAAT-3'. Primer set B produced control band at 409 bp and inner band for wildtype allele C at 212 bp.

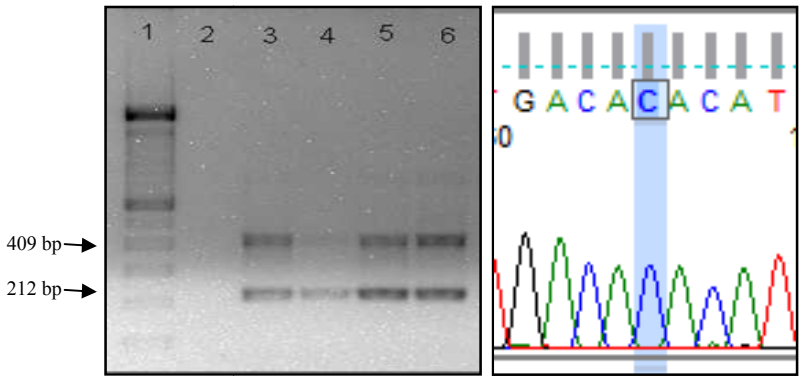

Figure 11. Electrophoresis result of rs7677751 ARMS PCR using primer set B. **Left:** Wildtype allele C shown as control band at 409 bp and inner band at 212 bp. (Lane 1: marker ladder 100bp, 2: blank, 3: positive control, 4-6: wildtype allele positive). **Right:** positive control was having allele C shown in sequencing result.

To genotyped mutant allele T, primer set C was using for all reaction (forward outer: 5'- TCTCCCTTCTTCACTTTCTCTATTG-3', reverse outer: 5'- TTTCAATGGTCCTTTAAACCATTT-3', forward inner: 5'- CAAAGGCCTCCTGTAAATGAAAT-3') that produced control band at 409 bp and inner band for mutant allele T at 250 bp.

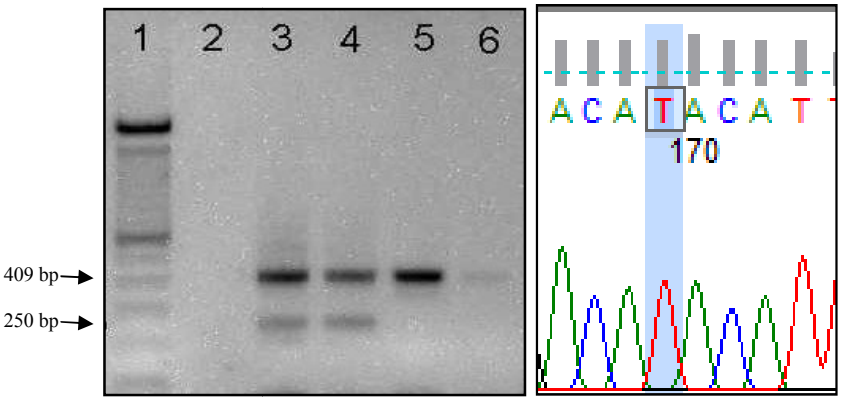

Figure 12. Electrophoresis result of rs7677751 ARMS PCR using primer set C**. Left:** Mutant allele T shown as control band at 409 bp and inner band at 250 bp. (Lane 1: marker ladder 100bp, 2: blank, 3: positive control, 4: mutant allele positive, 5-6: mutant allele negative). **Right:** positive control was having allele T shown in sequencing result.

Mutant allele was recognized by inner band product at 250 bp. Subject who did not have that inner band product classified as had no mutant allele.

Table 26. Allele distribution of rs7677751 C>T

Allele	Eye Status		Total
	Myop	Normal	
C/C	18 (34.0%)	35 (66.0%)	53 (53.0%)
T/T	0 (0.0%)	0 (0.0%)	0 (0.0%)
C/T	32 (68.1%)	15 (31.9%)	47 (47.0%)
Total	50 (50.0%)	50 (50.0%)	100 (100.0%)

There were 68% of wildtype allele C and 32% of mutant type allele T of rs7677751 *PDGFRA* gene polymorphism in South Sumatera tribes with early-onset myopia. There were 85% of wildtype allele C and 15% of mutant type allele T of rs7677751 *PDGFRA* gene polymorphism in South Sumatera tribes with normal eyes.

Table 27. Allele Frequency of rs7677751 C>T

Allele	Frequency (n)	Percentage (%)
C	153	76.5
T	47	23.5
Total	200	100

Allele frequency of rs7677751 was dominated by wildtype allele (76.5%). Mutant allele frequency only 0.23 (23.5%). There was no correlation between allele mutant in rs7677751 and corneal curvature because the *p* value was >0.05 for both eyes. There was a correlation between myopic status and the mutant allele in rs7677751 (*p* value 0.001).

Prevalence risk for subject who had mutant allele in rs7677751 was 2 times greater chance for having myopia.

5.1.3.4 SNP rs2307049 G>A

PCR reaction for rs2307049 using ARMS PCR technique for A allele was hold 1 95°C 3', 40 times (95°C 10", 60°C 30", 72°C 30"), and hold 2 72°C 2'. PCR reaction for G allele was only differ in annealing temperature (55°C). PCR reagents mix was 5 µl KAPA SYBR Fast Universal qPCR kit, 1 µl for each 5 pmol/ µl primer, 1 µl DNA sample 10 µg/ µl, and 1 µl H_2O PCR grade.

Primer set A (forward outer: 5'-TCTAGGAATGACGGATTATTTAGTCA-3', reverse outer: 5'-AGTCAGGAATGTGAATGAAAGTAAGA-3', reverse inner: 5'-GATTTAAAAAAAAAAAAAAATCCTAAAC-3') produced control band at 532 bp and inner band for wildtype allele G at 326 bp. Subject who did not have that inner band product classified as had no wildtype allele.

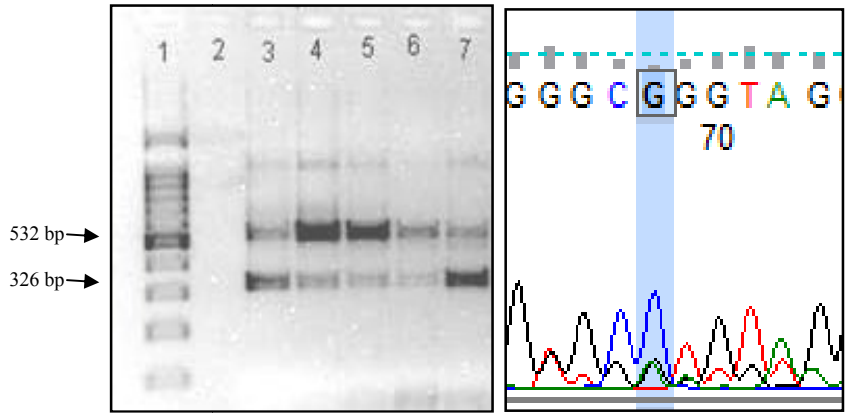

Figure 13. Electrophoresis result of rs2307049 ARMS PCR using primer set A. **Left:** Wildtype allele G shown as control band at 532 bp and inner band at 326 bp. (Lane 1: marker ladder 100bp, 2: blank, 3: positive control, 4-7: wildtype allele positive). **Right:** positive control was having allele G shown in sequencing result.

Primer set B (forward outer: 5'-TCTAGGAATGACGGATTATTTAGTCA-3', reverse outer: 5'-AGTCAGGAATGTGAATGAAAGTAAGA-3', forward inner: 5'-AGTAACAGGCAAAATCATAAGGTTCA-3') produced control band at 532 bp and inner band for mutant allele A at 260 bp.

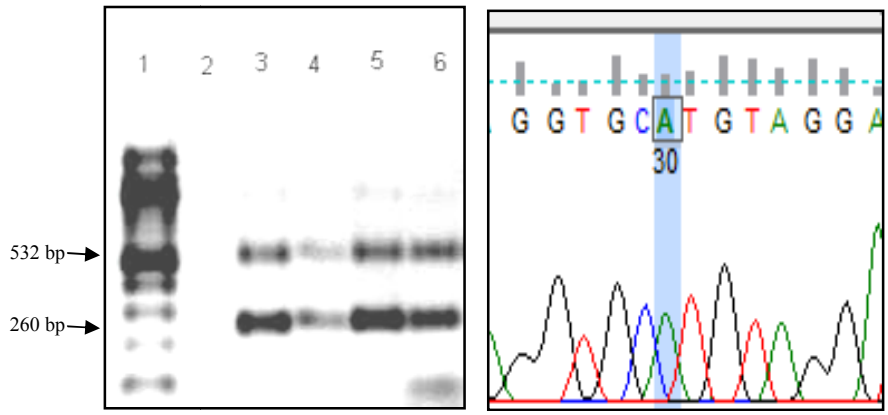

Figure 14. Electrophoresis result of rs2307049 ARMS PCR using primer set B. **Left:** Mutant allele A shown as control band at 532 bp and inner band at 260 bp. (Lane 1: marker ladder 100bp, 2: blank, 3: positive control, 4-6: mutant allele positive). **Right:** positive control was having allele A shown in sequencing result.

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Mutant allele was recognized by inner band product at 260 bp. Subject who did not have that inner band product classified as had no mutant allele.

Table 28. Allele distribution for rs2307049 G>A

Allele	Eye Status		Total
	Myop	Normal	
G/G	19 (61.3%)	12 (38.7%)	31 (31.0%)
A/A	9 (52.9%)	8 (47.1%)	17 (17.0%)
G/A	22 (42.3%)	30 (57.7%)	52 (52.0%)
Total	50 (50.0%)	50 (50.0%)	100 (100.0%)

There are 60% of wildtype allele G and 40% of mutant type allele A of rs2307049 *PDGFRA* gene polymorphism in South Sumatera tribes with early-onset myopia. There are 54% of wildtype allele G and 46% of mutant type allele A of rs2307049 *PDGFRA* gene polymorphism in South Sumatera tribes with normal eyes.

Table 29. Allele frequency of rs2307049 G>A

Allele	Frequency (n)	Percentage (%)
G	114	57
A	86	43
Total	200	100

Allele frequency of rs2307049 almost equal between wildtype (57%) and mutant allele (43%). There was no correlation between allele mutant in rs2307049 and corneal curvature because the *p* value was >0.05 for both eyes. There was no correlation between myopic status and the mutant allele in rs2307049 (*p* value 0.130).

5.1.3.5 SNP rs7682912 T>G

PCR reaction for rs7682912 using ARMS PCR technique: hold 1 95°C 3', 40 times (95°C 10", 71°C 45"), and hold 2 72°C 2'. PCR reagents mix was 5 µl KAPA SYBR Fast Universal qPCR kit, 1 µl for each 5 pmol/ µl primer, 1 µl DNA sample 10 µg/ µl, and 1 µl H_2O PCR grade.

Primer set A (forward outer: 5'-TGCTGAGGAGACAGACAGCAGAGGAGAGC-3', reverse outer: 5'-GGATCTGAGTGGGGGAATTCTGGGCT-3', forward inner: 5'-GCTACCGTGCCTGGCCTGATTCTTACCT-3') produced control band 522 bp and inner band for wildtype allele T at 200 bp. Subject who did not have that inner band was classified as had no wildtype allele for this SNP.

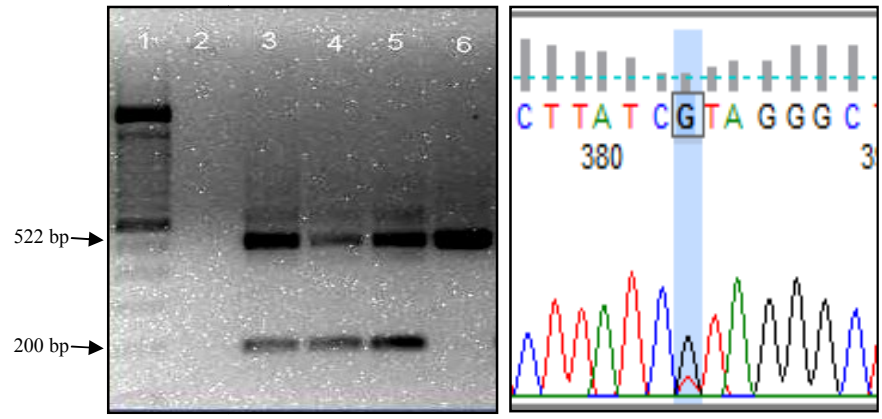

Figure 15. Electrophoresis result of rs7682912 ARMS PCR using primer set A. **Left:** Wildtype allele T shown as control band at 522 bp and inner band at 200 bp. (Lane 1: marker ladder 100bp, 2: blank, 3: positive control, 4-5: wildtype allele positive, 6: wildtype allele negative). **Right:** positive control was having allele heterozygote T and G shown in sequencing result.

Primer set B (forward outer: 5'-TGCTGAGGAGACAGACAGCAGAGGAGAGC-3', reverse outer: 5'-GGATCTGAGTGGGGGAATTCTGGGCT-3', reverse inner: 5'-ATGAAGGGTTCCCTCACAGCCCCAC-3') produced control band at 522 bp and inner band for mutant allele G at 376 bp.

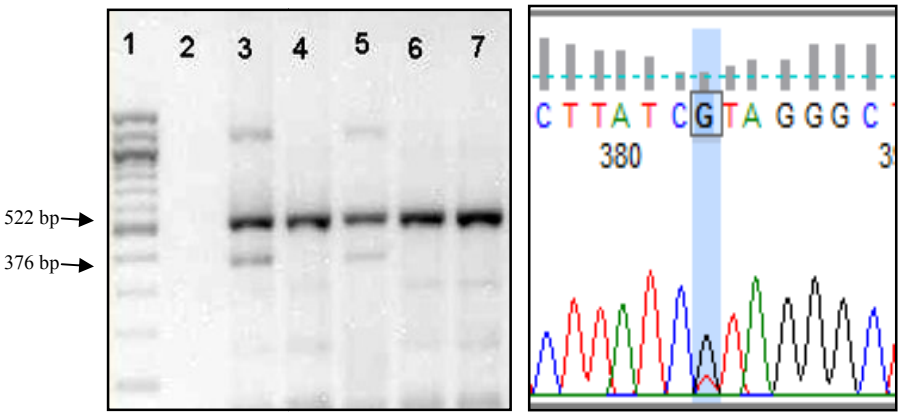

Figure 16. Electrophoresis result of rs7682912 ARMS PCR using primer set B. **Left:** Mutant allele G shown as control band at 522 bp and inner band at 376 bp. (Lane 1: marker ladder 100bp, 2: blank, 3: positive control, 4: mutant allele negative, 5: mutant allele positive, 6-7: mutant allele negative). **Right:** positive control was having allele heterozygote G and T shown in sequencing result.

Mutant allele was recognized by inner band product (376 bp). Subject who did not have that inner band product classified as had no mutant allele for this SNP.

Table 30. Allele distribution of rs7682912 T>G

Allele	Eye Status		Total
	Myop	Normal	
T/T	24 (43.6%)	31 (56.4%)	55 (55.0%)
G/G	0 (0.0%)	2 (100.0%)	2 (2.0%)
T/G	26 (60.5%)	17 (39.5%)	43 (43.0%)
Total	50 (50.0%)	50 (50.0%)	100 (100.0%)

There were 74% of wildtype allele T and 26% of mutant allele G of rs7682912 *PDGFRA* gene polymorphism in South Sumatera tribes with early-onset myopia. There were 79% of wildtype allele T and 21% of mutant allele G of rs7682912 *PDGFRA* gene polymorphism in South Sumatera tribes with normal eyes.

Table 31. Allele Frequency of rs7682912 T>G

Allele	Frequency (n)	Percentage (%)
T	110	55
G	90	45
Total	200	100

Allele frequency in rs7682912 was almost equal between wildtype (55%) and mutant allele (45%). There was no correlation between allele mutant in rs7682912 and corneal curvature because the *p* value was > 0.05 for both eyes (*p* value = 0.933). There was also no correlation between myopic status and the mutant allele in rs7682912 because the *p* value was >0.05 (*p* value = 0.159).

5.1.3.6 SNP rs7660560 G>A

PCR reaction for rs7660560 using ARMS PCR technique: hold 1 95°C 3', 40 times (95°C 10", 58°C 30", 72°C 30"), and hold 2 72°C 2'. PCR reagents mix for SNP 6 was 5 µl KAPA SYBR Fast Universal qPCR kit, 1 µl for each 5 pmol/ µl primer, 1 µl DNA sample 10 µg/ µl, and 1 µl H_2O PCR grade.

Primer set A was design to identify wildtype allele in rs7660560 (forward outer: 5'-TGCTTGAAATCACATCCCTTTAATG-3', reverse outer: 5'-GCCTCATGATATCATCTGATCCAATT-3', forward inner: 5'-AGAGTAGAGCCTGGCTAATCCGG-3') that produced control band at 550 bp and inner band for wildtype allele G at 214 bp. Subject who did not have that inner band product classified as had no wildtype allele.

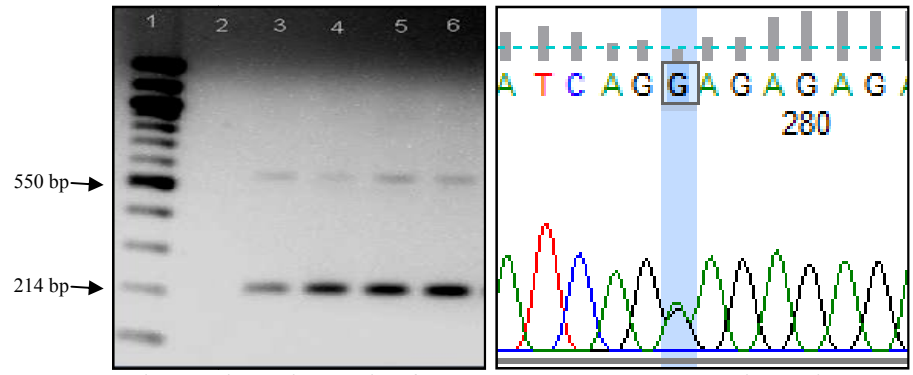

Figure 17. Electrophoresis result of rs7660560 ARMS PCR using primer set A. **Left:** Wildtype allele G shown as control band at 550 bp and inner band at 214 bp. (Lane 1: marker ladder 100bp, 2: blank, 3: positive control, 4-6: wildtype allele positive). **Right:** positive control was having allele G shown in sequencing result.

Primer set B was design to identify mutant allele in rs7660560 (forward outer: 5'-TGCTTGAAATCACATCCCTTTAATG-3', reverse outer: 5'-GCCTCATGATATCATCTGATCCAATT-3', reverse inner: 5'-CAAGCAACTCACTGCACATCTCTATT-3') that produced control band at 550 bp and inner band for mutant allele A at 385 bp. Subject who did not have that inner band product classified as had no mutant allele.

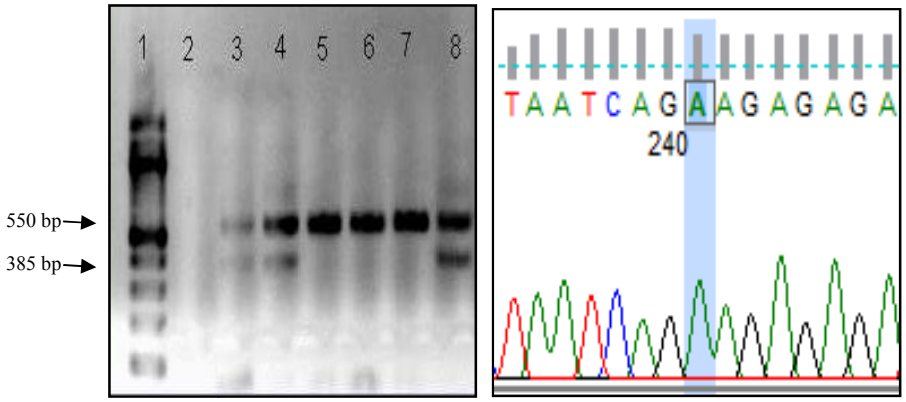

Figure 18. Electrophoresis result of rs7660560 ARMS PCR using primer set B. **Left:** Mutant allele A shown as control band at 550 bp and inner band at 385 bp. (Lane 1: marker ladder 100bp, 2: blank, 3: positive control, 4: mutant allele positive, 5-7: mutant allele negative, 8: mutant allele positive). **Right:** positive control was having allele A shown in sequencing result.

There were a few samples that could not be amplified with primer set B, so those DNA samples were amplified with primer set C and primer set D. Primer set C (forward outer: 5'- ACTAAAAGCTCTGGCCCCT-3', reverse outer: 5'- GGCTGAGGGGTGACTCATTC-3', reverse inner: 5'-CAAGCAACTCACTGCACATCTCTATT-3') produced control band at 380 bp and inner band at 298 bp for mutant allele A.

Primer set D (forward outer: 5'-ACTAAAAGCTCTGGCCCCT-3', reverse outer: 5'-GCCTCATGATATCATCTGATCCAATT-3', reverse inner: 5'-CAAGCAACTCACTGCACATCTCTATT-3') produced control band at 463 bp and inner band at 298 bp for mutant allele A.

Table 32. Allele distribution of rs7660560 G>A

Allele	Eye Status		Total
	Myop	Normal	
G/G	30 (49.2%)	31 (50.8%)	61 (61.0%)
A/A	0 (0.0%)	1 (100.0%)	1 (1.0%)
G/A	20 (52.6%)	18 (47.4%)	38 (38.0%)
Total	50 (50.0%)	50 (50.0%)	100 (100.0%)

There were 80% of wildtype allele G and 20% of mutant type allele A of rs7660560 *PDGFRA* gene polymorphism in South Sumatera tribes with early-onset myopia. There were 80% of wildtype allele G and 20% of mutant type allele A of rs7660560 *PDGFRA* gene polymorphism in South Sumatera tribes with normal eyes.

Table 33. Allele Frequency of rs7660560 G>A

Allele	Frequency (n)	Percentage (%)
G	160	80
A	40	20
Total	200	100

Allele frequency of rs7660560 was dominated by wildtype allele (71.5%). There was no correlation between allele mutant in rs7660560 and corneal curvature because the *p* value was > 0.05 for both eyes. There was no correlation between myopic status and the mutant allele in rs7660560 (*p* value 0.838).

5.1.3.7 SNP rs2114039 T>C

PCR reaction for rs2114039 using ARMS PCR technique: hold 1 95°C 3', 40 times (95°C 10", 69°C 30", 72°C 30"), and hold 2 72°C 2'. PCR reagents mix was 5 µl KAPA SYBR Fast Universal qPCR kit, 1 µl for each 5 pmol/ µl primer, 1 µl DNA sample 10 µg/ µl, and 1 µl H_2O PCR grade.

Primer set A was designed to identify wildtype allele in rs2114039 (forward outer: 5'-GAAGCGCCCTCAAAGCCAAAGGTGTG-3', reverse outer: 5'-GTACCCGGAGGAGAGGAGCGACATGC-3', reverse inner: 5'-AACTCCCGAATTGAGCCGGGCAGA-3') that produced control band at 516 bp and inner band at 318 bp for wildtype allele T. Subject who did not have that inner band was classified as have no wildtype allele for this SNP.

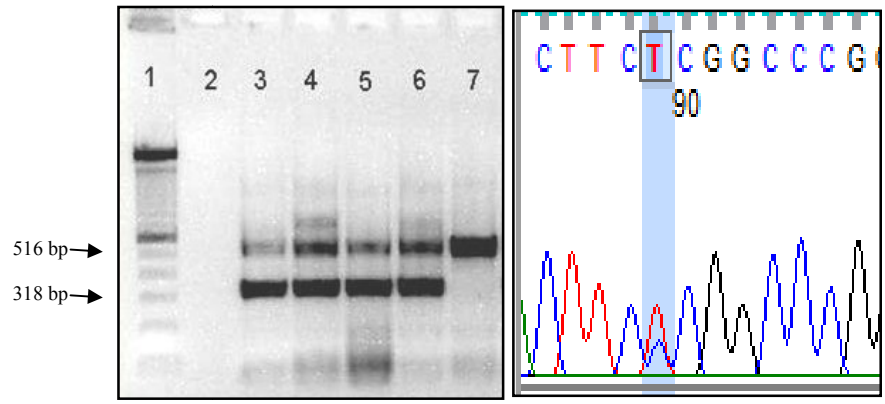

Figure 19. Electrophoresis result of rs2114039 ARMS PCR using primer set A. **Left:** Wildtype allele T shown as control band at 516 bp and inner band at 318 bp. (Lane 1: marker ladder 100bp, 2: blank, 3: positive control, 4-6: wildtype allele positive, 7: wildtype allele negative). **Right:** positive control was having allele T shown in sequencing result.

Primer set B (forward outer: 5'-GAAGCGCCCTCAAAGCCAAAGGTGTG-3', reverse outer: 5'-GTACCCGGAGGAGAGGAGCGACATGC-3', forward inner: 5'-CACAGTCCCTACCCTTTTCTCCCAACTGCC-3') produced control band at 516 bp and inner band at 252 bp for mutant allele C. Subject who did not have that inner band was classified as have no mutant allele for this SNP.

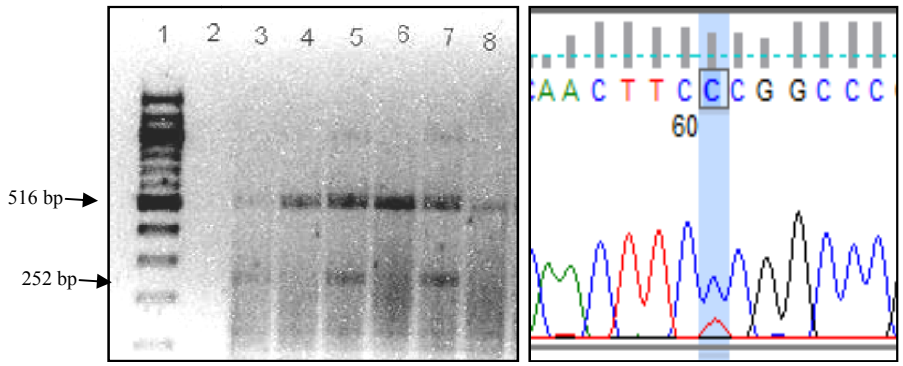

Figure 20. Electrophoresis result of rs2114039 ARMS PCR using primer set B. **Left:** Mutant allele C shown as control band at 516 bp and inner band at 252 bp. (Lane 1: marker ladder 100bp, 2: blank, 3: positive control, 4-5: mutant allele negative, 6-7: mutant allele positive, 8: mutant allele negative). **Right:** positive control was having allele C shown in sequencing result.

A few DNA samples could not be amplified with primer set B. So, they were amplified with primer set C to identify mutant allele in rs2114039 (forward outer: 5'-GCCCTCAATTTCATGTCGCC-3', reverse outer: 5'-AAGGTGGGGCTAGTTCTTGC-3', forward inner: 5'-CACAGTCCCTACCCTTTTCTCCCAACTGCC-3') that produced control band at 520 bp and inner band at 463bp. Subject who did not have that inner band was classified as have no mutant allele for this SNP.

Distribution of allele in rs2114039 T>C resumed in the table below:

Table 34. Allele distribution of rs2114039 T>C

Allele	Eye Status		Total
	Myop	Normal	
T/T	23 (43.4%)	30 (56.6%)	53 (53.0%)
C/C	9 (56.3%)	7 (43.7%)	16 (16.0%)
T/C	18 (58.1%)	13 (41.9%)	31 (31.0%)
Total	50 (50.0%)	50 (50.0%)	100 (100.0%)

There were 64% of wildtype allele T and 36% of mutant type allele C of rs2114039 *PDGFRA* gene polymorphism in South Sumatera tribes with early-onset myopia. There were 73% of wildtype allele T and 27% of mutant type allele C of rs2114039 *PDGFRA* gene polymorphism in South Sumatera tribes with normal eyes.

Table 35. Allele Frequency of rs2114039 T>C

Allele	Frequency (n)	Percentage (%)
T	137	68.5
C	63	31.5
Total	200	100

Allele frequency of rs2114039 was dominated by wildtype allele (68.5%). There was no correlation between mutant allele in rs2114039 and myopic status because the *p* value was >0.005 (*p* value=0.161). There was no correlation between mutant allele in rs2114039 and corneal curvature in South Sumatera population because the *p* value was >0.005 for both eyes (right eye= 0.423, left eye= 0.701).

Table 36. Association between mutant allele of SNPs and eye status

SNP	*p* value for myopic status	Prevalence Risk	Confidence Interval 95%
rs7676985	0.137	0.53	0.23-1.23
rs17084051	0.009	2.94	1.30-6.65
rs7677751	0.001	4.15	1.80-9.57
rs2307049	0.130	0.52	0.22-1.22
rs7682912	0.159	1.77	0.80-3.92
rs7660560	0.838	1.09	0.49-2.43
rs2114039	0.161	1.76	0.80-3.89

Table 36 showed the resume of *p* value for mutant allele in 7 SNPs in *PDGFRA* gene as a risk factor for myopia. There were 2 SNPs, rs17084051 and rs7677751, with *p* value <0.05 that showed a correlation between mutant allele in those SNPs and myopic status in South Sumatera tribes.

Table 37 showed the result of crosstabulation to find correlation between mutant allele in 7 SNPs and corneal curvature in South Sumatera tribes. The *p* value for all SNPs was >0.05 so there were no correlation between mutant allele in *PDGFRA* gene polymorphisms and corneal curvature in South Sumatera tribes.

Table 37. *p* value for mutant allele in SNPs and corneal curvature

SNP	*p* value for corneal curvature					
	Right eye	PR	CI 95%	Left eye	PR	CI 95%
rs7676985	0.958	0.98	0.41-2.33	0.958	0.98	0.41-2.33
rs17084051	0.738	1.15	0.51-2.61	0.738	1.15	0.51-2.61
rs7677751	0.423	1.40	0.61-3.19	0.701	1.17	0.52-2.67
rs2307049	0.331	0.64	0.26-1.59	0.331	0.64	0.26-1.59
rs7682912	0.933	1.04	0.46-2.35	0.933	1.04	0.46-2.35
rs7660560	0.384	1.45	0.62-3.42	0.657	1.21	0.52-2.81
rs2114039	0.423	1.40	0.61-3.19	0.701	1.17	0.52-2.67

PR: prevalence risk; CI 95%: confidence interval 95%

Minor allele frequency of 7 SNPs were resumed in table 38 and compared to the MAF of three major races in Singapore from previous study.

Table 38. Comparison between Minor Allele Frequency in South Sumatera and Singaporean population

SNP	MAF in South Sumatera population	MAF in Malay Singaporean population	MAF in Indian Singaporean population	MAF in Chinese Singaporean population
rs7676985	0.18	0.26	0.26	0.25
rs17084051	0.29	0.25	0.26	0.21
rs7677751	0.24	0.25	0.26	0.18
rs2307049	0.43	0.25	0.26	0.18
rs7682912	0.45	0.26	0.26	0.23
rs7660560	0.20	0.25	0.26	0.18
rs2114039	0.32	0.30	0.27	0.28

There were 4 SNPs with bigger MAF than the Singaporean which are rs17084051, rs2307049, rs7682912, and rs2114039. There was one SNP with lesser MAF, rs7676985, compare to three races in Singapore.

Table 39. Correlation between mutant allele in SNPs and pedigree, progressivity, and refractive power

SNP	*p* value for pedigree	*p* value for progressivity	*p* value for refractive power
rs7676985	0.110	0.091	0.251
rs17084051	0.910	0.197	0.340
rs7677751	0.639	0.131	0.224
rs2307049	0.326	0.409	0.122
rs7682912	0.614	0.517	0.192
rs7660560	0.058	0.875	0.173
rs2114039	0.099	0.599	0.244

Crosstabulation between mutant allele in every SNP and pedigree of case group gave no significant result for all SNP (*p* value >0.05) and so did with myopia progressivity group. Mutant allele and group of myopia classified by degree of refractive power was analysed with Kolmogorov Smirnov test and gave an insignificant *p* value for all SNPs. Correlation between mutant allele and myopic subject with steeper cornea found two significant result for rs17084051 (*p*= 0.029, PR= 1.697) and rs7677751 (*p*= 0.003, PR= 2.050).

5.2 Discussion

5.2.1 Sex

Sex was investigated as a risk factor for having myopia in some studies. Female had higher risk for having myopia than male.[43,44] In Beijing Childhood Eye Study, female sex was concluded as a risk factor for myopia (*p* value <0.001).[37] In Singapore Cohort Study of the Risk Factors for Myopia (SCORM), female had 1.21 times greater risk for having myopia

compare to males.[60] Among high school students in Beijing, females were having 1.31 times greater risk for having myopia (CI95%: 1.11-1.55).[61]

According to this study population where female population (67.3%) was more than male population (32.7%), this study could confirmed that female gender was a risk factor for having myopia (p value <0.05) and had a 4.21 times greater risk for having myopia compare to males (CI95%: 1.69-10.45).

Females were having higher risk to have myopia because of the female hormones. The experiment on porcine corneas showed oestrogen effect on corneal thickness and corneal biomechanics.[45] Level of estradiol (E2) was affecting spherical lens, cylindrical lens, axis, and interpupillary distance during menstrual cycle.[46] Because lens and axis also play a role in myopia development, the estradiol effect on lens and axis can cause the female's eye susceptibility on myopia.

5.2.2 Family History

Children with myopic parents are having high risk for myopia development.[32,33,34] In The Beijing Childhood Eye Study, children who had parents with myopia had a 1.45 greater risk to have myopia (p value < 0.05, CI 1.37-1.52).[37]

This study found positive association between parental, sibling, and paternal grandparent history of myopia with myopic status. The family history play a significant role as a risk factor of early myopia maybe because

of the genetics effect and or they share the same habit such as reading for pleasure.

There were 76% of case group with myopia history in ≥2 generation. This could be determined as a predictor for autosomal dominant mode of inheritance. Mutant allele in every SNP was more distributed in multi generation pedigree group. This can be assumed that, maybe, the myopia mode of inheritance play a role in distribution of mutant allele in nuclear family.

Myopia history only in the third generation could be determined as a predictor for multifactorial disease. The third generation was the millenium generation who had more times using computer or gadget, more channels to watch in television, less outdoor places for play, more indoor places for hangout, and more sources of information to read.

5.2.3 Outdoor Activity

The lower the outdoor activities, the bigger chance for having myopia in children.[33,38,40,41] The odds ratio was 0.93 (p value <0.001) for children with less daily exercise to have myopia. But, when the data adjusted for some parameters, this result became insignificant.[37]

In this study, there was no correlation between outdoor activity and myopia. Inadequate number of comfortable outdoor playground for children and the hot weather of Palembang city, Indonesia, make some parents and

children prefer to choose indoor activity. This situation makes no difference in outdoor activity habit between the normal and myopic subjects.

5.2.4 Lighting during near work

There was a correlation between myopia with reading in dim light in an investigation of risk factors for myopia in Greater Beijing school children (*p* value <0.005, OR 0.93). Eventhough it was correlated, the dim illumination is a protective factor for having myopia because the odds ratio was <1. The reason why myopic people prefer to do reading in dim illumination maybe because it was easier to read in such lighting.[37]

This study of South Sumatera population found an insignificant correlation between lighting while reading or watching and myopia.

5.2.5 Near Work

Reading, watching television, or using computer are categorized as near work. Myopic children were having more time studying and reading.[34] There was a positive association between myopia and the close distance while reading in Australian school children.[36] There was a correlation between myopia and shorter duration in watching television in The Beijing Childhood Eye Study.[37] One study in New England found that children with myopia was spent more time watching television compare to non-myopia children during school year.[38] The odds ratio for children who read more than 2 hours per day to have higher myopia was 2.16.[39]

This study found negative association between early-onset myopia, watching television and using computer/gadget. But, this study found positive association between frequency of reading ≥6 times per week and duration of reading >2 hours per day with early-onset myopia.

In Indonesia, computer or gadget still classified as a luxurious stuff so not everybody can have it and using it all the time like in the western countries. The disadvantage of this study was that economic status of some subjects are lower than other subjects. In future study, the economic status of the research subject supposed to be arranged into the same level as a middle up economic people.

Distance while doing near work was determined to be a risk factor for myopia in Beijing. High school student who had shorter near work distance had 1.87 greater risk for having myopia (CI 95%: 1.55-2.26).[61] This study also found a positive association between television viewing distance ≤60 cm and early-onset myopia (p = 0.0001, PR=3.35, CI 95% = 2.15-5.15).

5.2.6 Corneal Curvature

Mean value of corneal curvature radius in this study for right eye was 7.75 mm and for the left eye was 7.76. This value was bigger than mean value of corneal curvature in three major races in Singapore based on previous study. In Malay Singaporean mean value was 7.66 mm, Indian Singaporean was 7.62 mm, and Chinese Singaporean was 7.73 mm.[11]

Some studies found an association between corneal curvature and refractive anomaly. Myopic eye has steeper corneal curvature than normal eye.[23,25] But, this study found no difference between corneal curvature radius of myopic subjects and the normal one (p >0.05).

This study found no difference between male and female's corneal curvature radius. But if we looks into the mean value, male's corneal curvature radius was higher (right eye mean 7.79 ± 0.23 mm, left eye mean 7.83 ± 0.26 mm) than female's (both eye mean 7.72 ± 0.23mm). A study in Taiwan, Australia, and China also found that male had higher corneal curvature radius than female.[22,62,63] But, in Nigerians, female had higher corneal curvature radius.[64] A study of Taiwan school children found no difference in corneal curvature radius between boys and girls.[62]

5.2.7 *PDGFRA* Gene Polymorphisms

There was an association between corneal curvature and *PDGFRA* gene and *FRAP1* gene in Singaporean population. This study found 7 SNPs in *PDGFRA* gene whom correlated to corneal curvature.[11] In White Europeans, only rs17084051 was correlated to the corneal curvature.[13] In Australian, only rs2114039 was correlated to the corneal curvature.[12] The function of those mutant type allele were still unclear.

In this study, there were no SNPs of *PDGFRA* gene that had a correlation with corneal curvature in South Sumatera population. Because of the corneal curvature of myopic or normal people were not affected by 7

SNPs in *PDGFRA* gene, corneal curvature of South Sumatera population will not be lesser and become steeper cornea if these peoples have mutant allele in those 7 SNPs. Negative association between these two variables maybe caused by different technique of SNP genotyping, lesser subjects and ethnicity variation.

Eventhough Malay and South Sumatera population were said to be an ancestry of Taiwan farmer who expanded to West Indonesia and Malaysia, but this study showed a difference mutatn allele distribution in four SNP: rs17084051, rs2307049, rs7682912, and rs2114039. These SNPs were having bigger MAF compare to Malay Singaporean population. This condition described that the mutant allele in these SNPs were more distributed in South Sumatera population.

SNP rs7677751, rs7660560, and rs7676985 were having MAF 0.24, 0.20, and 0.18 respectively. These MAFs were lesser than MAF in Malay Singaporean population. This condition described that the mutant allele in these SNPs were existed more in Malay Singaporean than South Sumatera population.

Some factors may play a role in the difference MAF value between these populations, which are different ethnicity, different research method, different technique to genotyped the SNPs, and the amount of research samples.

Eventhough there was no correlation between mutant allele and corneal curvature, this study found a correlation between mutant allele in

rs17084051 and rs7677751 and early-onset myopia. Because these two SNPs were not correlated to corneal curvature, maybe these two SNPs were affecting another path of myopic pathophysiology, such as axial length or lens thickness. Based on Ocular Tissue Database, the lens had the highest expression of PDGFRA protein with 745.489 PLIER while cornea only had 88.85 PLIER.[65] PDGFRA protein also expressed in lens epithelium and conducted of hyperproliferation and ectopic differentiation into lens fiber cells.[16]

There were no correlation between mutant allele in every SNPs and myopia based on refractive power (low, medium, and hig myopia). High myopia was determined to be an effect of longer axial length. So maybe, these polymophisms were not affected the axial length that could cause high myopia.

Eventhough the result of this study were different with previous study in Singapore because of negative association between corneal curvature and *PDGFRA* gene polymorphism, but this is the first study in Indonesia who tried to find genetic susceptibility in corneal curvature and early-onset myopia while the previous study in Singapore studied the corneal curvature and corneal astigmatism. ARMS PCR technique was feasible to do in Indonesia, although it was more conventional compare to GWAS like previous studies did. This technique was easier than Restriction Fragment Length Polymorphism (RFLP) because only need one time gel

electrophoresis. ARMS PCR can be lot easier than RFLP if we can optimize two allele specific-primers in one tube.

The information of *PDGFRA* gene polymorphism can be useful in genetic counseling for myopic subjects to explain the possibility of mutant allele inheritance to their ancestry. Genetic counseling also can be given for peoples who are at risk of having early-onset myopia, such as females, high frequent readers and people with family history of myopia. So, they can reduce the risk and maintain the good visual acuity last longer.

5.2.8 Limitation of study

Measurement of corneal curvature radius was only done on the anterior side. So, this study address only the the corneal refractive power of the anterior corneal surface, but not the total refractive power of the cornea. Central corneal thickness and anterior chamber depth were not measured, so that the contribution of these biometric parameters to the optic system of the eyes could not be identified.