CHAPTER IV

RESULTS

Several potential SNPs were identified from the public databases and the literatures. A total of six SNPs were chosen for association study, because they result in amino acid changes and therefore may alter their functions. Of these, *TGFβ-3* 383A→G, *IRF6* 820G→A, and *MTHFD1* 1958G→A were obtained from previous reports[26,44], and *TGFβ-3* 179C→T was obtained from the Single Nucleotide Polymorphism (dbSNP) Database (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=4252315), rs4252315. In addition *SKI* SNPs, *SKI* 185C→G and *SKI* 1163C→T, were selected from submitted genomic sequences of SKI exon 1 (AY331180), and SKI exon 2 – 3 (AH013034) by Vieira, A.R. and Murray, J.C.

1. Genotyping

According to the RFLP pattern, since differences in restriction site by SNPs generated different fragments and thus a different band pattern, though other restriction site were internal control for RFLP manner.

1.1 *TGFβ-3* 179C→T, *TGFβ-3* 383A→G, and *SKI* 1163C→T

For these three SNPs, no variation was observed. Thirty-three CL/Ps and their twenty-two mothers were genotyped for *TGFβ-3* 179C→T. Additionally, fifty-six and ninety of controls were genotyped for *TGFβ-3* 383A→G and *SKI* 1163C→T, respectively.

1.2 *IRF6* 820G→A

*Dpn*II digested the G variance to 5 fragments (322bp, 177 bp, 80 bp, 33 bp and 35 bp), and it added the restriction site in A variance consequently digested 322 bp to 235 bp and 87 bp. Furthermore, heterozygous G/A consisted of 7 bands (322 bp, 235 bp, 177 bp, 87 bp, 80 bp, 33 bp and 35 bp). The *IRF6* 820G->A genotyping showed in figure 7 , except 33 bp and 35 bp don't appear on agarose gel.

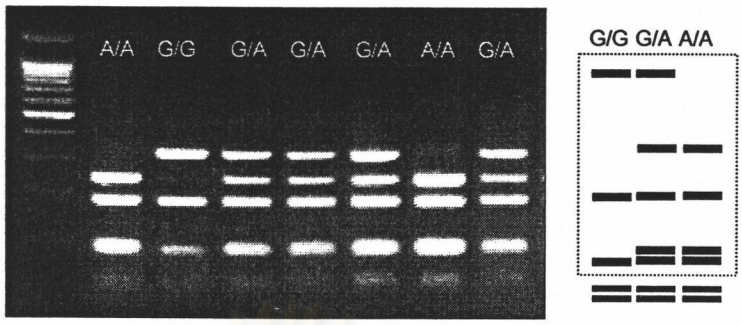

Figure 7 RFLP patterns of *IRF6* 820G→A. Lane 1 is 100 bp DNA marker. Lane 2 and 7 are homozygous A/A. Lane 3 is homozygous G/G. Lane 4, 5, 6, and 8 are heterozygous G/A.

1.3 *SKI* 185C→G

Genotyping of *SKI* 185C→G exhibited in figure 8, C variance consisted of 3 bands (227 bp,157 bp, and 51 bp) while G variance consisted of 4 bands (227 bp, 80 bp, 77 bp and 51 bp). *SKI* 185C→G increased the restriction site of *Nci*I in G allele, thus digested 157 bp of C allele to 80 bp and 77 bp. Moreover, heterozygous C/G consisted of 5 bands (227 bp,157 bp, 81, 77 bp, and 51 bp).

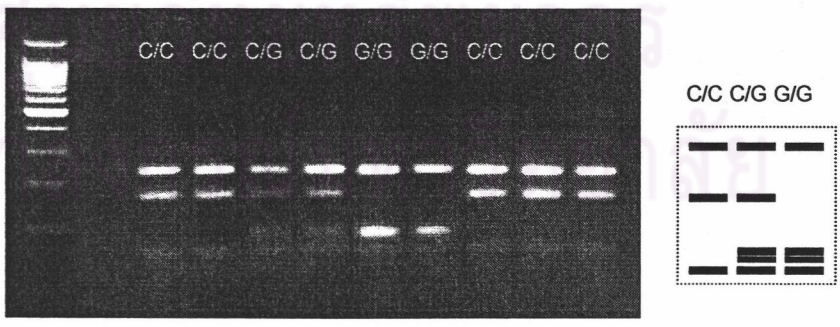

Figure 8 RFLP patterns of *SKI* 1163C→T. Lane 1 is 100 bp DNA marker. Lane 3 - 4 are homozygous C/C. Lane 5 - 6 are heterozygous C/G, and lane 7-8 are homozygous G/G.

1.4 *MTHFD1* 1958G→A

Genotyping of *SKI* 185C→G was performed by RFLP pattern of *Msp*I. G variance consisted of 4 bands (196 bp, 70 bp, 56 bp and 8 bp) while G variance decreased the digested bands, composed of unique 3 bands (266 bp, 56 bp and 8 bp). *MTHFD1* 1958G→A decreased the restriction site in A allele, thus arrested the 266 bp digested to 196 bp and 70 bp. Also heterozygous C/G consisted of 5 bands (266 bp, 196 bp, 70196 bp, 70 bp, 56 bp and 8 bp bp, 56 bp and 8 bp). In figure 9 revealed the pattern of each genotype, sinces 196 bp, 70 bp, 56 bp and 8 bp were presented on agarose gel.

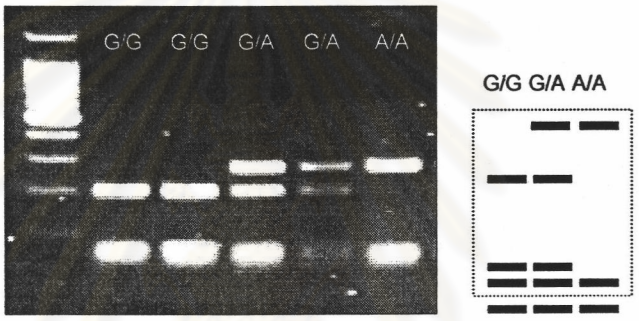

Figure 9 RFLP patterns of *MTHFD1* 1958G→A. Lane 1 is 100 bp DNA marker. Lane 2 and 3 are homozygous G/G. Lane 4 and 5 are heterozygous G/A. Lane 6 is homozygous A/A.

2.Genotype and allele frequencies

The observed frequencies of each SNPs in affected subjects, their mothers, their fathers, and controls were presented in each geontypic and allelic distribution table, classified by individual SNPs. There shown in 3 parts, consisted of CL/P, CPO and FEEM.

3.1 Cleft lip with or without cleft palate (CL/P)

The genotypic and allelic distribution of three polymorphic SNPs in patients, their parent groups, and controls were presented in tables 9, 10, and 11 . For the highest polymorphic SNPs, *IRF6* 820G->A, prevalence of 278 controls, 192 patients,

177 mothers, and 73 fathers were determined, only distribution in patient group was difference form control group (X^2 = 7.88, P-value = 0.02). The distribution of *MTHFD1* 1958G->A in each study groups was shown, there were 187 patient, 111 mothers, 67 fathers, and 279 controls. No significantly difference of genotype and allele frequencies was observed between members of family with CL/P and controls. Observation of *SKI* 185C->G in 269 controls, 186 patients, 109 mothers, and 63 fathers, only 2 homozygous GG were determined in 2 CL/P patients. Because low number and no occurrence of GG were presented, thus since allele frequencies were used to calculated chi square and there were not differ significantly between members of family with CL/P and controls.

Table 9 Genotypic and allelic distribution of the *IRF6* 820G→A in family with CL/P and controls

	Members of family with CL/P			Controls (n = 278)
	Patients (n = 192)	Mothers (n = 117)	Fathers (n = 73)	
Genotype:				
GG	93 (0.48)	46 (0.39)	31 (0.42)	100 (0.36)
GA	72 (0.38)	60 (0.51)	27 (0.37)	137 (0.49)
AA	27 (0.14)	11 (0.10)	15 (0.21)	41 (0.15)
X^2 (P-value, df = 2)	**7.88 (0.02)**	2.10 (0.35)	3.74 (0.15)	Ref.
Allele:				
G	258 (0.67)	152 (0.65)	89 (0.61)	337 (0.61)
A	126 (0.33)	82 (0.35)	57 (0.39)	219 (0.39)
X^2 (P-value, df = 1)	**4.23 (0.04)**	1.32 (0.25)	0.01 (0.94)	Ref.

Ref. = Reference category.

Table 10 Genotypic and allelic distribution of the *SKI* 185C→G in family with CL/P and controls

	Members of family with CL/P			Controls (n = 269)
	Patients (n = 186)	Mothers (n = 109)	Fathers (n = 63)	
Genotype:				
CC	177 (0.95)	103 (0.94)	60 (0.95)	258 (0.96)
CG	7 (0.04)	6 (0.06)	3 (0.05)	11 (0.04)
GG	2 (0.01)	NO	NO	NO
X^2 (P-value, df = 2)	ND	ND	ND	Ref.
Allele:				
C	361 (0.97)	212 (0.97)	123 (0.98)	527 (0.98)
G	11 (0.03)	6 (0.03)	3 (0.02)	11 (0.02)
X^2 (P-value, df = 1)	0.78 (0.38)	0.11 (0.75)*	0.01 (0.91)*	Ref.

Ref. = Reference category. NO = not observed. ND = not determined

* Calculated by Yates' extract.

Table 11 Genotypic and allelic distribution of the *MTHFD1* 1958G→A in family with CL/P and controls

	Members of family with CL/P			Controls (n = 279)
	Patients (n = 187)	Mothers (n = 111)	Fathers (n = 67)	
Genotype:				
GG	127 (0.68)	74 (0.67)	46 (0.69)	191 (0.68)
GA	53 (0.28)	34 (0.30)	18 (0.27)	80 (0.29)
AA	7 (0.04)	3 (0.03)	3 (0.04)	8 (0.03)
X^2 (P-value, df = 2)	0.08 (0.78)	0.07 (0.79)	0.04 (0.84)	Ref.
Allele:				
G	307 (0.82)	182 (0.82)	110 (0.82)	462 (0.83)
A	67 (0.18)	40 (0.18)	24 (0.18)	96 (0.17)
X^2 (P-value, df = 1)	0.08 (0.78)	0.07 (0.79)	0.01 (0.94)	Ref.

Ref. = Reference category.

3.2 Cleft palate only (CPO)

As for CPO study, there was a less number of subjects, the patient group there were 43 cases for *IRF6* 820G→A and *SKI* 185C→G genotyping, and 49 cases for *MTHFD1* 1958G→A genotyping. While mothers of them were 31, 32, and 36 cases for the three SNPs, respectively. In father group, a small number were observed in all SNPs. In case of *IRF6* 820G→A, 19 cases were included, while 18 and 23 cases were genotyped for *SKI* 185C→G, *MTHFD1* 1958G→A. According to chi square test for difference of genotype and allele frequencies between interesting subject groups and controls. No significantly were observed in all interesting subject groups, excepted in *MTHFD1* 1958G→A of patients showed significantly difference from controls. The data were shown in tables 12 - 14.

Table 12 Genotypic and allelic distribution of the *IRF6* 820G→A in family with CPO and controls

	Members of family with CPO			Controls (n = 278)
	Patients (n = 43)	Mothers (n = 31)	Fathers (n = 19)	
Genotype:				
GG	12 (0.28)	10 (0.32)	4 (0.21)	100 (0.36)
GA	19 (0.44)	15 (0.48)	13 (0.68)	137 (0.49)
AA	12 (0.28)	6 (0.19)	2 (0.11)	41 (0.15)
X^2 (P-value, df = 2)	3.47 (0.06)	0.50 (0.78)	2.64 (0.27)	Ref.
Allele:				
G	43 (0.50)	35 (0.56)	21 (0.55)	337 (0.61)
A	43 (0.50)	27 (0.44)	17 (0.45)	219 (0.39)
X^2 (P-value, df = 1)	3.47 (0.06)	0.40 (0.53)	0.43 (0.51)	Ref.

Ref. = Reference category.

Table 13 Genotypic and allelic distribution of the *SKI* 185C→G in family with CPO and controls

	Members of family with CPO			Controls (n = 269)
	Patients (n = 43)	Mothers (n = 32)	Fathers (n = 18)	
Genotype:				
CC	39 (0.91)	31 (0.97)	17 (0.94)	258 (0.96)
CG	4 (0.09)	1 (0.03)	1 (0.06)	11 (0.04)
GG	NO	NO	NO	NO
X^2 (P-value, df = 2)	ND	ND	ND	Ref.
Allele:				
C	82 (0.95)	212 (0.98)	123 (0.97)	527 (0.98)
G	4 (0.05)	6 (0.02)	3 (0.03)	11 (0.02)
X^2 (P-value, df = 1)	1.18 (0.28)*	0.05 (0.83)*	ND	Ref.

Ref. = Reference category. NO = not observed. ND = not determined

* Calculated by Yates' extract.

Table 14 Genotypic and allelic distribution of the *MTHFD1* 1958G->A in family with CPO and controls

	Members of family with CPO			Controls (n = 279)
	Patients (n = 49)	Mothers (n = 36)	Fathers (n = 23)	
Genotype:				
GG	27 (0.55)	18 (0.50)	14 (0.61)	191 (0.68)
GA	17 (0.35)	17 (0.47)	8 (0.35)	80 (0.29)
AA	5 (0.10)	1 (0.03)	1 (0.04)	8 (0.03)
X^2 (P-value, df = 2)	**7.29 (0.03)**	5.20 (0.07)	0.61 (0.74)	Ref.
Allele:				
G	71 (0.72)	53 (0.74)	36 (0.78)	462 (0.83)
A	27 (0.28)	19 (0.26)	10 (0.22)	96 (0.17)
X^2 (P-value, df = 1)	**5.86 (0.02)**	3.61 (0.06)	0.60 (0.44)	Ref.

Ref. = Reference category.

3.3 Frontoethmoidal encephalomeningocele (FEEM)

According to genotypic and allelic distribution data in tables 15 - 17, *IRF6* 820G→A, prevalence of 278 controls, 65 patients, 37 mothers, and 18 fathers were determined, no significantly difference of genotype and allele frequencies was observed. For distribution of *MTHFD1* 1958G→A, the same results as *IRF6* 820G→A were revealed in genotypic distribution of 279 controls, 64 patients, 40 mothers, and 17 fathers. And the number of allelic distribution of *SKI* 185C→G in 64 patients, 39 mothers, and 17 fathers did not differ from 269 controls.

Table 15 Genotypic and allelic distribution of the *IRF6* 820G→A in family with FEEM and controls

	Members of family with FEEM			Controls (n = 278)
	Patients (n = 65)	Mothers (n = 37)	Fathers (n = 18)	
Genotype:				
GG	22 (0.34)	14 (0.38)	4 (0.22)	100 (0.36)
GA	37 (0.57)	17 (0.46)	13 (0.72)	137 (0.49)
AA	6 (0.09)	6 (0.16)	1 (0.06)	41 (0.15)
X^2 (P-value, df = 2)	1.84 (0.40)	0.15 (0.93)	3.67 (0.16)	Ref.
Allele:				
G	81 (0.62)	45 (0.61)	21 (0.58)	337 (0.61)
A	49 (0.38)	29 (0.39)	15 (0.42)	219 (0.39)
X^2 (P-value, df = 1)	0.13 (0.72)	0.01 (0.97)	0.07 (0.79)	Ref.

Ref. = Reference category.

Table 16 Genotypic and allelic distribution of the *SKI* 185C→G in family with FEEM and controls

	Members of family with FEEM			Controls (n = 269)
	Patients (n = 64)	Mothers (n = 39)	Fathers (n = 17)	
Genotype:				
CC	62 (0.97)	35 (0.90)	16 (0.94)	258 (0.96)
CG	2 (0.03)	4 (0.10)	1 (0.06)	11 (0.04)
GG	NO	NO	NO	NO
X^2 (P-value, df = 2)	ND	ND	ND	Ref.
Allele:				
C	126 (0.95)	74 (0.98)	33 (0.97)	527 (0.98)
G	2 (0.05)	4 (0.02)	1 (0.03)	11 (0.02)
X^2 (P-value, df = 1)	0.01 (1.00)*	1.58 (0.21)*	0.07 (0.79)*	Ref.

Ref. = Reference category. NO = not observed. ND = not determined

* Calculated by Yates' extract.

Table 17 Genotypic and allelic distribution of the *MTHFD1* 1958G→A in family with FEEM and controls

	Members of family with FEEM			Controls (n = 279)
	Patients (n = 64)	Mothers (n = 40)	Fathers (n = 17)	
Genotype:				
GG	49 (0.77)	27 (0.68)	12 (0.71)	191 (0.68)
GA	13 (0.20)	12 (0.30)	5 (0.29)	80 (0.29)
AA	2 (0.03)	1 (0.02)	NO	8 (0.03)
X^2 (P-value, df = 2)	1.84 (0.40)	0.04 (0.98)	0.50 (0.78)	Ref.
Allele:				
G	111 (0.87)	66 (0.83)	29 (0.85)	462 (0.83)
A	17 (0.13)	14 (0.17)	5 (0.15)	96 (0.17)
X^2 (P-value, df = 1)	1.17 (0.28)	0.01 (0.95)	0.14 (0.71)	Ref.

Ref. = Reference category. NO = not observed

3. Test for Hardy Weinberg equilibrium (HWE)

The usual test for goodness of fit of observed data to HWE is chi-squares test, using the observed and expected numbers. Associated with any X^2 value of HWE, the degrees of freedom (df) is 1 for probability value (P-value). All groups were calculated by chi-squares test excepted number was below 1 or if the expected number was less than 5 in more than 20% of whole cells.

After genotyping, data of genotypic distribution of polymorphic SNPs in members of family with CL/P, CPO and FEEM, and controls were available (Tables 9 - 11). Regarding to the test for HWE, our population was consistent with HWE.

4. Odds ratio (OR)

The odds ratio is a useful measure of association for candidate SNPs and diseases. The effect of genotype calculate in four type: 1) by actual number of mutant alleles (co-dominant mode); 2) by an effect of having any mutant allele (dominant mode); 3) by an effect of having two mutant alleles (recessive mode); 4) by an effect of only heterozygous genotype. The results were shown by tables of genotypic distribution and odds ratio (OR), classified by the 3 entire disease.

4.1 Cleft lip with or without cleft palate (CL/P)

From the calculated odds ratio in tables 18 - 20, analysis of the *IRF6* 820G->A allele frequencies showed a significant excess of A allele in the patients with CL/P. There were GA group in codominance mode (**OR = 0.57 [0.37 <OR< 0.86]**), dominance mode (**0.60 [0.40 <OR< 0.88]**) and heterozygous genotype that compared with the other (**0.62 [0.42 <OR< 0.91]**). Whereas *MTHFD1* 1958G->A and *SKI* 185C->G exhibit no association with CL/P.

Table 18 Genotypic distribution and odds ratio (OR) of *IRF6* 820G→A in family with CL/P.

	Members of family with CL/P			Controls
	Patients	Mothers	Fathers	
	(n = 192)	(n = 117)	(n = 73)	(n = 278)
1.GG	1.00	1.00	1.00	
	(n = 93)[a]	(n = 46)	(n = 31)	(n = 100)
GA	**0.57[b] [0.37 <OR< 0.86][c]**	0.95 [0.58 <OR< 1.55]	0.64 [0.34 <OR< 1.18]	
	(n = 72)	(n = 60)	(n = 27)	(n = 137)
AA	0.71 [0.39 <OR< 1.29]	0.58 [0.26 <OR< 1.31]	1.18 [0.54 <OR< 2.55]	
	(n = 27)	(n = 11)	(n = 15)	(n = 41)
2.GG	1.00	1.00	1.00	
	(n = 93)	(n = 46)	(n = 31)	(n = 100)
AA or GA	**0.60 [0.40 <OR< 0.88]**	0.87 [0.54 <OR< 1.39]	0.76 [0.44 <OR< 1.33]	
	(n = 99)	(n = 71)	(n = 42)	(n = 178)
3.GA or GG	1.00	1.00	1.00	
	(n = 165)	(n = 106)	(n = 58)	(n = 237)
AA	0.95 [0.54 <OR< 1.65]	0.60 [0.28 <OR< 1.27]	1.49 [0.73 <OR< 3.02]	
	(n = 27)	(n = 11)	(n = 15)	(n = 41)
4.GG or AA	1.00	1.00	1.00	
	(n = 120)	(n = 57)	(n = 46)	(n = 141)
GA	**0.62 [0.42 <OR< 0.91]**	1.08 [0.69 <OR< 1.71]	0.60 [0.34 <OR< 1.06]	
	(n = 72)	(n = 60)	(n = 27)	(n = 137)

a = no of genotype, b = OR, c = 95% CI

Table 19 Genotypic distribution and odds ratio (OR) of *SKI* 185C→G in family with CL/P.

	Members of family with CL/P			Controls
	Patients	Mothers	Fathers	
	(n = 186)	(n = 109)	(n = 63)	(n = 269)
1.CC	1.00	1.00	1.00	
	(n = 177)[a]	(n = 103)	(n = 60)	(n = 258)
CG	0.93[b] [0.32 <OR< 2.64][c]	1.37 [0.40 <OR< 4.15]	1.17 [0.20 <OR< 4.63]	
	(n = 7)	(n = 6)	(n = 3)	(n = 11)
GG	ND	ND	ND	
	(n = 2)	NO	NO	NO
2.CC	1.00	1.00	1.00	
	(n = 177)	(n = 103)	(n = 60)	(n = 258)
GG or CG	1.19 [0.44 <OR< 3.17]	1.37 [0.40 <OR< 4.15]	1.17 [0.20 <OR< 4.63]	
	(n = 9)	(n = 6)	(n = 3)	(n = 11)
3.CG or CC	1.00	1.00	1.00	
	(n = 184)	(n = 109)	(n = 63)	(n = 269)
GG	ND	ND	ND	
	(n = 2)	NO	NO	NO
4.CC or GG	1.00	1.00	1.00	
	(n = 179)	(n = 103)	(n = 60)	(n = 258)
CG	0.92 [0.31 <OR< 2.61]	1.37 [0.40 <OR< 4.15]	1.17 [0.20 <OR< 4.63]	
	(n = 7)	(n = 6)	(n = 3)	(n = 11)

NO = not observed. ND = not determined, a = no of genotype, b = OR, c = 95% CI

Table 20 Genotypic distribution and odds ratio (OR) of *MTHFD1* 1958G→A in family with CL/P.

	Members of family with CL/P			Controls
	Patients	Mothers	Fathers	
	(n = 187)	(n = 111)	(n = 67)	(n = 279)
1.GG	1.00	1.00	1.00	
	(n = 127)[a]	(n = 74)	(n = 46)	(n = 191)
GA	1.00[b] [0.65 <OR< 1.54][c]	1.10 [0.66 <OR< 1.83]	0.93 [0.49 <OR< 1.78]	
	(n = 53)	(n = 34)	(n = 18)	(n = 80)
AA	1.32 [0.42 <OR< 4.11]	0.97 [0.16 <OR< 4.17]	1.56 [0.26 <OR< 6.80]	
	(n = 7)	(n = 3)	(n = 3)	(n = 8)
2.GG	1.00	1.00	1.00	
	(n = 127)	(n = 74)	(n = 46)	(n = 191)
AA or GA	1.03 [0.68 <OR< 1.56]	0.97 [0.16 <OR< 4.17]	0.99 [0.54 <OR< 1.82]	
	(n = 60)	(n = 37)	(n = 21)	(n = 88)
3.GA or GG	1.00	1.00	1.00	
	(n = 180)	(n = 108)	(n = 64)	(n = 271)
AA	1.32 [0.42 <OR< 4.08]	0.94 [0.16 <OR< 4.01]	1.59 [0.26 <OR< 6.84]	
	(n = 7)	(n = 3)	(n = 3)	(n = 8)
4.GG or AA	1.00	1.00	1.00	
	(n = 134)	(n = 77)	(n = 49)	(n = 199)
GA	0.98 [0.64 <OR< 1.51]	1.10 [0.66 <OR< 1.82]	0.91 [0.48 <OR< 1.73]	
	(n = 53)	(n = 34)	(n = 18)	(n = 80)

a = no of genotype, b = OR, c = 95% CI

4.2 Cleft palate only (CPO)

As for the results in tables the association was observed in A allele of *MTHFD1* 1958G->A in patients and their mother's group. In patient group OR = **4.42 (1.05 <OR< 16.52)** were revealed in AA group of codominance mode, while mother's group observed the significance in dominance mode (**OR = 2.17 [1.02 <OR< 4.62]**) and heterozygous mode (**OR = 2.23 [1.04 <OR< 4.75]**). Additionally GA group in condominance mode of mother's group presented significant OR (**OR = 2.25 [1.05 <OR < 4.86]**).

Table 21 Genotypic distribution and odds ratio (OR) of *IRF6* 820G→A in family with CPO.

	Members of family with CPO			Controls
	Patients	Mothers	Fathers	
	(n = 43)	(n = 31)	(n = 19)	(n = 278)
1.GG	1.00	1.00	1.00	
	(n = 12)[a]	(n = 10)	(n = 4)	(n = 100)
GA	1.16[b] [0.51 <OR< 2.67][c]	1.09 [0.44 <OR< 2.75]	2.37 [0.69 <OR< 10.25]	
	(n = 19)	(n = 15)	(n = 13)	(n = 137)
AA	2.44 [0.93 <OR< 6.40]	1.46 [0.44 <OR< 4.78]	.22 [0.11 <OR< 8.88]	
	(n = 12)	(n = 6)	(n = 2)	(n = 41)
2.GG	1.00	1.00	1.00	
	(n = 12)	(n = 10)	(n = 4)	(n = 100)
AA or GA	1.45 [0.68 <OR< 3.14]	1.18 [0.50 <OR< 2.81]	2.11 [0.65 <OR< 8.94]	
	(n = 31)	(n = 21)	(n = 15)	(n = 178)
3.GA or GG	1.00	1.00	1.00	
	(n = 31)	(n = 25)	(n = 17)	(n = 237)
AA	2.24 [0.99 <OR< 4.98]	1.39 [0.48 <OR< 3.85]	0.68 [0.07 <OR< 3.04]	
	(n = 12)	(n = 6)	(n = 2)	(n = 41)
4.GG or AA	1.00	1.00	1.00	
	(n = 24)	(n = 16)	(n = 6)	(n = 141)
GA	0.81 [0.41 <OR< 1.63]	0.96 [0.43 <OR< 2.15]	2.23 [0.76 <OR<7.35]	
	(n = 19)	(n = 15)	(n = 13)	(n = 137)

a = no of genotype, b = OR, c = 95% CI

Table 22 Genotypic distribution and odds ratio (OR) of *SKI* 185C→G in family with CPO.

	Members of family with CPO			Controls
	Patients	Mothers	Fathers	
	(n = 43)	(n = 32)	(n = 18)	(n = 269)
1.CC	1.00[a]	1.00	1.00	
	(n = 39)	(n = 31)	(n = 17)	(n = 258)
CG	2.41[b] [0.61 <OR< 8.75)][c]	0.76 [0.02 <OR< 5.54]	1.38 [0.03 <OR< 10.58]	
	(n = 4)	(n = 1)	(n = 1)	(n = 11)
GG	ND	ND	ND	
	NO	NO	NO	NO
2.CC	1.00	1.00	1.00	
	(n = 39)	(n = 31)	(n = 17)	(n = 258)
GG or CG	2.41 [0.53 <OR< 8.62]	0.76 [0.02 <OR< 5.54]	1.38 [0.03 <OR< 10.58]	
	(n = 4)	(n = 1)	(n = 1)	(n = 11)
3.CG or CC	1.00	1.00	1.00	
	(n = 43)	(n = 32)	(n = 18)	(n = 269)
GG	ND	ND	ND	
	NO	NO	NO	NO
4.CC or GG	1.00	1.00	1.00	
	(n = 39)	(n = 31)	(n = 17)	(n = 258)
CG	2.41 [0.53 <OR< 8.62]	0.76 [0.02 <OR< 5.54]	1.38 [0.03 <OR< 10.58]	
	(n = 4)	(n = 1)	(n = 1)	(n = 11)

NO = not observed. ND = not determined, a = no of genotype, b = OR, c = 95% CI

Table 23 Genotypic distribution and odds ratio (OR) of *MTHFD1* 1958G→A in family with CPO.

	Members of family with CPO			Controls
	Patients	Mothers	Fathers	
	(n = 49)	(n = 36)	(n = 23)	(n = 279)
1.GG	1.00	1.00	1.00	
	(n = 27)[a]	(n = 18)	(n = 14)	(n = 191)
GA	1.50[b] [0.74 <OR< 3.05][c]	**2.25 [1.05 <OR< 4.86]**	1.36 [0.50 <OR< 3.64]	
	(n = 17)	(n = 17)	(n = 8)	(n = 80)
AA	**4.42 [1.05 <OR< 16.52]**	1.33 [0.03 <OR< 10.88]	1.71 [0.04 <OR< 14.34]	
	(n = 5)	(n = 1)	(n = 1)	(n = 8)
2.GG	1.00	1.00	1.00	
	(n = 27)	(n = 18)	(n = 14)	(n = 191)
AA or GA	1.77 [0.91 <OR< 3.42]	**2.17 [1.02 <OR< 4.62]**	1.40 [0.53 <OR< 3.60]	
	(n = 22)	(n = 18)	(n = 9)	(n = 88)
3.GA or GG	1.00	1.00	1.00	
	(n = 44)	(n = 35)	(n = 22)	(n = 271)
AA	3.85 [0.94 <OR< 13.98]	0.97 [0.02 <OR< 7.59]	1.54 [0.03 <OR< 12.39]	
	(n = 5)	(n = 1)	(n = 1)	(n = 8)
4.GG or AA	1.00	1.00	1.00	
	(n = 32)	(n = 19)	(n = 15)	(n = 199)
GA	1.32 [0.66 <OR< 2.63]	**2.23 [1.04 <OR< 4.75]**	1.33 [0.49 <OR< 3.49]	
	(n = 17)	(n = 17)	(n = 8)	(n = 80)

a = no of genotype, b = OR, c = 95% CI

4.3 Frontoethmoidal encephalomeningocele (FEEM)

According to the OR of all candidate SNPs, no significance was observed in all study groups. The results were show in tables 24 - 26.

Table 24 Genotypic distribution and odds ratio (OR) of *IRF6* 820G→A in family with FEEM.

	Members of family with FEEM			Controls
	Patients (n = 65)	Mothers (n = 37)	Fathers (n = 18)	(n = 278)
1.GG	1.00 (n = 22)[a]	1.00 (n = 14)	1.00 (n = 4)	(n = 100)
GA	1.23[b] [0.66 <OR< 2.30][c] (n = 37)	0.89 [0.39 <OR< 2.01] (n = 17)	2.37 [0.70 <OR< 10.25] (n = 13)	(n = 137)
AA	0.67 [0.21 <OR< 1.86] (n = 6)	1.05 [0.31 <OR< 3.15] (n = 6)	0.61 [0.01 <OR< 6.43] (n = 1)	(n = 41)
2.GG	1.00 (n = 22)	1.00 (n = 14)	1.00 (n = 4)	(n = 100)
AA or GA	1.10 [0.60 <OR< 2.02] (n = 43)	0.92 [0.43 <OR< 1.99] (n = 23)	1.97 [0.59 <OR< 8.41] (n = 14)	(n = 178)
3.GA or GG	1.00 (n = 59)	1.00 (n = 31)	1.00 (n = 17)	(n = 237)
AA	0.59 [0.20 <OR< 1.49] (n = 6)	1.12 [0.36 <OR< 2.96] (n = 6)	0.34 [0.01 <OR< 2.30] (n = 1)	(n = 41)
4.GG or AA	1.00 (n = 28)	1.00 (n = 20)	1.00 (n = 5)	(n = 141)
GA	1.36 [0.76 <OR< 2.43] (n = 37)	0.87 [0.42 <OR< 1.83] (n = 17)	2.68 [0.86 <OR< 9.82] (n = 13)	(n = 137)

a = no of genotype, b = OR, c = 95% CI

Table 25 Genotypic distribution and odds ratio (OR) of *SKI* 185C→G in family with FEEM.

	Members of family with FEEM			Controls
	Patients	Mothers	Fathers	
	(n = 64)	(n = 39)	(n = 17)	(n = 269)
1.CC	1.00	1.00	1.00	
	(n = 62)[a]	(n = 35)	(n = 16)	(n = 258)
CG	0.76[b] [0.08 <OR< 3.60][c]	2.68 [0.59 <OR< 9.65]	1.47 [0.03 <OR< 11.31]	
	(n = 2)	(n = 4)	(n = 1)	(n = 11)
GG	ND	ND	ND	
	NO	NO	NO	NO
2.CC	1.00	1.00	1.00	
	(n = 62)	(n = 35)	(n = 16)	(n = 258)
GG or CG	0.76 [0.08 <OR< 3.60]	2.68 [0.59 <OR< 9.65]	.47 [0.03 <OR< 11.31]	
	(n = 2)	(n = 4)	(n = 1)	(n = 11)
3.CG or CC	1.00	1.00	1.00	
	(n = 64)	(n = 39)	(n = 17)	(n = 269)
GG	ND	ND	ND	
	NO	NO	NO	NO
4.CC or GG	1.00	1.00	1.00	
	(n = 62)	(n = 35)	(n = 16)	(n = 258)
CG	0.76 [0.08 <OR< 3.60]	2.68 [0.59 <OR< 9.65]	1.47 [0.03 <OR< 11.31]	
	(n = 2)	(n = 4)	(n = 1)	(n = 11)

NO = not observed. ND = not determined, a = no of genotype, b = OR, c = 95% CI

Table 26 Genotypic distribution and odds ratio (OR) of *MTHFD1* 1958G→A in family with FEEM.

	Members of family with FEEM			Controls
	Patients	Mothers	Fathers	
	(n = 64)	(n = 40)	(n = 17)	(n = 279)
1.GG	1.00	1.00	1.00	
	(n = 49)[a]	(n = 27)	(n = 12)	(n = 191)
GA	0.63[b] [0.31 <OR< 1.28][c]	1.06 [0.48 <OR< 2.31]	0.99 [0.27 <OR< 3.16]	
	(n = 13)	(n = 12)	(n = 5)	(n = 80)
AA	0.97 [0.1 <OR< 5.10]	0.88 [0.02 <OR< 7.05]	ND	
	(n = 2)	(n = 1)	NO	(n = 8)
2.GG	1.00	1.00	1.00	
	(n = 49)	(n = 27)	(n = 12)	(n = 191)
AA or GA	0.66 [0.34 <OR< 1.30]	1.05 [0.48 <OR< 2.23]	0.90 [0.24 <OR< 2.86]	
	(n = 15)	(n = 13)	(n = 5)	(n = 88)
3.GA or GG	1.00	1.00	1.00	
	(n = 62)	(n = 39)	(n = 17)	(n = 271)
AA	1.09 [0.11 <OR< 5.66]	0.87 [0.02 <OR< 6.78]	ND	
	(n = 2)	(n = 1)	NO	(n = 8)
4.GG or AA	1.00	1.00	1.00	
	(n = 51)	(n = 28)	(n = 12)	(n = 199)
GA	0.63 [0.31 <OR< 1.28]	1.07 [0.48 <OR< 2.31]	1.04 [0.28 <OR< 3.29]	
	(n = 13)	(n = 12)	(n = 5)	(n = 80)

NO = not observed. ND = not determined, a = no of genotype, b = OR, c = 95% CI

4. Transmission disequilibrium test (TDT)

The Transmission Disequilibrium Test (TDT), family-base case-control study, considers only the mating in which at least one parent is heterozygous for the marker. Thus subjects were decreased, except CL/P group had enough number of subjects for performed TDT. Analysis of *IRF6* 820G→A and *MTHFD1* 1958G→A, no difference transmitted between two alleles was observed. The results were shown in tables 27 - 28.

Table 27 TDT analysis of *IRF6* 820G->A in CL/P patients

Genotype	Transmitted	Untransmitted	X^2	P-value, df = 1)
G	20	18		
A	18	20	0.10	0.75

Table 28 TDT analysis of *MTHFD1* 1958G->A in CL/P patients

Genotype	Transmitted	Untransmitted	X^2	P-value, df = 1)
G	14	18		
A	18	14	0.50	0.49