relationships between occupational pesticide exposure and male reproductive function. Pesticide factory workers in China exposed to ethylparathion and methamidophos exhibited sperm aneuploidy (rate ratio of 1.51) [85] and reduction of sperm concentration and motility [86], suggesting a moderately adverse effect on semen quality. Danish researchers studied greenhouse workers with known exposure to more than 60 pesticides under the framework of endocrine disruptors. The high-level exposure group showed lower sperm concentration, morphology and viability under WHO guidelines [87]. A study was conducted on Mexican males exposed to DDT spraying for malaria control, despite the cease of its use in industrialized countries. The body burden of DDT metabolite was inversely correlated to semen volume and sperm count [88]. Such findings raise concern on the situation of developing countries, similar in nature to the report on DBCP.

Offspring Effects

For this section, the offspring effect can be defined as an endpoint that can be evaluated among the offspring of the occupationally exposed male, *i.e.*, paternal exposure. The offspring effects include changes in the sex ratio, cancer, accelerated puberty, and among males, cryptorchidism (undescended testis) and hypospadia. The central question to be addressed here is whether a common etiology exists among the offspring effect and other effects including reproductive function, hormonal changes and cancer among the exposed. Testicular dysgenesis syndrome [89] is the specific terminology applied in instances where only the exposure and effects among males are considered, and a common etiology is hypothesized for hypospadias, cryptorchidism, testicular cancer and infertility [19]. In particular, cryptorchidism and hypospadia have been characterized by Sharpe and Shakkebaek [1] as "representing mild degrees of feminization", and "important in the ongoing debate regarding the significance of endocrine disruptors or other environmental influences on male development".

Many researchers have argued that the incidence of cryptorchidism and hypospadia increased over the years in many countries [17, 18, 90, 91]. Although the secular trend certainly merits investigation, the reservations expressed in accompaniment with such observations are important. The reservations include the ethnic and racial differences in the underlying rates, inconsistencies in diagnostic procedures and criteria [17, 18] and inefficiencies with reporting [17]. This is particularly true with cryptorchidism, because the defect resolves spontaneously by the first birthday in $> 70\%$ of affected infants [19]. There is a contradictory observation that the rates of these diseases may, in fact, have decreased [92]. The issue is thus still inconclusive, but the possible increase of cryptorchidism and hypospadias as an effect of EDC cannot be dismissed. It is not only theoretically possible but can be deduced from the increased rate of cryptorchidism and hypospadia observed in the sons of women treated with the synthetic estrogen diethylstilbestrol [93].

Whereas the number of papers on temporal analyses is abundant, few studies have directly

evaluated the risk of cryptorchidism and hypospadia. Cryptorchidism rates (evaluated by orchidopexi rates) tended to be higher in districts where intensive farming is widespread [94]. The risk of cryptorchidism was significantly increased among the offspring of female (but not male) gardeners [92, 95]. Another study inferred a causal relationship between hypospadia and maternal occupational exposure to EDC [96]. In a recent case-control study from China, risk factors for cryptorchidism were sought, and paternal exposure to pesticides emerged with an OR of 12.8 (95% CI 2.9−56.4) [97].

Sex ratio and testicular cancer of offspring have been evaluated as an end-point in several observational studies. A predominance of female offsprings have been observed in relation to paternal exposure among Israeli DBCP production workers [71], Seveso accident survivors exposed to 2, 4, 5-trichlorophenol [18, 98] and glass workers [99], but such a relation was negated in a study of male employees exposed to inorganic borate compounds [100]. While one study showed an increase of testicular cancer in the offspring of farmers' sons (notably non-seminoma) [101], another study found no association between parental occupation in agriculture and testicular cancer in the offspring [102].

In conclusion, although it is often difficult to draw a distinct line between an endocrine disrupting process and a more general toxicological process, some occupational epidemiologic studies have begun to implicate the presence of EDCs in relation to the risk for cancer of the reproductive tract, hormonal changes, reproductive function and offspring effects. Progress has been made in areas such as adherence to standardized techniques in evaluating the male reproductive function and more sensitive study designs. However, epidemiologic findings are still constrained by difficulties in the identification of occupationally-exposed populations and the evaluation of exposure [103]. There is thus a need to combine knowledge from related disciplines and widen the scope of epidemiologic research targeting occupationally exposed populations under a carefully-designed protocol.

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References

1. Sharpe RM & Shakkebaek NE (1993): Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? Lancet 341: 1392−1395
2. Joffe M (2001): Are problems with male reproductive health caused by endocrine disruption? Occup Environ Med 58: 281−287
3. Waalkes MP & Rehm S (1994): Cadmium and prostate cancer. J Toxicol Environ Health 43:

251−269

4. Keller-Byrne JE, Khuder SA & Schaub EA (1997): Meta-analyses of prostate cancer and farming. Am J Ind Med 31: 580−586
5. Morrison H, Savitz D, Semenciw R, Hulka B, Mao Y, Morison D & Wigle D (1993): Farming and prostate cancer mortality. Am J Epidemiol 137: 270−280
6. Costantino JP, Redmond CK & Bearden A (1995): Occupationally related cancer risk among coke oven workers: 30 years of follow-up. J Occup Environ Med 37: 597−604
7. Stewart RE, Dennis LK, Dawson DV & Resnick MI (1999): A meta-analysis of risk estimates for prostate cancer related to tire and rubber manufacturing operations. J Occup Environ Med 41: 1079−1084
8. Van der Gulden JW, Kolk JJ & Verbeek AL (1995): Work environment and prostate cancer risk. Prostate 27: 250−257
9. Van der Gulden JW & Vogelzang PF (1996): Farmers at risk for prostate cancer. Br J Urol 77: 6−14
10. Sharma-Wagner S, Chokkalingam AP, Malker HS, Stone BJ, McLaughlin JK & Hsing AW (2000): Occupation and prostate cancer risk in Sweden. J Occup Environ Med 42: 517−525
11. Dich J & Wiklund K (1998): Prostate cancer in pesticide applicators in Swedish agriculture. Prostate 34: 100−112
12. Seidler A, Heiskel H, Bickeboller R & Elsner G (1998): Association between diesel exposure at work and prostate cancer. Scand J Work Environ Health 24: 486−494
13. Fleming LE, Bean JA, Rudolph M & Hamilton K (1999): Mortality in a cohort of licensed pesticide applicators in Florida. Occup Environ Med 56: 14−21
14. Janssens JP, Van Hecke E, Geys H, Bruckers L, Renard D & Molenberghs G (2001): Pesticides and mortality from hormone-dependent cancers. Eur J Cancer Prev 10: 459−467
15. Bullman TA, Watanabe KK & Kang HK (1994): Risk of testicular cancer associated with surrogate measures of Agent Orange exposure among Vietnam veterans on the Agent Orange Registry. Ann Epidemiol 4: 11−16
16. Pollan M, Gustavsson P & Cano MI (2001): Incidence of testicular cancer and occupation among Swedish men gainfully employed in 1970. Ann Epidemiol 11: 554−562
17. Giwercman A, Carlsen E, Keiding N & Skakkebaek NE (1993): Evidence for increasing incidence of abnormalities of the human testis: a review. Environ Health Perspect (101 Suppl 2): 65−71
18. Toppari J & Skakkebaek NE (1998): Sexual differentiation and environmental endocrine disrupters. Baillieres Clin Endocrinol Metab 12: 143−156
19. Moline JM, Golden AL, Bar-Chama N, Smith E, Rauch ME, Chapin RE, Perreault SD, Schrader SM, Suk WA & Landrigan PJ (2000): Exposure to hazardous substances and male reproductive health: a research framework. Environ Health Perspect 108: 803−813
20. Hardell L, Ohlson CG & Fredrikson M (1997): Occupational exposure to polyvinyl chloride as a risk factor for testicular cancer evaluated in a case-control study. Int J Cancer 73: 828−830
21. Ohlson CG & Hardell L (2000): Testicular cancer and occupational exposures with a focus on

xenoestrogens in polyvinyl chloride plastics. Chemosphere 40: 1277−1282

22. Hjollund NH, Bonde JP, Jensen TK, Ernst E, Henriksen TB, Kolstad HA, Giwercman A, Skakkebaek NE & Olsen J (1998): Semen quality and sex hormones with reference to metal welding. Reprod Toxicol 12: 91−95
23. McGregor AJ & Mason HJ (1990): Chronic occupational lead exposure and testicular endocrine function. Hum Exp Toxicol 9: 371−376
24. Gennart JP, Bernard A & Lauwerys R (1992): Assessment of thyroid, testes, kidney and autonomic nervous system function in lead-exposed workers. Int Arch Occup Environ Health 64: 49−57
25. Alexander BH, Checkoway H, van Netten C, Muller CH, Ewers TG, Kaufman JD, Mueller BA, Vaughan TL & Faustman EM (1996): Semen quality of men employed at a lead smelter. Occup Environ Med 53: 411−416
26. Benoff S, Jacob A & Hurley IR(2000): Male infertility and environmental exposure to lead and cadmium. Hum Reprod Update 6: 107−121
27. Telisman S, Cvitkovic P, Jurasovic J, Pizent A, Gavella M & Rocic B (2000): Semen quality and reproductive endocrine function in relation to biomarkers of lead, cadmium, zinc, and copper in men. Environ Health Perspect 108: 45−53
28. Bonde JP & Ernst E (1992): Sex hormones and semen quality in welders exposed to hexavalent chromium. Hum Exp Toxicol 11: 259−263
29. Li H, Chen Q, Li S *et al* (2001): Effect of Cr(VI)exposure on sperm quality: human and animal studies. Ann Occup Hyg 45: 505−511
30. Erfurth EM, Schutz A, Nilsson A, Barregard L & Skerfving S (1990): Normal pituitary hormone response to thyrotrophin and gonadotrophin releasing hormones in subjects exposed to elemental mercury vapour. Br J Ind Med 47: 639−644
31. McGregor AJ & Mason HJ (1991): Occupational mercury vapour exposure and testicular, pituitary and thyroid endocrine function. Hum Exp Toxicol 10: 199−203
32. Zeng X, Lin T, Zhou Y & Kong Q (2002): Alterations of serum hormone levels in male workers occupationally exposed to cadmium. J Toxicol Environ Health 65: 513−521
33. Zacharewski T(1998): Identification and assessment of endocrine disruptors: Limitations of *in vivo* and *in vitro* assays. Environ Health Perspect 106: 577−582
34. Svensson BG, Nise G, Erfurth EM, Nilsson A & Skerfving S (1992): Hormone status in occupational toluene exposure. Am J Ind Med 22: 99−107
35. Svensson BG, Nise G, Erfurth EM & Olsson H (1992): Neuroendocrine effects in printing workers exposed to toluene. Br J Ind Med 49: 402−408
36. Chia *et al* (1996): Semen parameters of workers exposed to trichloroethylene. Reproductive Toxicology 10: 295−299
37. Goh VH, Chia SE & Ong CN (1998): Effects of chronic exposure to low doses of trichloroethylene on steroid hormone and insulin levels in normal men. Environ Health Perspect 106: 41−44
38. Vanhoorne M, Vermeulen A & De Bacquer D (1993): Epidemiological study of endocrinological effects of carbon disulfide. Arch Environ Health 48: 370−375

39. Padungtod C, Lasley BL, Christiani DC, Ryan LM & Xu X (1998): Reproductive hormone profile among pesticide factory workers. J Occup Environ Med 40: 1038−1047
40. Akingbemi BT & Hardy MP (2001): Oestrogenic and antiandrogenic chemicals in the environment: effects on male reproductive health. Ann Med 33: 391−403
41. Hoyer PB (2001): Reproductive toxicology: current and future directions. Biochem Pharmacol 62: 1557−1564
42. Hanaoka T, Kawamura N, Hara K & Tsugane S (2002): Urinary bisphenol A and plasma hormone concentrations in male workers exposed to bisphenol A diglycidyl ether and mixed organic solvents. Occup Environ Med 59: 625−628
43. Bonde JP, Joffe M, Apostoli P *et al* (2002): Sperm count and chromatin structure in men exposed to inorganic lead: lowest adverse effect levels. Occup Environ Med 59: 234−242
44. Apostoli P, Porru S & Bisanti L (1999): Critical aspect of male fertility in the assessment of exposure to lead. Scand J Work Environ Health 25 supplement 1: 40−43
45. Robins TG, Bornman MS, Ehrlich RI, Cantrell AC, Pienaar E, Vallabh J & Miller S (1997): Semen quality and fertility of men employed in a South African lead acid battery plant. Am J Ind Med 32: 369−376
46. Kuo HW, Wang CS & Lai JS (1997): Semen quality in workers with long-term lead exposure: a preliminary study in Taiwan. Sci Total Environ 204: 289−292
47. Viskum S, Rabjerg L, Jorgensen PJ & Grandjean P (1999): Improvement in semen quality associated with decreasing occupational lead exposure. Am J Ind Med 35: 257−263
48. Alexander BH, Checkoway H, Costa-Mallen P, Faustman EM, Woods JS, Kelsey KT, van Netten C & Costa LG (1998): Interaction of blood lead and delta-aminolevulinic acid dehydratase genotype on markers of heme synthesis and sperm production in lead smelter workers. Environ Health Perspect 106: 213−216
49. Figa-Talamanca I, Traina ME & Urbani E (2001): Occupational exposures to metals, solvents and pesticides: recent evidence on male reproductive effects and biological markers. Occup Med (Lond) 51: 174−188
50. Bonde JP (1990): Semen quality and sex hormones among mild steel and stainless steel welders: a cross sectional study. Br J Ind Med 47: 508−514
51. Bonde JP (1990): Semen quality in welders before and after three weeks of non-exposure. Br J Ind Med 47: 515−518
52. Xiao G, Pan C, Cai Y, Lin H & Fu Z (1999): Effect of benzene, toluene, xylene on the semen quality of exposed workers. Chin Med J 112: 709−712
53. Xiao G, Pan C, Cai Y, Lin H & Fu Z (2001): Effect of benzene, toluene, xylene on the semen quality and the function of accessory gonad of exposed workers. Ind Health 39: 206−210
54. Lemasters GK, Olsen DM, Yiin JH, Lockey JE, Shukla R, Selevan SG, Schrader SM, Toth GP, Evenson DP & Huszar GB (1999): Male reproductive effects of solvent and fuel exposure during aircraft maintenance. Reprod Toxicol 13: 155−166
55. Cherry N, Labreche F, Collins J & Tulandi T (2001): Occupational exposure to solvents and male

infertility. Occup Environ Med 58: 635−640

56. Bukowski JA (2001): Review of the epidemiological evidence relating toluene to reproductive outcomes. Regul Toxicol Pharmacol 33: 147−156
57. Kim Y, Park J & Moon Y (1999): Hematopoietic and reproductive toxicity of 2-bromopropane, a recently introduced substitute for chlorofluorocarbons. Toxicol Lett 108: 309−313
58. Ichihara G, Ding X, Yu X, Wu X, Kamijima M, Peng S, Jiang X & Takeuchi Y (1999): Occupational health survey on workers exposed to 2-bromopropane at low concentrations. Am J Ind Med 35: 523−531
59. Vanhoorne M, Comhaire F & De Bacquer D (1994): Epidemiological study of the effects of carbon disulfide on male sexuality and reproduction. Arch Environ Health 49: 273−278
60. Veulemans H, Steeno O, Masschelein R & Groeseneken D (1993): Exposure to ethylene glycol ethers and spermatogenic disorders in man: a case-control study. Br J Ind Med 50: 71−78
61. Welch LS, Plotkin E & Schrader S (1991): Indirect fertility analysis in painters exposed to ethylene glycol ethers: sensitivity and specificity. Am J Ind Med 20: 229−240
62. Chia SE, Ong CN, Tsakok MF & Ho A (1996): Semen parameters in workers exposed to trichloroethylene. Reprod Toxicol 10: 295−299
63. Eskenazi B, Wyrobek AJ, Fenster L, Katz DF, Sadler M, Lee J, Hudes M & Rempel DM (1991): A study of the effect of perchloroethylene exposure on semen quality in dry cleaning workers. Am J Ind Med 20: 575−591
64. Li Y, Jiang QG, Yao SQ, Liu W, Tian GJ & Cui JW (1993): Effects of exposure to trinitrotoluene on male reproduction. Biomed Environ Sci 6: 154−160
65. Tielemans E, Burdorf A, te Velde ER, Weber RF, van Kooij RJ, Veulemans H & Heederik DJ (1999): Occupationally related exposures and reduced semen quality: a case-control study. Fertil Steril 71: 690−696
66. Grajewski B, Whelan EA, Schnorr TM, Mouradian R, Alderfer R & Wild DK (1996): Evaluation of reproductive function among men occupationally exposed to a stilbene derivative: I. Hormonal and physical status. Am J Ind Med 29: 49−57
67. Whelan EA, Grajewski B, Wild DK, Schnorr TM & Alderfer R (1996): Evaluation of reproductive function among men occupationally exposed to a stilbene derivative: II. Perceived libido and potency. Am J Ind Med 29: 59−65
68. Petrelli & Mantovani A (2002): Environmental risk factors and male fertility and reproduction. Contraception 65: 297−300
69. Whorton D, Krauss RM, Marshall S & Milby T (1977): Infertility in male pesticide workers. Lancet 2: 1259−1261
70. Lahdetie J (1995): Occupation- and exposure-related studies on human sperm. J Occup Environ Med 37: 922−930
71. Goldsmith JR (1997): Dibromochloropropane: epidemiological findings and current questions. Ann N Y Acad Sci 837: 300−306
72. Teitelbaum DT (1999): The toxicology of 1,2-dibromo-3-chloropropane (DBCP): a brief review.

Int J Occup Environ Health 5: 122−126

73. Olsen GW, Lanham JM, Bodner KM, Hylton DB & Bond G (1990): Determinants of spermatogenesis recovery among workers exposed to 1,2-dibromo-3-chloropropane. J Occup Med 32: 979−984
74. Slutsky M, Levin JL & Levy BS (1999): Azoospermia and oligospermia among a large cohort of DBCP applicators in 12 countries. Int J Occup Environ Health 5: 122−126
75. Petrelli G, Taggi F, Tropeano R, Fondi G, Meli P & Figa-Talamanca I (1999): The use of time to pregnancy in male infertility studies. A method to evaluate couple concordance of reproductive data. Eleventh Conference of the International Society for Environmental Epidemiology (ISEE). Ninth conference of the International Society of Exposure Analysis (ISEA). Epidemiology 10: 124
76. Lerda D & Rizzi R (1991): Study of reproductive function in persons occupationally exposed to 2,4-dichlorophenoxyacetic acid (2,4-D). Mutat Res 262: 47−50
77. Baird DD, Wilcox AJ & Weinberg CR (1986): Use of time to pregnancy to study environmental exposure. Am J Epidemiol 124: 470−480
78. de Cock J, Westveer K, Heederik D, te Velde E & van Kooij R (1994): Time to pregnancy and occupational exposure to pesticides in fruit growers in the Netherlands. Occup Environ Med 51: 693−699
79. Thonneau P, Larsen SB, Abell A, Clavert A, Bonde JP, Ducot B & Multigner L (1999): Time to pregnancy and paternal exposure to pesticides in preliminary results from Danish and French studies. Asclepios. Scand J Work Environ Health 25(suppl 1): 62−63
80. Petrelli G & Figa-Talamanca I (2001): Reduction in fertility in male greenhouse workers exposed to pesticides. Eur J Epidemiol 17: 675−677
81. Heacock H, Hogg R, Marion SA, Hershler R, Teschke K, Dimich-Ward H, Demers P, Kelly S, Ostry A & Hertzman C (1998): Fertility among a cohort of male sawmill workers exposed to chlorophenate fungicides. Epidemiology 9: 56−60
82. Harkonen K, Viitanen T, Larsen SB, Bonde JP & Lahdetie J (1999): Aneuploidy in sperm and exposure to fungicides and lifestyle factors. Asclepios. A European Concerted Action on Occupational Hazards to Male Reproductive Capability. Environ Mol Mutagen 34: 39−46
83. Tomenson JA, Taves DR, Cockett AT, McCusker J, Barraj L, Francis M, Pastoor TP, Wickramaratne GA & Northrop HL (1999): An assessment of fertility in male workers exposed to molinate. J Occup Environ Med 41: 771−787
84. Larsen SB, Spano M, Giwercman A & Bonde JP (1999): Semen quality and sex hormones among organic and traditional Danish farmers. Asclepios Study Group. Occup Environ Med 56: 139−144
85. Padungtod C, Hassold TJ, Millie E, Ryan LM, Savitz DA, Christiani DC & Xu X (1999): Sperm aneuploidy among Chinese pesticide factory workers: scoring by the FISH method. Am J Ind Med 36: 230−238
86. Padungtod C, Savitz DA, Overstreet JW, Christiani DC, Ryan LM & Xu X (2000): Occupational pesticide exposure and semen quality among Chinese workers. J Occup Environ Med 42: 982−

992

87. Abell A, Ernst E & Bonde JP (2000): Semen quality and sexual hormones in greenhouse workers. Scand J Work Environ Health 26: 492−500
88. Ayotte P, Giroux S, Dewailly E, Hernandez Avila M, Farias P, Danis R & Villanueva Diaz C (2001): DDT spraying for malaria control and reproductive function in Mexican men. Epidemiology 12: 366−367
89. Skakkebaek NE (2002): Endocrine disrupters and testicular dysgenesis syndrome. Horm Res 57 (suppl 2): 43
90. Toppari J, Haavisto AM & Alanen M (2002): Changes in male reproductive health and effects of endocrine disruptors in Scandinavian countries. Cad Saude Publica 18: 413−420
91. Chia SE (2000): Endocrine disruptors and male reproductive function--a short review. Int J Androl 23 (suppl 2): 45−46
92. Safe SH (2000): Endocrine disruptors and human health–is there a problem? An update. Environ Health Perspect 108: 487−493
93. Jensen TK, Toppari J, Keiding N & Skakkebaek NE (1995): Do environmental estrogens contribute to the decline in male reproductive health? Clin Chem 41 (12 Pt 2): 1896−1901
94. Garcia-Rodriguez J, Garcia-Martin M, Nogueras-Ocana M, de Dios Luna-del-Castillo J, Espigares Garcia M, Olea N & Lardelli-Claret P (1996): Exposure to pesticides and cryptorchidism: geographical evidence of a possible association. Environ Health Perspect 104: 1090−1095
95. Weidner IS, Moller H, Jensen TK & Skakkebaek NE (1998): Cryptorchidism and hypospadias in sons of gardeners and farmers. Environ Health Perspect 106: 793−796
96. Van Tongeren M, Nieuwenhuijsen MJ, Gardiner K, Armstrong B, Vrijheid M, Dolk H & Botting B (2002): A job-exposure matrix for potential endocrine-disrupting chemicals developed for a study into the association between maternal occupational exposure and hypospadias. Ann Occup Hyg 46: 465−477
97. Wang J & Wang B (2002): Study on risk factors of cryptorchidism. Zhonghua Liu Xing Bing Xue Za Zhi 23: 190−193
98. Mocarelli P, Brambilla P, Gerthoux PM *et al* (1996): Change in sex ratio with exposure to dioxin. Lancet 348: 409
99. Wingren G & Persson B (1998): Male reproductive pattern in a glass producing area. Int J Occup Med Environ Health 11: 227−234
100. Whorton D, Haas J & Trent L (1994): Reproductive effects of inorganic borates on male employees: birth rate assessment. Environ Health Perspect 102 (Suppl 7): 129−132
101. Kristensen P, Andersen A, Irgens LM, Bye AS & Vagstad N (1996): Testicular cancer and parental use of fertilizers in agriculture. Cancer Epidemiol Biomarkers Prev 5: 3−9
102. Moller H (1997): Work in agriculture, childhood residence, nitrate exposure, and testicular cancer risk: a case-control study in Denmark. Cancer Epidemiol Biomarkers Prev 6: 141−144
103. Nelson P (2003): Epidemiology, biology, and endocrine disruptors. Occup Environ Med 60: 541−542

内分泌撹乱およびその他の化学物質の職業性曝露に関連する男性リプロダクティブ・ヘルスー疫学的知見

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要　　旨：職業性曝露に関連するヒト，特に男性生殖系影響に関する研究の趨勢は，1990年代初頭頃に内分泌撹乱化学物質(endocrine disrupting chemicals, EDC)の概念が普及して以来分岐している．それ以前の研究の過半は限られた範囲の化学物質を対象に実施され，単一かつ比較的高濃度の曝露による生殖毒性の評価という枠組みを有していた(従来の枠組み)．この範疇の研究は現在も認められるが，EDCの概念の普及以来男性生殖系に関する研究については，内分泌撹乱作用の可能性をもった広範な化学物質の探索および化学物質の複合微量曝露の影響評価という新しい枠組みが与えられている．また両方の枠組みをもった研究もある．新しい枠組みをもった近年の研究では，男性生殖機能を評価するための標準的検査手法やより鋭敏な検出力をもった研究計画が採用されるようになった．その結果，職業環境中に内分泌撹乱化学物質の存在が示唆されている．しかしながら，職業性曝露を有する集団の同定や曝露評価に伴う困難から，疫学的知見の解釈は制約を受ける．今後は，知見の統合とともに慎重なプロトコールに立脚し，職業曝露集団に関する疫学研究の範囲を拡大する必要がある．

キーワード：内分泌撹乱，職業性曝露，疫学，生殖毒性．
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Active and passive smoking and breast cancer risk in middle-aged Japanese women

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To examine the hypothesis that tobacco smoke is associated with the risk of female breast cancer, we estimated the relative risks of active and passive smoke in middle-aged Japanese women in a population-based prospective study. The cohort consisted of residents in 4 public health center areas, aged 40 to 59 years. A self-administered questionnaire survey was conducted in 1990. This analysis included 21,805 subjects, 180 of whom had developed breast cancer by December 31, 1999. When the reference was defined as never-active smokers without passive smoking, adjusted relative risks (RRs) were 1.9 (95% confidence interval [CI] = 1.0–3.6) in current active smokers, 1.2 (95% CI = 0.4–4.0) in ex-active smokers and 1.2 (95% CI = 0.8–1.6) in never-active smokers with passive smoking. The elevated risk for ever-smokers was clearly observed in premenopausal women at baseline (RR = 3.9, 95% CI = 1.5–9.9) but not in postmenopausal women (RR = 1.1, 95% CI = 0.5–2.5). In never-active smokers, the adjusted RR for passive smoking, residential or occupational/public tobacco smoke exposure was 1.1 (95% CI = 0.8–1.6). In premenopausal women, passive smoking increased the risk (RR = 2.6; 95% CI = 1.3–5.2) but not in postmenopausal women (RR = 0.7; 95% CI = 0.4–1.0). We conclude that tobacco smoking increases the risk of female breast cancer in premenopausal women.
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Key words: breast neoplasms; smoking; passive smoking; cohort study

Because most established risk factors for female breast cancer cannot be modified, the etiological role of tobacco smoking has been of interest in the public health field. As shown in a recent general comment by WHO's Executive Director, the link between smoking and breast cancer has been elusive; some studies have suggested a positive link, others found no relationship and a few have suggested that smoking has protective effects.[1] A positive association has been observed in some previous case-control studies.[2–7] In contrast, little relationship has been reported by cohort studies.[8–11] Theoretically, a cohort study provides better evidence compared to a case-control study, but the limitations, *e.g.*, reference category and misclassification of smoking habits, in recent cohort studies are still under dispute.[12–15]

Tobacco smoke is well known to contain numerous possible carcinogens.[16] Although they do not directly contact mammary cells, many studies utilizing biomarkers have demonstrated that tobacco-related carcinogens reach human breast tissue.[17–19] On the other hand, antiestrogenic effects of tobacco smoke have been suggested by many published observations.[20–23] Thus, the exposure may decrease the breast cancer risk, especially in postmenopausal women.[24,25]

The objective of our study was to examine the hypothesis that tobacco smoking is associated with the risk of female breast cancer. We estimated the risks of active and passive smoking among middle-aged Japanese women in a population-based cohort study. The influence of tobacco smoke as a breast cancer risk was elucidated by menopausal status at the baseline survey of the study.

Material and methods

Study cohort

The study cohort is part of the Japan Public Health Center (JPHC)-based prospective study on cancer and cardiovascular diseases (JPHC Study, cohort I) established on January 1, 1990. The study population was defined as Japanese residents aged 40–59 years, 27,063 men and 27,435 women, in 14 administrative districts in 4 PHC areas across Japan.[26] After the initiation of the study, 37 women were found to be ineligible and were excluded, leaving 27,398 women eligible for the study. Study procedures were approved by the ethics committee of the National Cancer Center, Tokyo, Japan.

Baseline survey

A self-administered questionnaire was distributed mostly by hand and partly by mail to the subjects in 1990. They were asked about their personal and familial medical histories, smoking habit, alcohol consumption, dietary habits and other lifestyle factors. A total of 22,482 women responded to the survey (82.1% response rate). Although the date of questionnaire completion ranged from January 1990 to May 1992, 54% responded between February 1990 and March 1990. Only 4% of questionnaires were completed after October 1990. The questions on active smoking consisted of current and former smoking status, age at initiation of smoking, average number of cigarettes smoked per day and age at cessation of smoking for former smokers. Questions on passive smoking were in 2 parts: a) "Have you lived with any regular smokers?" and age at exposure (≤ 20 years old, > 20 years old, both) and b) " In places outside the home, *e.g.*, at work, how often are you exposed to environmental tobacco smoke ≥ 1 hr/day?" (almost never, 1 to 3 days/month, 1 to 4 days/week, almost everyday).

Follow-up and identification of breast cancer

We followed the subjects from recruitment until December 31, 1999. In Japan, all death certificates are submitted to a local government office and forwarded to the PHC in the area of residence. Mortality data are then sent to the Ministry of Health, Labour and Welfare and coded for inclusion in the National Vital Statistics. The registration of deaths in Japan is required by the Family Registration Law and is theoretically complete. Therefore, all deaths of the subjects were based upon death certificates from each PHC, when they remained in the original area. Changes in residence status were identified annually through the residential registry in each area. Collection of cancer incidence data and migration data was described in a previous report.[27] Briefly, on January 1, 1990, a specific cancer registry for the JPHC Study was

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established to collect cancer incidence data on the study subjects living within the study area *via* voluntary reports from local major hospitals, on-site visits to the hospitals and records from the prefecture-wide population-based cancer registry, if available (Akita and Nagano Prefectures do not have a prefecture-wide cancer registry). Cancer incidence data were collected only for subjects who were living within the study area. Site of origin and histologic type were coded using the International Classification of Disease for Oncology, second edition (ICD-O-2). By December 31, 1999, 226 new breast cancer cases had been identified. Twelve carcinoma *in situ* were not included among these breast cancer cases. A diagnosis of breast cancer was histologically confirmed in 97% of the cases. The incidence/mortality ratio in the cancer registration was 5.4, and no cases were ascertained by death certificate alone [Death Certificate Only (DCO)]. In 1.1% of cases the subjects' death certificates were used as a supplementary information source for the registry [Death Certificate Notification (DCN)]. The estimated completeness of the registration was 91.8%, which suggested that the completeness for this cohort was reasonably high.[28,29]

Migration data were obtained from residential registries. Among non-case study subjects, 1,837 (6.7%) moved out of the study area and 34 (0.1%) were lost to follow-up within the study period.

Data analysis

From the 22,482 subjects, we excluded 612 more (including 12 breast cancer cases) with a past history of cancer in any site. Consequently, after excluding still another 53 subjects who submitted incomplete information on active or passive smoking status, a total of 21,805 subjects, 180 of whom developed breast cancer, were included in this analysis. Person-years of follow-up were counted from the date of questionnaire completion until the dates of a diagnosis of breast cancer, migration out of the study areas, death or the end of the study (December 31, 1999), whichever came first.

The relative risk (RR) and 95% confidence interval (CI) were estimated by the Cox proportional hazards model, adjusting for age and area according to the SAS PHREG procedure (SAS Institute, Inc., Cary, NC). For further adjustment, we incorporated additional possible confounders into the model; education level (≥ high school and < high school), employment status (employed and unemployed), body mass index (< 22, < 25, and ≥ 25), family history of breast cancer in mother or sisters, history of past benign breast disease, age at menarche, number of births (0, ≥ 1), menopausal status (pre and post), hormone use and alcohol consumption per week (< once/week, < 250 g/week, ≥ 250 g/week). Concerning body mass index and the number of births, influence on the estimates was similar between the categorical and continuous variables. Height, weight, fruit and vegetable intake and physical activity had little influence on the estimates and thus were omitted from the adjustment in the final analysis. Breast-feeding was not incorporated in the adjustment factors because it was not included in the questionnaire. We coded current occupations recorded in an open-end column in the questionnaire according to a major occupational category (Standard Occupational Classification for Japan, the third revision of 1997, Statistic Bureau, The Ministry of Public Management). The occupational categories consisted of professionals and technicians; managers; clerks; shop and market sales workers; service workers; security workers; agricultural, forestry and fishery workers; transport and communication workers; assemblers and manual laborers; workers unclassified and unemployed. Most agricultural, forestry and fishery workers were farmers. In the analysis concerning active smoking, passive smoking was defined as a history of exposure to residential sidestream smoke in any period or exposure to sidestream smoke (almost everyday) in any occupational and/or public setting.

After excluding from the analyses 6 cases whose pathological information was uncertain, we obtained results similar to those presented.

Results

Among the 21,805 women, the prevalence of current, ex- and never-active smokers was 5.7%, 1.7% and 92.6%, respectively. Among never-active smokers, 69% reported that they had been exposed to sidestream smoke (Table I). Table II compares known risk factors and possible confounders for breast cancer among 4 categories of smoking status. These factors included characteristics reported in the literature to be risk factors, and most of them served as adjustment factors in further statistical analyses. Table III shows RRs of incidence according to active smoking. Without taking account of passive smoking in the reference category, the adjusted RR for current active smokers was 1.7 (95% CI = 1.0–3.1). When the reference condition was defined as never-active smokers without passive smoking, a 2-fold risk was observed among current active smokers (adjusted RR = 1.9; 95% CI = 1.0–3.6). Stratified analyses by employment status showed the following adjusted RRs: 1.0 (95% CI = 0.5–2.0) for unemployed women with passive smoking, 0.8 (95% CI = 0.2–3.9) for unemployed women with active smoking, 1.2 (95% CI = 0.8–1.9) for employed women with passive smoking and 2.3 (95% CI = 1.1–4.8) for employed women with active smoking. After omitting the first 3 years after the study baseline to exclude possibly ill subjects, we observed similar results (data not shown).

In premenopausal women at baseline, ever-active smokers showed a 4-fold increased risk (adjusted RR = 3.9; 95% CI = 1.5–9.9); never-active smokers with passive smoking also exhibited a significantly increased risk (adjusted RR = 2.6; 95% CI = 1.3–5.2)compared to never-active smokers without passive smoking. Stratified analyses by employment status showed increased risk for active and passive smoking in both unemployed and employed women; adjusted RR = 4.4 (95% CI = 0.6–34.6) for unemployed women with passive smoking; 7.9 (95% CI = 0.7–90.8) for unemployed women with ever-active smoking, 2.3 (95% CI = 1.1–4.9) for employed women with passive smoking and 3.3 (95% CI = 1.2–9.4) for employed women with ever-active smoking.

In postmenopausal women at baseline, no significant increased risk was observed for ever-active smokers (adjusted RR = 1.1; 95% CI = 0.5–2.5). Stratified analyses by employment status showed the following adjusted RRs; 0.6 (95% CI = 0.3–1.3) for unemployed women with passive smoking, 0.3 (95% CI = 0.04–2.6) unemployed women with ever-active smoking, 0.7 (95% CI = 0.4–1.2) for employed women with passive smoking and 1.5 (95% CI = 0.6–3.9) for employed women with ever-active smoking. When ex-smokers were eliminated from the statistical model because of the small number of cases and person-years, the risk of smoking remained essentially unchanged (data not shown).

TABLE I – SMOKING STATUS IN FEMALE STUDY SUBJECTS: JPHC STUDY COHORT I

Passive smoking	Active smoking		
	Never-smokers (n = 20169)	Ex-smokers (n = 374)	Current smokers (n = 1238)
Residential passive smoking (%)[1]			
Never	6175 (31.0)	79 (21.4)	234 (19.1)
Ever			
Before age 20	2231 (11.2)	54 (14.6)	225 (18.4)
After age 20	6957 (35.0)	136 (36.8)	444 (36.3)
Both	4536 (22.8)	101 (27.3)	320 (26.2)
Passive smoking in occupational and/or public settings (%)[2]			
Almost never	13626 (68.0)	199 (53.6)	553 (44.8)
1–3 days/month	1534 (7.7)	29 (7.8)	76 (6.2)
1–4 days/week	1057 (5.3)	25 (6.7)	76 (6.2)
Almost everyday	3811 (19.0)	118 (31.8)	529 (42.9)

[1]Missing and unavailable answers were omitted from the calculation; 270 in never-smokers, 4 in ex-smokers, 15 in current smokers.– [2]Missing were omitted from the calculation: 141 in never-smokers, 3 in ex-smokers, 4 in current smokers.

TABLE II – DISTRIBUTION OF KNOWN RISK FACTORS AND POSSIBLE CONFOUNDERS FOR BREAST CANCER BY SMOKING STATUS: JPHC STUDY COHORT 1

	Never-smokers				
	Without passive smoking (n = 5660)	With passive smoking (n = 14533)	Ex-smokers (n = 374)	Current smokers (n = 1238)	p for trend[1]
Age (mean)	49.9	49.6	49.1	48.6	<0.0001
Occupation, farmer (%)[2]	1281 (23.4)	3,014 (21.2)	46 (12.5)	131 (10.9)	<0.0001
Occupation, unemployed (%)[2]	2850 (52.1)	6,423 (45.2)	164 (44.6)	494 (41.2)	<0.0001
Education (> high school, %)[2]	597 (10.9)	1,746 (12.4)	68 (18.7)	140 (11.8)	0.02
Height (mean)	151.1	151.8	152.3	152.2	<0.0001
Weight (mean)	54.3	54.2	55.8	54.2	0.58
Body mass index (mean)	23.7	23.5	24.1	23.3	<0.0001
Family history of breast cancer in mother or sisters (%)[2]	18 (0.3)	90 (0.6)	3 (0.8)	5 (0.4)	0.18
History of past benign breast disease (%)[2]	455 (8.0)	1,525 (10.5)	40 (10.7)	98 (7.9)	0.08
Age at menarche (mean)	14.7	14.6	14.4	14.8	0.30
Parous women (%)[2]	4,922 (93.3)	13,063 (95.2)	307 (89.5)	1,043 (90.7)	0.04
Age at first delivery among parous women (mean)	25.0	24.9	25.5	24.5	<0.0001
Number of deliveries among parous women (mean)	2.9	2.9	2.8	2.9	0.29
Menopausal status (postmenopausal, %)[2]	3,045 (55.2)	7,734 (54.2)	189 (51.9)	602 (49.4)	<0.001
Previous and/or current hormone use (%)[2]	1,114 (21.0)	2,786 (20.4)	82 (23.0)	258 (22.1)	0.58
Alcohol consumption per week (mean grams)	79.2	115.7	164.0	239.3	<0.0001

[1]p for trend was calculated by Cochran-Mantel-Haenszel test.– [2]Missing were omitted from the calculation; 619 in occupation, 743 in education, 53 in family history of breast cancer, 53 in history of past benign breast disease, 1,369 in child birth, 473 in menopausal status and 1,369 in hormone use.

TABLE III – RELATIVE RISK OF FEMALE BREAST CANCER ACCORDING TO ACTIVE SMOKING; 10-YEAR FOLLOW-UP IN JPHC STUDY COHORT 1

Exposure	Number of case	Person-years	RR[1] (95% CI)	RR[2] (95% CI)
Pre-and post-menopausal women at baseline:				
Never-smoker	162	187,063	1.0	1.0
Ex-smoker	4	3,344	1.4 (0.5 to 3.8)	1.1 (0.4 to 3.5)
Current smoker	14	10,901	1.5 (0.9 to 2.6)	1.7 (1.0 to 3.1)
Pre-and post-menopausal women at baseline:				
Never-smoker without passive smoking	40	52,884	1.0	1.0
Never-smoker with passive smoking	122	134,178	1.2 (0.8 to 1.7)	1.1 (0.8 to 1.6)
Ex-smoker	4	3,344	1.6 (0.6 to 4.5)	1.2 (0.4 to 4.0)
Current smoker	14	10,901	1.7 (0.9 to 3.1)	1.9 (1.0 to 3.6)
Premenopausal women at baseline:				
Never-smoker without passive smoking	9	22,982	1.0	1.0
Never-smoker with passive smoking	68	60,272	2.9 (1.4 to 5.8)	2.6 (1.3 to 5.2)
Current- + ex-smoker	11	6,907	4.1 (1.7 to 9.9)	3.9 (1.5 to 9.9)
Postmenopausal women at baseline:				
Never-smoker without passive smoking	31	28,583	1.0	1.0
Never-smoker with passive smoking	52	71,602	0.7 (0.4 to 1.0)	0.6 (0.4 to 1.0)
Current- + ex-smoker	7	7,056	0.9 (0.4 to 2.1)	1.1 (0.5 to 2.5)

[1]Relative risks adjusted for public health center (4 areas) and age (4 5-year age groups).– [2]Relative risks adjusted for public health center, age, employment status (employed and unemployed), education level (≥high school and <high school), body mass index (<22, 22 ≤<25 and ≥25), family history of breast cancer in mother or sisters, history of past benign breast disease, age at menarche, number of births (0 and ≥1), menopausal status (pre and post), hormone use and alcohol consumption per week (<once/week, <250 g/week and ≥250 g/week).

Table IV shows RRs of incidence according to passive smoking status. Adjusted RR for any passive smoking was 1.1 (95% CI = 0.8–1.6). In premenopausal women at baseline, those with any passive smoking revealed a significantly increased risk (adjusted RR = 2.6; 95% CI = 1.3–5.2), and exposure to sidestream smoke in occupational and/or public settings itself showed increased risk (adjusted RR = 2.3; 95% CI = 1.4–3.8). Concerning passive smoking in occupational and/or public settings in premenopausal women, a dose-dependent increase was found (adjusted RR = 1.0 for "almost none"; 0.6 [95% CI = 0.4–2.4] for "1 to 3 days/month", 2.2 [95% CI = 1.4–3.7] for "≥ 1 days/week", p for trend 0.002). Past exposure to sidestream smoke at home did not show an increased risk. Among postmenopausal women at baseline, RRs for passive smoking were 0.7 (95% CI = 0.4–1.0), and those exposed to sidestream smoke in an occupational and/or public setting showed a marginal decreased risk (adjusted RR = 0.5; 95% CI = 0.2–1.0).

Discussion

In the present population-based prospective study of middle-aged Japanese women, an increased risk for active premenopausal smoking women was observed, especially when the reference was defined as never-active smokers without exposure to sidestream smoke. A subgroup analysis revealed that only premenopausal women at the study baseline showed increased risks from passive smoking. These findings were independent of reproductive risk factors and other potential confounders. In previous case-control studies, the risk for active and passive smoking was equivalent,[3,4,6,7] which seems to be implausible. However, the estimated risk for active smoking was larger than that for passive smoking in our study.

Breast cancer risks differ based on menopausal status.[30] Thus, the risk factors and the magnitude of their risk may be different before and after menopause. The etiological roles of endogenous

TABLE IV – RELATIVE RISK OF FEMALE BREAST CANCER ACCORDING TO PASSIVE SMOKING IN FEMALE NEVER-SMOKERS; 10-YEAR FOLLOW-UP IN JPHC STUDY COHORT 1

		Passive smoking		
	Never	(A) Past residential exposure (in any period)	(B) Occupational and/or public exposure (everyday)	(A) or (B)
All never-smokers				
Number of cases	40	114	37	122
Person-years	50,662	127,309	35,258	134,299
RR[1] (95% CI)	1.00	1.1 (0.8 to 1.5)	1.3 (0.9 to 1.8)	1.2 (0.8 to 1.7)
RR[2] (95% CI)	1.00	1.0 (0.7 to 1.4)	1.3 (0.9 to 1.9)	1.1 (0.8 to 1.6)
Premenopausal women at baseline:				
No. of cases	9	61	28	68
Person-years	22,263	56,896	17,884	60,320
RR[1] (95% CI)	1.00	1.7 (1.0 to 3.0)	2.1 (1.3 to 3.4)	2.9 (1.4 to 5.8)
RR[2]	1.00	1.6	2.3	2.6
(95% CI)		(0.9 to 2.7)	(1.4 to 3.8)	(1.3 to 5.2)
Postmenopausal women at baseline:				
Number of cases	31	51	8	52
Person-years	27,345	68,364	16,625	71,674
RR[1] (95% CI)	1.00	0.7 (0.4 to 1.1)	0.5 (0.3 to 1.1)	0.6 (0.4 to 1.0)
RR[2] (95% CI)	1.00	0.7 (0.4 to 1.1)	0.4 (0.2 to 1.0)	0.7 (0.4 to 1.0)

[1]Relative risks adjusted for public health center (4 areas) and age 4 5-year age group).– [2]Relative risks adjusted for public health center, age, employment status (employed and unemployed), education level (≥high school and <high school), body mass index (<22, 22 ≤<25 and ≥25), family history of breast cancer in mother or sisters, history of past benign breast disease, age at menarche, number of births (0 and ≥4), menopausal status (pre and post), hormone use and alcohol consumption per week (<once/week, <250 g/week and ≥250 g/week).

hormones admit of no doubt, and a causal model of breast cancer suggested that hormones increased the breast cancer risk in adults by increasing cell proliferation and the number of target cells, and also heightened the risk of the retention of spontaneous somatic mutations.[31] Therefore, higher levels of estrogens in premenopausal women may act jointly with exogenous carcinogens in breast carcinogenesis. The carcinogenic effects of tobacco smoke may result from a balance between its carcinogenic and anti-estrogenic effects.[6] Therefore, premenopausal women are likely to be affected by tobacco carcinogens because their estrogen levels are higher, thereby possibly canceling out the anti-estrogenic effects of tobacco smoke.

Smoking was reported to be associated with a decrease in the incidence of endometrial neoplasia in postmenopausal women.[23] The net effect of tobacco smoke may be antiestrogenic in the endometrium. However, available evidence, excluding 1 prospective study in Japan,[32] indicates that smoking has no beneficial effects in the breast. We did not observe statistically significant beneficial effects in the present study. However, our data suggest that at least the carcinogenic effects of tobacco smoke are not present in postmenopausal women.

Active and passive smoking are influenced by socioeconomic status.[33,34] Occupation is in fact related to smoking habits especially in women; working women generally smoke more and are exposed to sidestream smoke more frequently. Indeed, smoking status differed among several occupation-related factors in this cohort. A stratified analysis by employment status revealed interesting findings. In postmenopausal women, increased risk was observed only in employed women, although the small numbers of cases in the subgroup analyses precluded firm conclusions. Their pack-years were comparable (employed 10 ± 11 and unemployed 13 ± 13). These findings suggest that there were unknown residual confounders or different smoking behavior in these 2 groups. Risks for passive smoking were not increased in either employed or unemployed postmenopausal women. However, in premenopausal women, risks for active or passive smoking were increased in both employed and unemployed women. These findings suggest that any tobacco smoke exposure elevated the risk in premenopausal women no matter what their occupation. Educational level can be a surrogate indicator of socioeconomic status and has been reported as one of the important risk factors for breast cancer. Although we incorporated employment status and educational level into our statistical models, unknown residual confounders concerning socioeconomic status might not necessarily have been excluded from our analysis.

In our study, past exposure to sidestream smoke at home showed different effects from those by the occupational/social exposure. Residential exposure was defined as "a smoker(s) who had lived with a subject", although the current occupational/social exposure was assessed semi-quantitatively by self-report. Intensity or duration of daily exposure could not be estimated for the residential exposure. Previous cohort studies in Japanese women also used the smoking status of husbands as an index of passive smoking and did not observe elevated risk.[32,35]

The limitations of previous case-control studies were that recall and selection bias would tend to produce spurious positive association.[11] On the other hand, the limitations of previous cohort studies including misclassification of exposure and reference category have also been pointed out.[12–15] However, a well-designed prospective study is known to provide persuasive evidence. Our prospective study design also has some advantages in estimating the risks of smoking. Although recall bias may exist with information concerning passive smoking in a case-control study, there was no recall bias in our study because of its prospective nature. Never-active smokers without passive smoking were assigned to the reference, allowing for more accurate classification of exposure. Nonresidential passive smoking, *i.e.*, occupational or public exposure to tobacco smoke, was taken into account in the analyses. Subgroup analyses concerning menopausal status were done because the combined analyses may dilute the risk estimation.

On the other hand, there are some admitted limitations. Because the exposure assessment was done at 1 point (at baseline), a misclassification of the exposure might have occurred, thereby diluting the effects if some smoking women had quit smoking during the follow-up period. Information on the menopausal status was obtained at baseline. Therefore, we did not examine the risks for pre- and post-menopausal cancer. The relatively small number of incidence cases precluded further subgroup analyses. Results of the subgroup analyses according to menopausal status in this report should be confirmed by continued follow-up.

Different effects of active or passive smoking regarding breast cancer risk had been shown in premenopausal and postmenopausal women.[7,36] In a recent study, the risk of breast cancer among smokers has been clearly reported to be elevated in premenopausal women.[36] Immature breast cells are suggested to have especially increased susceptibility to smoking-related carcinogens.[6] In our

study, 94% of subjects had delivered children, but the effect of smoking in strata defined by age of full-term birth could not be examined. On the other hand, in postmenopausal women, the risk of breast cancer among smokers has been reported not to be elevated.[36] These previous observations are consistent with our observations regarding both active and passive smoking. Race is also an important factor in the interpretation of our results. To our knowledge, this is the first prospective study to link active smoking to breast cancer risk in Asian women, although recent large-scale cohort studies in America did not detect any increased risk of breast cancer.[10,11] Genetic differences concerning important metabolic enzymes, for example, higher frequency of a variant allele of cytochrome P450 1A1 gene, were reported,[37] and endogenous estrogen levels and the number of estrogen receptors have been reported to differ between Japanese and Caucasians.[38,39] Thus, an association between smoking and breast cancer might appear more readily in Japanese. The incidence of breast cancer among premenopausal women (88/90,161 person-year) was almost the same as that among postmenopausal women (90/107,241 person-year), and the association observed in premenopausal women was strong. These might be why we observed an elevated risk due to tobacco smoking in the overall subjects.

In conclusion, tobacco smoking increases the risk of female breast cancer in premenopausal women. Both active and passive smoking are promising targets in the prevention of breast cancer.

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References

1. Crabb C. Is breast cancer linked to smoking? Bull World Health Organ 2003;81:74–4.
2. Wells AJ. Breast cancer, cigarette smoking, and passive smoking. Am J Epidemiol 1991;133:208–10.
3. Smith SJ, Deacon JM. Chilvers CE. Alcohol, smoking, passive smoking and caffeine in relation to breast cancer risk in young women: UK National Case-Control Study Group. Br J Cancer 1994;70:112–9.
4. Morabia A, Bernstein M, Heritier S, Khatchatrian N. Relation of breast cancer with passive and active exposure to tobacco smoke. Am J Epidemiol 1996;143:918–28.
5. Millikan RC, Pittman GS, Newman B, Tse CK, Selmin O, Rockhill B, Savitz D, Moorman PG, Bell DA. Cigarette smoking, N-acetyltransferases 1 and 2, and breast cancer risk. Cancer Epidemiol Biomarkers Prev 1998;7:371–8.
6. Lash TL, Aschengrau A. Active and passive cigarette smoking and the occurrence of breast cancer. Am J Epidemiol 1999;149:5–12.
7. Johnson KC, Hu J, Mao Y. Passive and active smoking and breast cancer risk in Canada, 1994–97. The Canadian Cancer Registries Epidemiology Research Group. Cancer Causes Control 2000;11:211–21.
8. London SJ, Colditz GA, Stampfer MJ, Willett WC, Rosner BA, Speizer FE. Prospective study of smoking and the risk of breast cancer. J Natl Cancer Inst 1989;81:1625–31.
9. Jee SH, Ohrr H, Kim IS. Effects of husbands' smoking on the incidence of lung cancer in Korean women. Int J Epidemiol 1999;28:824–8.
10. Wartenberg D, Calle EE, Thun MJ, Heath CW, Jr., Lally C, Woodruff T. Passive smoking exposure and female breast cancer mortality. J Natl Cancer Inst 2000;92:1666–73.
11. Egan KM, Stampfer MJ, Hunter D, Hankinson S, Rosner BA, Holmes M, Willett WC, Colditz GA. Active and passive smoking in breast cancer: prospective results from the Nurses' Health Study. Epidemiology 2002;13:138–45.
12. Morabia A. Active and passive smoking in breast cancer. Epidemiol 2000;13:744–5.
13. Johnson KC, Wells AJ. Active and passive smoking in breast cancer. Epidemiol 2000;13:745–6.
14. Wells AJ. Re: Passive smoking exposure and female breast cancer mortality. J Natl Cancer Inst 2001;93:717–9; author reply 20–1.
15. Johnson KC. Re: Passive smoking exposure and female breast cancer mortality. J Natl Cancer Inst 2001;93:719–20; author reply 20–1.
16. International Agency for Research on Cancer. Tobacco smoking. IARC monographs on the evaluation of carcinogenic risk of chemicals to humans, vol. 38. Lyon: IARC, 1986.
17. Petrakis NL, Maack CA, Lee RE, Lyon M. Mutagenic activity in nipple aspirates of human breast fluid. Cancer Res 1980;40:188–9.
18. Li D, Wang M, Firozi PF, Chang P, Zhang W, Baer-Dubowska W, Moorthy B, Vulimiri SV, Goth-Goldstein R, Weyand EH, DiGiovanni J. Characterization of a major aromatic DNA adduct detected in human breast tissues. Environ Mol Mutagen 2002;39:193–200.
19. Firozi PF, Bondy ML, Sahin AA, Chang P, Lukmanji F, Singletary ES, Hassan MM, Li D. Aromatic DNA adducts and polymorphisms of CYP1A1, NAT2, and GSTM1 in breast cancer. Carcinogenesis 2002;23:301–6.
20. McKinlay SM, Bifano NL, McKinlay JB. Smoking and age at menopause in women. Ann Intern Med 1985;103:350–6.
21. Ross RK, Pike MC, Vessey MP, Bull D, Yeates D, Casagrande JT. Risk factors for uterine fibroids: reduced risk associated with oral contraceptives. Br Med J (Clin Res Ed) 1986;293:359–62.
22. Parazzini F, La Vecchia C, Negri E, Cecchetti G, Fedele L. Epidemiologic characteristics of women with uterine fibroids: a case-control study. Obstet Gynecol 1988;72:853–7.
23. Brinton LA, Barrett RJ, Berman ML, Mortel R, Twiggs LB, Wilbanks GD. Cigarette smoking and the risk of endometrial cancer. Am J Epidemiol 1993;137:281–91.
24. MacMahon B, Trichopoulos D, Cole P, Brown J. Cigarette smoking and urinary estrogens. N Engl J Med 1982;307:1062–5.
25. Baron JA, La Vecchia C, Levi F. The antiestrogenic effect of cigarette smoking in women. Am J Obstet Gynecol 1990;162:502–14.
26. Sasazuki S, Sasaki S, Tsugane S. Cigarette smoking, alcohol consumption and subsequent gastric cancer risk by subsite and histologic type. Int J Cancer 2002;101:560–6.
27. Yamamoto S, Sobue T, Kobayashi M, Sasaki S, Tsugane S. Soy, isoflavones, and breast cancer risk in Japan. J Natl Cancer Inst 2003;95:906–13.
28. Parlkin D, Chen V, Ferlay J, Galceran J, Storm H, Whelan S. Comparability and quality control in cancer registration. IARC Technical Report No.19. Lyon: IARC, 1994.
29. International Agency for Research on Cancer. Cancer incidence in five continents, vol. VIII, IARC Scientific Publications vol. 155. Lyon: IARC, 2002.
30. Key TJ, Verkasalo PK, Banks E. Epidemiology of breast cancer. Lancet Oncol 2001;2:133–40.
31. Adami HO, Persson I, Ekbom A, Wolk A, Ponten J, Trichopoulos D. The aetiology and pathogenesis of human breast cancer. Mutat Res 1995;333:29–35.
32. Nishino Y, Tsubono Y, Tsuji I, Komatsu S, Kanemura S, Nakatsuka H, Fukao A, Satoh H, Hisamichi S. Passive smoking at home and cancer risk: a population-based prospective study in Japanese nonsmoking women. Cancer Causes Control 2001;12:797–802.
33. Tseng M, Yeatts K, Millikan R, Newman B. Area-level characteristics and smoking in women. Am J Public Health 2001;91:1847–50.
34. Stamatakis KA, Brownson RC, Luke DA. Risk factors for exposure

to environmental tobacco smoke among ethnically diverse women in the United States. J Womens Health Gend Based Med 2002;11: 45–51.
35. Hirayama T. Cancer mortality in nonsmoking women with smoking husbands based on a large-scale cohort study in Japan. Prev Med 1984;13:680–90.
36. Band P, Le N, Fang R, Deschamps M. Carcinogenic and endocrine disrupting effects of cigarette smoke and risk of breast cancer. Lancet 2002;360:1044.
37. Garte S, Gaspari L, Alexandrie AK, Ambrosone C, Autrup H, Autrup JL, Baranova H, Bathum L, Benhamou S, Boffetta P, Bouchardy C, Breskvar K, et al. Metabolic gene polymorphism frequencies in control populations. Cancer Epidemiol Biomarkers Prev 2001;10:1239–48.
38. Shimizu H, Ross RK, Bernstein L, Pike MC, Henderson BE. Serum oestrogen levels in postmenopausal women: comparison of American whites and Japanese in Japan. Br J Cancer 1990;62:451–3.
39. Nomura Y, Kobayashi S, Takatani O, Sugano H, Matsumoto K, McGuire WL. Estrogen receptor and endocrine responsiveness in Japanese versus American breast cancer patients. Cancer Res 1977; 37:106–10.

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ORIGINAL ARTICLE

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Association of habitual smoking and drinking with single nucleotide polymorphism (SNP) in 40 candidate genes: data from random population-based Japanese samples

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Abstract Basic information on the association between lifestyle factors and candidate genes is valuable for genetic–environmental study. We screened the association of habitual smoking or drinking with polymorphism in 40 candidate genes for a total of 153 single nucleotide polymorphisms (SNPs) using a sample of 339 middle-aged, randomly selected Japanese men. Smoking and drinking statuses were elicited during questionnaire-based interviews. Genes were selected based on their possible involvement in genetic–environmental, life-style interactions and constitute the genes expressing xenobiotic metabolism enzymes, DNA repair enzymes, and other stress-related proteins. The *P* values of odds ratios to habitual smoking for CYP17A1, ESR1, EPHX1, GSTT2, ALDH2, NOS2A, OGG1, and SLC6A4 and those of odds ratios to habitual drinking for CYP1B1, ESR1, HSD17B3, GSTM3, COMT, ADH1C, ALDH2, NOS3, and NUDT1 were under 0.05. These variables were included in a stepwise logistic analysis in order to develop a predictive model for smoking or drinking behavior. In the final model, the only significant variables selected for smoking were OGG1, SLC6A4, EPHX1, ESR1, and CYP17A1, and for drinking, ALDH2 and NUDT1. The findings of the present study suggest that polymorphism in associated candidate genes plays a role in the habitual use of tobacco and alcohol among Japanese men.

Keywords Smoking · Drinking · Single nucleotide polymorphism · Candidate gene · Japanese men · Association study

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Introduction

Basic information on whether lifestyle factors and candidate genes are independent of each other is valuable for genetic–environmental study. Such information is essential for future studies to reveal the interaction of lifestyle factors and genetics. Furthermore, an understanding of genes associated with lifestyle factors will contribute to more accurate risk identification and to establishing tailor-made prevention measures.

Two of the most common and important lifestyle factors, cigarette smoking and alcohol drinking, are related to many diseases, including lung cancer, cardiovascular disease, and other chronic diseases. Hence, genetic influence on the use of tobacco has been strongly implicated by cross-sectional studies in twins, association studies, and numerous other genetic epidemiology data (Carmelli et al. 1992; Hussain et al. 2001; Pianezza et al. 1998; Sabol et al. 1999; Yoshida et al. 2001). It is also recognized that the use of tobacco is often accompanied by alcohol consumption (Hopfer et al. 2001). Alcohol consumption is found to be as heritable as smoking (Heath et al. 1991; McGue et al. 2001), and the contribution of ALDH2 and ADH1C genes to habitual alcohol drinking has been well known. However, current

knowledge on genetic polymorphism related to smoking or drinking behavior is far from sufficient. Information on associated genetic factors could provide a more rational basis for developing smoking or drinking cessation programs, including identification of persons at high risk and the introduction of suitable interventions to prevent smoking-related or drinking-related diseases.

We examined the relationship between the smoking and drinking habits of 339 Japanese men and their genotypes in 153 single nucleotide polymorphisms (SNPs) in 40 selected genes. The genes were selected based on possible involvement in genetic–environmental, life-style interactions and constitute the genes for xenobiotic metabolism enzymes, DNA repair enzymes, and other stress-related proteins. This population-based association study will provide a list of genes independent of habitual smoking and drinking, which may be included in a future case study. It will also establish the role of genetic influence on smoking and drinking behavior and thus contribute to the future development of genotype-based prevention of smoking-related and drinking-related diseases.

Subjects and methods

Subjects

The selection of subjects is described in detail by Tsugane et al. (1992a, b). Briefly, from 1989 to 1991, using a random sampling method employing resident registration rolls, we recruited men aged 40–49 years from five areas having a population of approximately 100,000 people. Between 170 and 195 subjects were selected from each area. The selected individuals were initially invited to participate in the study by e-mail, and a subsequent letter, telephone call, and home visit encouraged participation. A questionnaire-based interview elicited lifestyle details and health check-up information, as well as yielding blood and urine samples. The overall participation rate was 72%, or 634 out of 880.

Smoking and drinking habits

Subjects were asked whether they had ever used tobacco regularly (habitual smoking) and were then classified as "past smokers," "current smokers," or "never smokers." With regard to alcohol drinking, those who did not habitually consume alcohol once or more per month were considered "nondrinkers;" otherwise subjects were considered "drinkers." Whether they flush on drinking was recorded as well.

DNA samples and SNPs analyses

A total of 25 ml of blood was drawn by venipuncture, and genomic DNA was extracted from the buffy coat layer using a commercial kit (Wako, Osaka, Japan). Some samples were used in another study (Sugimura et al. 1998) and exhausted before the present study, leaving 339 cases with a representative sample of 0.5 μg or more of DNA. For each gene, 5–8 SNPs were chosen from public databases or published papers, with a total of 289 SNPs being typed for 44 genes using the mass spectroscopy-based technique, Mass ARRAY (Ross et al. 1998). The 44 were selected from genes encoding xenobiotic metabolic enzymes, DNA repair enzymes, and other stress-related proteins. Only SNPs in the Hardy–Weinberg equilibrium with a >0.05 chi-square and SNPs having a minor allele frequency of at least 1% were selected for analysis. Thus, 153 SNPs from 40 genes were finally included. Details of SNP selection, including allele frequency in the 153 SNPs, is described by Yoshimura et al. (2003). The genes included in the present study were cytochrome P450 genes (CYP1A1, CYP1B1, CYP2C9, CYP2C19, CYP2E1, CYP17A1, and CYP19A1), the aryl hydrocarbon receptor gene (AHR), estrogen receptor genes (ESR1, ESR2, and ERRRG), the progesterone receptor gene (PGR), epoxide hydrolase genes (EPHX1, and EPHX2), hydroxysteroid (17-beta) dehydrogenase genes (HSD17B2 and HSD17B3), glutathione S-transferase genes (GSTM2, GSTM3, GSTT2, and GSTP1), N-acetyltransferase genes (NAT1 and NAT2), the catechol-O-methyltransferase gene (COMT), alcohol dehydrogenase genes (ADH1A, ADH1B, and ADH1C), the aldehyde dehydrogenase gene (ALDH2), nitric oxide synthase genes (NOS2A and NOS3), interleukin genes (IL1A and IL1B), repair genes for oxidative DNA damage [OGG1 and NUDT1 (MTH1)], dopamine receptor genes (DRD2, DRD3, and DRD4), the serotonin transporter gene (SLC6A4), the glucocorticoid receptor gene [NR3C1 (GCCR)], the folate metabolizing enzyme gene (MTHFR), and the quinone oxidoreductase gene (NQO1).

Ethical issues

All DNA samples were anonymous and unlinked to specific individual information, i.e., any ID, name, or address. The protocol of the present study was approved by the ethics review committee of the National Cancer Center (protocol number G12-02).

Statistical analysis

First, each SNP was analyzed independently to screen the SNPs possibly related with smoking or drinking. In order to show the ratio of the odds of becoming a smoker or drinker in those exposed to targeted genotype relative to the unexposed individuals, we used an odds ratio as an indicator of the strength of association between genotypes and the smoking or drinking habit. A logistic regression analysis was used to obtain odds ratios, and 95% confidence intervals were calculated using

the standard errors of the logistic regression coefficients. P values were calculated from the χ^2 test. We also calculated the statistical power of the analyses by using the formula of Schlesselman (Schlesselman 1982). We then included all significant SNPs in the above analyses simultaneously in a stepwise logistic regression analysis to select substantially significant ones. We tested the statistical significance once and did not use a correction of multiple testing because we used a multivariate analysis. All computations were performed using the SAS software package Version 8.2 (SAS Institute, Inc., Cary, NC, USA).

Results

The number of never smokers, past smokers, and current smokers was 55, 71, and 213, respectively. We combined never smokers and past smokers as nonsmokers in order to gain greater investigative power. The number of nondrinkers and drinkers was 71 and 268, respectively. All 153 SNPs in the 40 genes examined are listed in Table 1. Among them, 11 SNPs of five genes (CYP17A1, ALDH2, NOS2A, OGG1, and SLC6A4) in the dominant model and three SNPs of three genes (ESR1, EPHX1, and GSTT2) in the recessive model were found to be associated with habitual smoking in a statistically significant manner (Table 2). Similarly, ten SNPs of five genes (HSD17B3, COMT, ADH1C, ALDH2, and NOS3) in the dominant model and five SNPs of four genes (CYP1B1, ESR1, GSTM3, and NUDT1) in the recessive model were found to be associated with habitual drinking in a statistically significant manner (Table 3). No relationship with smoking and drinking behavior was observed for 32 genes and 31 genes, respectively.

Next, a multivariate analysis was performed using variables including drinking status and the genotype of 14 SNPs listed in Table 2. The odds ratios of five genes for smoking, selected by stepwise logistic regression analysis, are given in Table 4. While a negative association was found in the dominant model between habit-

Table 1 List of 153 SNPs in 40 genes selected for association study

Gene symbol	SNP (rs number)
CYP1A1	rs1048943
CYP1B1	rs10012, rs1056827, rs1056836, rs10916
CYP2C9	rs1505
CYP2C19	rs1322179
CYP2E1	rs3813867, rs2031920, rs2070673
CYP17A1	rs6162, rs6163, rs743572
CYP19A1	rs700518, rs700519, rs4646
AHR	rs2066853, rs713150, rs2074113, rs2237297, rs2237299, rs2282886
ESR1	rs1913474, rs932479, rs2011885, rs974276, rs1062577
ESR2	rs1256054, rs1256049, rs1256027, rs944459, rs2274705, rs1256030
ESRRG	rs1498283, rs1339343
PGR	rs484389
EPHX1	rs1051741, rs1051740, rs6965, rs2292566, rs2234922, rs2292568
EPHX2	rs751141, rs1042032, rs891401, rs2291635, rs1126452, rs747276
HSD17B2	rs2042429, rs1017243, rs1424151, rs996752
HSD17B3	rs2066480, rs375944, rs280654, rs912462, rs2066479, rs867807
GSTM2	rs655315, rs428434
GSTM3	rs1332018
GSTT2	rs1622002, rs2719, rs2267047, rs140186
GSTP1	rs947894, rs762803
NAT1	rs15561
NAT2	rs1801280, rs1799929, rs1799930, rs1495744
COMT	rs4633, rs4680, rs6267, rs2097603, rs2020917, rs2239393
ADH1A	rs931635, rs1229967, rs1229970, rs975833, rs1618572, rs2276332
ADH1B	rs17033, rs1159918, rs1042026
ADH1C	rs1789924, rs1693430, rs2009181, rs2298755, rs3216150
ALDH2	rs671, rs2238151, rs2238152, rs441
NOS2A	rs1060826, rs1060822, rs2072324, rs2297518, rs2297520
NOS3	rs1800783, rs1549758, rs1799983, rs1800780, rs1800779
IL1A	rs17561, rs1800587, rs1800794, rs2071374
IL1B	rs1143627, rs16944, rs1071676, rs1143637, rs1143629, rs1143634
OGG1	rs2075747, rs1052133, rs2072668, rs1801129
NUDT1	rs4866, rs1062492
DRD2	rs1076560, rs1124491, rs6277, rs6275, rs1076563, rs1079596, rs1801028, rs1116313
DRD3	rs6280, rs1800828
DRD4	rs1800955, rs936460, rs752306
SLC6A4	rs1042173, rs2020939, rs2020936, rs1872924, rs25528, rs717742
NR3C1	rs6194, rs258751, rs6196, rs33388, rs33389, rs174050
MTHFR	rs2066470, rs1801133, rs1801131, rs2066471, rs2274976
NQO1	rs1800566

Table 2 Odds ratios (OR) and 95% confidence intervals (CI) of SNPs associated with smoking behavior. Allele A represents major allele; allele a represents minor allele

Gene symbol	SNP (rs number)	Reference allele/ variant allele	Amino acid change	Major allele/minor allele	Genotype of nonsmokers			Genotype of smokers			OR (95% CI)aa versus Aa+AA	*P* value	OR (95% CI)aa+ Aa versus AA	*P* value
					AA	Aa	aa	AA	Aa	aa				
ESR1	rs1913474	gttc(T/C)aaga	Intron	C/T	36	66	19	59	92	53	1.9 (1.1–3.4)	0.03	1.0 (0.6–1.7)	0.87
EPHX1	rs2292566	ctaa(G/A)attg	Lys → Lys	G/A	52	57	17	104	97	12	0.4 (0.2–0.8)	0.01	0.7 (0.5–1.1)	0.18
GSTT2	rs140186	ttag(G/A)ggat	Locus	G/A	42	46	22	73	96	20	0.5 (0.2–0.9)	0.02	1.0 (0.6–1.6)	0.94
CYP17A1	rs743572	ccac(T/C)gctg	Untranslated region	T/C	29	69	28	72	100	40	0.8 (0.5–1.4)	0.46	0.6 (0.4–1.0)	0.03
ALDH2	rs2238152	caaa(C/A)agat	Intron	C/A	81	42	3	160	48	3	0.6 (0.1–3.0)	0.52	0.6 (0.4–0.9)	0.02
	rs441	tgag(A/G)ccga	Intron	A/G	80	42	3	162	48	3	0.6 (0.1–2.9)	0.51	0.6 (0.3–0.9)	0.02
NOS2A	rs2072324	ttat(C/A)ttct	Intron	C/A	68	45	9	94	98	21	1.4 (0.6–3.1)	0.44	1.6 (1.0–2.5)	0.04
OGG1	rs1052133	caat(C/G)ccgc	Ser → Cys	C/G	29	69	28	72	105	36	0.7 (0.4–1.2)	0.23	0.6 (0.4–1.0)	0.04
	rs2072668	catt(C/G)tgtg	Intron	C/G	29	68	28	71	104	38	0.8 (0.4–1.3)	0.31	0.6 (0.4–1.0)	0.05
	rs1801129	tact(A/G)cggg	Untranslated region	A/G	107	17	1	197	13	1	0.6 (0.0–9.6)	0.71	0.4 (0.2–0.9)	0.02
SLC6A4	rs2020936	agcg(A/G)tctc	Locus	A/G	117	8	1	173	38	2	1.2 (0.1–13.2)	0.87	3.0 (1.4–6.4)	0.008
	rs1872924	gtca(T/C)ctag	Intron	T/C	115	8	1	168	35	2	1.2 (0.1–13.5)	0.89	2.8 (1.3–6.1)	0.003
	rs25528	tggt(T/G)tcgc	Locus	T/G	114	8	1	166	34	2	1.2 (0.1–13.7)	0.90	2.8 (1.3–5.9)	0.003
	rs717742	gcgc(A/T)gaca	Intron	A/T	104	6	1	155	34	2	1.2 (0.1–13.0)	0.88	3.5 (1.5–8.1)	0.006

ual smoking and the minor allele of SNPs in the OGG1 and CYP17A1 genes, a negative association was found in the recessive model for the minor allele of the SNP in EPHX1. In ESR1 and SLC6A4, minor alleles of SNPs were related to an increased risk for habitual smoking in the recessive and dominant models, respectively.

Dividing nonsmokers into never and past smokers indicated that the genotype frequencies containing the minor allele of the SNP in OGG1 (Cys) were similar among current and past smokers but much higher among never smokers. While past smokers have a higher genotype frequency containing the minor allele of the SNP in CYP17A1 than do current smokers, a lower frequency of the genotype containing the minor allele of the SNP in ESR1 was observed among past smokers compared with current smokers.

When the covariates, including smoking status and genes presented in Table 3, were selected using stepwise logistic regression analysis, polymorphisms in the ALDH2 and NUDT1 genes were chosen as the only variants significantly associated with alcohol drinking in the dominant and recessive heritable models for the minor alleles of their SNPs. We further examined the influence of NUDT1 and ALDH2 on alcohol-induced response. While NUDT1 had no association with alcohol-induced flushing, the odds ratio of having the Lys allele of ALDH2 was as high as 33 for alcohol-induced flushing (data not shown).

Discussion

Among 153 SNPs in the 40 genes, we found that SNPs in five genes (EPHX2, ESR1, hOGG1, SLC6A4, and CYP17A1) were related to habitual smoking and SNPs in two genes (ALDH2 and NUDT1) with habitual drinking. No common allele responsible for both habits was found among the 40 genes investigated in the present study. We also found that 32 genes were independent of smoking and 31 genes were independent of habitual alcohol consumption. However, due to the limitation of sample size, the statistical power was generally not large enough to detect an association that might actually exist for some genes. Further study is required to confirm the results.

A previous Japanese study noted that polymorphism of the SLC6A4 gene in the 5'-flanking region influences smoking behavior. Individuals with the homozygous S (a 44-bp deletion) genotype were less inclined to smoke and/or could more easily stop smoking than L allele (a 44-bp insertion) carriers (Ishikawa et al. 1999), although opposite data also exist (Lerman et al. 1998). In the present study, the presence of the minor allele rs717742 SNP of the same gene appears to confer a more than three-fold increased risk for smoking. Moreover, a significantly increased frequency of the minor allele of the SNP in current smokers compared with that in past smokers suggests that individuals with the minor allele may experience difficulty quitting. The location of the

Table 3 Odds ratios (OR) and 95% confidence intervals (CI) of SNPs associated with drinking behavior. Allele A represents major allele; allele a represents minor allele

Gene symbol	SNP (rs number)	Reference allele/ variant allele	Amino acid change	Major allele/ minor allele	Genotype of nondrinkers			Genotype of drinkers			OR (95% CI)aa versus Aa + AA	*P* value	OR (95% CI)aa + Aa versus AA	*P* value
					AA	Aa	aa	AA	Aa	aa				
CYP1B1	rs1056836	ccca(C/G)tgaa	Leu → Val	C/G	45	20	5	194	68	5	0.2 (0.1–0.9)	0.02	0.7 (0.4–1.2)	0.17
ESR1	rs932479	tatc(C/T)ctca	Intron	T/C	22	41	7	70	132	64	2.9 (1.2–6.5)	0.01	1.3 (0.7–2.3)	0.39
	rs1062577	attc(T/A)tttt	Untranslated region	T/A	30	25	13	126	102	26	0.5 (0.2–1.0)	0.05	0.8 (0.5–1.4)	0.42
GSTM3	rs1332018	atgt(C/A)gggt	Untranslated region	A/C	41	19	4	149	83	2	0.1 (0.0–0.7)	0.01	1.0 (0.6–1.8)	0.95
NUDT1	rs4866	tgca(C/T)gtcc	Val → Met	C/T	55	11	3	212	49	1	0.1 (0.0–0.8)	0.01	0.9 (0.5–1.8)	0.82
HSD17B3	rs2066479	cagc(G/A)gtgc	Gly → Ser	G/A	31	33	7	154	96	17	0.6 (0.2–1.5)	0.31	0.6 (0.3–1.0)	0.04
COMT	rs4680	tggc(G/A)tgaa	Val → Met	G/A	40	24	6	115	124	29	1.3 (0.5–3.2)	0.58	1.8 (1.0–3.0)	0.03
ADH1C	rs1789924	gtta(T/C)gaag	Locus	C/T	32	10		162	15				0.3 (0.1–0.7)	0.01
	rs1693430	aaat(T/C)ggtg	Intron	C/T	32	9		160	15				0.3 (0.1–0.8)	0.01
	rs2298755	cttt(G/C)acaa	Intron	C/G	37	11		172	18	1		0.62	0.4 (0.2–0.8)	0.02
	rs3216150	aaaa(A/-)tcac	Intron	*/A	38	11		169	18	1		0.61	0.4 (0.2–0.9)	0.02
ALDH2	rs671	cact(G/A)aagt	Glu → Lys	G/A	26	29	9	199	58	3	0.1 (0.0–0.3)	< 0.001	0.2 (0.1–0.4)	< 0.0001
	rs2238152	caaa(C/A)agat	Intron	C/A	58	13		183	77	6		0.20	2.0 (1.0–3.9)	0.03
	rs441	tgag(A/G)ccga	Intron	A/G	58	13		184	77	6		0.20	2.0 (1.1–3.9)	0.03
NOS3	rs1800780	gtcc(T/C)gggt	Locus	C/T	19	41	9	114	105	40	1.2 (0.6–2.7)	0.62	0.5 (0.3–0.9)	0.01

SNP of the SLC6A4 gene in the present study is about 10 kb to the polymorphism in the 5'-flanking region of previous studies. The possibility may exist that the two loci are in linkage disequilibrium.

Previous studies indicated that polymorphisms of the genes in the dopaminergic system are candidate genetic markers for habitual smoking; a relationship between the Taq I A or Taq I B RFLP polymorphisms of DRD2 and a predisposition to smoking was suggested, although the results were controversial (Wu et al. 2000; Yoshida et al. 2001). In addition, the DRD4 VNTR polymorphism was noted as another candidate marker for habitual smoking (Shields et al. 1998). No relationship between the selected SNPs of DRD2, DRD3, or DRD4 and habitual smoking was found in the present studies; albeit, the negative findings may narrow the research field to target SNPs in the dopaminergic system for future studies searching for genetic determinants of habitual smoking.

We found that the OGG1 Ser/Cys or Cys/Cys genotypes were associated with a decreased risk for smoking compared with homozygous Ser carriers. However, the OGG1 Cys allele polymorphism is considered to be related to an increased risk for lung cancer and other smoking-related cancers (Goode et al. 2002; Le Marchand et al. 2002). Since smoking is the most potent carcinogenic factor in lung cancer, the negative association of Cys carriers with habitual smoking and the positive association of Cys carriers with lung cancer risk seem controversial. However, a similar paradoxical relationship has been observed between ALDH2 polymorphism, alcohol consumption, and liver cancer incidence. The ALDH2 Lys allele has been related to an elevated risk for liver cancer, and Lys carriers lacking ALDH2 isozyme activity ought to reduce alcohol consumption, a well-known factor in hepatocellular carcinoma (Eriksson 2001; Yokoyama and Omori 2001). However, when those individuals with the Lys allele do drink alcohol habitually, they are at much higher risk for different forms of cancer of the digestive tract, liver, and upper respiratory tract than those without the allele (Munaka et al. 2003; Yokoyama and Omori 2001). The Cys allele may preclude the use of tobacco but may exacerbate the impact of smoking on smoking-related cancer development.

Many previous studies pointed out that the single base mutation from glutamic acid (glutamate) to lysine at residue 504 in ALDH2 was the best-characterized genetic factor influencing alcohol consumption behavior (Goedde et al. 1992; Muramatsu et al. 1995). The findings of the present study are in agreement with the original studies showing Lys allele carriers suffer the alcohol-flush reaction.

None of the previous studies examined whether the EPHX2, ESR1, and CYP17A1 genes contribute to smoking behavior or the NUDT1 gene to alcohol use. We observed an increased frequency of the genotype homozygous for the minor allele of the rs1913474 SNP in ESR1, as well as a decreased frequency of the minor