

Genetic component of lung cancer: cohort study of twins

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Summary

Epidemiological and molecular epidemiological findings suggest that inherited predisposition may be a component of lung cancer risk and an important modulator of the carcinogenic effects of cigarette smoke. We have carried out a genetic analysis of lung cancer mortality on the National Academy of Sciences/National Research Council Twin Registry.

The registry is composed of 15 924 male twin pairs who were born in the USA between 1917 and 1927 and who served in the armed forces during World War II. As evidence for a genetic effect on lung cancer, we required concordance for lung cancer death to be greater among monozygotic than among dizygotic twin pairs. No genetic effect on lung cancer mortality was observed. The ratio of observed to expected concordance among monozygotic twins did not exceed that among dizygotic twins (overall rate ratio 0.75 [95% CI 0.35–1.6]), even though monozygotic twin pairs are more likely to be concordant for smoking than dizygotic twin pairs in this population. A cohort analysis (accounting for age, sex, race, and smoking intensity) of lung cancer mortality found no lung cancer deaths during 300 person-years of follow-up (observed to expected ratio 0 [0–0.09]) among 47 monozygotic twin smokers whose smoking twins had died of lung cancer, even though smoking histories were very similar within twin pairs.

In our study, there is little if any effect of inherited predisposition on development of lung cancer. Genetic factors are not likely to be strongly predictive of lung cancer risk in most male smokers older than 50, the age group in which the vast majority of cases occur.

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Introduction

Advances in molecular biology have permitted the mapping and characterisation of several genes aetiologically related to hereditary syndromes of increased cancer susceptibility. In families with such syndromes, this knowledge holds the promise of better prevention of cancer in individual family members identified as having the genetic defect.

Although cancers in families with cancer syndromes constitute only a small proportion of all cancers, genetically determined factors may also have a role in the aetiology of lung and other cancers in the general population.¹ For example, case-control studies have shown an excess risk of lung cancer in first-degree relatives of cases,^{2–5} and a segregation analysis was compatible with a mendelian co-dominant autosomal gene related to early onset of the disease.⁶ These epidemiological findings could be due to differential elimination of respiratory carcinogens determined by glutathione-S-transferase gene polymorphisms,⁷ differential activation of respiratory carcinogens determined by cytochrome p450 enzyme *CYP2D6*⁸ or *CYP1A1* gene polymorphisms, or oncogene^{9,10} or tumour suppressor gene¹¹ polymorphisms. Many of these mechanisms, which illustrate the great variability in human xenobiotic metabolism,¹² have been put forward to explain the observation that more than 80% of smokers living to old age do not die of smoking-induced lung cancer.¹³

There is much interest in developing techniques of screening for inherited predisposition to cancer. A USA cohort of 31 848 twins, with 45 years of mortality follow-up, provided the opportunity to study the genetic component of lung cancer and to gain insight into the potential value of screening for inherited predisposition to a common cancer with a known environmental cause.

Patients and methods

The National Academy of Sciences/National Research Council Twin Registry has been described in detail elsewhere.^{14,15} About 93% of all white male twin births in the USA between 1917 and 1927 were identified. The twins composing the registry are the 15 924 pairs born during this time who had records in the master index file of the Department of Veterans Affairs (VA), which showed that they had passed an armed forces medical screening examination and served in the armed forces during World War II. Zygosity has been determined for 13 487 pairs of twins in the panel, largely on the basis of questionnaire data. This method has been validated by blood typing and is about 95% accurate in this and other twin cohorts.^{16,17} Most of the 2437 twin pairs classified as unknown zygosity are so classified because they did not respond to the questionnaire.¹⁵

For the concordance analysis, follow-up for lung cancer mortality for all the 15 924 twin pairs began at entry into the armed forces and ended at the time of death or Dec 31, 1990, whichever was earlier. Expected numbers of pairs concordant for lung cancer death in each of the zygosity groups were calculated by multiplying the square of the proportion of twins in the zygosity group who died from lung cancer by the total of pairs in the zygosity group. These expected frequencies do not take account of smoking habits. The ratio of the observed to expected number of pairs concordant for

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amniocentesis in the European and Danish studies; the Canadian study did not provide any such data.²⁻⁴ There were no cases of the oromandibular-limb hypogenesis syndrome; however, in 2 cases of the CVS group and 1 of the EA group there was shortening of some of the toes in one of the feet. The incidence of talipes equinovarus (1.63%), was higher in the EA group than in the CV group (0.56%), but this difference was not significant. A previous randomised study of second-trimester amniocentesis has shown that the incidence of talipes (0.8%) was not significantly different from controls that did not have invasive testing.¹

Our data suggest that EA and CVS, done in a fetal medicine centre with experience in transabdominal ultrasound-guided techniques, are equally effective in providing conclusive cytogenetic results. On the basis of these findings it might appear that EA rather than CVS is likely to become the established first-trimester technique for fetal karyotyping. The widespread experience with second-trimester amniocentesis can be more easily adapted to do EA rather than CVS. EA has the additional advantage over CVS that the processing of samples requires less experienced laboratory staff, can be done in batches, and is less labour intensive. However, it is more likely that CVS will become the established technique because EA may be associated with 2-3% excess risk of fetal loss and possibly a higher incidence of talipes among survivors.

References

- 1 Tabor A, Philip J, Madsen M, Bang J, Obel EB, Norgaard-Pedersen B. Randomised controlled trial of genetic amniocentesis in 4606 low-risk women. *Lancet* 1986; **i**: 1287-93.
- 2 MRC working party on the evaluation of chorion villus sampling. Medical Research Council European trial of chorion villus sampling. *Lancet* 1991; **337**: 1491-99.
- 3 Canadian collaborative CVS-amniocentesis clinical trial group. Multicentre randomised clinical trial of chorion villus sampling and amniocentesis. *Lancet* 1989; **i**: 1-6.
- 4 Smidt-Jehnsen S, Permin M, Philip J, et al. Randomised comparison of amniocentesis and transabdominal and transcervical chorion villus sampling. *Lancet* 1992; **340**: 1237-44.
- 5 Nevin J, Nevin NC, Dornan JC, Sim D, Armstrong MJ. Early amniocentesis: experience of 222 consecutive patients, 1987-1988. *Prenat Diagn* 1990; **10**: 79-83.
- 6 Stripparo L, Buscaglia M, Longatti L, et al. Genetic amniocentesis: 505 cases performed before the sixteenth week of gestation. *Prenat Diagn* 1990; **10**: 3359-64.
- 7 Penso CA, Sandstrom MM, Garber MF, Ladoulis M, Stryker JM, Benacerraf BB. Early amniocentesis: report of 407 cases within neonatal follow-up. *Obstet Gynecol* 1990; **76**: 1032-36.
- 8 Hanson FW, Tennant F, Hune Stacy, Brookhyser K. Early amniocentesis: outcome, risks, and technical problems at ≤ 12.8 weeks. *Am J Obstet Gynecol* 1992; **166**: 1707-11.
- 9 Wald NJ, Cuckle HS, Densm JW, et al. Maternal serum screening for Down's syndrome in early pregnancy. *BMJ* 1988; **297**: 883-97.
- 10 Nicolaides KH, Snijders RJM, Gosden CM, Berry C, Campbell S. Ultrasonographically detectable markers of fetal chromosomal abnormalities. *Lancet* 1992; **340**: 704-07.
- 11 Nicolaides KH, Azar G, Byrne D, Mansur C, Marks K. Fetal nuchal translucency: ultrasound screening for chromosomal defects in first trimester of pregnancy. *BMJ* 1992; **304**: 867-69.
- 12 Byrne D, Marks K, Azar G, Nicolaides KH. Randomised study of early amniocentesis versus chorionic villus sampling: a technical and cytogenetic comparison of 650 patients. *Ultrasound Obstet Gynecol* 1991; **1**: 235-40.
- 13 Firth HV, Boyd PA, Chamberlain P, MacKenie IZ, Lindenbaum RH, Huson SM. Severe limb abnormalities after chorion villous sampling at 56-66 days' gestation. *Lancet* 1991; **337**: 762-63.
- 14 Yudkin PL, Aboulfa M, Eyre JA, Redman CWG, Wilkinson AR. New birthweight and head circumference centiles for gestational ages 24-42 weeks. *Early Hum Dev* 1987; **15**: 45-52.
- 15 Snijders RJM, Holzgreve W, Cuckle H, Nicolaides KH. Maternal age-specific risks for trisomies at 9-14 weeks gestation. *Pren Diagn* (in press).

Twins in pair dying from lung cancer	No (%) of pairs		
	Monozygotic (n=5933)	Dizygotic (n=7554)	Unknown zygosity (n=2437)
None	5661 (95.4)	7176 (95.0)	2312 (94.9)
One	262 (4.4)	357 (4.7)	120 (4.9)
Both	10 (0.2)	21 (0.3)	5 (0.2)

Table 1: Concordance for lung cancer death

lung cancer death was compared for monozygotic and dizygotic twins. Because monozygotic twins have virtually identical genomes, whereas dizygotic twins are about 50% matched, a higher ratio among monozygotic twins than among dizygotic twins is regarded as evidence for possible genetic effects. Conversely, a lower ratio among monozygotic twins is evidence against genetic effects. We considered that familial effects were present if the observed/expected ratio of concordance frequency for lung cancer death in dizygotic twins exceeded 1.0.

A cohort analysis was undertaken to assess the predictability of the development of lung cancer among smokers who could be suspected of having a strong inherited predisposition to the disease. By contrast with the concordance analysis, the cohort analysis specifically took into account smoking and duration of follow-up. If genetic susceptibility explains why some smokers develop lung cancer while others with similar smoking exposure do not, a higher frequency of lung cancer death would be expected in monozygotic twin smokers who survived their smoking twins' lung cancer death. The cohort analysis used data from a questionnaire mailed in 1967 to 7372 intact twin pairs aged 40–50 years, who had responded to the earlier mail survey about zygosity. 84% of contacted twins replied to the questionnaire. Because its primary purpose was to find out about risk factors for cardiovascular and pulmonary disease, the questionnaire obtained information about smoking history and usual occupation.

The cohort analysis examined a subset of the twin registry that met the following two conditions: both members of the twin pair stated in the 1967 questionnaire that they currently smoked cigarettes; and after the questionnaire, one twin in the pair died from lung cancer and was survived by his brother. Surviving twins were followed as a cohort for mortality and cause of death. For calculation of time at risk, follow-up for the surviving twin began on the day of the twin's lung cancer death and extended to the surviving twin's date of death or Dec 31, 1990, whichever was earlier.

Lung cancer mortality rates specific for age, sex, and smoking intensity were obtained from the Dorn cohort study of 248 000 white US veterans.^{18,19} Using these rates, we calculated the number of lung cancer deaths that could be expected in the cohort of twin veterans who survived a twin's lung cancer death. Calendar time-specific rates through 1980 obtained from continued follow-up of the Dorn study cohort were used, and rates for 1976 to 1980 were applied to the period 1981 to 1990. Rates for smoking intensity were based on stratification into lower (20 or fewer cigarettes per day) or higher (21 or more cigarettes per day) intensity groups.

The Beneficiary Identification and Records Locator Subsystem (BIRLS) computer file of the VA was used to determine vital status of the twins in the cohort. Causes of death were obtained from death certificates filed with the VA as a prerequisite for the disbursement of death benefits. Death certificates are very accurate for determination of primary lung cancer death.²⁰ Additional ascertainment of mortality used computer vital status data of the Social Security Administration. Used together, these databases allow virtually complete mortality ascertainment.²¹

For the 15 924 twin pairs in the concordance analysis, the completeness of mortality assessment was evaluated. Up to 1990, 9364 (29.4%) of the 31 848 subjects in the cohort were recorded as dead in the BIRLS computer file of the VA. A match of 20 823 twins, whose social security numbers were recorded in the twin registry, to the computer mortality data of the Social Security Administration showed that the BIRLS computer file had ascertained 98.1% of deaths in the cohort. Information on cause of death was obtained for 8902 (95.1%) of the 9364 deaths.

	Monozygotic (n=5933)	Dizygotic (n=7554)	Unknown (n=2437)
Observed no of pairs	10	21	5
Expected no of pairs	3.35	5.27	1.73
Observed/expected ratio (95% CI)	2.98 (1.55–5.56)	3.99 (2.35–5.79)	2.89 (1.15–6.87)

Table 2: Observed and expected concordance for lung cancer death

For the twin pairs studied in the cohort analysis, follow-up for mortality and cause of death was essentially complete. Mortality data of the Social Security Administration were in agreement with the BIRLS system for all of the twins whose social security numbers were available. For all the twins reported by BIRLS as having died, information on cause of death was obtained.

Results

Concordance analysis

Concordance for lung cancer death among the 15 924 twin pairs is shown in table 1. Up to 1990, one or both members of about 5% of twin pairs had died of lung cancer. Compared with the number of concordant pairs that would be expected if lung cancer death were randomly distributed among the twin pairs (without taking smoking into account), there was a significant excess concordance of lung cancer death for all the zygosity groups (table 2). We decided that familial effects were present because the observed to expected ratio of the frequency of concordant pairs for lung cancer death in dizygotic twins (3.99) exceeded 1.0. However, the ratio was lower (2.98) among the monozygotic twins. The overall rate ratio (observed/expected concordance in monozygotic twins divided by observed/expected concordance in dizygotic twins) was 0.75 (0.35–1.6); this ratio does not provide evidence for a genetic component of lung cancer.

Cohort analysis

Lung cancer was strongly associated with cigarette smoking. Among all respondents to the 1967 questionnaire, the lung cancer mortality rate of smokers was 19.8 times that of non-smokers. Among the 83 smoking pairs in which a man survived his twin's lung cancer death; 2 of the "survivors" also died of lung cancer (table 3). The observed to expected ratio of lung cancer deaths (1.29) does not differ significantly from 1.0. No lung cancer deaths occurred in 300 person-years of follow-up among the monozygotic twin pairs, whereas 0.90 such deaths could be expected in male veterans with similar ages and smoking histories.

To assess whether the absence of substantially elevated lung cancer mortality was related to characteristics of smoking behaviour, we examined the twins' responses to the 1967 questionnaire (before the development of lung cancer). Twins dying of lung cancer and their survivors had quite similar smoking behaviour, and none of the differences between the groups was significant (table 4).

Zygosity	Person-years of follow-up	Lung cancer deaths		
		Observed	Expected	Observed/expected ratio (95% CI)
All (n=83)*	501	2	1.55	1.29 (0.16–4.67)
Monozygotic (n=47)	300	0	0.90	0 (0–4.09)
Dizygotic (n=34)	178	2	0.58	3.44 (0.41–12.4)

*Includes 2 pairs of unknown zygosity (23 person-years of follow-up).

Table 3: Observed to expected frequency of lung cancer death in twins who survived co-twin's lung cancer death, among twin pairs who reported they smoked in 1967

Characteristic	Twins dying of lung cancer (n=83)*	Survivors of co-twin's lung cancer (n=83)*	Monozygotic twins		Dizygotic twins	
			Dying of lung cancer (n=47)	Survivors of co-twin's lung cancer (n=47)	Dying of lung cancer (n=34)	Survivors of co-twin's lung cancer (n=34)
Mean (SE)						
Age started smoking	17.6 (0.3)	17.4 (0.3)	17.5 (0.4)	17.6 (0.3)	17.4 (0.4)	17.1 (0.6)
No of cigarettes smoked daily	31.8 (1.3)	30.3 (1.2)	30.8 (1.7)	30.9 (1.6)	33.4 (2.1)	30.1 (1.9)
Depth of inhalation (%)						
Deep	24	24	28	22	19	28
Moderate	71	65	67	63	75	66
Slight/none	5	11	4	15	6	6
Type of cigarette (%)						
Filter	37	30	40	31	33	27
Non-filter	63	70	60	69	67	73

*Includes 2 pairs of unknown zygosity.

Table 4: Smoking-related characteristics according to lung cancer mortality status and zygosity

The mean age of starting smoking was about 17 years in both groups, and therefore by the time of the 1967 questionnaire, the twins in the cohort had on average already smoked for 23–33 years. The number of cigarettes usually smoked per day was nearly identical among the monozygotic twins who subsequently died of lung cancer and their survivors; among dizygotic twins, there was a slightly, but not significantly, greater smoking intensity among the twins dying of lung cancer.

Possible occupational differences were also investigated. Among the 47 monozygotic twins, occupations with possible asbestos exposure were reported in the 1967 questionnaire by 3 men who subsequently died of lung cancer and 1 who did not die of lung cancer. Exclusion from the analysis of these 4 twin pairs did not affect the study's findings. Occupations with possible exposures to other, weaker carcinogens were not associated with lung cancer in this study (data not shown).

Discussion

Our concordance analysis of the National Academy of Sciences/National Research Council Twin Registry is the largest so far of lung cancer deaths in a twin study. The findings substantially strengthen a previous concordance analysis of lung cancer death up to 1978 in the same twin registry, when only 23% of the lung cancer deaths in our study had occurred.²² The cohort analysis complements the concordance analyses because it specifically accounts for smoking exposure and duration of follow-up. Neither analysis provided evidence that inherited predisposition contributes to lung cancer risk.

In the concordance analysis, the overall rate ratio was 0.75, which suggests that monozygotic twins were less likely to be concordant for lung cancer than dizygotic twins (table 2). This ratio is substantially lower than overall rate ratios observed for some common diseases considered to have both genetic and environmental components, which have been examined in the same twin registry. The overall rate ratio for schizophrenia was 5.4, hypertension 2.4, diabetes 2.4, peptic ulcer disease 1.7, ischaemic heart disease 1.6, and chronic obstructive pulmonary disease 1.5.²³

Smoking data were not available for all the twins in the concordance analysis. However, previous studies in the cohort have shown that concordance for smoking is high among both monozygotic and dizygotic twins.^{24,25} This finding probably accounts at least partly for the high concordance for lung cancer death observed among both types of twins. However, since concordance for smoking among monozygotic twins significantly exceeds that among dizygotic twins,²⁴ even in the absence of a genetic effect on

lung cancer death, monozygotic twin pairs could be expected to have greater concordance for lung cancer death than dizygotic twin pairs. Knowledge of smoking concordance among twins increases our confidence in the finding of no excess concordance for lung cancer death among monozygotic compared with dizygotic twins.

The cohort study findings on inherited predisposition to lung cancer among smokers are restricted to men older than 50 because smoking status is unknown for twins who died of lung cancer before the 1967 questionnaire, when they were aged 40–50. Brothers of such twins consequently did not meet the conditions required for inclusion in the cohort analysis. However, we are reassured about this exclusion by the rarity of lung cancer death before age 50.²⁶ Such mortality represents less than 10% of lung cancer mortality in the whole cohort up to 1990, and this proportion will continue to decline as additional lung cancer deaths accrue in the now elderly subjects. Therefore, even if half of lung cancer deaths before age 50 could be attributed to inherited predisposition, such deaths would represent fewer than 5% of lung cancer deaths overall.

Smoking cessation by survivors of a co-twin's lung cancer death cannot fully explain our findings. After smoking cessation by long-term heavy smokers who started smoking at an early age, such as the twins in our cohort analysis, the annual risk of lung cancer death remains roughly constant.^{27,28} For survivors in the cohort analysis who stopped smoking, the average annual risk during follow-up therefore is substantially higher (about 15 times) than that for never smokers, but about two-thirds that for current smokers.²⁹ Moreover, although we do not know the smoking status in the cohort analysis of all the survivors after their co-twin's death, of the 9 monozygotic twins with known status, 6 reported in a 1985 questionnaire that they continued to smoke.

Although our study does not entirely rule out an aetiological role for inherited predisposition in smoking-induced lung cancer, it suggests that inherited predisposition does not have a substantial role in the vast majority of smoking-induced lung cancers in men older than 50 years. At this time a random process probably best explains the development of lung cancer among a group similarly exposed to smoking.

Our study's results have implications for the value of testing for inherited predisposition in cancer risk assessment. It has been suggested that it may soon be possible for advances in molecular biology to be applied among the general population to develop a cancer-risk profile that will take into account an individual's genes and environmental exposures.¹ Inherited predisposition has

been envisaged to have an important role in prediction by this profile of an individual's likelihood of developing cancer. However, our findings suggest that for lung cancer, the leading cause of cancer death in the USA,³⁰ inherited predisposition will probably be of limited predictive value. For purposes of lung cancer prevention, the message of these findings is that smoking-induced lung cancer should be attributed to smoking, not to inherited predisposition.

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References

- Shields PG, Harris CC. Molecular epidemiology and the genetics of environmental cancer. *JAMA* 1991; **266**: 681-87.
- Tokuhata GK, Lilienfeld AM. Familial aggregation of lung cancer in humans. *J Natl Cancer Inst* 1963; **30**: 289-312.
- Ooi WL, Elston RC, Chen VW, Bailey-Wilson JE, Rothschild H. Increased familial risk for lung cancer. *J Natl Cancer Inst* 1986; **76**: 217-22.
- Samet JM, Humble CG, Pathak DR. Personal and family history of respiratory disease and lung cancer risk. *Am Rev Respir Dis* 1986; **134**: 466-70.
- Shaw GL, Falk RT, Pickle LW, Mason TJ, Buffler PA. Lung cancer risk associated with cancer in relatives. *J Clin Epidemiol* 1991; **44**: 429-37.
- Sellers TA, Bailey-Wilson JE, Elston RC, et al. Evidence for mendelian inheritance in the pathogenesis of lung cancer. *J Natl Cancer Inst* 1990; **82**: 1272-79.
- Nakachi K, Imai K, Hayashi S, Kawajiri K. Polymorphisms of the CYP1A1 and glutathione S-transferase genes associated with susceptibility to lung cancer in relation to cigarette dose in a Japanese population. *Can Res* 1993; **53**: 2994-99.
- Caporaso NE, Hayes RB, Dosemici M, et al. Lung cancer risk, occupational exposure, and the debrisoquine metabolic phenotype. *Cancer Res* 1989; **49**: 3675-79.
- Krontiris TG, Devlin B, Karp DD, Robert NJ, Risch N. An association between the risk of cancer and mutations in the HRAS1 minisatellite focus. *N Engl J Med* 1993; **329**: 517-23.
- Sugimura H, Caporaso NE, Modali RV, et al. Association of rare alleles of the Harvey ras protooncogene locus with lung cancer. *Cancer Res* 1990; **50**: 1857-62.
- Vahakangas KH, Samet JM, Metcalf RA, et al. Mutations of p53 and ras genes in radon-associated lung cancer from uranium miners. *Lancet* 1992; **339**: 576-80.
- Harris CC, Mulvihill JJ, Thorgeirsson SS, Minna JD. Individual differences in cancer susceptibility. *Ann Intern Med* 1980; **92**: 809-25.
- Mattson ME, Pollack ES, Cullen JW. What are the odds that smoking will kill you? *Am J Public Health* 1987; **77**: 425-31.
- Jablou S, Neel JV, Gershowitz H, Atkinson GF. The NAS-NRC twin panel: methods of construction of the panel, zygosity diagnosis, and proposed use. *Am J Hum Genet* 1967; **19**: 133-61.
- Hrubec Z, Neel JV. The National Academy of Sciences-National Research Council Twin Registry: ten years of operation. In: Nance WE, ed. Twin research: proceedings of the Second International Congress on Twin Studies, Washington DC, Aug 29-Sept 1, 1977. Part B: Biology and epidemiology. New York: Alan R Liss, 1978: 153-72.
- Cederlof R, Friberg L, Jonsson E, Kaij L. Studies on similarity diagnosis in twins with the aid of mailed questionnaires. *Acta Genet* 1961; **11**: 338-62.
- Nichols RC, Bilbro WC. The diagnosis of twin zygosity. *Acta Genet Stat Med* 1966; **16**: 265-75.
- Dorn HF. Tobacco consumption and mortality from cancer and other diseases. *Public Health Rep* 1959; **74**: 581-93.
- Kahn HA. The Dorn study of smoking and mortality among US veterans: report on eight and one-half years of observation. *NCI Monogr* 1966; **19**: 1-125.
- Percy C, Stanek E, Gloeckler L. Accuracy of cancer death certificates and its effect on cancer mortality statistics. *Am J Public Health* 1981; **71**: 242-50.
- Boyle CA, Decoufle P. National sources of vital status information: extent of coverage and possible selectivity in reporting. *Am J Epidemiol* 1990; **131**: 160-68.
- Hrubec Z, Neel JV. Occurrence of cancer before old age in twin veterans. *Am J Hum Genet* 1982; **34**: 658-71.
- Kendler KS, Robinette CD. Schizophrenia in the National Academy of Sciences-National Research Council Twin Registry: a 16-year update. *Am J Psychiatr* 1983; **140**: 1551-63.
- Carmelli D, Swan GE, Robinette D, Fabsitz R. Genetic influence on smoking: a study of male twins. *N Engl J Med* 1992; **327**: 829-33.
- Carmelli D, Swan GE, Robinette D, Fabsitz R. Heritability of substance abuse in the NAS-NRC twin registry. *Acta Genet Med Gemellol* 1990; **39**: 91-98.
- Ries LAG, Hankey BF, Miller BA, Hartman AM, Edwards BK. Cancer statistics review 1973-88. National Cancer Institute. NIH pub no 91-2789, 1991.
- Halpern MT, Gillespie BW, Warner KE. Patterns of absolute risk of lung cancer mortality in former smokers. *J Natl Cancer Inst* 1993; **85**: 457-64.
- Peto R, Doll R. The control of lung cancer. In: Mizell M, Correa P, eds. Lung cancer: causes and prevention. Deerfield Beach, Florida: Verlag Chemie International, 1984: 1-19.
- International Agency for Research on Cancer. IARC Monographs on the evaluation of carcinogenic risk of chemicals to humans. Vol 38. Tobacco smoking. Lyon: International Agency for Research on Cancer: 1986.
- Boring CC, Squires TS, Tong T. Cancer statistics, 1993. *CA Cancer J Clin* 1993; **43**: 7-26.

Short reports

Serodiagnosis of *Penicillium marneffe* infection

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Diagnosis of *Penicillium marneffe* infection is often made late. We evaluated an indirect immunofluorescent antibody test for *P marneffe* in serum from 103 patients with persistent fever and from 78 normal subjects. Germinating conidia (initial tissue-invasion phase) and yeast-hyphae (tissue multiplication phase) forms were used as antigen. All 8 documented *P marneffe* cases (8%) had an IgG titre of 160 or more; the other 95 patients and all the healthy controls had an IgG titre of 40 or below. Blood culture was positive in only 1 case with HIV infection. Biopsy and culture of tissues were necessary for confirmation in the other 7 cases. The test could provide rapid presumptive diagnosis and supplement conventional culture.

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Penicillium marneffe causes progressive systemic diseases in normal and immunosuppressed hosts.^{1–4} The gold standard of diagnosis is a positive culture from appropriate tissues, although positive blood cultures are not unusual in HIV positive patients.⁵ Unfortunately, invasive biopsies are often delayed and patients are often treated empirically for other diseases that present as persistent pyrexia despite multiple antimicrobial therapy. Because disseminated *P marneffe* infection has a high mortality and effective antifungal agents are available, we designed a rapid indirect immunofluorescent antibody test (IFAT) to expediate diagnosis and treatment.

Serum samples from 103 patients who were admitted for persistent fever despite multiple antimicrobials,⁶ and those from 78 normal blood donors were tested. All samples were tested for IgG, IgM, and IgA titres against *P marneffe* by IFAT. Two *P marneffe* antigens were evaluated: the yeast-hyphae and the germinating conidia forms, which represent the tissue multiplication phase and initial tissue invasion phase, respectively. For IFAT,⁷ 10⁸ germinating conidia or yeast-hyphae forms were suspended per mL (figure).

8 patients were found to have *P marneffe* infection (cases). The final diagnosis in the other 95 is shown in the table. IFAT for *P marneffe* antibody showed no positives at a 1 in 10 dilution for IgM. Geometric mean titres for IgG and IgA were much higher in the cases. IgG titre was higher than IgA titre in the cases but there was little difference between the germinating conidia or yeast-hyphae forms.

Clinical diagnosis of *P marneffe* infection is difficult because most patients present with fever without any localising symptom or sign. Most cases are empirically treated as typhoid fever. When pulmonary infiltrates, lymphadenopathy, or cutaneous or osteolytic lesions subsequently appear, tuberculosis or melioidosis are often imputed. When broad-spectrum antibiotics and anti-tuberculosis therapy fails, tissue diagnosis is attempted. Although each of our 8 cases had 3–15 blood cultures taken during their course of disease, only 1 case with HIV

infection yielded a positive blood culture. Tissue biopsies (lymph node, bone marrow, skin) were needed to confirm the diagnosis in the other 7. The mean delay from initial presentation to definitive diagnosis was 11 weeks.

An immunodiffusion test⁸ with *P marneffe* exoantigen from mycelial culture filtrate has been tested in 1 patient.⁹ We used yeast-hyphae and germinating conidia forms as antigens because they are more relevant to the in-vivo situation of the diseased host than the mycelial form. Our simple IFAT had good specificity and sensitivity since none of the cases had an IgG titre under 160 and all controls had titres of 40 or lower.

Because our test was being evaluated in an area of low endemicity in a small group, differentiation between the cases and controls was not unexpected. In places with high frequency of symptom-free exposure to *P marneffe*, and especially with HIV infection which may blunt the response¹⁰ (the titres were lowest in the 2 HIV-positive cases), the test may only supplement conventional fungal

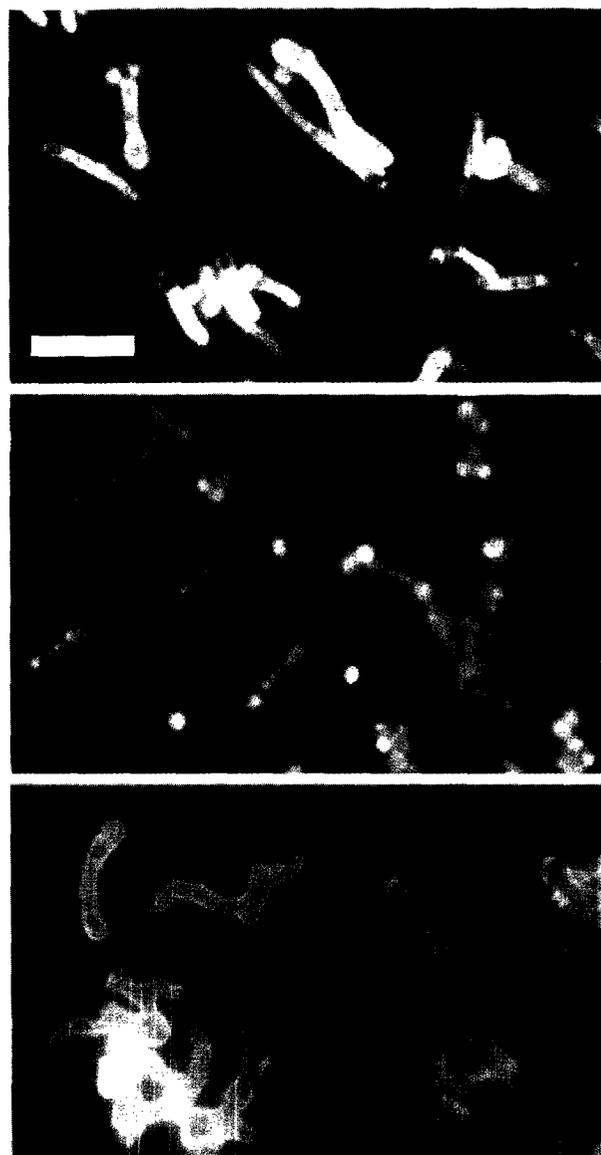


Figure: Immunofluorescent micrograph of *P marneffe*

Upper = germinating conidia form: positive apple-green fluorescence of germ tube arising from conidia, positive case. Middle = germinating conidia form: negative control. Lower = yeast-hyphae form: positive apple-green fluorescence of septate pseudohyphae and yeast cells, positive case. (Bar = 10 μ m.)