A pilot study testing the genetic polymorphism of N-acetyltransferase 2 as a risk factor in lung cancer*

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NAT2 as phase II enzyme is involved in the detoxification/activation of various drugs, environmental substances and carcinogenic compounds. A genotyping approach has been used to investigate NAT2 genotype with putative relevance in lung cancer in population of 110 Slovak-Caucasians patients and 167 non-malignant individuals from the same region. Slow acetylation was not observed to be a significant risk factor of lung cancer development (OR=1.19; 95% CI: 0.71–1.99). However, one genotype responsible for slow acetylation (NAT2*5B/*6) was observed significantly more frequently in lung cancer patients with squamous cell carcinoma compared with control subjects (OR=2.24; 95% CI: 1.14–4.34). Stratified analysis showed an increasing impact of the specific allelic combination NAT2*5B/*6 in non-smokers (OR=6.5; 95% CI: 1.25–15.08). In the case of squamous lung carcinoma an analysis revealed a tendency to adversely affect cancer risk in the individuals with the mentioned genotype in younger than 60 years (OR=3.14; 95% CI: 0.98–9.72) non-smokers (OR=10.40; 95% CI: 1.35–118.89) and in females (OR=4.25; 1.08–16.25). Additional studies are needed to confirm the results we observed and to assess the impact of other effects (specific allelic combinations, sex differences and histological subtype of lung cancer) on NAT2 susceptibility in lung carcinogenesis.

Key words: NAT2, lung cancer, susceptibility, and genetic polymorphism

Globally, lung cancer is the most frequent cancer today. In Slovak republic, its incidence is 80/100,000 in male and 11/100, 000 in female. The etiology of bronchogenic carcinoma involves the interplay of multiple environmental and host factors, and the relative contribution of each of them is not completely clear. Enzymatic activation and detoxification is a major principle in chemical carcinogenesis. Biotransformation plays a crucial role in carcinogen activity and many genetic polymorphisms in xenobiotic metabolizing enzymes have been associated with an increased risk of cancer [9, 31]. Acetylation, phase II-pathway, is involved in the detoxification of a large number of arylamine and hydrazine drugs and chemical carcinogens. It also takes part in the metabolic activation of a wide range of occupational, food-derived and environmental chemicals into DNA-binding electrophiles that have been postulated to be the causative agents in the development of different types of cancers.

This study focuses on the N-acetyltransferase 2 (NAT2), one of the two NAT isoenzymes in humans, as enzyme participating in metabolism of the aromatic/heterocyclic amines in tobacco, industrial smoke [13, 32] and in diet as protein paralysis products formed when meat is cooked "well-done" or in fumes from meat cooking [19, 26]. Polymorphic N-acetyltransferase-2 locus on the short arm of chromosome 8 has been related to posses several alleles containing point mutations in coding and non-coding regions, leading to altered rates of metabolism and hence influencing individual susceptibility to arylamine-induced cancers [4]. The fact that slow NAT2 status may represent a risk factor for cancer due to diminished potential to detoxify aryl amine substrates, or to be protective due to diminished activation of heterocyclic amine substrates, makes it difficult to specify the relations between cigarette smoking, NAT2 genotype and risk of lung cancer. Several case-control studies have compared slow or

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rapid acetylators in lung cancer case series, with its prevalence in controls with mixed results. These discrepancies prompted us to investigate the association of NAT2 polymorphism with lung cancer in Slovak population.

Material and methods

Blood samples were collected from 110 patients with histologically proven diagnosis of lung carcinoma recruited in the Clinic of Pneumology and Phtiseology at the Teaching Hospital Košice. Controls under age of 65 years were healthy blood donors from the Clinic of Hematology and Blood Transfusion, those over 65 years from Geriatric Centrum, both at the Teaching Hospital Košice. Controls and cases were interviewed and asked about histories of cancer, chronic diseases, occupation and smoking status. Only individuals without history of cancer and chronic respiratory disease were eligible to participate as controls. The main medical diagnoses in the control over 65 years were rheumatological or cardiovascular diseases. All cases and controls were Slovak people (Caucasians) from the general population of Eastern Slovakia. Participants were given an explanation of the nature of the study and informed consent was obtained.

Genomic DNA was prepared from peripheral blood leukocytes by salting out method [21]. Genotyping for rapid NAT2*4 and slow NAT2*5A, NAT2*5B, NAT2*5C, NAT2*6 and NAT2*7 alleles was carried out using the PCR-RFLP method [6]. At least 35 variant NAT2 alleles consisting of one or more of 13 single nucleotide polymorphisms have been detected in the human population [3], but the NAT2*5, NAT2*6, NAT2*7 alleles account for virtually all of the slow acetylator alleles in Caucasian populations, providing evidence for a high concordance between genotype and phenotype [6]. Individuals with both low activity alleles were classified as slow acetylators, whereas individuals with at least one rapid allele were classified as rapid acetylators.

The distribution of the NAT2 genotypes between cases and controls was evaluated using the Fisher's exact test. The crude Gart's odds ratio (OR) and 95% confidence intervals (CI) was used to approximate relative risk for the case-control studies. All computations were undertaken using statistical software Arcus Quickstat Biomedical ver. 1.1.

Results

The data for all calculated genotypes are presented in Table 1. The frequency of the investigated alleles varied only slightly and without statistical significance between cases and control individuals (Fisher's exact test, $p>0.05$). The frequency of individuals with slow acetylators status in control group is in close agreement with the incidence among Caucasians reported by international collaborative study – GSEC [8] (Tab. 1). The prevalence of the slow acetylators in lung cancer patients (56.36%) was similar to that found in the population controls (52.00%) (Fisher's exact test, $p>0.05$). Compared with the rapid acetylators, the crude OR of lung cancer

Table 1. Distribution of NAT2 genotypes and lung cancer risk

Genotype	GSEC[1] study	Our study									
	Control	Control		Lung cancer		Squamous lung cancer		Lung cancer		Squamous lung cancer	
	%	N	%	N	%	N	%	OR	95% C.I.	OR	95% C.I.
4*4	7.25	11	6.58	11	10.00	7	9.45	1.58	(0.59–4.17)	1.48	(0.46–4.39)
4*5a	3.06	4	2.40	0	0.00	0	0.00	–		–	
4*5b	16	37	22.15	17	15.45	12	16.22	0.64	(0.32–1.25)	0.68	(0.30–1.45)
4*5c	1.7	4	2.40	4	3.64	1	1.35	1.54	(0.28–8.43)	0.56	(0.01–5.78)
4*6	13.3	22	13.17	16	14.55	9	12.16	1.12	(0.52–2.37)	0.91	(0.35–2.20)
4*7	1.04	2	1.20	0	0.00	0	0.00	–		–	
Total rapid	42.35	80	47.90	48	43.64	29	39.20	0.84	(0.50–1.40)	0.70	(0.39–1.27)
5a*5a	5.96	2	1.20	0	0.00	0	0.00	–		–	
5a*5b	2.40	3	1.80	3	2.73	2	2.70	1.53	(0.20–11.64)	1.52	(0.12–13.53)
5a*6	2.27	3	1.80	3	2.73	2	2.70	1.53	(0.20–11.64)	1.52	(0.12–13.53)
5b*5b	11.70	17	10.18	11	10.00	5	6.77	0.98	(0.40–2.33)	0.64	(0.18–1.91)
5b*5c	0.83	5	2.99	2	1.82	1	1.35	0.60	(0.06–3.75)	0.44	(0.01–4.08)
5c*5c	0.36	6	3.59	0	0.00	0	0.00	–		–	
5b*6	21.60	31	18.56	29	26.36	25	33.78	1.57	(0.84–2.91)	2.24	(1.14–4.34)
5b*7	1.74	2	1.20	1	0.90	1	1.35	0.76	(0.01–14.72)	1.13	(0.02–22.02)
5c*6	1.60	4	2.40	2	1.82	2	2.70	0.75	(0.07–5.38)	1.13	(0.10–8.10)
6*6	6.55	14	8.38	11	10.00	7	9.45	1.21	(0.48–3.01)	1.14	(0.37–3.19)
6*7	1.69	0	0.00	0	0.00	0	0.00	–		–	
Total slow	56.70	87	52.10	62	56.36	45	60.80	1.19	(0.71–1.99)	1.28	(0.70–2.31)
Total		167		110		74					

was 1.19 (95% CI: 0.71–1.99) for slow acetylator status. Based on the role of tobacco smoking in etiology of squamous or small cell lung carcinoma an analysis by histological type of cancer was performed. The slow acetylator status among the all followed histological subtypes was similar to that found in the population controls (data not shown), but only in squamous cell subtype significantly higher proportion slow NAT2 allelic combinations NAT2*5B/*6 (OR=2.24; 95% CI: 1.14–4.34) was observed. There were no subjects with the mentioned specific genotype among patients with adenocarcinoma and large cell carcinoma genotype.

Stratified analysis by age, smoking status and gender (Tab. 2) showed an increasing impact of the specific slow acetylator allelic combination NAT2*5B/*6 in non-smokers (OR=6.5; 95% CI: 1.25–15.08). In the case of squamous lung carcinoma an analysis revealed a tendency to adversely affect cancer risk in the individuals younger than 60 years (OR=3.14; 95% CI: 0.98–9.72) in non-smokers (4 out of 6; OR=10.40; 95% CI: 1.35–118.89) and in females (7 out of 14; OR=4.25; 1.08–16.25), although the last two subgroups were small.

Discussion

The relevance of drug metabolism polymorphisms for cancer risk, as shown in several studies, is likely to be based on the ability of the polymorphic enzymes to activate and deactivate carcinogens and mutagens. Several cytochrome P450 enzymes are involved in the activation of environmental and food carcinogens. These include mutagens and carcinogens such as polycyclic aromatic hydrocarbons, heterocyclic amines and nitrosamines that can induce lung cancer. Lung tissue plays important role in metabolism of inhaled foreign compounds. The relevance of the NAT2 polymorphisms in lung cancer risk is of particular importance since that enzyme is expressed in lung [33] together with others drug-metabolizing enzymes including CYP1A1, CYP1A2, CYP1B1, CYP2B7, CYP2E1, CYP2A, CYP4B1, NADH cytochrome P450 reductase, aryl sulfotransferase and glutathione S-transferase [10, 23, 27, 30].

This study presents the first data on the frequency of the NAT2 acetylator genotype among Slovak Caucasians lung cancer patients. By molecular genetic analysis was found that NAT2 specific slow acetylator genotype NAT2*5B/*6 might have been a risk factor for development of the squamous cellular lung cancer. The association of specific NAT2 genotypes and lung cancer risk has not been extensively reported in other studies. The Spanish study described higher prevalence of mutant allelic variants 590A and 341C+481T+803G i.e. alleles 6 and 5B (nomenclature by VATSIS et al [28]) in lung cancer patients [20]. *In vitro* assessment showed that defective NAT2 alleles have different significance on function of enzymes. As an example, for NAT2*5B allele reduced enzyme activity and for NAT2*6 allele reduced half-life of protein [4] has been showed. This might explain the suggested link of some allelic variants of NAT2 gene to cancer [1, 20, 29].

In our study, the NAT2*5B/*6 genotype occurred more frequently in younger squamous lung cancer patients and in non-smokers. The impact of age and/or smoking dose on lung cancer risk was demonstrated in some recent studies. HOU et al [16] have reported a clear association between the slow NAT2 genotype and non-operable tumors in younger individuals who smoked less. Japanese study described a prevalence of slow acetylators among younger non-small-cell lung cancer patients [24]. ZHOU et al [34] in their gene-environment interaction analysis revealed that the adjusted ORs of the rapid versus slow NAT2 genotype increased significantly as pack-years increased. For non-smokers, the fitted OR for rapid acetylators was 0.66 (95% CI: 0.44–0.99). The NAT2-rapid homozygote genotype was associated with an increased risk only in a German lung cancer patients, however NAT2*4/*4 genotype was significantly over represented in patients with higher smoked dose [7]. In non-smokers, environmental pollutants or professional exposure may impact on the role of NAT2 polymorphism in the development of lung cancer. This enzyme catalyses the acetylation of arylamines, that are ubiquitous chemicals present in industry, cooked food and as environmental contaminants. Examples include heterocyclic arylamines formed during heat processing of meat, polycyclic nitroaromatic hydrocarbons in diesel exhaust or 4-aminobiphenyl and 2-naphthylamine 4-amino in dye manufacturing and in cigarette smoke [13–15]. For non-smokers, NAT2 appears to provide a competitive pathway to the cytochrome P4501A2 and P4501A1-catalyzed

Table 2. Distribution of specific allelic combination NAT2*5B/*6 stratified by age, smoking and gender

		Controls			Lung cancer					Squamous cell cancer				
		Total	N	%	Total	N	%	OR	95% C.I.	Total	N	%	OR	95% C.I.
Age	<60 years	79	12	15.19	45	10	22.22	1.60	(0.56–4.48)	25	9	36.00	3.14	(0.98–9.72)
	>60 years	88	19	21.59	65	19	29.23	1.50	(0.67–3.35)	49	16	32.65	1.76	(0.74–4.13)
Smoking st.	Non-smokers	124	20	16.13	9	5	55.56	6.50	(1.25–35.08)	6	4	66.67	10.40	(1.35–118.89)
	Smokers	43	11	25.58	101	24	23.78	0.91	(0.37–2.30)	68	21	48.84	1.30	(0.51–3.41)
Gender	Males	83	15	18.07	87	20	22.99	1.35	(0.60–3.10)	60	18	30.00	1.94	(0.82–4.61)
	Females	84	16	19.05	23	9	39.13	2.73	(0.87–8.20)	14	7	50.00	4.25	(1.08–16.25)

N-oxidation pathway for reactive hydroxylamines without an alternative mechanism [2]. NAT2 *N*-acetylates and detoxifies these aromatic amine compounds and it is a possible explanation why the rapid acetylator genotypes showed a possible protective effect in non-smokers. The hypothesis of association of NAT2 phenotype and air quality has been indirectly confirmed in Scandinavia. Non-smokers with the combination of NAT2 slow and GSTM1-negative genotype among bus maintenance workers exposed to diesel exhaust demonstrated higher aromatic DNA adducts levels in peripheral blood [17]. In Denmark study, the NAT2 slow genotype alone, showed elevated chromosomal aberration counts not only in bus drivers but also in postal workers. Authors suggest that NAT2 genotype effect is not due to exposure to high-level urban air pollution but due to something else common to all subjects [18].

Our preliminary data outlined significant increasing of lung cancer risk for squamous lung cancer in females. Several epidemiological studies have indicated that for given number of cigarettes smoked, females may be at higher risk of lung cancer compared with males. The data sets from the studies of BROWNSON [5], HARRIS [11] and RISCH [25] and their collaborators show that women were at 1.3 to 2.9-fold risk to develop lung cancer than men. In addition, MOLLERUP et al [22] found that female smokers exhibited a significantly higher expression level of lung *CYP1A1* than men and simultaneously, the level of PAH-DNA adducts were related to expression of *CYP1A1* mRNA in target tissue. These findings indicate that the *CYP1A1* expression may be an important factor in influencing sex difference in aromatic/hydrophobic DNA adducts levels in the lung. HAUGEN et al [12] outlined that although the mechanisms of sex difference are still unknown, important elements in lung carcinogenesis may be hormonal regulation of genes involved in the metabolism of tobacco carcinogens and DNA repair, interactions of smoking and hormone status, hormones and the activation of growth promoting pathways, cross talk between various signalling pathways and the interaction between stroma and epithelial cells during tumor development.

In conclusion, our finding suggests that NAT2 acetylator polymorphism probably affects susceptibility to lung cancer in Slovak region. Age and/or smoking status appear to play a role in altering the direction of the association between NAT2 polymorphisms and risk of lung cancer. Additional studies are needed to confirm the results we observed and to assess the impact of other effects (specific allelic combinations, sex differences and histological subtype of lung cancer) on NAT2 susceptibility in lung carcinogenesis.

References

[1] AGUNDEZ JA, LADERO JM, OLIVERA M, ABILDUA R, ROMAN JM, BENITEZ J. Genetic analysis of the arylamine N-acetyltransferase polymorphism in breast cancer patients. Oncology 1995; 52: 7–11.

[2] ALDRIDGE JE, HOU SM, RYBERG D, FALT S, DEVERILL A et al. GSTM1 and NAT2 polymorphisms in operable and non-operable lung cancer patients. Carcinogenesis 2000; 21: 49–54.

[3] Arylamine *N*-acetyltransferase (NAT) Nomenclature. *http://www.louisville.edu/medschool/pharmacology/NAT.html*. Last update October 16, 2004.

[4] BLUM M, GRANT DM, MC BRIDE W, HEIM M, MEYER UA. Human arylamine *N*-acetyltransferase genes: isolation, chromosomal localization and functional expression. DNA Cell Biol 1990; 9: 193–203.

[5] BROWNSON RC, CHANG JC, DAVIS JR. Gender and histologic type variations in smoking-related risk of lung cancer. Epidemiology 1992; 3: 61–64.

[6] CASCORBI I, DRAKOULIS N, BROCKMÖLLER J, MAURER A, SPERLING K, ROOTS I. Arylamine N-acetyltransferase (NAT2) mutations and their allelic linkage in unrelated Caucasian individuals: correlation with phenotypic activity. Am J Hum Genet 1995; 57: 581–592.

[7] CASCORBI I, BROCKMOLLER J, MROZIKIEWICZ PM, BAUER S, LODDENKEMPER R, ROOTS I. Homozygous rapid arylamine N-acetyltransferase (NAT2) genotype as a susceptibility factor for lung cancer. Cancer Res 1996; 56: 3961–3966.

[8] GARTE S, GASPARI L, ALEXANDRIE AK, AMBROSONE CH, AUTRUP H et al: Metabolic gene polymorphism frequencies in control populations. Cancer Epidemiol Biomarkers Prev 2001; 10: 1239–1248.

[9] GIBBONS JA, FLAHERTY MM, KREIDER ML, ROMANO JA, LEVIN ED. Heterogeneity of toxicant response: sources of human variability. Toxicol Sci 2003; 76: 3–20.

[10] HALL PM, STUPANS I, BURGESS W, BIRKETT DJ, MCMANUS ME. Immunohistochemical localization of NADPH-cytochrome P450 reductase in human tissues. Carcinogenesis 1989; 10: 521–530.

[11] HARRIS RE, ZANG EA, ANDERSON JI, WYNDER EL. Race and sex differences in lung cancer risk associated with cigarette smoking. Int J Epidemiol 1993; 22: 529–599.

[12] HAUGEN A. Women who smoke: are women more susceptible to tobacco-induced lung cancer? Carcinogenesis 2002; 23: 227–229.

[13] HEIN DW. Molecular genetics and function of NAT1 and NAT2: role in aromatic amine metabolism and carcinogenesis. Mutat Res 2002; 506–507: 65–77.

[14] HEIN DW, DOLL MA, RUSTAN TD, GRAY K, FENG Y et al. Metabolic activation and deactivation of arylamine carcinogens by recombinant human NAT1 and polymorphic NAT2 acetyltransferases. Carcinogenesis 1988; 14: 1633–1638.

[15] HIRVONEN A. Polymorphic NATs and cancer predisposition. In: Vineis P, Caporaso N, Cuzick J, Lang M, Malats N, Boffetta P, edditors. Genetic susceptibility to cancer: metabolic polymorphisms. IARC Scientific Publication No. 148. International Agency for Research on Cancer, Lyon, 1999: 251–270.

[16] HOU SM, RYBERG D, FALT S, DEVERILL A, TEFRE T et al. GSTM1 and NAT2 polymorphisms in operable and non-operable lung cancer patients. Carcinogenesis 2000; 1: 49–54.

[17] HOU SM, FALT S, YANG K, NYBERG F, PERSHAGEN G et al. Differential interactions between GSTM1 and NAT2 genotypes on aromatic DNA adduct level and HPRT mutant frequency

in lung cancer patients and population controls. Cancer Epidemiol Biomarkers Prev 2001; 10: 133–140.

[18] KNUDSEN LE, NORPPA H, GAMBORG MO, NIELSEN PS, OKKELS H et al. Chromosomal aberrations in humans induced by urban air pollution: Influence of DNA repair and polymorphisms of glutathione S-transferase M1 and N-acetyltransferase 2. Cancer Epidemiol Biomarkers Prev 1999; 8: 303–310.

[19] LAYTON DW, BOGEN KT, KNIZE MG, HATCH FT, JOHNSON VM, FELTON JS. Cancer risk of heterocyclic amines in cooked foods: an analysis and implication for research. Carcinogenesis 1995; 16: 39–52.

[20] MARTINEZ C, AGUNDEZ JA, OLIVERA M, MARTIN R, LADERO JM, BENITEZ J. Lung cancer and mutations at the polymorphic NAT2 gene locus. Pharmacogenetics 1995; 5: 207–214.

[21] MILLER SA, DYKES DD, POLESKY HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988; 16: 1215.

[22] MOLLERUP S, RYBERG D, HEWER A, PHILLIPS DH, HAUGEN A. Sex differences in lung CYP1A1 expression and DNA adduct levels among lung cancer patients. Cancer Res 1999; 59: 3317–3320.

[23] NISHIMURA M, YAGUTI H, YOSHITSUGU H, NAITO S, SATOH T. Tissue distribution of mRNA expression of human cytochrome P450 isoforms assessed by high-sensitivity real-time reverse transcription PCR. Yakugaku Zasshi 2003; 123: 369–375.

[24] OYAMA T, KAWAMOTO T, MIZOUE T, YASUMOTO K, KODAMA Y, MITSUDOMI T. N-acetylation polymorphism in patients with lung cancer and its association with p53 gene mutation. Anticancer Res 1997; 17: 577–581.

[25] RISCH HA, HOWE GR, JAIN M, BURCH JD, HOLOWATY EJ, MILLER AB. Are female smokers at higher risk for lung cancer than male smokers? A case-control analysis by histologic type. Am J Epidemiol 1993; 138: 281–293.

[26] ROBBANA-BARNAT S, RABACHE M, RIALLAND E, FRADIN J. Heterocyclic amines: occurrence and prevention in cooked food. Environ Health Perspect 1996; 104: 280–288.

[27] TOUSSAINT C, ALBIN N, MASSAAD L, GRUNENWALD D, PARISE O JR et al. Main drug- and carcinogen-metabolizing enzyme systems in human non-small cell lung cancer and peritumoral tissues. Cancer Res 53; 1993: 4608–4612.

[28] VATSIS KP, WEBER WW, BELL DA, DUPRET JM, EVANS DA et al. Nomenclature for *N*-acetyltransferases. Pharmacogenetics 1995; 5: 1–17.

[29] VAZIRI SA, HUGHES NC, SAMPSON H, DARLINGTON G, JEWETT MA, GRANT DM. Variation in enzymes of arylamine procarcinogen biotransformation among bladder cancer patients and control subjects. Pharmacogenetics 2001; 11: 7–20.

[30] VINEIS P. Individual susceptibility to carcinogens. Oncogene. 2004; 23: 6477–6483.

[31] VINEIS P, FORASTIERE F, HOEK G, LIPSETT M. Outdoor air pollution and lung cancer: recent epidemiologic evidence. Int J Cancer. 2004; 111: 647–652.

[32] WILLEY JC, COY E, BROLLY C, UTELL MJ, FRAMPTON MW et al. Xenobiotic metabolism enzyme gene expression in human bronchial epithelial and alveolar macrophage cells. Am J Respir Cell Mol Biol 1996; 14: 262–271.

[33] WINDMILL KF, GAEDIGK A, HALL PM, SAMARATUNGA H, GRANT DM, MCMANUS ME. Localization of N-acetyltransferases NAT1 and NAT2 in human tissues. Toxicol Sci 2000; 54: 19–29.

[34] ZHOU W, LIU G, THURSTON SW, HU LL, MILLER DP et al. Genetic polymorphisms in N-acetyltransferase-2 and microsomal epoxide hydrolase, cumulative cigarette smoking and lung cancer. Cancer Epidemiol Biomarkers Prev 2002; 11: 15–21.