RESEARCH ARTICLE

TP53 Codon 72 Polymorphisms and Lung Cancer Risk in the Bangladeshi Population

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Abstract

Objective: To assess associations between codon 72 polymorphisms (Pro or B and Arg or b alleles) of the *TP53* gene and lung cancer risk among Bangladeshis. Materials and Methods: The distribution of the BB, Bb, and bb genotypes and the frequencies of the B and b alleles were determined by PCR-RFLP method using DNA extracted from leucocytes of 50 confirmed lung cancer patients and 50 age-matched controls and the data were analysed. Results: The ratio of BB, Bb, and bb genotypes were in Hardy-Weinberg equilibrium except for the male patients ($\chi 2=4.6$). The B allele is overrepresented among all patients (OR=2.0, p=0.02) and the female patients (OR=4.1, p≤0.01) compared to the controls. The BB/bb ratio was also higher among the patients (OR=3.0, p=0.03). The relative risk of cancer for having BB over bb genotype was 1.8 (p=0.04) but no effect was observed for the Bb genotype. The B allele was overrepresented among patients with adenocarcinomas (OR=2.4, p≤0.01) and squamous cell carcinomas (OR=2.7, p≤0.01) over the controls but the difference was not significant for those with small cell lung carcinomas (OR=1.1, p=0.66). The B allele was overrepresented among patients age 50 or younger (OR=2.7, p≤0.01), but not for older patients (OR=1.7, p=0.07), and among smokers compared to the controls (OR=1.8-10.0, p≤0.01-0.03). However, no correlation between increasing pack-years and lung cancer was observed. Conclusions: The Pro/Pro (BB) genotype and the B allele are risk factors for lung cancer among Bangladeshis, particularly for people under age 50, women and smokers.

Keywords: Lung cancer - *TP53* gene - codon 72 polymorphism - PCR-RFLP analysis - Bangladeshis

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Introduction

Lung cancer is an uncontrolled growth of cells in the tissues of the lung, usually the epithelial lining of the lower respiratory tract. In 2012, lung cancer was the most prevalent type of all cancers, accounting for 13% of the annual global cancer incidence of 14.1 million (Cancer Research UK, 2014). About 55% of lung cancer cases occur in the developing countries and males outnumber females in lung cancer by a ratio of 2.5:1 (Farlay et al., 2010). Bangladesh is a densely populated developing nation with an annual lung cancer incidence of about 0.2 million (WHO, 2004). Lung cancer is the cause of highest cancer mortality in Bangladesh and it is the 9th most prevalent cause of all deaths in the country (WordHealthRanking, 2014). The majority of cancer patients admitted at the National Institute of Cancer Research and Hospital (NICRH), Dhaka, Bangladesh in 2005-2007 had lung cancer. During that period, a total of 3,209 lung cancer patients were admitted at NICRH, of them about 86% were males (Cancer Registry Report, 2009). Reported lung cancer incidence in Dhaka, the capital of the country, had increased from 902 in 2005 to 1,231 in 2007, which is a 36% increase (Cancer Registry Report, 2009). A significant fraction of lung cancer incidences in Bangladesh is never diagnosed or reported but the male female ratio of the reported cases is 6.53:1 (Akhtar et al., 2011).

The *TP53* gene is a major tumor suppressor gene located in the human chromosome 17 region 17p13. The gene codes for a transcription factor known as p53 protein involved in diverse cellular functions including cell cycling, DNA repair, genome stability, and apoptosis (reviewed in Hoe et al., 2014). The *TP53* gene has about 200 single nucleotide polymorphisms (IARC, 2014) but the majority of the single nucleotide polymorphisms (SNPs) are in the introns. Of the 19 exonic SNPs, eight are synonymous and 11 are non-synonymous and four of the non-synonymous SNPs have been validated (Naccarati et al., 2011). The SNP at codon 72 in exon 4 of the *TP53* gene has been of particular interest because this SNP has been reported to be associated with various types of cancers

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(reviewed in Bellini et al., 2012; Qiao and Hu, 2013). The p53 codon 72 polymorphism (c.215C/G) creates two morphs, TP53-Pro (TP53-P72) or TP53-Arg (TP53-R72) in the general population (Matlashewski et al., 1987). These genetic variations are considered polymorphisms and not mutations because the variations are present in ≥1% of the general population.

In vitro studies indicated that p53 Arg/Arg and p53 Pro/Pro variants differ in their abilities in binding various components of the transcriptional machinery, activating transcription, inducing apoptosis, and suppressing the transformation of primary cells (Thomas et al., 1999). Thus one of the morphs of p53 can be associated with increased risk of cancers but epidemiologic studies have failed to conclusively establish the point. For example, some studies have reported higher frequency of Pro/Pro genotype among lung cancer patients (Fan et al., 2000; Wang et al., 1999; Kawajiri et al., 1993) but other studies found increased frequency of Arg/Arg genotype among lung cancer patients (Jain et al., 2005; Devi et al., 2010) and yet other studies observed no significant difference in the frequency of the two genotypes among cancer patients and the control populations (Birgander et al., 1995; Pierce et al., 2000). This may be due to racial and ethnic differences (Dianat et al., 2009) and the exposure of different populations to different risk factors.

In the present study we investigated the association of p53 codon 72 polymorphisms and lung cancer risk among Bangladeshis. To our knowledge, this is among the few initial genetic association studies ever conducted on Bangladeshi/Bengalese patients with confirmed lung cancers of different histological types. We also investigated the association of codon 72 polymorphisms and lung cancer risks of smokers and people of different age groups.

Materials and Methods

Patients and controls

The study consisted of 50 clinically confirmed lung cancer patients (mean age 55±10 years) and 50 closely age-matched controls (mean age 46±11 years). The patients were admitted at the National Institute of Cancer Research and Hospital (NICRH), Dhaka and the controls were from the general population and of various professions. A clinician diagnosed the type of lung cancer and graded the severity of the disease. Informed written consent to participate in the study, donation of blood for DNA extraction, storage, and using the DNA samples for molecular research was obtained from each of the subjects in accordance with the Declaration of Helsinki and following the guidelines of the Institutional Review Board of the University of Dhaka.

All participants also completed a questionnaire and participated in an interview that obtained detailed information of the subjects' cigarette smoking or other tobacco usage, alcohol consumption, location of residence, occupation, religion, and history of cancers and other diseases among first-degree family members. Non-smokers were defined as subjects who had smoked fewer than 100 cigarettes in their lifetime.

Blood sampling and DNA extraction

Blood was drawn in EDTA vacutainers (BD, Oxford, UK). Erythrocytes of the samples were lysed by osmotic shock using 20 mM Tris-HCl (pH 8.0). Leucocytes were separated by centrifugation and the cell pellet was washed with phosphate buffered saline. The washed leucocyte pellet was suspended in a lysis buffer (10 mM Tris-HCl pH 8.1, 1 mM EDTA, 0.1% weight percentage of sodium dodecyl sulphate) containing 1 mg/ml of proteinase K (Sigma-Aldrich, St. Louis, MO). The tube was incubated at 55°C for 2-4 hours. The tube was then cooled in ice and then the content was extracted with equal volume of phenol-chloroform-isoamyl alcohol (25:24:1). The aqueous phase was separated and extracted again with an equal volume of chloroform-isoamyl alcohol (24:1). The aqueous phase was separated again and mixed with an equal volume of ice-cold isopropanol. DNA was precipitated by centrifugation and washed twice with 70% ethanol. The pellet was air-dried and then suspended in (DNA, RNA and nuclease-free) 10 mM Tris-HCl pH 7.5. The DNA concentration was determined using a Nanodrop spectrophotometer (Thermo Scientific, Wilmington, DE). Acceptable DNA samples (A260/A280 of 1.8-2.0) were diluted to 20 ng/μl and stored at -40°C until used.

PCR amplification and RFLP analysis

A fragment of DNA around exon 4 of the *TP53* gene (the location of the codon 72) was PCR-amplified using the following primer pair: 5´-TTGCCGTCCCAAGCAATGGATGA-3´, and 5´-TCTGGGAAGGGACAGAAGATGAC-3´, as described previously (Ara et al., 1990) with slight modifications. Briefly, the PCR mixture contained 1x reaction buffer (Life Technologies, Grand Island, NY), 1.75 mM $MgCl_2$, 0.2 mM dNTP, 20 picomoles of the two primers, 20-40 ng of genomic DNA and 1.0 unit of *Taq* DNA polymerase (Life Technologies) in a total volume of 25 μl. The PCR cycling was as follows: 94°C for 5 minutes (one cycle), 94°C for 30 sec, 60°C for 30 sec and 72°C for 30 sec (40 cycles); 72°C for 7 minutes (one cycle), and soak at 4°C. The amplification product (199 bp) was visualized using a UV-transilluminator and documented using a digital camera after resolving the product along with DNA size markers (100 bp DNA ladder, Fermentas/Thermo, Wilmington, DE) in 2% agarose gel and staining the gel with ethidium bromide.

The genotype of each individual was determined by restriction fragment-length polymorphism (RFLP) analysis. Briefly, the amplification product was digested with 1.0 unit of BstUI (Fermentas/Thermo Scientific) in appropriate digestion buffer for four hours and then resolved in 8% polyacrylamide gel. The gel was stained with ethidium bromide and documented using a digital camera. The BstUI (also AccII or FnuDII) restriction site (5'CG/CG3') is located in codon 72 and the cutting would generate two DNA fragments of sizes 113 bp and 87 bp. By convention, having the restriction endonuclease site is considered the recessive genotype. Thus the RFLP pattern was grouped as follows: BB (i.e. Pro/Pro)-199 bp, Bb (i.e. Pro/Arg)- 199 bp, 113 bp and 87 bp, and bb (i.e. Arg/Arg)-113 bp and 87 bp.

Statistical analysis

Whether the frequencies of the codon 72 genotypes in lung cancer patients and the control subjects are in agreement with the Hardy-Weinberg equilibrium was tested by χ^2 statistics at 0.05 % level of significance. The hypothesis that the population is in Hardy-Weinberg equilibrium was rejected if the χ^2 value was >3.84. Association between lung cancer and the genotypes was estimated by calculating the odds ratio (OR) and the 95% confidence interval (CI) as previously described (Bland and Altman, 2000). In addition, relative risk (RR) of lung cancer for having certain genotypes was calculated following a method described by Sheshkin (2004). MedCalc software (MedCalc, Mariakerke, Belgium) was used in calculating OR and RR as well as 95% CI and the p values. A p value of ≤0.05 was considered significant.

Results

DNA extracted from peripheral blood of 50 clinically confirmed lung cancer patients including 40 male and 10 female patients and 50 closely age-matched healthy control subjects including 39 males and 11 females was analysed in this study. The genotype distribution of the codon 72 locus was in agreement with Hardy-Weinberg equilibrium except in the male lung cancer patients (Table 1). Among the male lung cancer patients, the ratio of BB, Bb and bb was significantly different from the expected ratio (χ^2=4.6).

Table 1. Allele Distribution of Codon 72 Genotypes of the Controls and the Subjects with Lung Cancer

Group	BB(%)	Bb(%)	bb(%)	χ2	RorNR*
Patients (50)	19 (38)	19 (38)	12 (24)	2.5	NR
Males (40)	16 (40)	13 (33)	11 (28)	4.6	R
Females (10)	3 (30)	6 (60)	1 (10)	0.6	NR
Controls (50)	11 (22)	18 (36)	21 (42)	3.1	NR
Males (39)	9 (23)	16 (41)	14 (36)	1.1	NR
Females (11)	2 (18)	2 (18)	7 (64)	3.2	NR

*-R: hypothesis rejected, NR: hypothesis not rejected

Table 2. Allelic Frequencies of Codon 72 Genotypes of the Controls and the Subjects with Lung Cancer

Categories		Control	Patients	OR	95% CI	p
All subjects	B	40	57	1	Ref	
	b	60	43	2	1.1-3.5	0.02*
Males	B	44	56	1	Ref	
	b	57	44	1.7	0.9-2.1	0.08
Females	B	27	60	1	Ref	
	b	73	40	4.1	2.2-7.4	<0.01*

*-significant at the 0.05% level of significance

The frequency of B and b alleles among the male and female lung cancer patients and the control subjects are shown in Table 2. The B allele is significantly more prevalent among all the patients (OR=1.9, 95% CI: 1.1.3-3.5, p=0.02) as well as the female patients (OR=4.1, 95% CI: 2.2-7.4, p≤0.01) compared to the corresponding controls. The B allele is also more prevalent among the male lung cancer patients compared to the controls although the difference is not statistically significant (OR=1.7, 95% CI: 0.9-2.1, p=0.08).

The ratio of BB, Bb, and bb genotypes among the lung cancer patients and the control subjects is shown in Table 3. There is no significant difference in the ratio of BB/Bb and Bb/bb among the lung cancer patients and the controls. However, the ratio of BB/bb is significantly higher among cancer patients compared to the controls (OR=3.0, 95% CI=1.1-8.4, p=0.03 and the corresponding RR=1.8, 95% CI: 1.0-3.1, p=0.04). The ratio of (BB+Bb)/bb and BB/(Bb+bb) is substantially different for the lung cancer patients compared to the controls, although the differences are not quite statistically significant (for (BB+Bb)/bb, OR=2.3, 95% CI: 1.0-5.4, p=0.06, RR=1.3, 95% CI: 1.0-1.7, p=0.06; for BB/(Bb+bb), OR=3.3, 95% CI: 1.4-7.7, p=0.01, RR=1.7, 95% CI: 0.9-3.2, p=0.09).

Histological analysis of the lung tissues biopsied from the patients revealed that the patient cohort had 14 (or 28%) patients with small cell lung carcinoma (SCLC) and 36 (or 72%) patients with non-small cell lung carcinoma (NSCLC). Of the patients with NSCLC, 14 (or 38.9%) patients had adenocarcinoma (AC) and 22 (or 61.1%) patients had squamous cell carcinoma (SCC). The distribution of B and b alleles among the patients is shown in Table 4. There is no statistically significant difference in the ratio of B/b in the patients with SCLC and the controls (OR=1.1, 95% CI: 0.6-2.0, p=0.66). However, the B/b ratio was significantly higher for the patients with AC (OR=2.4, 95% CI: 1.3-4.1, p≤0.01) and SCC (OR=2.7, 95% CI: 1.5-4.7, p≤0.01) compared to the controls.

Patients sorted by age indicated that 18 (or 36%) of the patients were 50 years of age or younger and 32 (or 64%) of the patients were older than 50 years (Table 5). The distribution of BB, Bb, and bb genotypes of the younger and older groups was in Hardy-Weinberg equilibrium (χ^2=0.4 and 1.9, respectively). However, as shown in Table

Table 3. Genotypic Frequencies of Codon 72 among Lung Cancer Patients (Pat.) and the Control (cont.) Subjects

Genotype	Patients	Controls	OR	95% CI	p	RR	95%CI	p
BB/Bb	(19/19)	(11/18)	1	Ref	-	1	Ref	-
			1.6	0.6-4.4	0.3	1.3	0.8-2.3	0.3
BB/bb	(19/12)	(11/21)	1	Ref	-	1	Ref	-
			3	1.1-8.4	0.03*	1.8	1.0-3.1	0.04
Bb/bb	(19/12)	(18/21)	1	Ref	-	1	Ref	-
			1.8	0.7-4.8	0.21	1.3	0.9-2.1	0.2
(BB+Bb)/bb	(38/12)	29/21	1	Ref	-	1	Ref	-
			2.3	0.7-4.8	0.06	1.3	1.0-1.7	0.06
BB/(Bb+bb)	(19/31)	(11/39)	1	Ref	-	1	Ref	-
			3.3	1.4-7.7	0.01*	1.7	0.9-3.2	0.09

*-significant at the 0.05% level of significance

Table 4. Frequency of TP53 Codon 72 Genotypes among the Patients with Different Histological Lung Cancers and the Control Subjects

Cancer types	BB	Bb	bb	B/b	Control B/b	OR	95% CI	p
AC	5	7	2	61/39	40/60	1	Ref	-
						2.4	1.3-4.1	<0.01*
SCC	10	8	4	64/36	40/60	1	Ref	-
						2.7	1.5-4.7	<0.01*
SCLC	4	4	6	43/57	40/60	1	Ref	-
						1.1	0.6-2.0	0.66

*AC- adenocarcinoma, SCC- squamous cell carcinoma, SCLC- small cell lung carcinom, *-significant at the 0.05% level of significance

Table 5. Distribution of TP53 Codon72 Genotypes By Age, Smoking Habit and Smoking Pack Years (PY) among Lung Cancer Patients and the Control Subjects

	BB	Bb	bb	B/b	Control B/b	OR	95% CI	p
Age ≤50 (18)	8	7	3	64/36	40/60	1	Ref	-
						2.7	1.5-4.7	<0.01*
Age >50 (32)	11	12	9	53/47	40/60	1	Ref	-
						1.7	1.0-2.9	0.07
Pack years ≤10 (4)	3	1	0	87/13	40/60	1	Ref	-
						10	4.9-20.4	<0.01*
Pack years 10-30 (19)	7	7	5	55/45	40/60	1	Ref	-
						1.8	1.0-3.2	0.03*
Pack years ≥31 (21)	9	6	6	57/43	40/60	1	Ref	-
						1.9	1.0-3.5	0.02*

*-significant at the 0.05% level of significance

5, the ratio of B and b alleles of the younger patients was significantly different from the controls (OR=2.7, 95% CI: 1.5-4.7, p≤0.01). The ratio of B and b alleles of the older patients was substantially but not significantly different from the controls (OR=1.7, 95% CI: 1.0-2.9, p=0.07).

Patients grouped by smoking habit showed that 44 of the 50 patients (or 88%) were smokers and, as shown in Table 5, the heavy smokers (≥31 pack-years) outnumbered the moderate smokers (11-30 pack-years) and occasional smokers (≤10 pack-years). The ratio of the B and b alleles for all the groups (Table 5) was significantly higher than the control group (OR: 1.8-10.04, 95% CI: 1.0-20.4 and p<0.01-0.03). The crude OR for cancer development among smokers having BB genotype compared to the other genotypes was 2.7 (95% CI: 1.1-6.6; p=0.02). No significant correlation was observed between increasing pack-years and development of cancer.

Discussion

Lung cancers, particularly SCC and AC are significant public health concerns in Bangladesh (Akhtar et al., 2011). Several factors, such as cigarette and cigar (locally known as bidi) smoking, second hand smoking, fossil fuel exhausts, particulate pollutants, use of talcum powder, arsenic in drinking water, and tuberculosis, that increase the risk of lung cancer (American Cancer Society, 2014; Cancerquest 2014), are common places in Bangladesh. Low literacy rate, inadequate enforcement of the tobacco use laws and fossil fuel emission control laws, shortage of safe drinking water, and inadequate public health infrastructures in the country further exacerbate the conditions. Early detection of lung cancer is an important public health goal in Bangladesh. For all practical purposes, lung cancer research in Bangladesh is in its infancy. In this report, we analysed DNA extracted from peripheral blood leucocytes of 50 confirmed lung cancer patients and 50 age-matched controls in an attempt to identify biomarkers of diagnostic and prognostic significances.

We analysed the distribution of the *TP53* codon 72 polymorphism genotypes Pro/Pro (B/B), Pro/Arg (B/b) and Arg/Arg (b/b) and the frequency of B and b alleles in these population samples. Our analysis indicates that the distribution of BB, Bb, and bb genotypes in these population samples is in agreement with the Hardy-Weinberg equilibrium except the male patient population (χ^2=4.6). The male patient group had an overrepresentation of the BB and bb genotypes. Further analyses of the distribution of B and b alleles indicated that the B allele is overrepresented in the overall patient group (p=0.02) and the female lung cancer patients (p<0.01) compared to the controls. The B allele is also substantially, but not significantly, more common among the male lung cancer patients compared to the controls (p=0.08). This result indicates that the B allele is a potential risk factor for lung cancer among younger subjects. A previous study (Wang et al., 1999) indicated that the B allele increases the risk for lung cancer among Taiwanese females. Our analysis indicated that the BB genotype increases the relative risk of developing lung cancer by 1.8 fold (p=0.04) over the bb genotype. Our study found no significant difference in relative risk of developing lung cancer for having Bb genotype over BB genotype (p=0.33) or bb genotype (p=0.20). However, Devi and colleagues (2010) observed the association of the Bb genotype with an increased risk of lung cancer among smokers. Overall, our analysis indicates that the B allele and BB genotype are risk factors for developing lung cancer among Bangladeshis. This result supports and further confirms results of many previous studies (Kawajiri et al., 1993; Wang et al., 1999; Fan et al., 2000; Wang et al., 2013; Zhao et al., 2013; Lu et al., 2014). Our results also indicate that although the bb genotype has protective significance, heterozygosis (i.e. having one b allele) may not have any protective effect.

The majority (i.e. 72%) of the patients in our study had NSCLC such as AC (28%) and SCC (44%) and the rest of the patients had SCLC (28%). A previous study indicated that NSCLC, particularly SCC and AC are the most common lung cancers in Bangladesh (Akhtar et al., 2011). Our results indicate that there was no association of codon 72 polymorphisms and the risk for SCLC (OR=1.1, 95% CI: 0.6-2.0, p=0.66). However, the results also indicate that the B allele is a significant risk factor for AC (OR=2.4,

95% CI: 1.3-4.1, p<0.01) and SCC (OR=2.7, 95% CI: 1.5-4.7, p<0.01). To the best our understanding, there is no previous report of such a stark difference in the association the codon 72 polymorphisms and the risk for different types of lung cancers. It is tempting to hypothesize that the development of SCLC is associated with other p53 mutations and other gene mutations. However, we have not completed the study of other mutations that may be present in the p53 genes and other genes of the patients. Our study also suffers from small sample size, particularly female patients, because in Bangladesh, lung cancer is predominantly a disease of the males.

Our study indicates that the B allele is a significant risk factor for subjects 50 years or younger (p<0.01) but less of a risk for subjects older than 50 (p=0.07). A previous study (Jin et al., 1995) observed that the BB genotype is associated with higher risk of lung cancers among younger African Americans. The majority (88%) of the lung cancer patients we examined were smokers and most of the subjects were moderate to heavy smokers. Our study indicated that the B allele is a significant risk factor for lung cancer for all three groups of smokers (p<0.03) but we found no relationship between increasing pack-year and lung cancer risk. A previous report (Fan et al., 2000) indicated that the prevalence of the BB homozygous genotype increased in frequency with increasing pack-years among lung cancer patients and the risk associated with smoking was higher for the population with the combined BB+Bb genotypes over the bb genotype.

The codon 72 polymorphism is located in the proline-rich domain of the p53 protein and the domain is associated with apoptotic activity of p53 protein (Venot et al., 1998). Since the p53 polymorphism alters the amino acid sequence of this important domain and perhaps the overall structure of the protein, it is expected that this particular polymorphism may be associated with cancer risk. Early studies by Thomas and colleagues (Thomas et al., 1999) showed that p53-Pro and p53-Arg forms are structurally similar and both are equally effective in binding DNA but the p53-Pro form is more effective in binding certain transcription factors and in overall transcriptional activation. However, the p53-Arg form was found to be more effective in suppressing cell transformation and inducing apoptosis (Thomas et al., 1999). A large number of studies, however, have failed to establish a clear-cut relationship of the codon 72 polymorphisms and cancer risk. Some investigators observed the B allele and the B B genotype associated with increased risk of cancers (Lu et al., 20013; Zhao et al., 2013; Kawajiri et al., 1993), some investigators observed the b allele or bb genotype associated with increased risk of cancers (Perez-Perez et al., 2005; Yamashita et al., 2014), some investigators observed that the Bb heterozygotes are more susceptible to lung cancer (Devi et al., 2010), and yet other investigators found no association between the codon 72 polymorphisms and cancer risk (Birgander et al., 1995; Pierce et al., 2000; Alqumber et al., 2014). Alqumber and colleagues (2014) attempted to reconcile such inconsistencies by invoking the effects of different genetic backgrounds for cases and controls, diverse genotype distribution of codon 72 Arg to Pro in different ethnic groups and uneven selection criteria for the cases and controls in different studies for different cancers. Three meta-analyses (Wang et al., 2013; Zhao et al., 2013; Lu et al., 2014) indicated that the majority of the reports showed an association of the BB genotype and the B allele as a risk factor for lung cancer.

Genetic polymorphisms are somewhat tolerated mutations and thus effects of individual SNPs are generally minor. Besides, each gene may contain multiple polymorphisms and some of the polymorphisms could have beneficial effects and some polymorphisms could have harmful effects (Latil et al., 2001). Furthermore, every protein works with a number of partners in almost any given pathway (von Mering et al., 2002) and the majority of the partners would be polymorphic given that single nucleotide polymorphisms (SNPs) are extremely common (Oros et al., 2013). Therefore, a system biology approach is likely to be more suitable to investigate the association of gene polymorphisms and diseases risks (Luo et al., 2001; Prakash et al., 2002). The approach is now known as the genome-wide association studies (GWAS) of SNP and disease risks (Patnala et al., 2013). Till the GWAS approach evolves as a routine high-resolution method, investigation of the association of certain SNPs and disease risks will remain an important tool to identify appropriate candidate genes and their polymorphisms in association with disease risks.

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