用される病院や研究施設がある。

2　有機溶剤

シンナーの主成分であるトルエン、キシレンなどの有機溶剤は粘膜刺激作用と麻酔作用があり原因物質の一つと考えられている。トルエンは有機溶媒としてしばしば用いられ、接着剤の主成分として広く建材、建具などに用いられている。キシレンの主な発生源は、塗料、芳香剤、油性マーカー、のりなどである。

3　有機リン

シロアリ駆除剤として使用されてきたクロルピリフォスや衣料、繊維、カーテン、絨毯などに含まれる殺虫剤も原因と考えられている。

V　MCSの発症メカニズムについての仮説

発現機序については、免疫学的機序や神経学的機序、心因学的機序など多方面から研究が行われているが、いずれも決定的な病態解明には至っていない。臨床症状がアレルギー様であることから、当初は免疫学的研究が多く報告されたが、明確な異常は示されていない。現在は中枢神経系の機能的・器質的研究と、心因学説に立脚した研究報告が多数なされている（表6）[19]。そのなかから代表的な仮説を紹介する。

1　神経学的機序

Millerは①化学物質の毒性により惹起される化学物質への耐性の喪失（Toxicant-induced loss of tolerance：TILT）と、②その後の微量化学物質暴露による症状の発現、という2段階のプロセスを述べている[6]。また、Bellらは神経系統の変化による症状形成の過程の観点から、キンドリング（Kindling）や時間－依存性感作（Time-Dependent sensitization：TDS）などの動物研究をもとにした仮説を提唱している[4]。

キンドリングとは、初めは何の変化も起こさないような、弱い電気刺激または化学物質による刺激を毎日繰り返し与えつづけると、10日間から14日間後には激しいてんかん様けいれん発作を起こすようになるものをいう。キンドリングはけいれん発現閾値量以下の薬剤を投与することでも成立することや、神経系に明らかな病理学的障害が認められないことが、MCSの特徴である微量化学物質への高感受性と、身体的検査所見に異常が認められないという点で一致することから、可能性のある仮説とされている[20]。

時間—依存性感作（TDS）とは、（薬理学的あるいは心理的な）刺激やストレスにさらされると、その刺激やストレスに対する感度が徐々に時間の経過にしたがって亢進する現象をいう。これはMCSの微量化学物質への慢性暴露による過敏性の獲得という過程に類似しており、仮説の1つに取り上げられている[21]。

2　免疫学的機序

MCS患者では化学物質の暴露経路として吸入経路が有力であり、化学物質の吸入により上気道の炎症が惹起される。その炎症がアレルギー反応あるいは類似反応を引き起こし、産生された炎症性蛋白質が、中枢神経系・免疫系に影響を与え、全身症状を引き起こすという仮説がある。MCSの臨床症状はアレルギー症状と類似しているが、アレルギーの特徴であるIgEの増加やそれに伴うインターロイキン等のサイトカインの上昇、ヒスタミンの異常放出などの客観的な診断指標はMCSの場合は変動しないとされる。一方、MCSの発症者の64%にアレルギー疾患の既往歴があったという報告があり[22]、種々

表6　MCSのメカニズムについての仮説〔文献19〕

メカニズム仮説	コメント	文献
気道反応性	鼻炎や気道反応性はMCS患者の共通症状である。また、MCSの複合症状は、”反応性上気道異常諸侯群”と呼ばれ、化学物質暴露に引き続いて喘息や鼻炎症状を呈する患者にも認められる。	26,27
アレルギー	ほとんどのアレルギー反応は臨床的な症状と関連性をもった免疫学的メカニズムが背後にある。しかし、MCS患者においては免疫学的変化はほとんど観察されないか、されたとしてもその変化は軽度である。これらの結果は、MCSがアレルギーのメカニズムを介さないことを示唆している。しかし、アレルギーとMCSとの関連性は否定できない。	28
不安反応	トリガー物質によるチャレンジテストによってMCS患者に観察される臨床症状は、過換気症候群による不安反応に一致した症状や兆候である。	25
自己暗示	疾病や原因の存在に対する信念が、症状の原因である可能性がある。そして、ある特定のサポート集団、医学アドバイザー、メディアによってMCSという信念が継続・強化されていく。	8
異臭症	嗅覚感度の変化。化学物質の臭いが自律神経の興奮を促進し、時間経過とともに増強される。臭いによるパニック症状のようにもみえる。	29,30
条件反射	化学物質の臭いが症状を引き起こすような行動反応の原因となる。この基本的メカニズムは”パブロフの条件反射”である。しかし、ほとんどのMCS患者は初めに症状を自覚し、次に暴露に気づくようである。	31,32
希望スコアー	希望スコアー(Herth Hope Scale)の低値が、MCS患者に報告されている。	33
疾病信念システム	化学物質に過敏な患者の疾病の原因に関する信念が、治療を行う医師によって強化され臨床症状のレベルにまで達する。鉱油に汚染された土壌に暴露されたトンネル作業者の研究によれば、MCSはナイーブで議論を好まない人に発生していることが報告されている。	34
免疫学的メカニズム	免疫学的データの変化が、しばしばMCS患者に認められる。しかし、時に有意差が認められず、MCS患者すべてに共通な指標とはなっていない。その変化はウイルス感染のようなエピソードとしばしばリンクしている。	35
エネルギー産生に関与する生化学的経路の障害	MCS患者にみられる疲労は細胞の基礎的なエネルギー代謝の障害を示唆している。筋肉や神経系のようなエネルギー要求性の大きな組織が影響を受けやすい。	
神経伝達経路の障害	疲労症状は、2次的に重要な神経伝達物質であるGABAレセプターの感受性を変化させる。	36
辺縁系キンドリング	キンドリングとは、初めは何の変化を起こさないような弱い電気刺激を短時間、1日1回与えると、2～3週間後同レベルの刺激によって間代性痙攣を特徴とする辺縁系発作を起こす現象をいう。キンドリングが、微量化学物質に対して成立している。この仮説は、MCSの多臓器にわたる症状や、時間依存的な感受性の亢進を説明しているといわれる。	4,20,37
詐病	患者はMCSの症状によって休職したり、補償をうけとることができる。しかし、この仮説はほとんどありえないことだとは思われる。なぜならMCS患者間の症状の幅があまりに一定であり、意図的に仕事を忌避する目的で多くの人に用いられるランダムな症状ではないからである。	38
神経感作	化学物質の継続的かつ間欠的な暴露に対する反応の進行的な増幅。このメカニズムは、イニシエーションと感作の2段階あると考えられる。感作獲得仮説を支持するMCSの動物モデルの結果がある。	39,40,41
神経原性炎症（上気道炎症に由来）	上気道の炎症がサイトカインや伝達ペプチドを産生し、気道に位置する神経細胞過敏を引き起こすことが知られている。部位特異的な神経過敏のメカニズムが示唆されている。	
嗅覚閾値感度	MCS患者は嗅覚閾値の低下が示唆されている。この事実に関しては結論は出ていない。ある研究は、嗅覚刺激物質に暴露後のMCS患者の神経生理学的機能の変化を示唆しているが、別の研究は、MCS患者群とマッチした対照群との試験実験に差が認められなかったことを報告している。しかし、正常な被験者の脳波研究によれば、低濃度の臭い物質への暴露が臭い自覚以前に脳波活動度の変化と関連していることを示している。そして、異臭症の患者でより大きな影響があったとされる。	42,43,44
生体内変化パスウエイのオーバーロード	生体内変化許容量の機能的予備量は個人によって異なる。もし、この予備量が飽和に近い場合や減少した場合、身体はより一定以上の暴露を処理することができない。ほとんどのMCS患者は生体内変化プロセスが一部崩壊している。低濃度に対する感度増加や、反応する化学物質の種類増加がこの仮説を支持している。例として、硫酸抱合パスウエイの障害が報告されている。	45
パニック障害	化学物質の臭いが臭気性パニック発作を誘発する。	46
心理学的・精神医学的疾病	心理学的あるいは精神医学的な誤診の副産物である可能性。心理学的疾患はMCSの診断からははずされるべきである。症状のある個人が精神病理学的な兆候を示すことに関する明白なデータはない。忌避治療が有効である反面、行動療法が無効であることがこの仮説を支持している。	47,48
心身医学的状況	症状は生理学的あるいは心理学的原因を示唆している。非常に低濃度の化学物質暴露であり、従来型中毒では説明が困難。また、MCSの症状は心理学的介入によって完全に治療することができない。	49,50
神経システムの過敏	”神経原性スィッチング(Neurogenic switching)”：一つの局所刺激が、別の局所刺激を誘発する。	51
身体的うつ症状	MCSは、しばしば　うつと関連しており、うつの身体的愁訴と重なっている	52
時間依存性感作	薬剤・環境からの軽度の刺激に対し、時間依存的に感度が増加し、生理学的・行動学的反応が徐々に亢進する	21,53
有毒物質惹起性耐性損失症(Toxicant-Induced Loss of Tolerances, TILT)	MCSが2段階のプロセス、すなわち化学物質に対する高感受性者が様々な毒物に対する耐性を喪失し、次に耐性を喪失した極微量の毒物によって引き起こされる症候であるという仮説	6

の抗原に対する皮内反応に対して陽性を示す患者の存在も報告されている[23]。しかし、統計的な有意性への言及がなく、適正な対象群の欠如や再現性が得られた報告がないなど、その評価には注意が必要である[24]。現状では、MCSと免疫学的異常との関連性は想定されているものの、免疫学的な機序のみでMCSの病態を説明することは困難である。

3　心因的機序

MCSの心因性については、原因とされる化学物質との因果関係を説明できるような身体的検査所見や病理学的所見に乏しいこと、既知の精神疾患と類似していることなどが、主な根拠となっている。

Leznoffは、15名のMCS患者それぞれに対し、症状がもっとも出る誘発物質を暴露させ、その前後での肺機能、血中のCO_2とO_2の分圧、O_2飽和度（Oxygen saturation）を測定する誘発実験を行った[25]。その結果、被験者15名のうち誘発物質により症状を再現した11名全員に過呼吸（hyperventilation）を伴った急激なCO_2分圧の低下が観察された。この実験結果からLeznoffは、MCS患者は環境汚染物質により不安が引き起こされ、その不安を症状として発現しているのだと考え、少なくとも症状のある部分は過呼吸により引き起こされると結論づけている。ただし、これは一部のMCS患者に過呼吸をベースとする心因性集団が存在することを示唆するがMCS全体に心因性を適応する根拠としては不十分と思われる。

Ⅵ　まとめ

MCS及び化学物質過敏症については、病態等について未解明な点が多く、また、その発生頻度など基本的な疫学情報が不足しており、それらの収集が第一の課題である。そのためには、臨床診断が困難な状況ではあるが、MCSに関する啓発活動を行いながら、一般診療家が使えるような“明確で判定しやすい診断基準”の設定が必要である。さらに、化学物質暴露との因果関係の証明、そのメカニズムの解明、治療法を確立するなどが緊急の課題であり、多くの研究者の参加と協力が不可欠だと考える。

参考文献

1　Shorter E : Multiple chemical sensitivity : Psedudodisease in historical perspective. Scad J Work Environ Health 23 (suppl 3) : 35–42, 1997.

2　Cullen MR : The worker with multiple chemical sensitivities : An overview. Occup Med 2 : 655–661, 1987.

3　Staudenmayer H, Selner JC, Buhr MP : Double-blind provocation chamber challenges in 20 patients presenting with "Multiple Chemical Sensitivity". Reg Toxicol Pharmacol 18 : 44–53, 1993.

4　Bell IR, Schwartzs GE, Baldwin CM, Hardin EE, Klimas NG, Kline JP, Patarca R, and Zhi-Ying Song : Individual differences in neural sensitization and the role of context in illness from low-level environmental chemical exposures. Environ Health Perspect 105 (Suppl 2) : 457–466, 1997.

5　西岡　清：化学物質過敏症—はじめに—。アレルギー・免疫　6：964—968、1999。

6　Miller CS : Toxicant-induced loss of tolerance : an emerging theory of disease. Environ Health Perspect 105 (suppl 2) 445–453, 1997.

7　Sparks PJ, Daniell W, Black DW, Kipen HM, Altman LC, Simon GE, Terr AI : Multiple chemical sensitivity syndrome : a clinical perspective. JOM 36 : 718–730, 1994.

8　Altenkirch H : Multiple chemical sensitivity (MCS)-Differential diagnosis in clinical neurotoxicology : A German perspective. NeuroToxicology 21 : 589–598, 2000.

9　石川　哲：化学物質過敏症．医学のあゆみ　188：785—788，1999。

10　石川　哲、宮田幹夫、難波龍人、西本浩之：化学物質過敏症診断基準について。日本医事新報3857：25－29、1998。

11　Buchwald D, Garrity D : Comparison of patients with chronic fatigue syndrome, fibromyalgia, and multiple chemical sensitivities. Arch Intern Med 154 : 2049–2053, 1994.

12　宮田幹夫、難波龍人：多種化学物質過敏症（multiple chemical sensitivity）の臨床。自律神経、33：257—261、1996。

13　環境庁環境保健部環境安全課：本態性多種化学物質過敏状態の調査報告書、平成12年２月２日。
14　Meggs WJ, Cunn KA, Bloch RM, Goodman PE, Davidoff AL: Prevalence and nature of allergy and chemical sensitivity in a general population. Arch Environ Health 51 : 275–282, 1996.
15　Miller CS: The compelling Anomaly of Chemical Intolerance. In: The role of Neural Plasticity in Chemical Intolerance (ed by Sorg BA, Bell IR), pp 1–23, The New York Academy of Sciences, New York, 2001.
16　Kreutzer R, Neutra RR, Lashuay N: Prevalence of people reporting sensitivities to chemicals in a population-based survey. Am J Epidemiol 150 : 1–12, 1999.
17　内山巌夫、村山留美子、平成11年度厚生科学研究費補助金報告書—公衆衛生学的立場から見た化学物質過敏症、１－５、2000。
18　北條祥子、日本における QEESI を使った疫学的研究、平成11年度厚生科学研究費補助金報告書—シックハウス症候群の病態解明、診断治療法に関する研究、134—152、2002。
19　Winder C: Mechanisms of multiple chemical sensitivity. Toxicol Lett 128 : 85–97、2002.
20　相澤好治：化学物質過敏症（本態性環境不寛容状態）について—労働衛生とのかかわり—。中央労働災害防止協会委託研究報告書　化学物質過敏症予防のための労働衛生管理技法に関する研究：３—16、2000。
21　Miller C: Are we on the threshold of a new theory of disease? Toxicant-induced loss of tolerance and its relationship to addiction and abdiction. Toxicol Ind Health 15 : 284–294, 1999.
22　市辺義章、宮田幹夫：化学物質過敏症：眼科医の立場から。Current Therapy 17 : 503–509、1999。
23　石川哲、宮田幹夫（編）生命と環境21　化学物質過敏症　ここまできた診断・治療・予防法、かもがわ出版、京都、2000。
24　Salvaggio JE: Understanding clinical immunological testing in alleged chemically induced environmental illness. Regul Toxicol Pharmacol 24 (1 Pt 2) : S 16–27, 1996.
25　Leznoff A: Provocative challenges in patients with multiple chemical sensitivity. J Allergy Clin Immunol 99 : 438–442, 1997.
26　Meggs WJ, Cleveland CH: Rhinolaryngoscopic examination of patients with the multiple chemical sensitivity syndrome. Arch Environ Health 48 : 14–18, 1993.
27　Meggs WJ: Multiple chemical sensitivities–Chemical sensitivities as a symptom of airway inflammation. J Toxicol Clin Toxicol 33 : 107–110, 1995.
28　Ross GH: Clinical characteristics of chemical sensitivity: An illustrative case histry of asthma and MCS. Environ Health Perspect 105 (Suppl.2) : 437–441, 1997.
29　Schusterman D, Balmes J, Cone J: Behavioral sensitization to irritants and odorants after acute exposure. J Occup Med 30 : 565–567, 1988.
30　Dalton P, Wysecki CJ, Brady MJ, Lawley HJ: The influence of cognitive bias on the perceived odor, irritation and health symptoms from chemical exposure. Int Arch Occup Environ Health 69 : 407–417, 1997.
31　Bolta–Wilson K, Wilson R, Bleecker ML: Conditioning of physical symptoms after neurotoxic exposure. J Occup Med 30 : 684–686, 1988.
32　Seigel S: Multiple chemical sensitivity. Multiple chemical sensitivity as a conditioned response. Toxicol Ind Health 15 : 323–330, 1998.
33　Gibson PR: Hope in multiple chemical sensitivity: Social support and attitude towards healthcare delivery as a predictors of hope. J Clin Nurs 8 : 275–283, 1999.
34　Davidoff AL, Keyl PM, Meggs W: Development of multiple chemical sensitivities on laborers after acute operation. Arch Environ Health 53 : 183–189, 1998.
35　Mitchell CS, Donnay A, Hoover DR, Margolick JB: Immunologic parameters of multiple chemical sensitivity. Occup Med 15 : 647–665, 2000.
36　Corrigan FM, MacDonald S, Brown A, Armstrong K, Armstrong EM: Neurasthenic fatigue, chemical sensitivity and GABA receptor toxins. Med Hypothesis 43 : 195–200, 1994.
37　Bell IR, Miller CS, Schwartz GE: Limbic system alteration in multiple chemical sensitivities patients. Biol Psychiatry 32 : 218–242, 1992.
38　McSherry J: Chronic fatigue syndrome. A fresh look at an old problem. Can Fam Physician 39 : 336–340, 1993.
39　Bell IR, Baldwin CM, Fernandez M, Schwartz GE: Neural sensitization model for multiple chemical sensitivity: Overview of theory and empirical evidence. Toxicol Ind Health 15 : 295–304, 1998.
40　Arnetz BB: Model development and research vision for the future of multiple chemical sensitivity. Scand J Work Environ Health 25 : 569–573.
41　Sorg BA: Multiple chemical sensitivity: Potential role for neural sensitization. Crit Rev Neurobiol 13 : 283–316, 1999.
42　Callender TJ, Duhon D, Ristovv M: Olfactory intolerance and dynamic nurophysiological abnormalities in patients with toxic encephalopathy. Adv Biosci 93 : 611–619, 1994.
43　Doty RL: Olfaction and multiple chemical sensitivity. Toxicol Ind Health 10 : 359–368, 1994.
44　Schwartz GE, Bell IR, Dikman ZV, Fernandez M, Kline JP, Peterson JM, Wright KP: EEG responses to low level chemicals in normals and cacosmics. Toxcol Ind Health 10 : 633–640, 1994.

45　Mcfadden SA : Phenotypic variation in xenobiotic metabolism and adverse environmental response : focus on sulfur dependent detoxification pathways. Toxicology 111 : 34–65, 1996.
46　Dager SJ, Holland JP, Cowley DS : Panic disorder precipitated by exposure to organic solvents in the work–place. Am J Psychiatry 144 : 1056–1058, 1987.
47　Davidoff LL : Models of mutiple chemical sensitivities syndrome : using empirical data (especially interview data) to focus investigations. Toxicol Ind Health 8 : 229–247, 1992.
48　Simon GE : Psychiatric symptoms in multiple chemical sensitivity. Toxicol Ind Health 10 : 487–496, 1994.
49　Davidoff LL : Illnesses from chemical odours : Is the health signficance understood?. Arch Environ Health 47 : 88–91, 1992.
50　Miller CS : Possible models for multiple chemical sensitivity : conceptual issues and role of the limbic system. Toxicol Ind Health 10 : 181–202, 1994.
51　Meggs W : Neurogenic switching, a hypothesis for a mechanism for shifting the site of inflammation in allergy and chemical sensitivity. Environ Health Perspect 103 : 54–65, 1995.
52　Brown-DeGagne AM, McGlone J : Multiple chemical sensitivity : A test of the olfactory–limbic model. J Occup Environ Med 41 : 366–377, 1999.
53　Antelman SM : Time dependent sensitization in animals : a possible model of multiple chemical sensitivity in humans. Toxicol Ind Health 10 : 335–342, 1994.

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ORIGINAL PAPER

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Genetic polymorphisms of tobacco- and alcohol-related metabolizing enzymes and the risk of hepatocellular carcinoma

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Abstract The effect of genetic polymorphisms for glutathione S-transferase (*GST*) *M1*, *GSTT1*, *GSTP1-1* (*GSTP1*), cytochrome P450 2E1 (*CYP2E1*) and aldehyde dehydrogenase 2 (*ALDH2*) on the risk of hepatocellular carcinoma (HCC) was observed in 78 Japanese patients with HCC and 138 non-cancer hospital controls. We found a positive association between cumulative amounts of alcohol consumption (≧600,000 ml in a lifetime) and the risk of HCC (OR = 4.52, 95% CI 2.39–8.55). However, cigarette smoking was not significantly related to the risk of HCC (OR = 1.23, 95% CI 0.57–2.68). The allelic frequencies of *GSTM1*, *GSTT1*, *GSTP1*, *CYP2E1* and *ALDH2* of HCC patients were not significantly different from those of controls when odds ratios were only adjusted for age and gender except for any *2* alleles of *ALDH2* in drinkers (OR = 2.53, 95% CI 1.21–5.31). However, the frequency of any *C2*alleles of *CYP2E1* and any *2* alleles of *ALDH2* were significantly higher than those of controls (OR = 5.77, 95% CI 1.24–27.39, OR = 9.77, 95% CI 1.63–58.60) when covariates including viremia were selected by using stepwise logistic regression analysis. We conclude that habitual alcohol drinking is likely to lead to an increased risk of HCC, and any *C2* alleles of *CYP2E1* as well as any two alleles of *ALDH2* were also associated with an increased risk of HCC.

Keywords Genetic polymorphism · GSTM1 · CYP2E1 · ALDH2 · Hepatocellular carcinoma

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Introduction

Primary liver cancer usually complicates several chronic liver diseases, mainly those induced by hepatitis B virus (HBV) and hepatitis C virus (HCV) (Ruiz et al. 1992; Tsai et al. 1994). Especially, HBV and HCV prevalence was found to be associated with 95% of hepatocellular carcinoma (HCC) patients in Japan (The Liver Cancer Study Group of Japan 2000). The proportions of HBV and HCV were 16.5% and 74.8%, respectively. Not only is HCC an inevitable consequence of chronic HBV or HCV infection, but also other HCC risk factors, such as tobacco smoking, alcohol drinking and aflatoxin exposure (Ross et al. 1992), are related to susceptibility to HCC. Epidemiological studies have shown a possible correlation between ethanol abuse and the development of HCC (Chen et al. 1991; Mohamed et al. 1992). Otherwise, the possibilities of a relationship between tobacco smoking and the occurrence of HCC are controversial (Trichopoulos et al. 1987; Tsukuma et al. 1993; Kuper et al. 2000; Tanaka et al. 1992; Hadziyannis et al. 1995).

Many chemical carcinogens are also metabolically converted into active forms that have harmful effects on the liver. The metabolizing enzymes, including

glutathione S-transferases (GSTs), cytochrome P-450s (CYPs) and aldehyde dehydrogenase 2 (ALDH2), play an important role in the detoxification or activation of carcinogens. This metabolic activation depends on genetic variations, which may be responsible for individual differences. *GSTM1*, *GSTT1* and *GSTP1* may play a part in the activation and detoxification of procarcinogens in tobacco smoke (Guengerich 1991; Mannervik and Danielson 1988). Individual variations in enzyme activities have been demonstrated for several GSTs. Some of these variations are genetically linked and may affect individual cancer risk.

When drinking alcohol, some of the proposed mechanisms for ethanol-related carcinogenesis are closely linked to the action of acetoaldehyde. Approximately half of the Japanese population lacks ALDH2 activity because of a structural point mutation in the *ALDH2 gene*. This genetic polymorphism, which is seen in Asians, including Japanese, but not in Caucasians, results in catalytic deficiency of aldehyde metabolism (Harada and Zhang 1993). Besides ALDH2, the ethanol inducible CYP2E1 catalyses the oxidation of ethanol itself. In addition, CYP2E1 is of critical importance in the metabolic activation of many carcinogens, including N-nitrosamines, benzene and aniline, that are present in tobacco smoke. Therefore, previous reports have shown that *CYP2E1* might modulate the risk of HCC (Ladero et al. 1996).

In this study, we have made the hypothesis that alcohol abuse and/or tobacco smoking is a risk factor for the development of HCC, and we have examined the effects of the *GSTs* (*M1*, *T1*, *P1-1*), *CYP2E1* and *ALDH2* polymorphism on the susceptibility of HCC among Japanese people in relation to their smoking or alcohol-drinking status.

Materials and methods

Subjects

A total of 78 HCC patients seen in the University of Occupational and Environmental Health (UOEH) Hospital in Japan from June 1997 to April 1998 were enrolled in the present study. Acid-citrate-dextrose-anti-coagulated blood was drawn from 78 patients with HCC and from 138 hospital controls with no evidence of cancer in any organ. Cases and controls were unmatched. The demographic data of both case and control groups are shown in Table 1. All study subjects completed a questionnaire administrated by a trained interviewer, covering medical, residential, occupational and smoking and drinking history. The lifetime amount of cigarette smoking was quantified by the Brinkman-Coates index, which is the product of the daily number of cigarettes smoked and years of smoking. The cumulative amount of ethanol consumption was quantified by drink-years, which was calculated by multiplying the volume of ethanol a year by the number of drinking-years. None of the subjects had had any exposure to carcinogens, heavy metals or radiation in their occupational history.

This study was approved by the ethics committee of medical care and research of the University of Occupational and Environmental Health (UOEH) under the guidelines of the Ministry of Education, Culture, Sports, Science and Technology in Japan.

Table 1 Distribution of demographic variables for cases and controls

	Variables	Cases	Controls
Gender	Males (%)	61 (78.2%)	94 (68.1%)
	Females (%)	17 (21.8%)	44 (31.9%)
Age	Mean age (± SD)	66.1 ± 7.7	67.2 ± 10.5
	Range	47–84	34–92
Smoking status	Non smokers (%)	20 (25.6%)	50 (36.2%)
	Smokers (%)	58 (74.4%)	88 (63.8%)
Drinking status	Non drinkers (%)	25 (32.1%)	56 (40.6%)
	Drinkers (%)	53 (67.9%)	82 (59.4%)
Viremias	Non viremias (%)	2 (2.6%)	127 (92.0%)
	HBV (%)	14 (17.9%)	1 (0.7%)
	HCV (%)	54 (69.2%)	10 (7.3%)
	HBV + HCV (%)	8 (10.3%)	0 (0%)

Genotyping

Genomic DNA was isolated from peripheral leukocytes by proteinase K digestion and phenol/chloroform extraction and ethanol precipitation. A multiplex polymerase chain reaction (PCR) method was used to detect the presence or absence of *GSTM1* and *GSTT1* (Katoh et al. 1996). The genotype of *GSTP1* (A to G substitution at nucleotide 313) was determined by PCR/RFLP according to Watson et al. (1998). The genetic polymorphism in the 5′-flanking region of *CYP2E1*was determined by PCR amplification followed by digestion with *RsaI*, using the method described previously (Adami et al. 1992). The dominant allele (*c1*) was sensitive to *RsaI* digestion and the *c2* allele was resistant to *RsaI* digestion. The genotypes of *ALDH2* were identified as the homozygous genotype of a normal *ALDH2* (*1/1*), the homozygous genotype of an inactive *ALDH2* (*2/2*) and the heterozygous genotype of normal and inactive *ALDH2* (*1/2*) by the method of Harada and Zhang (1993).

Statistical analysis

Statistical analysis was performed by comparing each gene polymorphism of five metabolic enzymes in HCC patients with the hospital controls. Odds ratios and 95% confidence intervals were adjusted for age and gender by multiple logistic regression analysis with the SPSS for Windows Medical Pack (SPSS Inc., Chicago). Needing to combine heterozygous genotypes (*GSTM1/T1/P1*, *CYP2E1*ALDH2) to examine the interaction between environmental and genetic factors as well as smoking or drinking status, we carried out stratification analysis of HCC risk associated with genotypes.

Results

Table 2 demonstrates the risk of HCC by drinking, smoking habits and viremias. The age- and gender-adjusted OR of heavy drinkers, who consumed alcohol above a threshold of 600,000 ml during their lifetime, was significantly higher (OR = 5.19, 95% CI 2.53–10.64) than in non-drinkers and light drinkers who consumed alcohol under 600,000 ml during their lifetime. On the other hand, there was no tendency of increased risk in the smoker strata (OR = 1.23, 95% CI 0.57–2.68). We also confirmed a strong association between viremia and HCC (OR = 805.17, 95% CI 134.37–4,824.52).

Table 2 Odds ratio of hepatocellular carcinoma by drinking, smoking and viremias

		Odds ratio (95% CI)
Drinking status	Non drinkers	1
	Drinkers	1.45 (0.81–2.60)
Alcohol consumption	1–<200,000 ml	0.31 (0.15–0.62)
	≦200,000–<600,000 ml	0.79 (0.40–1.57)
	≧600,000 ml	4.52 (2.39–8.55)
Smoking status	Non smokers	1
	Smokers	1.23 (0.56–2.67)
Smoking exposure (Blinkman-Coates index)	0–<400	1.14 (0.58–2.25)
	400≦–<800	1.09 (0.56–2.14)
	≧800	1.09 (0.56–2.15)
Viremias	Non viremias	1
	HBV or HCV	438.72 (94.69–2,032.61)

Odds ratios were adjusted for age and gender

Table 3 Relationship between *GSTM1*, *GSTT1*, *GSTP1*, *CYP2E1* and *ALDH2* genotypes and HCC

		Controls (n = 138) % (n)	Cases (n = 78) % (n)	Odds ratio (95% CI)
GSTM1	Positive genotype	50.7% (70)	61.5% (48) [a]	1
	Null genotype	49.3% (68)	38.5% (29) [a]	0.59 (0.33–1.05)
GSTT1	Positive genotype	52.2% (72)	48.7% (38) [a]	1
	Null genotype	47.8% (66)	51.3% (39) [a]	0.97 (0.57–1.76)
GSTP1	*A/A*	66.7% (92)	76.9% (60)	1
	Any *G*	33.3% (46)	23.1% (18)	0.66 (0.35–1.27)
CYP2E1	*C1/C1* homozygote	64.5% (89)	57.7% (45) [a]	1
	Any *C2*	35.5% (49)	42.3% (32) [a]	1.22 (0.68–2.17)
ALDH2	*1/1* homozygote	55.1% (76)	43.6% (34)	1
	Any *2* allele	44.9% (62)	56.4% (44)	1.54 (0.87–2.71)

Odds ratio of *GSTM1*, *GSTT1*, *GSTP1*, *CYP2E1* and *ALDH2* were adjusted for age and gender.
[a]The total number is different from the total of cases (n = 78) because it was impossible to obtain PCR products for some patients

Table 4 Odds ratio for the genotypes related to HCC by drinking or smoking status

	Non smokers	Smokers
	OR (95%CI)	OR (95%CI)
GSTM 1 null type vs. positive type	0.48 (0.16–1.48)	0.59 (0.29–1.22)
GSTT1 null type vs. positive type	1.19 (0.41–3.47)	0.93 (0.47–1.87)
GSTP1 A/A genotype vs. any *G* allele	0.50 (0.15–1.69)	0.73 (0.33–1.59)
*CYP2E1*C1/*C1* genotype vs. any *C2* allele	0.89 (0.30–2.61)	1.33 (0.65–2.73)
	Non drinkers	Drinkers
	OR (95%CI)	OR (95%CI)
CYP2E1 C1/*C1* genotype vs. any *C2* allele	2.10 (0.79–5.64)	0.85 (0.40–1.81)
ALDH2 any *2* allele vs. *1/1* genotype	0.75 (0.24–2.34)	2.53 (1.21–5.31)

ORs were adjusted for age and gender

The age- and gender-adjusted frequencies of *GSTM1*, *GSTT1*, *GSTP1*, CYP2E1 and *ALDH2* genotypes associated with HCC are shown in Table 3. There was no significant difference between controls and HCC in terms of frequency distribution of their genes. To evaluate the interaction between the genotypes, we analyzed the combination of the genes. No significant association was observed for any interaction of genes (data not shown).

Furthermore, we calculated the OR for data that was classified by smoking or drinking to evaluate the effect of the gene in combination with smoking or drinking. The summarized data and the ORs are shown in Table 4, together with the 95% confidence interval. The frequency of any *2* allele of *ALDH2* had a significant correlation with increased risk of HCC among alcohol drinkers (OR = 2.53, 95% CI 1.21–5.31). However, other genotype distributions of HCC were not significantly different from those of the controls (data not shown).

Table 5 Logistic regression analysis output

Factor	Odds ratio	95% confidence interval
CYP2E1 C1/*C1* genotype vs. any *C2* allele	5.77	1.24–27.39
ALDH2 any *2* allele vs. *1/1* genotype	9.77	1.63–58.60

Covariates were selected by using stepwise logistic regression; variable available for selection include age, gender, drinking status, smoking status, viremia, *GSTM1*, *GSTT1*, *GSTP1*, *CYP2E1* and *ALDH2* genotypes. ORs were adjusted for age, gender, drinking status and viremia

Finally, we had a multivariate analysis including viremias; variables available for selection include age, gender, drinking status, smoking status, viremia and each genotype of five enzymes (Table 5). The frequencies of *C2* alleles of *CYP2E1* (OR = 5.77, 95% CI 1.24–27.39) and *2* alleles of *ALDH2* (OR = 9.77, 95% CI

1.63–58.60) were significantly higher than those of controls (Table 5).

Discussion

We have observed the correlation between habitual alcohol drinking and the risk of HCC for many years. Our results showed that there was a significant association between heavy alcohol drinking, which is over 600,000 ml in a lifetime, and an increase in the risk of HCC: the OR for alcohol drinkers was 4.52 (95% CI 2.39–8.55) in our HCC patients (Table 2). This relationship is in agreement with most of the many previous reports on this topic (Mohamed et al. 1992; Kuper et al. 2000). This seems to be a valid finding because alcohol has been assumed to be a promoter or growth enhancer of HCC (Adami et al. 1992).

We also examined the association between tobacco smoking and the risk of HCC. Some risk excess was observed among tobacco smokers (OR = 1.23, 95% CI 0.57–2.68) compared with non-smokers, but it was not significant.

Otherwise, the data on smoking and risk of HCC are contradictory (Trichopoulos et al. 1987; Tsukuma et al. 1993; Kuper et al. 2000; Tanaka et al. 1992; Hadziyannis et al. 1995). Our data revealed that there was likely to be no positive relationship between tobacco smoking and HCC. If tobacco smoking is one of the causes of HCC, this discrepancy could be due to some biases. The first one was that the smoking histories were excessively error prone. The second was that it was impossible to distinguish between two kinds of non-smoker. One of them had never smoked in their life, and another had quit smoking, but had a past history of smoking. The last was that the alcohol habit confounded it in the present study. We need a further examination without biases such as smoking history, alcohol and viremia.

We present data on the frequency of the *ALDH2* genotype in HCC. A significant relationship between the occurrence of certain cancers and the *ALDH2* polymorphism has been reported, particularly in alcoholics (Hori et al. 1997). Other reports also indicated that the differences of *ALDH2* genotypes has no association with HCC development (Takeshita et al. 2000). However, in a multivariate analysis including the viral factor, the frequency of any *2* allele of *ALDH2* was significantly different from controls (OR = 9.77, 95%CI 1.63–58.60). Moreover, we found evidence of a significant effect of drinking depending on the difference of the genetic polymorphism of *ALDH2*. Statistically, there was an association between any *2* allele of *ALDH2* and HCC patients in habitual drinkers (OR = 2.53, 95% CI 1.12–5.31). It is likely that alcoholic liver diseases with the *ALDH2* heterozygote (*1/2*) are more severe than those with the *ALDH2* homozygote (*1/1*) (Enomoto et al. 1991), since those with the *ALDH2* heterozygote (*1/2*) would have higher internal exposure to acetaldehyde after drinking alcohol (Takeshita et al. 1997). Ohhira et al. (1996) studied primary hepatocellular carcinoma associated with alcoholic liver disease without hepatitis virus infection. In the analysis of genetic polymorphism of *ALDH2*, all of the subjects had the *ALDH2* homozygote (*1/1* or *2/2*). Otherwise, Shibata et al. (1998) showed that ORs resulting from the *ALDH2* homozygote and some accumulated amount of alcohol intake by age 40 based on community controls were statistically significant in HCC. Although it is inconsistent which is a risk factor, the homozygote or heterozygote gene, these results might imply that individual differences of *ALDH2* genotypes change the risk of HCC by alcohol consumption.

A multivariate analysis showed that an increase of risk for HCC also was found to a significant degree in the difference of *CYP2E1* genotypes (OR = 5.77, 95% CI 1.24–27.39). The rate of *CYP2E1* activity increases in the liver after alcohol induction. This means that the *c2 CYP2E1* gene increases in habitual drinkers, especially those with chronic liver disease (Ladero et al. 1996; Tsutsumi et al. 1994a, 1994b). As a result, the activation of carcinogens increases in the liver. It is possible that the CYP2E1 activity in the human liver is associated with the susceptibility of HCC. There are two different mechanisms that influence its rate of activity. One of them is the genetic functional difference between *c1* and *c2* alleles. The other depends on environmental factors, mainly ethanol or other inducers, which also frequently show a carcinogenic potential in the liver. Earlier reports have suggested the *CYP2E1* polymorphisms may play an important role in smoking-related HCC. Homozygosity for the *c1/c1* genotype significantly increased the risk of developing HCC in cigarette smokers (Yu et al. 1995). In contrast, there was no significant association between HCC risk and genotype *c1/c2* or *c2/c2* in all HCC patients (Lee et al. 1997).

In this study, the possible effects of GSTs metabolic enzymes in modulating the development of HCC were not confirmed among alcohol drinkers or tobacco smokers. Members of the GST family are important candidates for involvement in susceptibility to commonly occurring forms of cancer, because they may regulate an individual's ability to metabolize environmental carcinogens. Normal or increased GST enzyme activity or levels may protect susceptible tissues from somatic mutations in DNA by facilitating the conjugation and subsequent elimination of electrophilic carcinogens. Absent or deficient GST enzyme activity may result in poorer elimination of electrophilic carcinogens, particularly in the presence of very active electrophilic activation by phase1 enzymes. If an individual's inherited genotype at a GST locus does not permit the efficient metabolism of compounds involved in carcinogens, then that individual may be at increased cancer risk.

For example, the *GSTM1/ GSTT1* is polymorphic in humans. *GSTM1* has been shown to be polymorphic and is absent in 35–60% of individuals (Bell et al. 1993; Katoh et al. 1995). Similarly, *GSTT1* is also polymorphic and is absent in 10–65% of human populations

(Chenevix-Trench et al. 1995). The lack of GSTM1 activity is due to the inherited homozygous deletion of the genes, and *GSTM1* deficiency has been linked with risk for various cancers (Bell et al. 1993; Brockmoller et al. 1996; Rebbeck 1997). Less is known about the association between *GSTT1* and cancer risk, but persons with the *GSTT1* null type show reduced ability to detoxify metabolites of 1,3-butadiene (Pemble et al. 1994) and ethylene oxide (Wiencke et al. 1995). A report suggested that the *GSTT1* null type might be a risk modifier in the occurrence of colorectal cancer (Deakin et al. 1996). Also, the difference of *GSTM1/T1* polymorphisms may be subject to increased risk of urothelial cancer in tobacco smokers (Katoh et al. 1998).

The *GSTP1* is also widely expressed in normal epithelial tissue and is particularly abundant in the urinary, respiratory and digestive tracts, suggesting a possible role for *GSTP1* in the detoxification and elimination of toxic products in these tissues. GSTP1 is a major enzyme involved in the inactivation of carcinogens in cigarette smoke, such as benzo(a)pyrene diol epoxide and acrolein, as well as other cigarette smoke toxins. The gene is also suggested to be involved in the development of acquired resistance towards anti-cancer drugs. The *GG* genotype of *GSTP1* was significantly more frequent among patients with oral squamous cell carcinoma and lung cancer (Katoh et al. 1999; Ryberg et al. 1997).

Overall, the differences of genetic polymorphisms on GST enzymes have no association with the development of HCC, although alcohol drinking showed a significant association with it. The discrepancy between our results and previous reports could be explained by the following suggestions: first, there was a racial difference in the frequencies of each genotype (Kato et al. 1992); for another reason, the risk of genetic polymorphism to HCC could be overshadowed by the great etiologic role of HBV and HCV viremia in the development of HCC (Tsukuma et al. 1990; Yu et al. 1994; Donato et al. 1998). However, HBV positive patients had significantly lower GST activity than those who were HBV negative (Zhou et al. 1997). These results suggest that the risk of HCC is not only associated with *GST* polymorphism, but also GST activity.

In conclusion, we found that there was a significant association between *CYP2E1* and *ALDH2* polymorphisms with the interaction of alcohol and the risk of HCC in Japanese people.

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References

Adami HO, Hsing AW, McLaughlin JK, Trichopoulos D, Hacker D, Ekbom A, Persson I(1992) Alcoholism and liver cirrhosis in the etiology of primary liver cancer. Int J Cancer 51:898–902

Bell DA, Taylor JA, Paulson DF, Robertson CN, Mohler JL, Lucier GW(1993) Genetic risk and carcinogen exposure: a common inherited defect of the carcinogen-metabolism gene glutathione S-transferase M1 (GSTM1) that increases susceptibility to bladder cancer. J Natl Cancer Inst 85:1159–1164

Brockmoller J, Cascrbi I, Kerb R, Roots I(1996) Combined analysis of inherited polymorphisms in arylamine N-acetyltransferase 2, glutathione S-transferases M1 and T1; microsomal epoxide hydolase, and cytochrome P450 enzymes as modulators of bladder cancer risk. Cancer Res 56:3915–3925

Chen CJ, Liang KY, Chang AS, Chang YC, Lu SN, Liaw YF,-Chang WY, Sheen MC, Lin TM(1991) Effects of hepatitis B virus, alcohol drinking, cigarette smoking and familiar tendency on hepatocellular carcinoma. Hepatology 13:398–406

Chenevix-Trench G, Young J, Coggan M, Board P(1995) Glutathione S-transferase M1 and T1 polymorphisms: susceptibility to colon cancer and age onset. Carcinogenesis 16:1655–1657

Deakin M, Elder J, Hendrickse C (1996) Glutathione S-transferase GSTT1 genotypes and susceptibility to cancer: Studies of interactions with GSTM1 in lung, oral, gastric and colorectal cancers. Carcinogenesis 17:881–884

Donato F, Boffetta P, Puoti M(1998) A meta-analysis of epidemiological studies on the combined effect of Hepatitis B and C virus infections in causing hepatocellular carcinoma. Int J Cancer 75:347–354

Enomoto N, Takase S, Takada N, Takada A (1991) Alcohol liver disease in heterozygotes of mutant and normal aldehyde dehydrogenase-2 genes. Hepatology 13:1071–1075

Guengerich FP (1991) Oxidation of toxic and carcinogenic chemicals by human cytochrome P-450 enzyme. Chem Res Toxicol 4:391–407

Hadziyannis S, Tabor E, Kaklamani E, Tzonou A, Stuver S, Tassopoulos N, Mueller N, Trichopoulos D (1995) A case-control study of hepatitis B and C virus infections in the etiology of hepatocellular carcinoma. Int J Cancer 60:627–631

Harada S, Zhang S (1993) New strategy for detection of ALDH2 mutant. Alchol Alchohol [Suppl] 1A:11–13

Hori H, Kawano T, Endo M, Yuasa Y (1997) Genetic polymorphisms of tobacco- and alcohol-related metabolizing enzymes and human esophageal squamous cell carcinoma susceptibility. J Clin Gastroenterol 25:568–575

Kato S, Shields PG, Caporaso NE, Hoover RN, Trump BF, Sugimura H, Weston A, Harris CC (1992) Cytochrome P450IIE1 genetic polymorphisms, racial variation, and lung cancer risk. Cancer Res 52:6712–6715

Katoh T, Inatomi H, Nagaoka A, Sugita A (1995) Cytochrome P450 1A1 gene polymorphism and homozygous deletion of the glutathione S-transferase M1 gene in urothelial cancer patients. Carcinogenesis 16:655–657

Katoh T, Nagata N, Kuroda Y, Itoh H, Kawahara A, Kuroki N, Ookuma R, Bell DA (1996) Glutathione S-transferase M1 (GSTM1) and T1 (GSTT1) genetic polymorphism and susceptibility to gastric and colorectal adenocarcinoma. Carcinogenesis 17:1855–1859

Katoh T, Inatomi H, Kim H, Yang M, Matsumoto T, Kawamoto T (1998) Effect of glutathione S-transferase(GST)M1 and GSTT1 genotypes on urothelial cancer risk. Cancer Letters 132:147–152

Katoh T, Kaneko S, Takasawa S, Nagata N, Inatomi H, Ikemura K, Itoh H, Matsumoto T, Kawamoto T, Bell DA (1999) Human glutathione S-transferase P1 polymorphism and susceptibility to smoking related epithelial cancer; oral, lung, gastric, colorectal and urothelial cancer. Pharmacogenetics 9:165–169

Kuper H, Tzonou A, Kaklamani E, Hsieh CC, Lagiou P, Adami HO, Trichopolous D, Stuver SO (2000) Tobacco smoking, alcohol consumption and their interaction in the causation of hepatocellular carcinoma. Int J Cancer 85:498–502

Ladero JM, Agundez JAG, Rodriguez-Lescure A,Diaz-Rubio M, Benitez J (1996) Rsalpolymorphism at the cytochrome P450 2E1 locus and risk of hepatocellular carcinoma. Gut 39:330–333

Lee HS, Yoon JH, Kamimura S, Iwata K, Watanabe H, Kim CY (1997) Lack of association of cytochrome P450 2E1 genetic polymorphisms with the risk of human hepatocellular carcinoma. Int J Cancer 71:737–740

Mannervik B, Danielson UH (1988) Glutathione transferases structure and catalytic activity. CRC Crit Rev Biochem 23:283–337

Mohamed AE, Kew MC, Groeneveld HT (1992) Alcohol consumption as a risk factor for hepatocellular carcinoma in urban southern Africa Blacks. Int J Cancer 51:537–541

Ohhira M, Fujimoto Y, Matsumoto A, Ohtake T, Ono M, Kohgo Y(1996) Hepatocellular carcinoma associated with alcoholic liver disease: a clinicopathological study and genetic polymorphism of aldehyde dehydrogenase 2. Alcohol Clin Exp Res 20 [Suppl 9):378A–382A

Pemble S, Schroeder KR, Spencer SR, Meyer DJ, Hallier E, Bolt HM, Ketterer B, Taylor JB (1994) Human glutathione S-transferase theta (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. Biochem J 300 (Pt 1):271–276

Rebbeck TR (1997) Molecular epidemiology of the human glutathione S-transferase genotypes GSTM1 and GSTT1 in cancer susceptibility. Cancer Epidemiol Biomarkers Prevent 6:733–743

Ross RK, Yuan JM, Yu MC, Wogan GN, Qian GS, Tu JT, Groopman JD, Gao YT, Henderson BE (1992) Urinary aflatoxin biomarkers and risk of hepatocellular carcinoma. Lancet 339:943–946

Ruiz J, Sangro B, Cuende JI, Beloqui O, Riezu-Boj JI, Herrero JI, Prieto J (1992) Hepatitis B and C viral infections in patients with hepatocellular carcinoma. Hepatology 16:637–641

Ryberg D, Skaug V, Hewer A, Phillips DH, Harries LW, Wolf CR, Øgreid D, Ulvik A, Vu P, Haugen A (1997) Genotypes of glutathione transferase M1 and P1 and their significance for lung DNA adduct levels and cancer risk. Carcinogenesis 18: 1285–1289

Shibata A, Fukuda K, Nishiyori A, Ogimoto I, Sakata R, Tanikawa K (1998) A case-control study on male hepatocellular carcinoma based on hospital and community controls. J. Epidemiology 8:1–5

Takeshita T, Kawai T, Morimoto K (1997) Elevated levels of hemoglobin-associated acetaldehyde related to alcohol drinking *in the atypical* genotype of low K_m aldehyde dehydrogenase. Cancer Res 57:1241–1243

Takeshita T, Yang X, Inoue Y, Sato S, Morimoto K (2000) Relationship between alcohol drinking, ADH2 and ALDH2 genotype, and risk for hepatocellular carcinoma in Japanese. Cancer Letters 149:69–76

Tanaka K, Hirohata T, Takeshita S, Hirohata I, Koga S, Sugimachi K, Kanematsu T, Ohryohji F, Ishibashi H (1992) Hepatitis B virus, cigarette smoking and alcohol consumption in the development of hepatocellular carcinoma.: a case control study in Fukuoka, Japan. Int J Cancer 51:509–514

The Liver Cancer Study Group of Japan(2000) Survey and follow-up study of primary liver cancer in Japan Report 14 (in japanese). Acta Hepatol Japonica 41:799–811

Trichopoulos D, Day NE, Kaklamani E, Tzonou A, Munoz N, Zavitsanos X, Koumantaki Y, Trichopoulou A (1987) Hepatitis B virus, tobacco smoking and ethanol consumption in the etiology of hepatocellular carcinoma. Int J Cancer 39: 45–49

Tsai JF, Chang WY, Jeng JE, Ho MS,Lin ZY, Tsai JH (1994) Hepatitis B and C virus infection as risk factors for liver cirrhosis and cirrhotic hepatocellular carcinoma. A case-control study. Liver 14:98–102

Tsukuma H, Hiyama T, Oshima A, Sobue T, Fujimoto I, Kasugai H, Kojima J, Sasaki Y, Imaoka S, Horiuchi N, Okuda S (1990) A case-control study of hepatocellular carcinoma in Osaka, Japan. Int J Cancer 45:231–236

Tsukuma H, Hiyama T, Tanaka S, Nakao M, Yabuuchi T, Kitamura T, Nakanishi K, Fujimoto I, Inoue A, Yamazaki H, Kawashima T (1993) Risk factors for hepatocellular carcinoma among patients with chronic liver disease. N Engl J Med 328:1797–1801

Tsutsumi M, Takada A, Wang JS(1994a) Genetic polymorphisms of cytochrome P450 2E1 related to the development of alcoholic liver disease. Gastroenterology 107:1430–1435

Tsutsumi M, Wang JS, Takase S, Takada A (1994b) Hepatic messenger RNA contents of cytochrome 450 2E1 in patients with different P450 2E1 genotypes. Alchol Alcohol *29* [Suppl 1]:29–32

Watson MA, Stewart RK, Smith GBJ, Massey TE,BellDA (1998) Human glutathione S-transferase P1 polymorphisms: relationship to lung tissue enzyme activity and population frequency distribution. Carcinogenesis 19:275–280

Wiencke JK, Pemble S, Ketterer B, Kelsey KT (1995) Gene deletion of glutathione transferase theta1: correlation with induced genetic damage and potential role in endogenous mutagenesis. Cancer Epidemiol Biomarkers Prev 4:253–259

Yu MW, Chen CJ, Luo JC, Brandt-Rauf PW, Carney WP, Santella RM (1994) Correlations of chronic hepatitis B virus infection and cigarette smoking with elevated expression of neu oncoprotein in the development of hepatocellular carcinoma. Cancer Res 54:5106–5110

Yu MW, Gladek-Yarborough A, Chiamprasert S, Santella RM, Liaw YF, Chen CJ (1995) Cytochrome P450 2E1 and glutathione S-transferase M1 polymorphisms and susceptibility to hepatocellular carcinoma. Gastroenterology 109:1266–1273

Zhou T, Evans AA, London WT, Xia X, Zou H, Shen F, Clapper ML (1997) Glutathione S-transferase expression in hepatitis B virus-associated human hepatocellular carcinogenesis. Cancer Res 57:2749–2753

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ORIGINAL PAPER

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Cytochrome P450 (CYP) 1A2, sulfotransferase (SULT) 1A1, and N-acetyltransferase (NAT) 2 polymorphisms and susceptibility to urothelial cancer

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Abstract *Purpose*: Arylamines are suspected to be the primary causative agent of urothelial cancer in tobacco smoke. In the human liver, arylamines are N-hydroxylated by a cytochrome P450 (CYP)1A2-catalyzed reaction, which produces a substrate for O-esterification that can be catalyzed by N-acetyltransferases (NAT) or sulfotransferases (SULT). Recently, several polymorphisms of CYP1A2, SULT1A1, and NAT2 that affect their activities have been reported. *Methods*: In this study, 306 Japanese patients with urothelial transitional cell carcinoma and 306 healthy controls were compared for frequencies of CYP1A2, SULT1A1, and NAT2 genotypes. *Results*: The frequencies of NAT2 intermediate or slow acetylator genotype were significantly higher in the urothelial cancer patients than in the healthy control subjects [odds ratio (OR) = 1.49, 95% confidence interval (95% CI) 1.06–2.09, OR = 3.23, 95% CI 1.72–6.08, respectively]. Stratifying by amount of smoking, among subjects who consumed >33.5 pack-years and carried the SULT1A1 *1/*1 or NAT2 slow acetylator genotype, the OR was 1.73 (95% CI 1.01–2.97) whereas it was 7.31 (95% CI 1.90–28.05) in non-smokers who carried the homozygous wild genotype, respectively. The relationships between CYP1A2, SULT1A1, and NAT2 polymorphisms and clinical findings including tumor differentiation, stage, and recurrence rate were analyzed. Only associations between NAT2 genotype and pathological findings were admitted, and the higher OR of NAT2 intermediate and slow acetylator genotype was more likely to present to a low-grade tumor (G1) among heavy-smokers. *Conclusions*: Our results suggest that SULT1A1 *1/*1 and NAT2 slow acetylator genotypes might modulate the effect of carcinogenic arylamines contained in tobacco smoke, and that the modulation of NAT2 intermediate and slow acetylator genotype has a tendency to present a higher risk for highly differentiated tumors among heavy-smokers.

Keywords CYP1A2 · SULT1A1 · NAT2 · Polymorphism · Urothelial cancer

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Introduction

It has been recognized that tobacco smoke is the main cause of human urothelial cancer (Silverman et al. 1992), and carcinogenic arylamines, including 4-aminobiphenyl (ABP), in tobacco smoke may represent one of the leading causes of urothelial cancer (Bartsch et al. 1993; Cohen et al. 2000). Studies of molecular epidemiology have suggested that the excess of urothelial cancer in smokers could be attributed to arylamines (Bartsch et al. 1990; Vineis et al. 1996). Arylamines are not carcinogenic in the parent form, but require metabolic activation to reactive electrophiles in order to exert their carcinogenic effects (Grant et al. 1997; Guengerich 1992). These can proceed via a two-step pathway involving cytochrome P450 (CYP) 1A2-catalyzed N-hydroxylation (Crofts et al. 1998; Williams et al. 1998) followed by an O-esterification step catalyzed by N-acetyltransferase(s) (NATs) and/or sulfotransferase(s) (SULTs) (Grant et al. 1997; Lewis et al. 1998; Yamazoe et al. 1999). As a consequence of these reactions, in urothelial cancers, the arylnitrenium ions generated from N-hydroxylamines are believed to be the ultimate reactive intermediates responsible for carcinogenic activity (Kadlubar et al. 1977).

Enzyme polymorphisms that have been previously associated with arylamine metabolic pathways include

CYP1A2 and NAT2 (Kadlubar 1994). Accordingly, recent NAT2 genotype studies show associations with urinary bladder cancer that are highest for particular NAT2 alleles associated with slow acetylator phenotypes, consistent with earlier phenotypic studies (Cartwright et al. 1982). Some meta-analyses of case-control studies that investigated associations between NAT2 and bladder cancer were reported and a weak interaction between smoking and NAT2 slow acetylation was observed (Green et al. 2000; Johns and Houlston 2000; Marcus et al. 2000a; Marcus et al. 2000b). Evidence linking elevated CYP1A2 activity to increased bladder cancer risk has been reported (Kaderlik and Kadlubar 1995; Lee et al. 1994). Several polymorphisms of CYP1A2 have been reported (Chida et al. 1999; Nakajima et al. 1999; Sachse et al. 1999; Chevalier et al. 2001), and a single nucleotide change from guanine (CYP1A2*1A) to adenine (CYP1A2*1C) at position −2964 in the 5'-flanking region, that caused a significant decrease of CYP1A2 activity, was reported by Nakajima et al (Nakajima et al. 1999). Therefore, categorization based on a combination of both NAT2 and CYP1A2 might be a more discerning biomarker for identification of high-risk individuals for urothelial cancer. Moreover, molecular dosimetry studies indicate that the slow NAT2/rapid CYP1A2 individual has the highest level of ABP-hemoglobin adducts and, conversely, the lowest level of ABP-hemoglobin adducts, as observed in individuals who are rapid NAT2/slow CYP1A2 (Bartsch et al. 1993; Kaderlik and Kadlubar 1995). Thus, those individuals who are rapid for CYP1A2 and slow for hepatic NAT2 might be at a higher risk for arylamine-induced urothelial cancer compared with those who are slow for CYP1A2 and rapid for NAT2 (Kloth et al. 1994).

Recently, single amino acid substitutions (^{213}Arg/His and ^{213}Met/Val) in polymorphic human phenol-sulfating phenol sulfotransferase were found (Raftogianis et al. 1997; Raftogianis et al. 1999). In a Japanese population, allele frequencies of ^{213}Arg (SULT1A1*1) and ^{213}His (SULT1A1*2) were 83.2% and 16.8%, respectively, but the ^{213}Val allele (SULT1A1*3) was not found (Ozawa et al. 1999). The SULT1A1*2 allele was associated with reduced sulfotransferase activity and thermostability in platelets (Raftogianis et al. 1997; Ozawa et al. 1998).

Given the role of CYP1A2, SULT1A1, and NAT2 in the metabolism of arylamines, in this paper we hypothesized that the rapid genotype of CYP1A2 and the slow genotypes of SULT1A1 or NAT2 might cause an increased risk of urothelial cancer among smokers. We previously reported the association of SULT1A1 and NAT2 genotypes with urothelial cancer in a preliminary case-control study, and the metabolic activation of 4-aminobiphenyl by recombinant STLT1A1*1 and SULT1A1*2 (Ozawa et al. 2002). We also found that low activity alleles of NAT2 were overall high-risk alleles (OR = 2.11, 95% CI 1.08–4.26), and recombinant SULT1A1*1 enzyme showed a tendency of catalyzing higher in vitro 3'-phosphoadenosine 5'-phosphosulfate-dependent DNA adduct formation than SULT1A1*2. In this work, we studied the CYP1A2, SULT1A1, and NAT2 genotypes with urothelial cancer in relation to smoking status among larger study populations. Additionally, we assessed CYP1A2, SULT1A1, and NAT2 genotypes in relation to clinical findings associated with the outcome. These include tumor differentiation, stage, and recurrence. We have demonstrated associations between SULT1A1, NAT2 genotype, and urothelial cancer susceptibility that are consistent with the smoking amount-related association with the SULT1A1*1/*1 genotype and NAT2 slow acetylator genotype. As clinical parameters, NAT2 intermediate and slow acetylator genotype tends to be more frequent among heavy-smokers with well-differentiated tumors, compared with moderately and poorly differentiated tumors.

Materials and methods

Subjects

The case groups were comprised of 306 patients with urothelial cancer (bladder $n = 236$, renal pelvis and ureter $n = 35$, overlap $n = 35$) (242 men, 64 women; mean age 69.4 years) in Kitakyushu city and Miyazaki city, Japan. No statistically significant differences were found between patients in Kitakyushu and Miyazaki for age, gender, and smoking habit. The patients were treated at the University of Occupational and Environmental Health Hospital ($n = 170$) and Miyazaki Medical College Hospital ($n = 136$), and had been histologically diagnosed for urothelial transitional cell carcinoma. Tumors were graded according to the criteria of the Japanese Urological Association (Japanese Urological Association and Japanese Society of Pathology 2001) and were staged according to the TNM classification system (Sobin and Wittekind 1997). Thirty-two patients (10.5%) had well-differentiated disease (G1), 138 (45.1%) had moderately differentiated (G2) and 136 (44.4%) had poorly differentiated disease (G3). We used the term 'superficial tumors' to refer to those which were limited to the mucosa (pTis, pTa) or the lamina propria (pT1); and 'invasive tumors' to refer to those which had invaded the muscle layer (pT2, pT3) or deeper (pT4). One hundred and eighty-four patients (60.1%) had superficial tumors, 117 (38.2%) had invasive tumors, and five (1.6%) had unknown stage tumors. A total of 306 controls, frequency-matched with cases for age (±5 years) and gender, were selected from the people who visited a medical institution located in Kitakyushu city for a general health check-up (242 men, 64 women, mean age 66.9 years). All study subjects completed a questionnaire administered by a trained interviewer covering medical, residential, and occupational exposures as well as smoking history. Smoking history was summarized as the total amount of cigarettes consumed during their lifetime up until the time of the interview. The amount of tobacco smoke exposure was calculated as pack-years [1 pack (20 cigarettes)/day × years of smoking]. The median value of smoking amounts among controls who had ever smoked was 33.5 pack-years. We used the term 'light-smokers' to refer to subjects who consumed ≦33.5 pack-years; and 'heavy-smokers' to refer to subjects who consumed >33.5 pack-years. All participants were given an explanation of the nature of the study, and informed consent was obtained. This study was approved by the ethics committees of the University of Occupational and Environmental Health and Miyazaki Medical College.

Genotyping

Genomic DNA was isolated from peripheral leukocytes by proteinase K digestion and phenol/chloroform extraction. The genetic polymorphism in the 5'-flanking region of CYP1A2 was determined

by polymerase chain reaction (PCR) amplification followed by digestion with DdeI and BslI using the method described previously (Nakajima et al. 1999). Genotyping of SULT1A1 for polymorphism at codon 213 (Arg/His) was performed according to the previously published methods of PCR-restriction fragment length polymorphism (RFLP) (Ozawa et al. 1999). A NAT2 high activity allele, NAT2*4, and NAT2 low activity alleles, NAT2*5B, NAT2*6A, and NAT2*7B, were determined according to a previously published PCR-RFLP method (Bell et al. 1993). A NAT2 variant allele (NAT2*14), which is very rare (less than 1% frequency) in the Japanese population, was not considered (Katoh et al. 1998). All of the genotyping was performed by laboratory personnel unaware of case-control status, and blinded quality control samples were inserted to validate genotyping identification procedures; concordance for blinded samples was 100%. None of the genetic polymorphisms departed significantly from the Hardy-Weinberg equilibrium.

Statistical analysis

Crude ORs and 95% CIs were calculated for CYP1A2, SULT1A1, and NAT2 genotypes. Odds ratios were adjusted for age, gender, and smoking status (ever and never-smokers), using multiple logistic regression analysis by SPSS Medical Pack for Windows. To examine the interaction between environmental and genetic factors, stratification analysis of urothelial cancer risk associated with CYP1A2, SULT1A1, and NAT2 genotypes was carried out for smoking amounts. All statistical tests were based on two-tailed probability. The Cochran-Armitage method was used for a trend analysis. A P value less than 0.05 (two-tail) was considered to be statistically significant.

Results

The frequencies of CYP1A2, SULT1A1, and NAT2 genotypes associated with urothelial cancer are shown in Table 1. There were no associations between CYP1A2 or SULT1A1 genotypes and urothelial cancer adjusted for age, gender, and smoking status. The NAT2 genotypes were categorized as homozygous mutant (slow), heterozygous wild-mutant (intermediate), and homozygous wild type (rapid). Compared with those who had the rapid acetylator genotype of NAT2, those who had intermediate or slow acetylator genotypes had an increased urothelial cancer risk after adjustment for age, gender, and smoking status. Those who had the intermediate or slow genotypes had an OR of 1.49 (95% CI 1.06–2.09) or 3.23 (95% CI 1.72–6.08) compared with those who had a rapid genotype, respectively, and these results provided evidence of a gene dosage effect (Cochran-Armitage test, $P=0.0001$).

In order to check the effect of the gene in combination with smoking, we calculated the OR for data that were classified by smoking status and cumulative cigarette dose (pack-years) and by gene genotypes (Table 2). Among non-smokers, there were no significant differences between CYP1A2, SULT1A1, and NAT2 wild genotypes and these variant genotypes. When the OR was investigated within the strata of cumulative cigarette dose, joint effects of tobacco smoking and SULT *1/*1 or NAT2 slow acetylator genotype were observed only among heavy-smokers. Among subjects who consumed >33.5 pack-years and carried the SULT1A1 *1/*1 or NAT2 slow acetylator genotype, the OR was 1.73 (95% CI 1.01–2.97) whereas it was 7.31 (95% CI 1.90–28.05) in non-smokers who carried the wild genotype as a reference, respectively.

The relationships between CYP1A2, SULT1A1, and NAT2 polymorphisms and pathological findings including differentiation and stage were analyzed. No statistically significant changes were observed when the CYP1A2 and SULT1A1 genotypes were examined relative to tumor differentiation or stage (data not shown). Table 3 shows the OR of developing urothelial cancer for NAT2 genotypes subdivided according to tumor differentiation and smoking status. Among non-smokers, there was no significant difference between NAT2 genotypes and tumor differentiation. Among light smokers, the OR of G1 patients with NAT2 intermediate and slow acetylator genotype was 4.16 (95% CI 0.81–21.45) compared to controls, but not significant.

Table 1 Relationship between CYP1A2, SULT1A1, and NAT2 genotypes and urothelial cancer

Genotype	Patients ($n=306$) % (n)	Controls ($n=306$) % (n)	Crude OR (95% CI)	OR[a] (95% CI)
CYP1A2 genotype				
*1A/*1A	59.8% (183)	58.5% (179)	1	1
*1A/*1C	34.0% (104)	36.9% (113)	0.90 (0.64–1.26)	0.89 (0.64–1.25)
*1C/*1C	6.2% (19)	4.6% (14)	1.33 (0.65–2.73)	1.31 (0.64–2.70)
*1A/*1C + *1C/*1C	40.2% (123)	41.5% (127)	0.95 (0.69–1.31)	0.93 (0.67–1.29)
SULT1A1 genotype				
*1/*1	77.8% (238)	79.1% (242)	1	1
*1/*2	20.3% (62)	19.6% (60)	1.05 (0.71–1.56)	1.05 (0.70–1.57)
*2/*2	2.0% (6)	1.3% (4)	1.53 (0.43–5.47)	1.74 (0.47–6.39)
*1/*2 + *2/*2	22.2% (68)	20.9% (64)	1.08 (0.74–1.59)	1.09 (0.74–1.61)
NAT2 genotype[b]				
Rapid acetylator	42.8% (131)	55.6% (170)	1	1
Intermediate acetylator	44.4% (136)	39.2% (120)	1.47 (1.05–2.06)[c]	1.49 (1.06–2.09)[c]
Slow acetylator	12.7% (39)	5.2% (16)	3.16 (1.69–5.91)[d]	3.23 (1.72–6.08)[d]
Intermediate + slow acetylator	57.2% (175)	44.4% (136)	1.67 (1.21–2.30)[e]	1.69 (1.23–2.34)[e]

[a]Odds ratios were adjusted for age, gender, and smoking status
[b]Trend analysis was carried out on the frequency of NAT2 genotype among urothelial patients and controls; $P=0.0001$
[c]$P<0.05$
[d]$P<0.001$
[e]$P<0.005$

Table 2 Odds ratio of developing urothelial cancer for CYP1A2, SULT1A1, and NAT2 genotypes stratified by smoking amounts

Genotype	Non-smokers			Smokers					
				≤ 33.5 pack-years			> 33.5 pack-years		
	No.[a]	Crude OR (95%CI)	OR[b] (95%CI)	No.[a]	Crude OR (95%CI)	OR[b] (95%CI)	No.[a]	Crude OR (95%CI)	OR[b] (95%CI)
All	94/98	1[c]	1[c]	83/104	0.83 (0.56–1.25)	0.88 (0.56–1.40)	129/104	1.29 (0.88–1.90)	1.42 (0.88–2.28)
CYP1A2									
*1A/*1A	60/59	1[c]	1[c]	46/60	0.75 (0.45–1.28)	0.75 (0.41–1.36)	77/60	1.26 (0.77–2.07)	1.30 (0.72–2.36)
*1A/*1C	30/34	0.87 (0.47–1.59)	0.80 (0.43–1.49)	30/40	0.74 (0.41–1.34)	0.71 (0.37–1.37)	44/39	1.11 (0.63–1.95)	1.04 (0.53–2.05)
*1C/*1C	4/5	0.79 (0.20–3.07)	0.76 (0.19–3.03)	7/4	1.72 (0.48–6.19)	1.69 (0.44–6.43)	8/5	1.57 (0.49–5.09)	1.49 (0.43–5.16)
SULT1A1									
*1/*1	71/77	1[c]	1[c]	63/87	0.79 (0.50–1.24)	0.91 (0.54–1.52)	104/78	1.45 (0.94–2.24)	1.73 (1.01–2.97)[d]
*1/*2 + *2/*2	23/21	1.19 (0.61–2.33)	1.23 (0.62–2.44)	20/17	1.28 (0.62–2.63)	1.46 (0.65–3.26)	25/26	1.04 (0.55–1.97)	1.25 (0.60–2.62)
NAT2									
Rapid acetylator	44/51	1[c]	1[c]	39/59	0.77 (0.43–1.36)	0.74 (0.39–1.41)	48/60	0.93 (0.53–1.61)	0.93 (0.48–1.79)
Intermediate acetylator	38/40	1.10 (0.60–2.01)	1.09 (0.59–2.00)	37/39	1.10 (0.60–2.01)	1.10 (0.57–2.14)	61/41	1.72 (0.98–3.03)	1.70 (0.85–3.40)
Slow acetylator	12/7	1.99 (0.72–5.49)	1.97 (0.71–5.48)	7/6	1.35 (0.42–4.33)	1.29 (0.38–4.42)	20/3	7.72 (2.15–27.72)[e]	7.31 (1.90–28.05)[e]

[a]Number of cases/number of controls
[b]Odds ratios were adjusted for age and gender
[c]Reference category
[d]$P < 0.05$
[e]$P < 0.005$

Among heavy-smokers, the OR of G1 patients who carried NAT2 intermediate and slow acetylator genotype was 6.21 (95% CI 1.26–30.59) when compared to the controls. Similarly, among heavy-smokers, the OR of G2 or G3 patients who carried NAT2 intermediate and slow acetylator genotype was 1.97 (95% CI 1.01–3.85) or 2.53 (95% CI 1.31–4.88), respectively. Table 4 shows the OR of developing urothelial cancer for NAT2 genotypes subdivided according to tumor stage and smoking status. A significant association was not found between NAT2 genotypes and tumor stage among non-smokers and light-smokers. Among heavy-smokers, the OR of superficial tumor or invasive tumor patients who carried NAT2 intermediate and slow acetylator genotype was 2.54 (95% CI 1.32–4.89) or 2.45 (95% CI 1.26–4.73) when compared to the controls. Furthermore we analyzed the interaction ORs between NAT2 genotypes and pathological findings among only case groups subdivided by smoking status (never, ever, light and heavy-smokers) (data not shown). A significant interaction of NAT2 intermediate and slow acetylator genotype was found only between G1 tumor patients and G2 tumor patients among ever-smokers ($P = 0.03$).

We analyzed the associations between CYP1A2, SULT1A1, and NAT2 polymorphisms and the recurrence rate of superficial tumor patients ($n = 142$), who had been treated with bladder reserving operation for the first time and been followed for more than 1 year after operations (mean follow-up, 69.8 months). However, no significant association between CYP1A2, SULT1A1, and NAT2 polymorphisms and recurrence rate of superficial tumor patients was found (data not shown).

Discussion

Recently, several polymorphisms of CYP1A2 have been reported (Chida et al. 1999; Nakajima et al. 1999; Sachse et al. 1999; Chevalier et al. 2001), and two variant alleles which affect CYP1A2 activity were reported. One variant allele was a point mutation from guanine (CYP1A2*1A) to adenine (CYP1A2*1C) at position −2964 in the gene, which caused a significant decrease in CYP1A2 activity (Nakajima et al. 1999). Another variant allele was a C → A transversion (CYP1A2*1F) in intron 1 at position 734 downstream of the first transcribed nucleotide, which has recently been associated with increased CYP1A2 inducibility (Sachse et al. 1999).

In this study, we did not find any significant association between CYP1A2*1C polymorphisms and urothelial cancer. Additional polymorphic variants could be implicated. Moreover, the relationship between genotype and phenotype may be complex and will have to be explored in detail.

Different alleles of SULT1A1 were reported as *2 (213Arg → His), *3 (223Met → Val) and *4 (37Arg → Gln) (Raftogianis et al. 1997; Raftogianis et al. 1999). In Oriental populations, the frequency of

Table 3 Relationship between NAT2 genotypes and pathological differentiation of urothelial cancer

Smoking status		NAT2 genotype	
		(Intermediate and slow) vs rapid	
		Crude OR[a] (95%CI)	OR[a,b](95%CI)
Nonsmokers			
	Controls ($n=98$)	1	1
	Differentiation		
	G1 ($n=11$)	1.90 (0.52–6.90)	1.91 (0.53–6.98)
	G2 ($n=42$)	1.19 (0.58–2.46)	1.20 (0.58–2.49)
	G3 ($n=41$)	1.14 (0.55–2.36)	1.15 (0.55–2.40)
Light-smokers			
≤ 33.5 pack-years	Controls ($n=104$)	1	1
	Differentiation		
	G1 ($n=9$)	4.59 (0.91–23.16)	4.16 (0.81–21.45)
	G2 ($n=40$)	1.19 (0.57–2.47)	1.23 (0.59–2.56)
	G3 ($n=34$)	1.48 (0.68–3.21)	1.45 (0.67–3.16)
Heavy-smokers			
> 33.5 pack-years	Controls ($n=104$)	1	1
	Differentiation		
	G1 ($n=12$)	4.09 (1.05–15.99)[c]	6.21 (1.26–30.59)[c]
	G2 ($n=56$)	1.82 (0.94–3.51)	1.97 (1.01–3.85)[c]
	G3 ($n=61$)	2.60 (1.35–5.01)[d]	2.53 (1.31–4.88)[d]

[a]A combination of NAT2 rapid acetylator genotype and controls was used as a referent group to calculate the OR and 95%CI of urothelial patients subdivided according to tumor differentiation
[b]ORs were adjusted for age and gender
[c]$P<0.05$
[d]$P<0.005$

Table 4 Relationship between NAT2 genotypes and pathological stage of urothelial cancer

Smoking status		NAT2 genotype	
		(Intermediate and slow) vs Rapid	
		Crude OR[a] (95% CI)	OR[a,b] (95%CI)
Nonsmokers			
	Controls ($n=98$)	1	1
	Stage		
	Superficial ($n=59$)	1.12 (0.59–2.14)	1.12 (0.59–2.15)
	Invasive ($n=32$)	1.23 (0.55–2.74)	1.28 (0.57–2.90)
Light-smokers			
≤ 33.5 pack-years	Controls ($n=104$)	1	1
	Stage		
	Superficial ($n=59$)	1.45 (0.76–2.76)	1.45 (0.76–2.78)
	Invasive ($n=24$)	1.55 (0.64–3.78)	1.55 (0.63–3.78)
Heavy-smokers			
> 33.5 pack-years	Controls ($n=104$)	1	1
	Stage		
	Superficial ($n=66$)	2.24 (1.19–4.21)[c]	2.54 (1.32–4.89)[d]
	Invasive ($n=61$)	2.42 (1.26–4.64)[d]	2.45 (1.26–4.73)[d]

[a]Combination of NAT2 rapid acetylator genotype and controls was used as a referent group to calculate the OR and 95%CI of urothelial patients subdivided according to tumor stage
[b]OR were adjusted for age and gender
[c]$P<0.05$.
[d]$P<0.01$

the SULT1A1*2 allele was 0.16–0.33 in previous studies (Ozawa et al. 1999; Carlini et al. 2001) and 0.11 in our controls. The variant SULT1A1*2 was associated with low SULT1A1 activity and thermostability (Ozawa et al. 1999). There are several reports which studied the association between SULT1A1 genotypes and cancer susceptibility. Two studies investigating the association between breast cancer and SULT1A1 genotype were reported. One study reported no effect of SULT1A1 genotype on the risk of breast cancer (Seth et al. 2000); however, another study reported a borderline association between SULT1A1*2/*2 genotype and breast cancer risk (OR = 1.80, 95% CI 1.0–3.2) (Zheng et al. 2001). It was also reported that the SULT1A1*1/*2 or *2/*2 genotype was associated with moderately elevated risk of lung cancer after adjusting for age, sex, and smoking status (OR = 1.41, 95% CI 1.04–1.91) (Wang et al. 2002). Furthermore, the risk was significantly higher in current smokers (OR = 1.74, 95% CI 1.08–2.29) and heavy smokers (OR = 1.45, 95% CI 1.05–2.00). These results of positive association between SULT1A1 polymorphism and cancer susceptibility suggested that

SULT enzymes are involved in numerous detoxification pathways and are responsible for metabolizing a wide range of procarcinogens to less reactive substrates. Therefore, reduced SULT1A1 activity and thermostability conferred by the polymorphism may result in inefficient metabolism and excretion of procarcinogens, which may cause DNA damage. In our study, both the urothelial cancer patients and the controls demonstrated similar frequencies of the SULT1A1*1/*2 or *2/*2 genotype. However, stratifying by amounts of smoking among subjects who consumed >33.5 pack-years and carried the SULT1A1*1/*1 genotype, the OR was significantly higher with non-smokers (OR = 1.73, 95% CI 1.01–2.97), but not among subjects who consumed >33.5 pack-years and carried the SULT1A1*1/*2 and *2/*2 genotype (OR = 1.25, 95% CI 0.60–2.62). This result is not consistent with previous results (Zheng et al. 2001; Wang et al. 2002) and the reason for this discrepancy is unknown at the present. However, a rational hypothesis can be invoked to explain this finding. SULT1A1 protein is found almost ubiquitously in human tissues (Gilissen et al. 1994), perhaps including urothelial mucosa, and particularly prevalent in the liver. Arylamines are detoxicated by SULT1A1 in the liver, and decreased SULT activity conferred by the SULT1A1 polymorphism prevents arylamines from being detoxicated. The quantity of SULT1A1 in the liver is abundant and detoxication of arylamines would not be so influenced by SULT1A1 polymorphism. After arylamines are metabolized in the liver through N-hydroxylation by CYP1A2, N-hydroxy arylamines enter the circulation and undergo renal filtration into the urothelial lumen, where they can be reabsorbed into the urothelial mucosa (Kaderlik and Kadlubar 1995). N-hydroxy arylamines are activated by NAT1 and SULT1A1, and form the arylnitrenium ions in urothelial mucosa. A polymorphism in the polyadenylation signal site of the NAT1 gene (NAT1*10 allele) that has been reported to have higher NAT1 enzyme activity in bladder tissue relative to NAT1 wild allele (NAT1*4 allele), was associated with an increased risk of urothelial cancer (Katoh et al. 1999). Similar to NAT1, the high activity SULT1A1*1 allozyme would activate N-hydroxy arylamines and form the arylnitrenium ions leading to DNA adducts, mutation, and neoplasia.

A number of studies have been conducted on urothelial cancer and the NAT2 polymorphisms. It has been suggested that NAT2 slow acetylators may be at increased risk of urothelial cancer when exposed to environmental arylamine carcinogens, due to their slower inactivation. Recently, some pooled meta-analyses of NAT2 status (phenotype and genotype) studies were reported (Green et al. 2000; Johns and Houlston 2000; Marcus et al. 2000a; Marcus et al. 2000b). Marcus et al. (2000a) reported that slow acetylators had an approximately 40% increase in risk compared with rapid acetylators (OR = 1.4, 95% CI 1.2–1.6) (22 studies, 2,496 cases, 3,340 controls). Studies conducted in Asia generated a summary OR of 2.1 (95% CI 1.2–3.8), in Europe, a summary OR of 1.4 (95% CI 1.2–1.6), and in the USA, a summary OR of 0.9 (95% CI 0.7–1.3). Vineis et al. (2001) reported a pooled analysis of NAT2 genotype-based studies in Caucasian populations (six studies, 1,530 cases, and 731 controls), and a significant association between NAT2 and bladder cancer (OR = 1.42, 95% CI 1.14–1.77). The risk of cancer was elevated in smokers and occupationally exposed subjects, with the highest risk among slow acetylators. They suggested that NAT2 was not a risk factor but modulated the effect of carcinogens contained in tobacco smoke (probably arylamines) or associated with occupational exposures. In our study, there is no significant association between NAT2 genotypes and urothelial cancer among non-smokers, but among smokers the ORs of developing urothelial cancer for NAT2 slow acetylator genotype were much higher according to smoking amounts. Our present results provide support for the findings of Vineis et al. (2001).

There are several inconsistent reports about the association between NAT2 polymorphism and pathological findings of urothelial cancer. Some have reported that NAT2 slow acetylator genotype was likely to have a higher risk for less-differentiated or advanced stage tumors (Cartwright et al. 1982; Inatomi et al. 1999; Mommsen and Aagaard 1986); however, others have reported a tendency towards a higher risk for highly-differentiated and superficial tumors (Hanssen et al. 1985). In our study, the higher OR of NAT2 intermediate and slow acetylator genotypes was more likely to present a well-differentiated tumor (G1) among heavy-smokers, although the risk of urothelial cancer for NAT2 intermediate and slow acetylator genotypes did not differ between superficial and invasive tumors. However, significant interaction ORs between NAT2 genotypes and pathological findings among the case group only were not found, with the exception of the interaction OR of NAT2 intermediate and slow acetylator genotypes between G1 tumor patients and G2 tumor patients among ever-smokers ($P = 0.03$). These non-significant interactions between NAT2 genotypes and pathological findings might be due to insufficient power, because urothelial cancer cases were subdivided into subgroups according to pathological findings and smoking status. Further investigation should clear up this issue.

Smoking is thought to be a moderate risk factor for recurrence of urothelial cancer (Aveyard et al. 2002), and 55.6% (79/142) of superficial tumor patients had recurrence in this study. We did not find any associations between CYP1A2, SULT1A1, and NAT2 polymorphisms and recurrence rate of superficial urothelial cancer patients. To our knowledge, there have been no reports on the recurrence rates of urothelial cancer patients in relation to polymorphisms of the NAT2 gene. Such associations become more difficult as, during the therapeutic process, patients are given intravesical Bacillus Calmette-Guerin, which helps to reduce the recurrence rate of superficial urothelial cancer tremendously (Morales et al. 2002).

In conclusion, our data also suggest a potentially modulating effects of the SULT1A1 polymorphism or NAT2 polymorphism on the association of smoking and urothelial cancer, and the modulation of NAT2 intermediate and slow acetylator genotypes has a tendency to present a higher risk for highly differentiated tumors among heavy-smokers.

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References

Aveyard P, Adab P, Cheng KK, Wallace DM, Hey K, Murphy MF (2002) Does smoking status influence the prognosis of bladder cancer? A systematic review. BJU Int 90:228–239

Bartsch H, Caporaso N, Coda M, Kadlubar F, Malaveille C, Skipper P, Talaska G, Tannenbaum SR, Vineis P (1990) Carcinogen hemoglobin adducts, urinary mutagenicity, and metabolic phenotype in active and passive cigarette smokers. J Natl Cancer Inst 82:1826–1831

Bartsch H, Malaveille C, Friesen M, Kadlubar FF, Vineis P (1993) Black (aircured) and blond (fluecured) tobacco cancer risk. IV: Molecular dosimetry studies implicate aromatic amines as bladder carcinogens. Eur J Cancer 29:1199–1207

Bell DA, Taylor JA, Butler MA, Stephens EA, Wiest J, Brubaker LH, Kadlubar FF, Lucier GW (1993) Genotype/phenotype discordance for human arylamine N-acetyltransferase (NAT2) reveals a new slow-acetylator allele common in African-Americans. Carcinogenesis 14:1689–1692

Carlini EJ, Raftogianis RB, Wood TC, Jin F, Zheng W, Rebbeck TR, Weinshilboum RM (2001) Sulfation pharmacogenetics: SULT1A1 and SULT1A2 allele frequencies in Caucasian, Chinese and African-American subjects. Pharmacogenetics 11:57–68

Cartwright RA, Glashan RW, Rogers HJ, Ahmad RA, Barham-Hall D, Higgins E, Kahn MA (1982) Role of N-acetyltransferase phenotypes in bladder carcinogenesis: a pharmacogenetic epidemiological approach to bladder cancer. Lancet 2:842–845

Chevalier D, Cauffiez C, Allorge D, Lo-Guidice JM, Lhermitte M, Lafitte JJ, Broly F (2001) Five novel natural allelic variants — 951A > C, 1042G > A (D348 N), 1156A > T (I386F), 1217G > A (C406Y) and 1291C > T (C431Y) — of the human CYP1A2 gene in a French Caucasian population. Hum Mutat 17:355–356

Chida M, Yokoi T, Fukui T, Kinoshita M, Yokota J, Kamataki T (1999) Detection of three genetic polymorphisms in the 5'-flanking region and intron 1 of human CYP1A2 in the Japanese population. Jpn J Cancer Res 90:899–902

Cohen SM, Shirai T, Steineck G (2000) Epidemiology and etiology of premalignant and malignant urothelial changes. Scand J Urol Nephrol Suppl 205:105–115

Crofts FG, Sutter TR, Strickland PT (1998) Metabolism of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine by human cytochrome P4501A1, P4501A2 and P4501B1. Carcinogenesis 19:1969–1973

Gilissen RA, Bamforth KJ, Stavenuiter JF, Coughtrie MW, Meerman JH (1994) Sulfation of aromatic hydroxamic acids and hydroxylamines by multiple forms of human liver sulfotransferases. Carcinogenesis 15:39–45

Grant DM, Hughes NC, Janezic SA, Goodfellow GH, Chen HJ, Gaedigk A, Yu VL, Grewal R (1997) Human acetyltransferase polymorphisms. Mutat Res 376:61–70

Green J, Banks E, Berrington A, Darby S, Deo H, Newton R (2000) N-acetyltransferase 2 and bladder cancer: an overview and consideration of the evidence for gene-environment interaction. Br J Cancer 83:412–417

Guengerich FP (1992) Metabolic activation of carcinogens. Pharmacol Ther 54:17–61

Hanssen HP, Agarwal DP, Goeddè HW, Bucher H, Huland H, Brachmann W, Ovenbeck R (1985) Association of N-acetyltransferase polymorphism and environmental factors with bladder carcinogenesis. Study in a north German population. Eur Urol 11:263–266

Inatomi H, Katoh T, Kawamoto T, Matsumoto T (1999) NAT2 gene polymorphism as a possible marker for susceptibility to bladder cancer in Japanese. Int J Urol 6:446–454

Japanese Urological Association and Japanese Society of Pathology (2001) General rules for clinical and pathological studies on bladder cancer. 3rd edn. Kanehara, Tokyo

Johns LE, Houlston RS (2000) N-acetyl transferase-2 and bladder cancer risk: a meta-analysis. Environ Mol Mutagen 36: 221–227

Kaderlik KR, Kadlubar FF (1995) Metabolic polymorphisms and carcinogen-DNA adduct formation in human populations. Pharmacogenetics 5:S108–S117

Kadlubar FF (1994) Biochemical individuality and its implications for drug and carcinogen metabolism: recent insights from acetyltransferase and cytochrome P4501A2 phenotyping and genotyping in humans. Drug Metab Rev 26:37–46

Kadlubar FF, Miller JA, Miller EC (1977) Hepatic microsomal N-glucuronidation and nucleic acid binding of N-hydroxy arylamines in relation to urinary bladder carcinogenesis. Cancer Res 37:805–814

Katoh T, Kaneko S, Boissy R, Watson M, Ikemura K, Bell DA (1998) A pilot study testing the association between N-acetyltransferases 1 and 2 and risk of oral squamous cell carcinoma in Japanese people. Carcinogenesis 19:1803–1807

Katoh T, Inatomi H, Yang M, Kawamoto T, Matsumoto T, Bell DA (1999) Arylamine N-acetyltransferase 1 (NAT1) and 2 (NAT2) genes and risk of urothelial transitional cell carcinoma among Japanese. Pharmacogenetics 9:401–404

Kloth MT, Gee RL, Messing EM, Swaminathan S (1994) Expression of N-acetyltransferase (NAT) in cultured human uroepithelial cells. Carcinogenesis 15:2781–2787

Lee SW, Jang IJ, Shin SG, Lee KH, Yim DS, Kim SW, Oh SJ, Lee SH (1994) CYP1A2 activity as a risk factor for bladder cancer. J Korean Med Sci 9:482–489

Lewis AJ, Walle UK, King RS, Kadlubar FF, Falany CN, Walle T (1998) Bioactivation of the cooked food mutagen N-hydroxy-2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine by estrogen sulfotransferase in cultured human mammary epithelial cells. Carcinogenesis 19:2049–2053

Marcus PM, Vineis P, Rothman N (2000a) NAT2 slow acetylation and bladder cancer risk: a meta-analysis of 22 case-control studies conducted in the general population. Pharmacogenetics 10:115–122

Marcus PM, Hayes RB, Vineis P, Garcia-Closas M, Caporaso NE, Autrup H, Branch RA, Brockmoller J, Ishizaki T, Karakaya AE, Ladero JM, Mommsen S, Okkels H, Romkes M, Roots I, Rothman N (2000b) Cigarette smoking, N-acetyltransferase 2 acetylation status, and bladder cancer risk: a case-series meta-analysis of a gene-environment interaction. Cancer Epidemiol Biomarkers Prev 9:461–467

Mommsen S, Aagaard J (1986) Susceptibility in urinary bladder cancer: acetyltransferase phenotypes and related risk factors. Cancer Lett 32:199–205

Morales A, Eidinger D, Bruce AW (2002) Intracavitary Bacillus Calmette-Guerin in the treatment of superficial bladder tumors. 1976. J Urol 167:891–893

Nakajima M, Yokoi T, Mizutani M, Kinoshita M, Funayama M, Kamataki T (1999) Genetic polymorphism in the 5'-flanking region of human CYP1A2 gene: effect on the CYP1A2 inducibility in humans. J Biochem 125:803–808

Ozawa S, Tang YM, Yamazoe Y, Kato R, Lang NP, Kadlubar FF (1998) Genetic polymorphisms in human liver phenol sulfotransferases involved in the bioactivation of N-hydroxy

derivatives of carcinogenic arylamines and heterocyclic amines. Chem Biol Interact 109:237–248

Ozawa S, Shimizu M, Katoh T, Miyajima A, Ohno Y, Matsumoto Y, Fukuoka M, Tang YM, Lang NP, Kadlubar FF (1999) Sulfating-activity and stability of cDNA-expressed allozymes of human phenol sulfotransferase, ST1A3*1 ((213)Arg) and ST1A3*2 ((213)His), both of which exist in Japanese as well as Caucasians. J Biochem 126:271–277

Ozawa S, Katoh T, Inatomi H, Imai H, Kuroda Y, Ichiba M, Ohno Y (2002) Association of genotypes of carcinogen-activating enzymes, phenol sulfotransferase SULT1A1 (ST1A3) and arylamine N-acetyltransferase NAT2, with urothelial cancer in a Japanese population. Int J Cancer 102:418–421

Raftogianis RB, Wood TC, Otterness DM, Van Loon JA, Weinshilboum RM (1997) Phenol sulfotransferase pharmacogenetics in humans: association of common SULT1A1 alleles with TS PST phenotype. Biochem Biophys Res Commun 239:298–304

Raftogianis RB, Wood TC, Weinshilboum RM (1999) Human phenol sulfotransferases SULT1A2 and SULT1A1: genetic polymorphisms, allozyme properties, and human liver genotype-phenotype correlations. Biochem Pharmacol 58:605–616

Sachse C, Brockmoller J, Bauer S, Roots I (1999) Functional significance of a C → A polymorphism in intron 1 of the cytochrome P450 CYP1A2 gene tested with caffeine. Br J Clin Pharmacol 47:445–449

Seth P, Lunetta KL, Bell DW, Gray H, Nasser SM, Rhei E, Kaelin CM, Iglehart DJ, Marks JR, Garber JE, Haber DA, Polyak K (2000) Phenol sulfotransferases: hormonal regulation, polymorphism, and age of onset of breast cancer. Cancer Res 60:6859–6863

Silverman DT, Hartge P, Morrison AS, Devesa SS (1992) Epidemiology of bladder cancer. Hematol Oncol Clin North Am 6:1–30

Sobin LH, Wittekind Ch (1997) UICC TNM Classification of malignant tumors. 5th edn. Wiley, New York

Vineis P, Talaska G, Malaveille C, Bartsch H, Martone T, Sithisarankul P, Strickland P (1996) DNA adducts in urothelial cells: relationship with biomarkers of exposure to arylamines and polycyclic aromatic hydrocarbons from tobacco smoke. Int J Cancer 65:314–316

Vineis P, Marinelli D, Autrup H, Brockmoller J, Cascorbi I, Daly AK, Golka K, Okkels H, Risch A, Rothman N, Sim E, Taioli E (2001) Current smoking, occupation, N-acetyltransferase-2 and bladder cancer: a pooled analysis of genotype-based studies. Cancer Epidemiol Biomarkers Prev 10:1249–1252

Wang Y, Spitz,M R, Tsou AM, Zhang K, Makan N, Wu X (2002) Sulfotransferase (SULT) 1A1 polymorphism as a predisposition factor for lung cancer: a case-control analysis. Lung Cancer 35:137–142

Williams JA, Stone EM, Millar BC, Gusterson BA, Grover PL, Phillips DH (1998) Determination of the enzymes responsible for activation of the heterocyclic amine 2-amino-3-methylimidazo[4,5-f]quinoline in the human breast. Pharmacogenetics 8:519–528

Yamazoe Y, Nagata K, Yoshinari K, Fujita K, Shiraga T, Iwasaki K (1999) Sulfotransferase catalyzing sulfation of heterocyclic amines. Cancer Lett 143:103–107

Zheng W, Xie D, Cerhan JR, Sellers TA, Wen W, Folsom AR (2001) Sulfotransferase 1A1 polymorphism, endogenous estrogen exposure, well-done meat intake, and breast cancer risk. Cancer Epidemiol Biomarkers Prev 10:89–94

〔原　著〕

解剖学実習室における気中ホルムアルデヒド濃度評価と自覚症状調査

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要　　旨：医学教育における系統解剖実習においては，ホルムアルデヒドに高濃度で曝露される可能性がある．解剖学実習における学生および教職員の安全性評価のために，系統解剖学実習時に環境中濃度測定と自覚症状についてのアンケート調査を実施し，解剖学実習の環境改善および防備体制などの充実に繋げる基礎データを蓄積することを目的とした．濃度評価は作業環境測定に準じ，2,4-dinitrophenylhydrazine（DNPH）含浸シリカゲルカラムに気中ホルムアルデヒドを捕集し，アセトニトリルで溶出後，高速液体クロマトグラフ（HPLC）にて分離・定量を行った．解剖実習開始前のホルムアルデヒド濃度の平均値は20～93 ppbであったが，実習開始後は実習の進展に伴い気中濃度は増加し最高時には1012～1380 ppbを示した．自覚症状調査においては，「喉が乾燥する」，「目がチカチカする」，「目がかゆい」，「気分が悪い」，「疲れている」などにおいて，普段に比べ解剖学実習室内において有意に高い訴えを認めた．

キーワード：ホルムアルデヒド，解剖学実習室，自覚症状，室内空気汚染．

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はじめに

ホルムアルデヒドは，無色で刺激臭を有し，室温で容易に重合する気体である．一般に市販されているホルマリン溶液は約37％のホルムアルデヒド水溶液で，重合を避けるために安定剤としてメタノールが加えられている．ホルムアルデヒドの急性症状は主に粘膜刺激症状であるが，慢性曝露においては発がん性が懸念されている．Kernsら[1]の，マウス・ラットを用いたホルムアルデヒド長期曝露発がん実験における陽性結果などを受けて，ホルムアルデヒドを取り扱う職域での疫学調査や動物実験の再現が幅広く行われて来た[2－4]．一方，医学教育における系統解剖実習においては，ホルムアルデヒドに高濃度に曝露される可能性がある．諸外国では早くからその生体影響について検討がなされ，遺体固定法の改良，全体あるいは局所換気など実習室内換気法の改善，保護具の使用などの種々の対応が取られて来た［5－9］．ただし，その際の影響としては粘膜刺激などの急性症状の誘発あるいは上記の発がんが主な検討課題とされていた．