

# Assessment of BicC family RNA binding protein 1 and Ras protein specific guanine nucleotide releasing factor 1 as candidate genes for high myopia: A case–control study

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**Purpose:** The aim is to evaluate the association between high myopia and genetic variant in the BicC family RNA binding protein 1 (BICC1) as well as its association with Ras protein specific guanine nucleotide releasing factor 1 (RASGRF1) genes in a Chinese Han population with a case–control study. **Methods:** Five TagSNPs in BICC1 and RASGRF1 genes were selected and genotyped in 821 unrelated subjects, which composed of 419 controls (spherical equivalent within  $\pm 1.00$  D in both eyes and axial length  $\leq 24.0$  mm) and 402 cases (spherical equivalent  $\leq -6.0$  D in at least one eye and axial length  $\geq 26.0$  mm). Statistical analysis was performed with SNPstats. **Results:** After an analysis adjusted by age and sex, rs4245599 in BICC1 was found to be significantly associated with high myopia under the codominant, dominant, recessive and log-additive model ( $P = 0.001, 0.0015, 0.0045$  and  $2e-04$ , odds ratio [OR] = 2.15, 1.59, 1.73 and 1.46, respectively), and rs10763559 in BICC1 was associated with high myopia and under the dominant and log-additive model ( $P = 0.032$  and  $0.036$ , OR = 0.72 and 0.78, respectively). Rs4778879 in RASGRF1 was found to be significantly associated with high myopia under codominant, dominant, recessive, and log-additive model ( $P = 0.0088, 0.0065, 0.026$ , and  $0.0021$ , OR = 1.87, 1.48, 1.56, and 1.37, respectively). However, no significant association was found between rs745030 in RASGRF1 and high myopia, neither was there any association of rs745029 in RASGRF1. **Conclusion:** The present study indicated that genetic variants in BICC1 and RASGRF1 are closely associated with high myopia, which appears to be a potential candidate for high myopia in Chinese Han population. Considering the small sample size of this study, further work is needed to validate our results. The function of BICC1 and RASGRF1 in the process of developing high myopia needs to be explored in the future.

**Key words:** BicC family RNA binding protein 1, case–control study, high myopia, polymerase chain reaction restriction fragment length polymorphism, Ras protein specific guanine nucleotide releasing factor 1

Myopia, or nearsightedness, is the most common global health problem which causes visual impairment, especially when accompanied by structural changes in the eye, which increase the risk of pathological complications such as cataract, glaucoma, retinal detachment, and macular degeneration.<sup>[1,2]</sup> High myopia (defined as spherical equivalent  $\leq -6.0$  D in at least one eye and axial length  $\geq 26.0$  mm) often has a higher increased axial length than myopia, which potentially increases the risk of ocular complications and leads to blindness.<sup>[3]</sup> The etiology of high myopia is complicated since both environmental and genetic factors can contribute to it.<sup>[4]</sup> To date, several loci have been mapped and are thought to be associated with high myopia, including 18p11.31 (MYP2), 12q23.1-q24 (MYP3), 11p13 (MYP7), 3q26 (MYP8), and 4q12 (MYP9).<sup>[5-7]</sup> These loci contain most of the candidate genes which have been identified as associated with high myopia, whereas the candidate genes of other loci have not been explored. The etiology of high myopia remains unclear. In the past 50–60 years, there has been a substantial rise in the number of myopic patients in several

East Asian countries.<sup>[8,9]</sup> More than 80% of junior high school students were myopic in Taiwan in 2000, particularly in the group of 18-year-old, of whom 21% have high myopia, which has a higher risk of pathological complications.<sup>[10]</sup> Therefore, there is an urgent need for study of the etiology of high myopia.

Recently, two large-scale genome-wide association studies (GWASs) conducted by CREAM (Consortium for Refractive Error and Myopia) and Kiefer *et al.* revealed several loci which may be associated with refractive error. Among these loci, two showed significant associations with myopia, including BicC family RNA binding protein 1 (BICC1) and Ras protein specific guanine nucleotide releasing factor 1 (RASGRF1).<sup>[11,12]</sup> So, we are curious to find whether if these two loci may contribute to high myopia.

Here, we performed a case–control study to determine whether these two loci are associated with high myopia in the Chinese Han population.

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**Table 1: Characteristics of high myopia cases and controls**

Group	Total number	Average age (years)	Male (%)	Female (%)	OD/OS	Mean±SD		
						RE (D)	AL (mm)	ACD (mm)
Controls	419	32.8±6.31	211 (50.4)	208 (49.6)	OD	0.06±0.31	23.62±0.71	3.50±0.32
						OS	0.06±0.52	23.70±0.83
Cases	402	28.09±8.42	241 (60.0)	161 (40.0)	OD	9.52±4.86	27.34±1.92	3.66±0.43
						OS	9.66±4.32	27.15±1.85

RE: Refractive errors, SD: Standard deviation, OD: Right eye, OS: Left eye, D: Diopter, AL: Axial length, ACD: Anterior chamber depth

**Table 2: Polymerase chain reaction primers of all TagSNPs and polymerase chain reaction products length**

SNP	Primer sequence	Size (bp)
rs4245599	Left primer: 5'TCCTGGAGTTGGTAATTGCCT3'	197
rs10763559	Right primer: 5'AAAGGATGAAGCAGGCCAAC3'	173
	Left primer: 5'AGCTTGTGGTTGGAGATGGT3' Right primer: 5'ACTCCGCCACTTTATCCTACA3'	
rs745029	Left primer: 5'CTGGTTTCCTGAGCTGCAAG3'	237
	Right primer: 5'GGCCCTATGGATGGAAGGTT3'	
rs4778879	Left primer: 5'CTGGTTTCCTGAGCTGCAAG3'	350
rs745030	Right primer: 5'GGCCCTATGGATGGAAGGTT3'	237
	Left primer: 5' CTGGTTTCCTGAGCTGCAAG3' Right primer: 5' GGCCCTATGGATGGAAGGTT3'	

## Methods

### Subjects

All participants were randomly selected from the Chinese Han population. All processes used in the study accorded with the tenets of the Declaration of Helsinki, and written informed consent was obtained from all subjects.

Ophthalmologic examinations were performed on all subjects, including retinoscopy, examination of anterior chamber depth, fundus examination, and measurement of refractive error and axial length. Those who had ocular diseases, a history of ocular procedures or systemic diseases which might have altered the results were excluded from the study. In all, 821 unrelated individuals participated in this study, comprising of 402 cases (spherical equivalent  $\leq -6.0$ D in at least one eye and axial length  $\geq 26.0$  mm) and 419 controls (spherical equivalent within  $\pm 1.00$  D in both eyes and axial length  $\leq 24.0$  mm). Associated characteristics are presented in Table 1.

### SNP selection and genotyping

We selected five TagSNPs (rs4245599, rs10763559, rs745029, rs4778879, and rs745030) in genes BICC1 and RASGRF1, which two loci have been previously reported associated with myopia. Venous blood of each participant was drawn from the ulnar vein and collected in an EDTA tube and preserved at  $-20^{\circ}\text{C}$ . Genomic DNA was extracted from leukocytes by serial phenol/chloroform extraction and ethanol precipitation. The design of primers was conducted by online program Prime3 (<http://primer3.ut.ee/>) [Table 2]. SNP genotyping was performed using ABI Prism SNaPshot Multiplex Kit. Polymerase chain reaction product and genotype results were confirmed and validated by the direct sequencing method.

### Statistical analysis

Analysis of the SNPs was conducted using SNPStats to ascertain their association with high myopia. All analysis was adjusted for age and sex as covariates, and corresponding  $P$  values were given.  $P < 0.05$  was considered to be suggestive of association. The Hardy-Weinberg equilibrium (HWE) was calculated by Chi-square  $t$ -test. Genetic power was calculated using software Quanto (<http://hydra.usc.edu/gxe>).

## Results

In the study, a total of 821 unrelated Han Chinese participants were included, comprising of 419 controls and 402 high myopia cases [Table 1]. Genotypes within HWE in both high myopia cases and controls ( $P > 0.05$ ) and genetic power of rs4245599, rs10763559, rs745029, rs4778879, and rs745030 calculated using software Quanto equals to 89.71%, 86.35%, 87.54%, 88.79%, and 88.21%, respectively. There was no significant association between high myopia and rs745030 in RASGRF1 gene, or between rs745029 in RASGRF1 gene; however, rs4245599 and rs10763559 in BICC1 gene were found to be significantly associated with high myopia with  $P = 0.0003$  and  $0.0185$ , respectively, and rs4778879 in RASGRF1 gene were significantly associated with high myopia as well, with  $P = 0.0023$  [Table 3].

Furthermore, the genotype distribution was conducted under different genetic models using SNPstats, including codominant, dominant, recessive, overdominant, and log-additive. After being adjusted by sex and age, rs4245599 showed significant association under codominant, dominant, recessive and log-additive model, rs10763559 showed association under dominant and log-additive model, in addition, rs4778879 showed significance association under codominant, dominant, recessive and log-additive model, however rs745030 and rs745029 were not found to be the same [Table 4].

## Discussion

The present study showed a significant association between BICC1 gene and high myopia in the Chinese Han population, as well as an association with RASGRF1 gene. Three SNPs were found to be significant, the A allele and genotype AG, AG-AA, AA (under the model of a codominant, dominant, and recessive model, respectively) of rs4245599 suggests the strong risk of high myopia, which previously reported an association with myopia in Europeans.<sup>[11]</sup> Interestingly, we found two genetic variants in BICC1 and RASGRF1 gene, respectively, which associates with high myopia in Han Chinese population, the genotype TT (under the model of dominant) of rs10763559 suggests low risk of high myopia, and the genotype A/A, A/A,

**Table 3: Association between associated allele of SNPs and high myopia cases in Chinese Han population**

Gene	SNP	Chromosome	Minor allele	MAF (case/control)	P* (case/control)	P
BICC1	rs10763559	10	C	0.425/0.483	0.61/0.38	0.0185
	rs4245599	10	A	0.452/0.363	0.61/0.60	0.0003
RASGRF1	rs745030	15	T	0.447/0.441	0.55/0.62	0.8388
	rs4778879	15	G	0.425/0.351	0.68/0.83	0.0023
	rs745029	15	A	0.471/0.432	0.76/0.49	0.1090

\*Hardy-Weinberg equilibrium. MAF: Frequency of minor allele, RASGRF1: Ras protein specific guanine nucleotide releasing factor 1, BICC1: BicC family RNA binding protein 1

**Table 4: Association between SNPs in the BicC family RNA binding protein 1 and Ras protein specific guanine nucleotide releasing factor 1 genes and high myopia in Chinese Han population (adjusted by age and sex)**

SNP (gene)	Model	Genotype	Controls (%)	Cases (%)	OR (95% CI)	P	AIC
rs4245599 (BICC1)	Codominant	GG	167 (39.9)	118 (29.4)	Reference	0.001	1130
		AG	200 (47.7)	205 (51)	1.45 (1.07-1.97)		
		AA	52 (12.4)	79 (19.6)	2.15 (1.41-3.28)		
	Dominant	GG	167 (39.9)	118 (29.4)	Reference	0.0015	1131.8
		AG-AA	252 (60.1)	284 (70.7)	1.59 (1.19-2.13)		
	Recessive	GG-AG	367 (87.6)	323 (80.3)	Reference	0.0045	1133.7
		AA	52 (12.4)	79 (19.6)	1.73 (1.18-2.53)		
	Overdominant	GG-AA	219 (52.3)	197 (49)	Reference	0.35	1140.9
		AG	200 (47.7)	205 (51)	NS <sup>s</sup>		
	Log-additive	-	-	-	-	1.46 (1.19-1.79)	2e-04
-		-	-	-	-	-	-
rs10763559 (BICC1)	Codominant	TT	107 (25.5)	130 (32.3)	Reference	0.054	1138
		CT	219 (52.3)	202 (50.2)	NS		
		CC	93 (22.2)	70 (17.4)	NS		
	Dominant	TT	107 (25.5)	130 (32.3)	Reference	0.032	1137.2
		CT-CC	312 (74.5)	272 (67.7)	0.72 (0.53-0.97)		
	Recessive	TT-CT	326 (77.8)	332 (82.6)	Reference	0.085	1138.8
		CC	93 (22.2)	70 (17.4)	NS		
	Overdominant	TT-CC	200 (47.7)	200 (49.8)	Reference	0.56	1141.5
		CT	219 (52.3)	202 (50.2)	NS		
	Log-additive	-	-	-	-	0.78 (0.64-0.96)	0.016
-		-	-	-	-	-	-
rs745030 (RASGRF1)	Codominant	CC	128 (30.6)	120 (29.9)	Reference	0.98	1143.7
		CT	212 (50.6)	205 (51)	NS		
		TT	79 (18.9)	77 (19.1)	NS		
	Dominant	CC	128 (30.6)	120 (29.9)	Reference	0.83	1141.7
		CT-TT	291 (69.5)	282 (70.2)	NS		
	Recessive	CC-CT	340 (81.2)	325 (80.8)	Reference	0.91	1141.8
		TT	79 (18.9)	77 (19.1)	NS		
	Overdominant	CC-TT	207 (49.4)	197 (49)	Reference	0.91	1141.8
		CT	212 (50.6)	205 (51)	NS		
	Log-additive	-	-	-	-	NS	0.84
-		-	-	-	-	-	-
rs4778879 (RASGRF1)	Codominant	A/A	175 (41.8)	131 (32.6)	Reference	0.0088	1134.3
		A/G	194 (46.3)	201 (50)	1.38 (1.03-1.87)		
		G/G	50 (11.9)	70 (17.4)	1.87 (1.22-2.87)		
	Dominant	A/A	175 (41.8)	131 (32.6)	Reference	0.0065	1134.4
		A/G-G/G	244 (58.2)	271 (67.4)	1.48 (1.12-1.97)		
	Recessive	A/A-A/G	369 (88.1)	332 (82.6)	Reference	0.026	1134.8
		G/G	50 (11.9)	70 (17.4)	1.56 (1.05-2.30)		
	Overdominant	A/A-G/G	225 (53.7)	201 (50)	Reference	0.29	1140.7
		A/G	194 (46.3)	201 (50)	NS		
	Log-additive	-	-	-	-	1.37 (1.12-1.68)	0.0021
-		-	-	-	-	-	-

Contd...

**Table 4: Contd...**

SNP (gene)	Model	Genotype	Controls (%)	Cases (%)	OR (95% CI)	P	AIC
rs745029 (RASGRF1)	Codominant	G/G	132 (31.5)	111 (27.6)	Reference	0.26	1141.1
		A/G	213 (50.8)	204 (50.8)			
		A/A	74 (17.7)	87 (21.6)			
	Dominant	G/G	132 (31.5)	111 (27.6)	Reference	0.22	1140.3
		A/G-A/A	287 (68.5)	291 (72.4)	NS		
	Recessive	G/G-A/G	345 (82.3)	315 (78.4)	Reference	0.15	1139.7
		A/A	74 (17.7)	87 (21.6)	NS		
	Overdominant	G/G-A/A	206 (49.2)	198 (49.2)	Reference	0.98	1141.8
		A/G	213 (50.8)	204 (50.8)	NS		
	Log-additive	-	-	-	NS	0.1	1139.2

OR: Odds ratio, CI: Confidence interval, AIC: Akaike information criterion, NS: No significance, RASGRF1: Ras protein specific guanine nucleotide releasing factor 1, BICC1: BicC family RNA binding protein 1

and A/A-A/G (under the model of codominant, dominant and recessive model, respectively) of rs4778879 suggests low risk of high myopia. Nevertheless, rs745030 and rs745029 in RASGRF1 showed no significant association with high myopia.

The BICC1 gene, i.e., the BicC family RNA binding protein 1 gene, is located on chromosome 10q21.1 (MYP15), which encodes a protein that can regulate gene expression by binding RNA. Nallasamy *et al.* showed 10q21.1 to be associated with high myopia in a Hutterite population.<sup>[13]</sup> Then, Kiefer *et al.* indicated that rs4245599 in BICC1 gene play an important role in the development of early onset myopia in Europeans,<sup>[11]</sup> and Yoshikawa *et al.* replicated the results of Kiefer *et al.* in the Japanese cohort.<sup>[14]</sup> In the present study, we achieved additional insight into its association with high myopia in the Chinese Han Population using a case-control study, where it was found that gene BICC1 could be considered a candidate gene contributing to high myopia. Furthermore, we found a novel genetic variant (rs10763559) in BICC1 to be significantly associated with high myopia, which has not been reported in previous studies. Opposite results of the association between high myopia and genotype distribution of two SNPs in BICC1 under different genetic models, which may indicate complex correlation with BICC1 gene and high myopia.

RASGRF1 gene, located on 15q24.2, encoding RASGRF1. RAS pathways linked to the pathological changes of the retina in diabetes.<sup>[15]</sup> Fernández-Medarde *et al.* found the expression of RASGRF1 in the retina of the mouse, and severe impairments of retinal photoreception in the RASGRF1 knock out mice.<sup>[16]</sup> CREAM found that rs4778879 near the locus was associated with refractive error and myopia in both European and Asian population,<sup>[12]</sup> Hysi *et al.* identified several genetic variants near RASGRF1 strongly associated with myopia in Caucasians.<sup>[17]</sup> Qiang *et al.* showed an association between high myopia and rs8027411 in the 5' untranslated region of RASGRF1.<sup>[18]</sup> Our study found no significant association between high myopia and rs745030, which is the intron variant of RASGRF1.

Given the large sample size of GWAS and case-control study recently, the limitation of our study contains a small number of subjects. This was true, despite the subjects being carefully selected and clinical information being collected to minimize the influence of other factors on the results. However, with multifactorial diseases such as high myopia,

the etiology is complicated and involves a range of genetic and environmental factors. One of the best roads for further research would be to enlarge the scale of the sample size and combine other means to conduct a large scale meta-analysis, thus hopefully achieving better insight into the association between BICC1 gene and high myopia, as well as its association with the RASGRF1 gene. Nevertheless, our results do indicate that BICC1 and RASGRF1 gene are significantly associated with high myopia. Surprisingly, we found a novel genetic variant (rs10763559) in the BICC1 gene, which is significantly associated with high myopia and has a protective genotype C/T-C/C under dominant model, and a novel genetic variant (rs4778879) in the RASGRF1 gene, which is significantly associated with high myopia and has protective genotype A/A, A/A and A/A-A/G under the model of codominant, dominant, and recessive model, respectively.

## Conclusion

In summary, BICC1 and RASGRF1 gene could be considered candidate genes for high myopia in the Chinese Han population, and more research is needed in the future.

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## Conflicts of interest

There are no conflicts of interest.

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