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Characterization of a new SNP c767A/T (Arg222Trp) in the candidate TSG *FUS2* on human chromosome 3p21.3: prevalence in Asian populations and analysis of association with nasopharyngeal cancer

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Abstract

The *FUS2* gene, encoding a novel cytoplasmic acetyltransferase, resides in the tumor suppressor gene region on human chromosome 3p21.3 and is considered a promising candidate tumor suppressor gene. We have identified a new single nucleotide polymorphism (SNP), c767A/T, in the coding region of the gene. The polymorphism leads to a non-conservative amino acid change (R222W) located between the acetyltransferase (GNAT) and the proline-rich domains of the protein. We have analyzed 254 subjects included in 14 sub-populations. The occurrence of the SNP varies with the ethnicity of the population, suggesting that this SNP could be a valuable biomarker for population genetics. It is most prevalent in various Asian populations (T allele frequency > 0.54), followed by the Canadian polar Inuit (T allele frequency $= 0.3$), African American (T allele frequency $= 0.17$), and Caucasian population (T allele frequency $= 0.1$). Since nasopharyngeal carcinoma (NPC) is frequent in Southern China, Taiwan, Borneo and polar Canada, we further tested for the possible association of the *FUS2* SNP with this form of endemic cancer. Our analysis, albeit limited, suggests no likely association between NPC and the *FUS2* gene polymorphism. Further large-scale case-control studies are necessary and warranted to prove the strength of this contention.

Keywords: Nasopharyngeal carcinoma; Single nucleotide polymorphism; FUS2; Genetic association

1. Introduction

The *FUS2* gene resides in the lung, breast, and head and neck tumor suppressor region on human chromosome 3p21.3, and is considered a promising candidate tumor suppressor gene [1–2]. *FUS2* is a single copy gene (accession # AF040705 and # AF040706) expressed as a ~1.9-kb mRNA in many normal human tissues [1]. The mRNA is well represented in EST databases of normal and tumor tissues [1]. Bioinformatics analyses [3] predict the *FUS2* protein to be a soluble nuclear protein with several interesting domains and motifs, namely an acetyltransferase (GNAT) domain (residues 66–189) and a proline-rich

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domain (residues 239–262), which overlaps with the Wilms' tumor protein signature (residues 234–249). The presence of these domains suggests the intriguing possibility that *FUS2* may be directly involved in nuclear activities. However, Zegerman et al. have recently demonstrated that the *FUS2* protein is a soluble cytoplasmic protein functioning as a *N*-acetyltransferase [4]. The occurrence of mutations in some lung cancers [1] and the demonstration of its biochemical activity make *FUS2* an attractive candidate TSG for several cancers that involve the 3p21.3 region.

Nasopharyngeal carcinomas (NPC) are rare among Caucasians in Western Europe and North America (<1 per 100,000 persons/year). However, they are endemic in Northern Africa and Southeast Asia including China, Taiwan, Hong Kong and Malaysia [5–6]. The disease also affects the Inuit populations of Canada and Greenland [5]. Familial forms of NPC have been reported in the Inuit [7–8] and among different Chinese [9]. Males in the high-risk regions of Southern China, Taiwan and Malaysia exhibit an incidence of over 24 per 100,000 persons/year. While in females, the incidence drops to 9 per 100,000 persons/year. Importantly, the incidence of NPC in these populations peaks at the relatively young age of 45 years [5–6].

Previous studies have demonstrated that the development of NPC may involve EBV latent infection [10] and multiple genetic changes including deletion of chromosome 3p [11]. The high frequency of 3p LOH suggested that the inactivation of tumor suppressor gene(s) in this chromosome might be an early event in the tumorigenesis of NPC. We have previously demonstrated, by mono-chromosome-mediated gene transfer into NPC cultured cells, the presence of growth-suppressive gene(s) located in the chromosome 3p region that overlaps with the lung cancer 3p21.3 deletion [1–2]. Therefore, the loss of 3p21.3 seems to play a crucial role in the development of this cancer. Indeed, in recent studies it was shown that the promoter of *RASSF1A*, a novel tumor suppressor gene isolated from the lung tumor suppressor locus at 3p21.3 [1], was highly methylated in primary NPCs (67–83%) [12]. In the present study, we tested for a possible association of NPC with the candidate TSG *FUS2,* also residing in 3p21.3 close to the *RASSF1* locus [1].

2. Materials and methods

2.1. Subjects and DNA Samples

Informed consent was obtained from each subject, and the study was performed with the approval of the ethics committees of each institution involved in this project. Blood samples and paraffin-embedded NPC tissue blocks were collected for isolation of genomic DNA. The 30 Caucasian subjects were unrelated individuals from the Utah subset of the CEPH [13]. Genomic DNAs were isolated from human peripheral blood, B cell lines or tumor tissues following standard procedures [1]. Information on all the analyzed sub-populations and their abbreviated designations is listed in Table 1.

Table 1
The 14 sub-populations and their basic statistics in the *FUS2*SNP c767 A/T study

Ethnicity (collection)	Designation	Status of Subjects	Source of Genomic DNA	Number of Subjects	T Allele Frequency	HWE Probability (p)	Heterozygosity
Japan	JPN	NI	PB	12	0.54	1	0.58
Taiwan/Chinese	TWN	NI	PB	29	0.59	1	0.48
Hong-Kong/Chinese	HKN	NI	PB	14	0.64	0.09	0.71
Caucasian/CEPH	CEPH	NI	BC	30	0.1	0.24	0.13
African American	AA	NI	PB	9	0.17	1	0.33
Canadian Inuit	INU	NI	PB	23	0.3	0.37	0.35
				117			
Chinese (C1)	CHID	NPCP	NPC tumor	9	0.83	1	0.33
Borneo/Malaysia (B1)	BRN1	NPCP	NPC tumor	5	1	Homozygous sample	
Borneo/Malaysia (B2)	BRN2	NPCP	NPC tumor	7	0.85	1	0.29
Chinese(C2)	CH2M	NPCP	PB	47	0.6	0.77	0.51
Chinese (C3)	CHC	NPCP	PB	8	0.81	1	0.38
Chinese (LF)	SCH*	NPCP	NPC tumor and PB	21	0.67	0.34	0.57
Hong-Kong/Chinese	HKD*	NPCP	NPC tumor and PB	17	0.66	0.26	0.29
				114			
Hong-Kong/Chinese	HKL*	LCP	LC tumor and PB	23	0.61	0.008	0.74
				23			

Abbreviations: NI, normal individuals; NPCP, nasopharyncheal carcinoma patients; LCP, lung cancer patients; PB, peripheral blood; BC, B-cell line; NPC, nasopharyncheal carcinoma. Numbers in bold are subtotal of normal individuals (117), NPC patients (114) and lung cancer patients (23). All sub-populations are in HWE ($p > 0.05$) except sub-population HKL ($p = 0.008$). There is no significant difference between tumor and peripheral blood in those sub-populations (marked with*) containing paired samples. The HWE probability and heterozygosity were estimated using the TFGPA software [14]. The Fisher's Exact Test was performed to compute p values.

2.2. Identification of the SNP

Overlapping primer sets covering the genome sequence of the *FUS2* locus were designed on the basis of size and overlap of PCR products. Genomic DNA was subjected to PCR amplification followed by Dye Terminator Cycle Sequencing (Applied Biosystems, Foster City, CA). Polymorphisms were first identified by the comparison of sequences from 24 chromosomes of Asian, Caucasian and African American subjects. They were then confirmed by re-amplification and re-sequencing. The remainder of the study subjects was sequenced only in the regions in where the SNP was identified.

2.3. SNP analysis

Genomic DNAs were PCR amplified with one of the following primer sets: 1F (5′ CGCCTGTCACGGGTGCTGAAC) and 1R (5′ GACTGTCGGGGCAGTCTATTG) or 2F (5′ TGACCAGGTGCACTTCTATAC) and 2R (5′ TGAGATGGTTCAGGCACTCAG). Primer set 1F and 1R produced a 543 bp amplicon; primer set 2F and 2R produced a 232 bp amplicon. All PCR were performed in a total volume of 50 μl with 50 ng of genomic DNA using a Gene Amp PCR System 9600 (PE Applied Biosystem, Foster City, CA) under the following conditions: 1 min at 94 °C; 30 s at 94 °C, 30 s at 56 °C, 1 min at 72 °C, 40 cycles; 10 min at 72 °C; hold at 4 °C.

Amplicons were sequenced on an ABI 373 stretch DNA sequencer (Applied Biosystems, Foster City, CA) with FUS2F504 (5′AGAGCCTCTTAGTGGAGACAG) or FUS2F651 (5′ATACCCACCTGGGCTACCAGC) primers. Sequence chromatograms were analyzed to determine genotypes at the position corresponding to codon # c767 (GenBank accession # AF040706).

2.4. Statistical methods

FUS2 cSNP Genotypes from 14 sub-populations (see Table 1) were collected for statistical analyses. In each sub-population, we examined the heterozygosity and Hardy–Weinberg Equilibrium (HWE); we reported simple heterozygosity as direct counts, showing the proportion of heterozygous genotypes using the TFPGA software [14]. HWE was estimated using pooled genotypes in a Fisher's Exact Test to account for the possibility of some genotypes failing to appear in this sampled data [15]; it was then verified using all genotypes in a Monte-Carlo Estimate. Both the Fisher's Exact Test and the Monte-Carlo Estimate were also performed using the TFPGA software [14].

We pooled the sub-populations that appeared identical in order to build a smaller number of pooled populations. We assumed that when the probability of groups being identical was greater than $p = 0.05$, the populations could be pooled. We completed binary comparisons for all sub-populations using the usual Contingency Table Analysis [16] and built pooled populations based on these results. All pooled populations were then subjected to a set of Contingency Table Analyses [16], ensuring that the pooled populations were, in fact, homogenous. These calculations were performed with Fisher's Exact Test using the SAS software [17].

Finally, we examined the allele frequencies and genotypes among the pooled populations using Contingency Table Analyses [16] to search for differences in the pooled populations. When the probability that two populations are the same is 0.01 or less, we define them as being significantly different. When the probability two populations are the same is 0.05 or between 0.05 and 0.01, we define them as being marginally (or possibly) different. These calculations were performed with Fisher's Exact Test using the S-Plus software [18].

3. Results

Three genotypes and corresponding sequences in the *FUS2* cSNP region are shown in Fig. 1. The 254 observations in 14 sub-populations used in the cSNP data analysis are summarized in Table 1. Genotype comparisons are valid when the population is in HWE at the locus used for analysis. Table 1 also shows the equilibrium status and heterozygosity of these sub-populations. All sub-populations are in HWE except the Hong Kong Lung Cancer patients. In the sub-populations (SCH, HKD, and HKL)

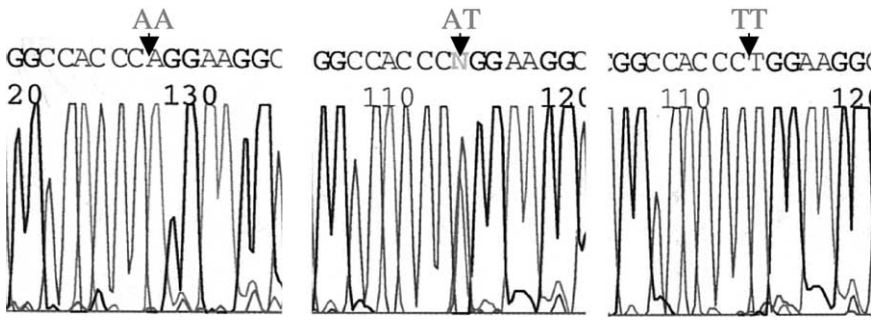

Fig. 1. Sequence chromatograms show c767A/T SNP in the *FUS2* gene. Genomic DNAs from three different Asian individuals were PCR amplified with primers 2F and 2R. Amplicons were sequenced on an ABI 373 stretch DNA sequencer with the primer FUS2F651.

Table 2
Binary comparisons of the *FUS2* SNP c767 A/T in 14 sub-populations

	JPN	TWN	HKN	CEPH	AA	INU	CH1D	BRN1	BRN2	CH2M	CHC	SCH	HKD	HKL
JPN	1	0.89	0.40	***0***	0.06	0.12	0.15	**0.03**	0.12	0.91	0.33	0.57	0.26	0.17
TWN		1	0.21	***0***	0.01	0.02	0.21	**0.03**	0.25	1	0.35	0.52	0.41	0.07
HKN			1	***0***	***0***	***0.002***	0.10	**0.01**	0.16	0.25	0.19	0.83	**0.04**	1
CEPH				1	0.48	0.05	***0***	***0***	***0***	***0***	***0***	***0***	***0***	***0***
AA					1	0.85	***0.003***	***0***	***0.003***	***0.004***	***0.004***	***0***	***0.008***	***0***
INU						1	***0.002***	***0.001***	***0.004***	***0.004***	***0.005***	***0.001***	0.02	***0***
CH1D							1	0.26	1	0.25	1	0.47	0.61	0.05
BRN1								1	0.47	0.02*	0.23	0.05*	0.25	***0.005***
BRN2									1	0.21	1	0.41	0.82	0.07
CH2M										1	0.31	0.61	0.28	0.08
CHC											1	0.57	0.69	0.09
SCH												1	0.21	0.42
HKD													1	***0.007***
HKL														1

Binary genotype comparisons between the sampled 14 sub-populations (see Table 1 for information and abbreviations). The white entries are those for normal individuals, light gray entries indicate NPC patients, and gray entries show lung tumor patients. The dark gray cells show small probability values ($p < 0.01$) suggesting that those populations differ. White cells with bold underlined numbers show possible differences ($p < 0.05$) between Asian NPC and normal populations. Light gray cells with marked * numbers show possible difference ($p \leq 0.05$) between Asian NPC sub-populations. Probabilities that are indicated as zero represent values too small to be measured reliably. Exact probabilities are reported here to avoid bias when the number of patients or individuals in a given group is small or zero. The comparisons were performed using Contingency Table Analysis [16]. Probability value (p) was computed with the Fisher's Exact Test.

containing pair samples, we found no significant difference between tumor and peripheral blood (data not shown).

The results of binary comparisons of these sampled populations are shown in Table 2. The white entries are normal individuals, light gray entries indicate NPC patients, and gray entries represent lung tumor patients. The dark gray cells contain small probability values ($p < 0.01$), suggesting that those populations differ. White cells with bold underlined numbers contain possible differences ($p < 0.05$) between the Asian NPC and Asian normal populations. Light gray cells with numbers marked with * contain possible differences ($p \leq 0.05$) between Asian NPC sub-populations. Probabilities indicated as zero represent values too small to be reliably measured. Exact probabilities are reported to avoid bias when the number of patients in a given group is small or zero. Comparisons between each of the Asian normal sub-populations (JPN, TWN, HKN), and between each of the NPC sub-populations (CH1D, BRN1, BRN2, CH2M, CHC, SCH, HKD) show no significant difference ($p > 0.01$).

As shown in Table 2, the results of binary comparisons indicate a possible difference between Asian normal sub-populations and some of the NPC sub-populations (HKN vs. HKD, JPN vs. BRN1, TWN vs. BRN1 and HKN vs. BRN1, $p < 0.05$.). These possible differences suggest that the NPC condition may be associated with a certain allele or genotype. Comparisons of allele frequencies between the pooled Asian normal (JPN, TWN, and HKN) and pooled NPC patients (CH1D, BRN1, BRN2, CH2M, CHC, SCH, and HKD) do not show any significant difference ($p = 0.1$, Table 3). Further comparisons of genotype distributions among pooled Asian normal and various combined NPC cases show no significant association overall, although marginal associations are observed between the 'TT' genotype and certain NPC cases. As shown in Table 4, genotype comparisons between the pooled Asian normal and the pooled NPC tumor cases show marginal differences ($p = 0.025$ when comparing all genotypes, and $p = 0.01$ when comparing pooled AA/AT with TT). Comparisons between the pooled Asian normal and three other pooled NPC cases show no significant difference ($p = 0.06$, $p = 0.07$, or $p = 0.2$ when comparing pooled AA/AT with TT).

It is clear from Table 2 that the African American (AA) and Caucasian (CEPH) populations are very different from the Asian populations, and the Inuit population seems to form a third group. As shown in Table 3, the 'T' allele is found much more prominently in pooled Asia than in AA or CEPH normal populations. Comparisons of the allele

Table 3
Comparisons of allele frequencies in the *FUS2* cSNP among 14 sub-populations

Sub-population(s)	A Allele frequency	T Allele frequency	vs. Asian normal (JPN + TWN + HKN)	vs. Inuit normal (INU)
CEPH normal	0.9	0.1	$p \sim 0$*	$p = 0.006$*
AA normal	0.83	0.17	$p = 0.002$*	$p = 0.24$
(CEPH + AA) normal	0.88	0.12	$p \sim 0$*	$p = 0.05$
Inuit normal	0.7	0.3	$p = 0.003$*	–
Asian normal (JPN + TWN + HKN)	0.41	0.59	–	–
Asian NPC (CHID + BRN1 + BRN2 + CH2M +CHC + SCH + HKD)	0.31	0.63	$p = 0.1$	$p \sim 0$*
Asian lung cancer (HKL)	0.37	0.63	$p = 0.7$	$p = 0.006$*

Comparisons of allele frequencies among normal populations, Asian NPC populations and Asian lung cancer population (see Table 1 for abbreviations). Those populations that significantly differ are indicated with * in p values. Probabilities (p values) were determined with the Fisher's Exact Test using the S-Plus software.

frequencies show a significant difference between the pooled CEPH/AA and the pooled Asian ($p \sim 0$), between the Inuit population and the CEPH population ($p = 0.006$), and between the Inuit population and the pooled Asian populations ($p = 0.003$).

4. Discussion

During mutation analysis of the candidate TSG *FUS2* that encodes a novel cytoplasmic acetyltransferase [1,4], we discovered a single nucleotide polymorphism c767A/T (R222W). This non-conservative change is located in a rather 'neutral' portion of the protein, between the acetyltransferase (GNAT) and proline-rich domains [1]. However, it is quite possible the SNP may affect either the protein function or RNA splicing. Exonic SNPs/mutations may be very prevalent, and cause disease by exclusion/inclusion of particular exons [19]. The SNP was characterized at the population level and shown to be in Hardy–Weinberg equilibrium in almost all the studied populations (Table 1). It is highly prevalent in Asian populations as compared to Caucasians and African Americans (Table 3). The fact that the occurrence of the SNP varies with the ethnicity of the population, suggests that this SNP could be a valuable biomarker for population genetics. Since NPC is prevalent in at risk Asian populations as compared to Caucasians in Europe and North America [5], we performed association analysis of the SNP and NPC. Population association between a particular locus (marked by a SNP) and disease may arise when the locus itself is causally related to the disease with different alleles carrying different risks, or is sufficiently close to a separate causal locus. Functional studies based on chromosome-mediated gene transfers have shown the presence of a NPC locus in 3p21.3 [2]. This locus

Table 4
The *FUS2* cSNP genotype comparisons between normal and NPC cases in the Asian populations

Comparisons	Normal vs. Total NPC cases	Normal vs. NPC cases (All tumors)	Normal vs. NPC cases (Tumor without Borneo cases)	Normal vs. Total NPC cases Without the Borneo cases
Sub-populations included	(JNP + TWN + HKN) vs. (CHID + BRN1 + BRN2 + CH2M + CHC + SCH + HKD)	(JNP + TWN + HKN) vs. (CHID + BRN1 + BRN2 + SCH + HKD)	(JNP + TWN + HKN) vs. (CHID + SCH + HKD)	(JNP + TWN + HKN) vs. (CHID + CH2M + CHC + SCH + HKD)
Subjects	55 vs. 114	55 vs. 59	55 vs. 47	55 vs.100
Genotypes				
AA	7 vs. 11	7 vs. 4	7 vs. 4	7 vs. 11
AT	31 vs. 49	31 vs. 22	31 vs. 20	31 vs. 46
TT	17 vs. 54	17 vs. 33	17 vs. 23	17 vs. 43
Comparisons				
AA vs. AT vs. TT	$p = 0.13$	$p = 0.025$**	$p = 0.19$	$p = 0.3$
AA vs. AT	$p = 0.8$	$p = 0.9$	$p = 0.9$	$p = 0.6$
(AA/AT) vs. TT	$p = 0.06$	$p = 0.01$**	$p = 0.07$	$p = 0.2$

Genotype comparisons between normal and NPC cases in Asian populations. Column two shows comparisons for the overall population including both blood and tumor samples. In column three, only NPC tumor tissue cases are included. In column four, Borneo cases are removed from the NPC tumor population. In column five, Borneo cases are removed from the total NPC population. There is no overall significant association between NPC and the genotypes ($p > 0.05$in columns 2, 4 and 5). Although, marginal associations (marked with **, $0.01 \leq p < 0.05$ in column 2) are observed between the NPC condition and the 'TT' genotype. The reported probability levels represent conservative estimates determined from both χ^2 and the Fisher's Exact Test results. (See Table 1 for abbreviations).

overlaps with the lung, and breast TSG region [1]. Indeed, controlled expression of *FUS2* transgenes in lung and NPC cells demonstrated suppression of growth in culture and tumor formation in mice (Stanbridge et al, unpublished observations). Methylation studies in NPC [12] have demonstrated the early inactivation of the TSG *RASSF1A* also residing in 3p21.3 region [1]. These findings further justify the association studies performed in this work, albeit in a limited number of cases.

To test for allelic association between *FUS2* and NPC, we genotyped the common *FUS2* polymorphism c767A/T (R222W) in the combined normal Asian population and in patients with NPC or lung cancer, and found no significant allele frequency differences. Genotype comparisons between pooled Asian normal and various pooled NPC cases also showed no overall significant association between genotypes and the NPC condition (column 2, 4 and 5, Table 4). Although, the 'TT' genotype is found to be marginally associated with the NPC condition especially when Borneo samples are included in the comparisons ($p = 0.025$ or 0.01, column 3, Table 4). The overall weak association may be simply due to the ethnicity of the Borneo tribes, since BRN1 is shown to be possibly different with CH2M ($p = 0.02$, Table 2) and SCH ($p = 0.05$, Table 2). The small subject number of Borneo sub-populations and the inability to obtain samples from Borneo normal individuals may also contribute biases to our study. 3p LOH affecting NPC and even histological normal-looking nasopharyngeal epithelia was well documented [6]. LOH in the gene locus showing preferential deletion of the 'A' allele would strengthen the contention that the 'T' allele could be a low penetrance allele predisposing to NPC. However, the sequencing data of the tumor samples were inconclusive to show clearly preferential loss of the 'A' allele. This is probably due to contamination with normal DNA originating from stoma and blood cells. Future analysis with quantitative real time PCR and micro-dissected samples would verify this explanation. It is well known that systemic errors have the potential to overwhelm (upset) weak disease associations [20]. Therefore, larger scale case-control studies in high risk Asian populations and carefully prepared samples to adjust for potential biases would be necessary to further explore the association between the *FUS2* locus and NPC.

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