Eur J Cancer, Vol. 29A, No. 10, pp. 1476–1478, 1993.
Printed in Great Britain

0964–1947/93 $6.00 + 0.00

Absence of Germline Mutations of Exons 5 to 8 of the P53 Gene in 26 Breast Cancer Families from the North of France

Claude Preudhomme, Pierre Fenaux, Jean Philippe Peyrat, Joëlle Fournier, Jacques Bonneterre and Philippe Vennin

We looked for germline mutations of exons 5 to 8 of the P53 gene in 27 female patients from 26 families originating from the north of France who had breast cancer and at least one first degree relative also affected with breast cancer. Detection of the mutations was made by single strand conformation polymorphism analysis. No mutation was found in any patient, confirming that germline mutations of the P53 gene are very rare in familial breast cancer (apart from Li Fraumeni families).
Eur J Cancer, Vol. 29A, No. 10, pp. 1476–1478, 1993.

INTRODUCTION

P53 GENE IS a tumour suppressor gene whose mutations have been found in many types of solid tumours, including 15 to 50% of breast carcinomas [1], and less often in haematological malignancies [2, 3]. In the majority of those tumours, point mutation of one P53 allele was associated with loss of the other P53 allele, leading to inactivation of both normal P53 alleles. The vast majority of point mutations were found in exons 5 to 8, and were clustered in four mutation "hotspots" situated between codons 120 and 280 [1].

Although most cases of breast carcinoma appear to be sporadic, familial clustering occurs in 5 to 10% of cases, suggesting an inherited predisposition [4]. Two studies have linked early onset familial breast cancer and familial breast-ovarian cancer to chromosome 17q12-q23, where one or several predisposing gene(s) may be present [5, 6]. Among familial syndromes of breast carcinoma is also the Li Fraumeni syndrome, a very rare autosomal dominant trait associated with a high incidence of early onset breast carcinomas (and also of other tumours) [7]. In the majority of cases of the Li Fraumeni syndrome, germ line mutation of a P53 allele, occurring in exon 7 between codons 245 and 258, is present [7].

The high incidence of somatic P53 gene mutations in sporadic breast carcinoma and of germline P53 gene mutations in familial breast carcinoma associated with the Li Fraumeni syndrome suggested a possible role for germline P53 gene mutations in other families with a strong history of breast cancer. Borresen *et al.* [8], Warren *et al.* [9] and Prosser *et al.* [10] very recently found no germline mutations in exons 5 to 9 of the P53 gene in 30, 25 and 5 breast cancer families, respectively. We also looked for such mutations of the P53 gene in 27 breast cancer patients who had at least one first degree relative also affected with breast cancer. Those patients were recognised among more than 400 cases of breast carcinoma diagnosed over 1 year in our cancer centre in the north of France. Detection of the mutations was made by single strand conformation polymorphism analysis (SSCP) [11] of exons 5 to 8, a sensitive method which can detect even a single nucleotide substitution in a short DNA fragment. Like other authors [8–10], we found no germline mutation in our patients.

Correspondence to P. Fenaux at the Service des Maladies du Sang, C.H.U., 1 Place de Verdun, 59037 Lille, France.
C. Preudhomme is at Inserm U_{124} and Service des Maladies du Sang, C.H.U., Lille; and J.P. Peyrat, J. Fournier, J. Bonneterre and P. Vennin are at the Centre Oscar Lambret, Lille, France.
Revised 18 Dec. 1992; accepted 23 Dec. 1992.

PATIENTS AND METHODS

Patients

From October 1990 to October 1991, 419 cases of breast carcinoma were diagnosed at the Centre Oscar Lambret, a multidisciplinary cancer centre whose catchment area covers most of the north of France (population of approximatively 4 million).

27 of those 419 patients had at least one first degree relative affected with breast cancer. All 27 were studied here. 2 of the patients belonged to the same family, so that 26 families were analysed. In six families, at least two first degree relatives of the patient were affected with breast cancer. Median age at diagnosis was 52.5 years (range 31–76) and 10 out of 27 patients were aged less than 50 at diagnosis. The tumour was bilateral in 3 cases.

Methods

DNA. Blood samples were collected from patients after informed consent, and DNA extracted from lymphocytes isolated by centrifugation over a Ficoll Isopaque (Nyegaard A/S, Oslo) gradient.

Polymerase chain reaction (PCR)–SSCP analysis. Oligonucleotide primers were purchased from Genset (Paris, France). The names and nucleotide sequences of the primers used in this work are listed in Table 1. Two genomic regions were amplified: region 1, encompassing exons 5 and 6, and intron 5, and measuring 408 bp; region 2, encompassing exons 7 and 8, and intron 7, and measuring 610 bp. Because SSCP analysis seems to require fragments of less than 400 bp [11], region 2 was digested, after amplification and before SSCP analysis, by Dra 1 enzyme, as a Dra 1 restriction site is present in intron 7. This led to two fragments, region 2a and region 2b, each encompassing the corresponding exon, and measuring 392 and 218 bp, respectively.

Genomic DNA (0.1 μg) were subjected to PCR in a 10 μl solution containing 200 μmol/l each of dATP, dGTP, dTTP,

Table 1. Primers used for the PCR of exons 5 to 8 of the P53 gene

DNA fragment amplified	Name of the primers	Size of the fragment	Sequence
Region 1	I_S	408 bp	5'TTCCTCTTCCTGCAGTACTC3'
	I_{AS}		5'AGTTGCAAACCAGACCTCAG3'
Region 2	II_S	610 bp	5'AGGTTGGCTCTGACTGTACC3'
	II_{AS}		5'ATTGTCCTGCTTGCTTACCTC3'

dCTP, 0.1 μl of 32PdCTP (Amersham, U.K., 10 mCi/ml), 0.1 μmol/l of 5' and 3' primer, 10 mmol/l Tris–HCl (pH 8.3), 50 mmol/l KCl, 10 mmol/l $MgCl_2$, 0.5 unit of Taq polymerase (Boehringer, Mannheim, Germany) in a thermocycler (Techne, Princeton, New Jersey, U.S.A.). PCR was performed as follows: 5 min at 94°C, then 30 cycles at 94°C for 1 min, 58°C for 1 min, 72°C for 2 min followed by final elongation at 72°C for 7 min. After amplification, 1 μl of the reaction mixture for region 1 was mixed with 19 μl of 0.1% SDS 20 mmol/l EDTA solution. For region 2, 1 μl of the reaction mixture was first digested by Dra 1 in 10 μl, and diluted in SDS-EDTA solution. Then 3 μl of the diluted region 1 on one hand and regions 2a and 2b, on the other hand, were mixed with 3 μl of a solution of 95% formamide 20 mmol/EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol, heated at 80°C and applied (2 μl/lane) to a 5% polyacrylamide gel containing 90 mmol/l Tris–borate pH 8.3, 4 mmol/l EDTA, and 10% glycerol. Electrophoresis was performed at 35 W for 5 to 6 h at room temperature, with cooling using a fan.

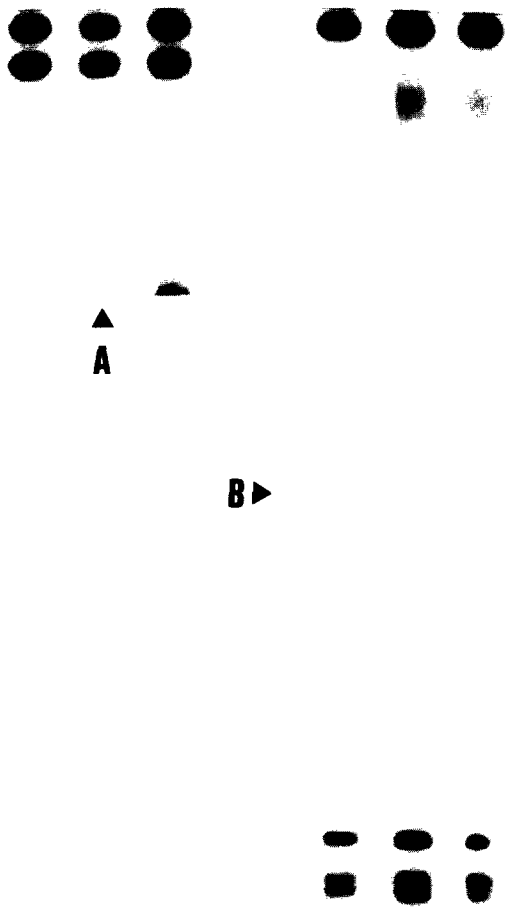

Fig. 1. SSCP analysis of regions 1 and 2. (A) Region 1, showing normal fragments in 1 control and 2 patients. (B) Region 2, showing normal fragments for region 2 after digestion with Dra 1 enzyme, which separated region 2a (upper band) and region 2b (lower bands). From left to right: 1 control and 2 patients.

RESULTS AND DISCUSSION

All 27 patients had a normal PCR-SSCP pattern for regions 1 (exons 5 and 6), 2a (exon 7) and 2b (exon 8). Examples of these normal results are shown in Fig. 1.

Our previous works, in which we performed both SSCP analysis and direct sequencing, had shown us the high sensitivity and specificity of SSCP in detecting mutations of exons 5 to 8 of the P53 gene [2, 12, 13]. The validity of this method for the P53 gene has also been demonstrated by other groups [3, 14]. Thus, it is improbable that we could have overlooked mutations in exons 5 to 8 of the P53 gene in our patients. In addition, because more than 90% of the P53 gene mutations reported so far in cancer (including breast cancer) were found in exons 5 to 8, the possibility that those patients had mutations in other exons is low [1].

Our findings, therefore, confirm that germline P53 gene mutations must be very rare in breast cancer families, except in the exceptional Li Fraumeni syndrome which, in addition to breast carcinoma, predisposes to other types of cancers. Germline mutations of the P53 gene have also been reported in 4 of 59 children or adults who had had two successive cancers [15], and in 8 of 196 patients with a sarcoma [16]. However, none of those patients had a familial history of breast cancer. Non-Li Fraumeni syndrome germline P53 gene mutation associated with a strong familial history of cancer, (including several cases of breast carcinoma) so far seem to have been reported in only one family [17]. Thus, other genes must be implicated in most cases of familial breast cancer. As yet unidentified tumour suppressor genes located in 17q [5, 6] or 17p [18] may be potential candidates.

1. Hollstein M, Sidransky D, Vogelstein B, Harris CC. P53 mutations in human cancers. *Science* 1991, **253**, 49–53.
2. Fenaux P, Jonveaux P, Quiquandon I, *et al.* P53 gene mutations in acute myeloid leukemia with 17p monosomy. *Blood* 1991, **78**, 1652–1657.
3. Gaidano G, Ballerini P, Gong J, *et al.* P53 mutations in human lymphoid malignancies: association with Burkitt lymphoma and chronic lymphocytic leukemia. *Proc Natl Acad Sci USA* 1991, **88**, 5413–5417.
4. Lynch HT. *Genetics and Breast Cancer.* New York, Van Nostrand Reinhold, 1981.
5. Hall JM, Lee MK, Newman B, *et al.* Linkage of early-onset familial breast cancer to chromosome 17q21. *Science* 1990, **250**, 1684–1689.
6. Narod SA, Feunteun J, Lynch HT, *et al.* Familial breast-ovarian cancer locus on chromosome 17q12-q23. *Lancet* 1991, **ii**, 82–83.
7. Malkin D, Li FP, Strong LC, *et al.* Germ line P53 mutations in a

familial syndrome of breast cancer, sarcomas and other neoplasms. *Science* 1990, **250**, 1233–1238.
8. Borresen AL, Andersen TI, Gaber J, *et al.* Screening for germ line TP53 mutations in breast cancer patients. *Cancer Res* 1992, **52**, 3234–3236.
9. Warren W, Eeles RA, Ponder BAJ, *et al.* No evidence for germline mutations in exons 5-9 of the P53 gene in 25 beast cancer families. *Oncogene* 1991, **7**, 1043–1046.
10. Prosser J, Elder PA, Condie A, Mac Fadyen I, Steel CM, Evans HJ. Mutations in P53 do not account for heritable breast cancer: a study in five affected families. *Br J Cancer* 1991, **63**, 181–184.
11. Orita M, Suzuki Y, Sekiya T, Hayashi K. Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. *Genomics* 1989, **5**, 874.
12. Fenaux P, Jonveaux P, Quiquandon I, *et al.* Mutations of the P53 gene in B cell acute lymphoblastic leukemia. A report on 60 cases. *Leukemia* 1992, **6**, 42–46.
13. Fenaux P, Preudhomme C, Laï J, *et al.* Mutations of the P53 gene in B cell chronic lymphocytic leukemia. A report on 39 cases with cytogenetic analysis. *Leukemia* 1992, **6**, 246–250.
14. Takahashi T, Nau MM, Chiba I, *et al.* P53: a frequent target for genetic abnormalities in lung cancer. *Science* 1989, **236**, 491–494.
15. Malkin D, Kent MD, Jolly MD, *et al.* Germline mutations of the P53 tumor-suppressor gene in children and young adults with second malignant neoplasms. *N Engl J Med* 1992, **326**, 1309–1315.
16. Togughida J, Yamaguchi T, Dayton SH, *et al.* Prevalence and spectrum of germline mutations of the P53 gene among patients with sarcoma. *N Engl J Med* 1992, **326**, 1301–1307.
17. Prosser J, Porter D, Coles C, *et al.* Constitutional p53 mutations in a non-Li-Fraumeni cancer family. *Br J Cancer* 1992, **65**, 527–528.
18. Coles C, Thompson AM, Elder PA, *et al.* At least two genes on chromosome 17p are implicated in human breast carcinogenesis. *Lancet* 1990, **ii**, 761.

Eur J Cancer, Vol. 29A, No. 10, pp. 1478–1479, 1993.
Printed in Great Britain

0964–1947/93 $6.00 + 0.00

Malignant Mesothelioma in the Rotterdam Area, 1987–1989

Ronald A.M. Damhuis and Teun van Gelder

Malignant mesothelioma is commonly regarded as a rare disease. This, however, does not apply to the Rotterdam area. Registry data show an age-standardised incidence rate (per 100 000 per year, world standard population) of pleural cancer in men of 6.2 for the period 1987–1989. This is substantially higher than thus far reported by other cancer registries. Similar observations may be expected in other areas with shipbuilding or asbestos industries.
Eur J Cancer, Vol. 29A, No. 10, pp. 1478–1479, 1993.

INTRODUCTION

THE RELATION between asbestos exposure and the occurrence of malignant mesothelioma is well-known. After the original publication by Wagner *et al.* in 1960 [1], numerous researchers have confirmed the carcinogenic potential of this 'magic mineral'. As a consequence many governments have issued regulations to prevent or diminish exposure to asbestos. In contrast with the U.K., where the Asbestos Regulations date from 1969, legal measures were not taken in The Netherlands until 1977. The use of crocidolite (blue asbestos) was forbidden while the use of chrysotile (white asbestos) was restricted. However, taking into consideration the long latency period between exposure and diagnosis, effects of these measures may not be expected before the end of this century.

Studies reporting an increase of the incidence of mesothelioma initially came from countries with asbestos mines, like Australia and South Africa [2]. Later reports mentioned a high risk for specific occupational groups using asbestos products, such as insulators, pipe fitters and shipyard workers.

For The Netherlands an increase of the mortality of pleural cancer has been reported by Meijers *et al.* [3]. As a consequence of the increased use of asbestos after World War II, mortality appeared to have tripled. Most cases were found in coastal areas with shipbuilding and other heavy industries. This report deals with the incidence of pleural cancer and malignant mesothelioma in one of those areas.

PATIENTS AND METHODS

In this report we present data from the Rotterdam Cancer Registry for the period 1987–1989. The current registry started in 1982 and covers the southwestern part of The Netherlands, an area known for its industrial activities and shipping industry. Data on newly diagnosed cancer patients are collected from hospital and pathology records by specially trained registrars. From 1987 registration is complete in the central part of the region with about 1.5 million inhabitants. For this part of the region, age-standardised incidence rates (to the world standard population) were calculated using population data provided by the Central Bureau of Statistics.

RESULTS

The incidence rates of pleural cancer are shown in Table 1. To represent the impact of possible misclassification of other primary cancers the number of histologically or cytologically verified cases of mesothelioma is also given. For men the incidence rates correspond to a cumulative rate over 0–74 years, being an approximation of the life-time risk, of 0.7%. Apart from pleural mesothelioma 19 cases of peritoneal mesothelioma were registered, 17 in men, 2 in women.

Correspondence to R.A.M. Damhuis at the Comprehensive Cancer Centre Rotterdam, P.O. Box 289, 3000 AG Rotterdam, The Netherlands.
T. van Gelder is at the Department of Internal Medicine I, University Hospital Dijkzigt, Rotterdam, The Netherlands.
Revised 31 July 1992; accepted 8 Feb. 1993.