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Acetylator Status and its Relationship to Breast Cancer and Other Diseases of the Breast

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Abstract—*The distribution of* N*-acetylation phenotypes has been investigated in* 410 *consecutive patients prior to diagnostic excision of a breast lump and compared to that in* 337 *healthy controls. Contrary to previously published data there was no excess incidence of fast acetylators in patients with malignant breast disease. There was, however, a significant trend for more rapid acetylator ratios to be associated with advanced disease at first presentation. There was a slight but non-significant excess of fast acetylators in patients with benign breast disease as compared to controls, but there were no significant differences in the proportion of the acetylator phenotypes between cystic breast diseases with or without epithelial hyperplasia. There were no significant associations between oestrogen and progestogen receptors and the acetylator phenotype. It is concluded that the acetylation phenotype has no important association with malignant breast disease.*

INTRODUCTION

EPIDEMIOLOGICAL studies have suggested environmental influences on the occurrence of breast cancer as manifested by changing patterns of incidence occurring in immigrating populations [1]. At the same time, the risk conferred by a family history of cancer is well known, despite its occurrence in a small fraction of cases. Besides the well-known risk factors (age and menstrual and reproductive history) few carcinogens have been implicated in the aetiology. The effect of rauwolfia derivatives as tumour promotors [2] is not clearly established and the effect of dietary factors, and in particular fat [3], remains controversial [4]. There also remain other constitutional factors, such as blood group [5], which appear to play an ill-defined role in prognosis.

One possible factor modulating the effect of carcinogens is the ability of the individual to metabolize such compounds. *N*-Acetylation of some substrates is largely effected in man by a non-inducible cytosolic hepatic enzyme: *N*-acetyltransferase. This enzyme catalyses the major route of arylamine metabolism found in most animal species and in man there exists a hereditary polymorphism in acetylation rate. Individuals may be categorized into the 'slow' and 'fast' parts of a bimodal distribution which has been attributed to a simple Mendelian genetic model determined by two major alleles [6]. This polymorphism has important consequences for drug therapy and toxicology [7] in that arylamine carcinogens are acetylated by *N*-acetyltransferase. This suggests that differences in acetylation capacity may confer different susceptibility to chemically or environmentally induced cancers [8]. We have shown, for example, that the development of bladder cancer following industrial exposure to benzidine was highly associated with the slow acetylator phenotype [9].

The present study was initiated to investigate further the report [10] that a group of patients with 'advanced' breast cancer included a higher proportion of rapid acetylators of sulphadimidine than a matched, but mixed, control group of patients with cardiovascular disease and normal subjects.

MATERIALS AND METHODS

1. *Subjects*

(a) *Patients.* Four hundred and ten females, presenting consecutively, the majority as new patients, to the Breast Unit at Guy's Hospital with a suspicious breast lump. A few patients had received previous therapy for malignant breast disease with complete response. Most patients were of Cauca-

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sian origin with very few of other ethnic origins. The study period was between June 1982 and August 1985. Approval to conduct this study was granted by the local Ethics Committee at Guy's Hospital. Informed consent was obtained from each subject.

Staging of malignant breast disease at first clinical presentation was according to TNM classification. Histological examination with immunohistochemistry was undertaken by a single pathologist (RRM). Analysis of biopsy mateial with malignant histology was performed for oestrogen and progesterone receptor content at the ICRF Laboratories, Lincoln's Inn Fields, London, as described by King *et al.* [11]. A receptor value less than 5 fmol receptor/mg cytosol protein was designated as negative and any value greater than this as positive.

(b) *Controls.* Three hundred and thirty-seven British whites of both sexes were recruited from staff and others at Guy's Hospital and Medical School.

Excluded from this study were individuals with a history of sensitivity to sulphonamides, glucose-6-phosphate dehydrogenase deficiency or heavy alcohol intake.

2. *Acetylator phenotyping*

Acetylator testing was carried out on the day preceeding biopsy of the breast lump. The technique was similar to that used earlier [9] involving acetylation phenotyping with dapsone. This is a useful population technique in that timed blood or urine specimens do not need to be collected and dapsone itself has little liability to produce adverse effects in the dose employed. Assignment of acetylator phenotype was made by determination of the ratio of monoacetyldapsone/dapsone (MADDS/DDS) concentrations in the plasma. Each subject received a single dose of 50 mg DDS orally, and a 5 ml blood sample was taken 2–6 h afterwards. Plasma was separated, identified by code number, and stored at −20°C pending analysis for MADDS and DDS by high-performance liquid chromatography [12]. Analyses were conducted without knowledge of the origin of the plasma.

The MADDS/DDS ratio rapidly achieves a constant value and remains constant for a considerable period following DDS administration so that the exact time of blood sampling is immaterial [12]. As the phenotypic expression of *N*-acetyltransferase activity probably represents several enzymic systems, MADDS/DDS ratios <0.30 are categorized as slow acetylators and those with ratios >0.30 are designated fast acetylators [13].

3. *Statistical methods*

An uncorrected chi-square test was used to investigate difference in distribution of acetylator phenotypes in different groups. The Jonckheere–Terpstra test for ordered alternatives [14] was used to test for trend.

RESULTS

The breakdown of the categories of breast diseases studied was as follows: 181 breast cancer, 136 benign breast disease, 26 normal histology and 67 with other miscellaneous non-malignant conditions (e.g. fat necrosis, fistula, abscess).

Table 1 shows the distribution of acetylator ratios in breast cancer, benign breast diseases and controls, 54.7%, 47.1% and 55.2% were slow acetylators, respectively. An apparent excess of fast acetylators in patients with benign disease did not shown significant difference from the controls ($0.10 > P > 0.05$).

Table 2 shows the MADDS/DDS ratios for the three most common types of benign breast disease. Despite the small excess of the fast acetylator phenotype in cystic diseases, no significant differences emerge when comparing the sub-groups with the controls. In addition, there was no difference in the proportion of acetylator phenotypes between cystic disease with or without epithelial hyperplasia.

The malignant disease group was further subdivided according to the age and stage at first presentation and sex hormone receptor status. Table 3 shows that age had no effect on the distribution of acetylation phenotypes. Table 4 demonstrates that there may be differences in acetylation ratios depending on stage of disease at first presentation, as there was a significant ($P > 0.001$) trend for 'more rapid' ratios to be associated with advanced disease.

Table 5 shows the results for patients in relation to oestrogen and progesterone receptor value. There were no significant associations between receptor values and acetylator phenotype.

DISCUSSION

It is well known that breast cancer may exhibit an hereditary component in aetiology as manifested by familial clustering. In a study of family histories in 225 consecutively ascertained patients with verified breast cancer [15] the findings were consistent with a hereditary breast cancer syndrome in 5% of the patients. The inheritance of metabolic polymorphisms is one mechanism whereby cancer could be hereditarily predetermined. Bulovskaya *et al.* [10] determined acetylator status in 41 patients with advanced breast cancer contrasting these with a control group of 38 healthy age-matched females. The breast cancer patients revealed a relatively greater proportion of cases with a rapid rate of acetylation (68%) compared to the controls (37%). Moreover, the rate of acetylation in cancer patients was relatively higher in both rapid and slow acetyl-

Table 1. Distribution of acetylation phenotypes in malignant breast disease, benign breast disease and controls

MADDS/DDS	Malignant breast disease		Benign breast* disease		Controls	
	n	%	*n*	%	*n*	%
0.01–0.09	9	5.0	11	8.1	12	3.6
0.10–0.19	65	35.9	34	25.0	113	33.5
0.20–0.29	25	13.8	19	14.0	61	18.1
0.30–0.39	20	11.0	19	14.0	37	11.0
0.40–0.49	13	7.2	16	11.8	44	13.1
0.50–0.59	12	6.6	15	11.0	22	6.5
0.60–0.69	13	7.2	6	4.4	16	4.7
0.70–0.79	4	2.2	7	5.1	14	4.2
0.80–0.89	7	3.9	5	3.7	8	2.4
0.90–0.99	4	2.2	2	1.5	5	1.5
1.00+	9	5.0	2	1.5	5	1.5
Total	181	100.0	136	100.0	337	100.0
Slow acetylators	99	54.7	64	47.1	189	55.2

*Cystic disease with or without epithelial hyperplasia and fibroadenoma.
Benign breast disease vs. control group: $\chi^2 = 3.17$, df = 1, $0.10 > P > 0.05$ (N.S.).

Table 2. Distribution of MADDS/DDS ratios in cystic disease, cystic disease with epithelial hyperplasia and fibroadenoma

MADDS/DDS	Cystic disease		Cystic disease with epithelial hyperplasia		Fibroadenoma	
	n	%	*n*	%	*n*	%
0.01–0.09	4	8.5	4	8.3	3	7.3
0.10–0.19	9	19.1	11	22.9	14	34.1
0.20–0.29	7	14.9	6	12.5	6	14.6
0.30–0.39	7	14.9	5	10.4	7	17.1
0.40–0.49	6	12.8	6	12.5	4	9.8
0.50–0.59	6	12.8	7	14.6	2	4.9
0.60–0.69	3	6.4	1	2.1	2	4.9
0.70–0.79	2	4.3	3	6.3	2	4.9
0.80–0.89	2	4.3	3	6.3	0	0.0
0.90–0.99	1	2.1	0	0.0	1	2.4
1.00+	0	0	2	4.2	0	0
Total	47		48		41	
Slow acetylators	20	42.6	21	43.8	23	56.1

Cystic disease vs. controls: $\chi^2 = 3.04$, df = 1, $0.10 > P > 0.05$ (N.S.); cystic disease with epithelial hyperplasia vs. controls: $\chi^2 = 2.58$, df = 1, $0.50 > P > 0.10$ (N.S.).

ator phenotypes than in the corresponding groups of controls. This study utilized the ratio of acetylated sulphadimidine in plasma to unchanged drug and categorized patients as fast or slow acetylators according to the criteria of Price Evans [16]. Assignment of acetylator phenotype using the MADDS/DDS ratio has been shown to correlate with that using sulphonamide as marker drug [13, 17].

In the present study 54.7% of breast cancer patients were slow acetylators, which is close to observations on other Caucasian populations [6] including the control group employed in this study. The original Russian study employed a smaller population size and included patients with 'advanced' breast cancer without a definition of the stage of the disease. It is thus conceivable that most or all of their patients had stage IV disease. The bulk of the patients in the present study had stage I and II disease with 18 and 12 patients in stages III and IV, respectively. It should be pointed out that even advanced cancer cases in our study were not in a cachectic state. However, there is no evidence that undernutrition alters the sulphonamide actylation phenotype [18].

There is a significant trend, however, for an increased proportion of rapid acetylators in advanced stage of disease at presentation. The numbers investigated with stages III and IV were

Table 3. The distribution of acetylation phenotype in the malignant breast disease group in relation to age at presentation

	Malignant breast disease			
	≤ 50 years		> 50 years	
MADDS/DDS	*n*	%	*n*	%
0.01–0.09	5	6.9	4	3.7
0.10–0.19	24	33.3	42	38.5
0.20–0.29	11	15.3	14	12.8
0.30–0.39	8	11.1	12	11.0
0.40–0.49	6	8.3	7	6.4
0.50–0.59	4	5.6	7	6.4
0.60–0.69	4	5.6	9	8.3
0.70–0.79	3	4.2	1	0.9
0.80–0.89	3	4.2	4	3.7
0.90–0.99	1	1.4	3	2.8
1.00+	3	4.2	6	5.5
Total	72		109	
Slow acetylators	40	55.5	60	55.0

small and further study of this phenomenon is warranted. It is important to remember, however, that the present series of cases is taken from the referral population of a specialist centre and as such may be unrepresentative of all patients with breast cancer. Such retrospective 'trohoc' studies, although commonly used in cancer research, are also susceptible to numerous sources of transition and chronological bias making prospective studies the ideal methods for investigation [19].

In this study we have failed to demonstrate a significant relationship between acetylator status and breast cancer of the type previously suggested. With such negative results it is important to determine the statistical power of the investigation which in this case may be calculated [20] as low (<0.4). Given the observed effect size, a study would require >2100 individuals in each group to achieve a two-sided probability of 0.05 and power 0.9.

A consistent clinical finding has been a two- to three-fold increased risk of breast cancer in women with a history of benign breast disease. It has been suggested that epithelial hyperplasia is the most critical abnormality which increases the cancer risk two to eight times [21]. Our study suggests a non-uniform distribution of DDS acetylation ratios among the three groups with the most common benign breast conditions. Approximately 43% of cystic diseases and 56% of fibroadenomas were slow acetylators, the latter having acetylation phenotype proportions similar to the controls. Nevertheless, this distribution fails to attain significant statistical difference, and would need many more subjects to be investigated to determine whether any significant difference exists between the groups.

The results of our study show no difference in distribution of acetylation phenotypes among breast cancer patients with tumours possessing or lacking sex hormone receptors. There is still controversy regarding the role of oestrogen and progesterone receptors in predicting recurrence of breast cancer following mastectomy and the duration of disease-free interval or survival after relapse [22, 23]. Positive receptor values have been suggested to be a favourable prognostic index: the acetylation phenotype cannot apparently contribute to this debate.

Table 4. Distribution of individuals in the different groups of stage at presentation in relation to MADDS/DDS

	Stage at presentation (TNM)							
	I		II		III		IV	
MADDS/DDS	*n*	%	*n*	%	*n*	%	*n*	%
0.01–0.09	4	8.3	3	3.3	1	5.6	0	0.0
0.10–0.19	15	31.3	36	40.0	7	38.9	4	33.3
0.20–0.29	11	22.9	9	10.0	0	0	0	0
0.30–0.39	3	6.3	10	11.1	5	27.8	0	0
0.40–0.49	5	10.4	4	4.4	0	0	4	33.3
0.50–0.59	4	8.3	6	6.7	0	0	1	8.3
0.60–0.69	1	2.1	10	11.1	1	5.6	0	0
0.70–0.79	0	0	4	4.4	0	0	0	0
0.80–0.89	0	0	2	2.2	3	16.7	2	16.7
0.90–0.99	0	0	3	3.3	0	0.0	1	8.3
1.00+	5	10.4	3	3.3	1	5.6	0	0
Total	48		90		18		12	
Slow acetylators	30	62.5	48	53.3	8	44.4	4	33.3

Trend of % slow acetylators in I > II > III > IV significant at $P < 0.001$.

Table 5. Distribution of MADDS/DDS ratios in relation to presence or absence of oestrogen and progesterone receptors

	Oestrogen receptors				Progesterone receptors			
	Positive		Negative*		Positive		Negative*	
MADDS/DDS	*n*	%	*n*	%	*n*	%	*n*	%
0.01–0.09	7	6.6	1	4.2	5	5.7	3	7.0
0.10–0.19	42	39.6	9	37.5	32	36.8	19	44.2
0.20–0.29	11	10.4	3	12.5	9	10.3	5	11.6
0.30–0.39	10	9.4	2	8.3	9	10.3	3	7.0
0.40–0.49	11	10.4	1	4.2	9	10.3	3	7.0
0.50–0.59	5	4.7	2	8.3	4	4.6	3	7.0
0.60–0.69	6	5.7	3	12.5	5	5.7	4	9.3
0.70–0.79	2	1.9	1	4.2	3	3.4	0	0
0.80–0.89	5	4.7	2	8.3	6	6.9	1	2.3
0.90–0.99	2	1.9	0	0	2	2.3	0	0
1.00+	5	4.7	0	0	3	3.4	2	4.7
Total	106		24		87		43	
Slow acetylators	60	56.6	13	54.2	46	52.9	27	62.8

*<5 fmol receptor/mg cytosol.

Oestrogen +ve vs. oestrogen −ve: $\chi^2 = 0.047$, df = 1, $P > 0.5$ (N.S.); progesterone +ve vs. progesterone −ve: $\chi^2 = 0.74$, df = 1, $P > 0.5$ (N.S.).

Some authors [24, 25] have suggested that a small number of subjects with MADDS/DDS ratios 0.30–0.35 cannot be consistently phenotyped and hence fall into an 'indeterminate acetylator group'. Re-analysis of the data with the omission of individuals with ratios 0.30–0.35 does not materially alter the interpretation of the results of this study.

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