Ten genetic polymorphisms in bladder cancer

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SUMMARY Data are presented on a group of cases of primary carcinoma of the bladder, detailing red cell surface blood group antigenic phenotypes, serum haptoglobin phenotypes, and some red cell isoenzyme phenotypes. Account is taken of the stage of the disease at presentation. The results are compared with corresponding phenotype frequencies in groups of presumed healthy persons originating either in Yorkshire or County Durham. Differences in relative incidences were found in the haptoglobin, phosphoglucomutase (PGM), and some other systems. These are both differences between all cases and controls and between particular stages at presentation and controls.

Various studies have been published since 1956 which investigate the relationship between the ABO and a few other blood group systems and bladder cancer.¹⁻¹¹ Some of these results are summarised by Mourant *et al*¹² and indicate an excess of the A gene of the ABO blood group system among bladder cancer cases.

Many of these studies might be criticised, however, on the grounds that they only examine a few genetic polymorphisms. One study includes esterase D subtypes¹³ and other reports exist which report on two other blood group systems.^{1,2} This paper reports a series of bladder cancer cases from Yorkshire whose ABO, Rhesus, and MNSs results have been reported elsewhere,¹⁴ giving information on ten further genetic polymorphisms.

Method

Patients with transitional cell carcinoma of the urothelium (ICD 9th edition 188 and 233.3) include cases previously described as 'papilloma' as well as 'carcinoma'. They are, with the exception of some patients from York and Huddersfield, all newly diagnosed (incident) cases of bladder cancer arising in six Yorkshire hospitals from October 1978 to October 1981. The other (prevalent) cases were diagnosed some time before. They have all been

involved in an epidemiological study of bladder cancer in West Yorkshire, some results of which have been reported elsewhere.^{15,16} The entire group is being carefully followed and the disease stage at presentation is known for the majority of patients. The staging is the result of both clinical and histopathological examination and follows the TNM system for bladder cancer¹⁷ as follows:

- Tis, pre-invasive flat carcinoma or carcinoma in situ.
- T1, freely mobile mass which does not invade beyond the lamina propria (this includes Ta).
- T2, induration of the bladder wall and superficial muscle invasion.
- T3, induration or mobile mass palpable after resection and deep muscle invasion.
- T4, tumour fixed to pelvis or extending to neighbouring structures.

Blood was collected from cases and sent to Durham University by post for analysis, usually arriving within 24 hours of venepuncture. Analysis of the red cell surface antigens took place using standard methods of agglutination, the exact technique depending on the nature of each antiserum. Serum was separated and electrophoresis on starch gels undertaken for haptoglobin variation after the method of Harris *et al*.¹⁸ Red cell haemolysates were prepared, and electrophoresis for the following polymorphisms undertaken, broadly

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following the techniques of the various authors quoted: phosphoglucomutase (PGM),¹⁹ acid phosphatase (AP),²⁰ adenylate kinase (AK),²¹ esterase D (EsD),¹³ and adenosine deaminase (ADA).²²

The comparison groups are all from the laboratory which tested the cases and represent control series built up from various normal populations in County Durham. They are detailed by Williams²³ and, in general, represent adults aged 25 to 65 years of age.

The results were recorded and gene frequencies analysed by gene counting or the use of the homozygous recessive frequency, depending on the polymorphic system.

Relative incidence was computed using the basic method of Woolf,²⁴ but also computing the Mantel-Haenszel chi statistic²⁵ and confidence limits, after the method of Miettinen.²⁶

Results

Table 1 indicates the number and sources of the bladder cancer cases used in this study. Not all results are available for each system and a few cases were at an indeterminate stage at diagnosis, so the numbers recorded in the subsequent tables are often fewer than this total.

The results by sex for the blood groups, serum haptoglobin, and red cell isoenzyme types have been computed, but as there are no statistical differences between the sexes for any system all subsequent results have the sexes pooled.

Table 2 gives the results of the phenotype determinations and the corresponding gene frequencies compared with those of the various corresponding Durham control series. The gene frequencies are presented together with a standard error of frequency.

Table 3 gives the relative incidence between case and control phenotypes along with the confidence limits and probability levels. The probability column indicates that there is an excess of the B acid phosphatase phenotype when contrasted with the A and BA types and that there are more 21 and 22 phenotypes in the phosphoglucomutase and adenosine deaminase systems. These latter two

TABLE 2 Bladder cancer cases and controls: blood group phenotypes and gene frequencies.

		All cases		Durham controls	
		No	%	No	%
Kidd					
Phenotypes	aa	17	32.1	7	25.9
	ab	19	35.8	13	48.1
	bb	17	32.1	7	25.9
	Total	53		27	
Genes	a	0.5002	±0.0687	0.4995	±0.0962
	b	0.4998		0.5005	
Duffy					
Phenotypes	aa	54	18.8	55	19.0
	ab	140	48.8	126	43.6
	bb	93	32.4	108	37.4
	Total	287		289	
Genes	a	0.4320	±0.0292	0.4080	±0.0289
	b	0.5680		0.5920	
Kell					
Phenotypes	KK	1	0.3	1	0.2
	Kk	25	8.7	34	6.6
	kk	262	91.0	479	93.2
	Total	288		514	
Genes	K	0.0465	±0.0124	0.0350	±0.0081
	k	0.9535		0.9650	
Penney					
Phenotypes	aa	0	—	0	—
	ab	3	1.5	6	1.4
	bb	191	98.5	417	98.6
	Total	194		423	
Genes	a	0.0075	±0.0062	0.0070	±0.0041
	b	0.9925		0.9930	
Haptoglobin					
Phenotypes	1-1	51	18.8	16	18.6
	2-1	117	43.2	44	51.2
	2-2	103	38.0	26	30.2
	Total	271		86	
Genes	1	0.4040	±0.0298	0.4420	±0.0536
	2	0.5960		0.5580	
Acid phosphatase					
Phenotypes	A	27	9.3	24	10.5
	BA	115	39.7	111	48.5
	B	122	42.1	80	34.9
	BC	14	4.8	10	4.4
	CA	12	4.1	4	1.7
	Total	290		229	
Genes	a	0.3120	±0.0272	0.3560	±0.0316
	b	0.6435		0.6135	
	c	0.0445		0.0305	
Phosphoglucomutase					
Phenotypes	1-1	158	59.0	79	68.7
	2-1	95	35.4	32	27.8
	2-2	15	5.6	4	3.5
	Total	268		115	
Genes	1	0.7670	±0.0259	0.8260	±0.0354
	2	0.2330		0.1740	
Adenylate kinase					
Phenotypes	1-1	256	91.8	363	91.9
	2-1	21	8.2	30	7.6
	2-2	0	—	2	0.5
	Total	277		395	
Genes	1	0.9590	±0.0119	0.9570	±0.0102
	2	0.0410		0.0430	
Esterase D					
Phenotypes	1-1	227	81.9	304	77.9
	2-1	48	17.3	82	21.0
	2-2	2	0.7	4	1.0
	Total	277		390	
Genes	1	0.9055	±0.0176	0.8850	±0.0162
	2	0.0945		0.1150	
Adenosine deaminase					
Phenotypes	1-1	240	87.9	96	94.1
	2-1	32	11.7	6	5.9
	2-2	1	0.4	—	—
	Total	273		102	
Genes	1	0.9375	±0.0146	0.9705	±0.0168
	2	0.0625		0.0295	

TABLE 1 Yorkshire bladder cancer: sources of cases.

Health districts	No of cases
Airedale	8
Calderdale	37
Huddersfield	143
Pontefract	26
Wakefield	34
York	40
Total	288

TABLE 3 *Relative incidence of bladder cancer phenotypes when compared with control phenotypes.*

Phenotype system	Comparison	Relative incidence	95% confidence limit	Chi (Mantel-Haenszel)	p
Kidd	aa/ab+bb	0.69	0.25-1.91	-0.71	0.239
Duffy	aa/ab+bb	0.99	0.65-1.50	-0.07	0.472
Kell	KK+Kk/kk	1.36	0.80-2.30	1.14	0.127
Penney	ab/bb	1.09	0.27-4.41	0.12	0.452
Haptoglobin	11/21+22	1.01	0.54-1.89	0.04	0.484
Acid phosphatase	A+BA/B	0.69	0.48-0.99	-1.98	0.024
Phosphoglucomutase	11/21+22	0.66	0.41-1.04	-1.80	0.036
Adenylate kinase	11/21+22	1.08	0.61-1.91	0.25	0.401
Esterase D	11/21+22	1.28	0.87-1.89	1.26	0.104
Adenosine deaminase	11/21+22	0.46	0.19-1.10	-1.75	0.040

TABLE 4 *Blood group haptoglobin and isoenzyme phenotypes and stage at presentation (sexes pooled).*

Phenotypes	T1 No %	T2 No %	T3 No %	T4 No %	Carcinoma in situ No %
Duffy					
aa	28 16.9	10 22.2	10 23.3	1 16.7	2 16.7
ab	85 51.2	23 51.1	19 44.2	2 33.3	7 58.3
bb	53 31.9	12 26.7	14 32.6	3 50.0	3 25.0
Total	166	45	43	6	12
Kell					
KK	1 0.6	0 —	0 —	0 —	0 —
Kk	13 7.8	2 4.4	5 11.6	1 16.7	2 16.7
kk	153 91.6	43 95.6	38 88.4	5 83.3	10 83.3
Total	167	45	43	6	12
Haptoglobin					
1-1	32 20.4	2 5.0	10 23.3	2 40.0	3 27.3
2-1	64 40.8	23 51.1	18 41.9	2 40.0	6 54.5
2-2	61 38.8	15 37.5	15 34.9	1 20.0	2 18.2
Total	157	40	43	5	11
Acid phosphatase					
A	16 9.6	6 13.0	4 9.1	0 —	1 8.3
BA	63 37.7	18 39.1	17 38.6	3 50.0	4 33.3
B	72 43.1	19 41.3	18 40.9	2 33.3	7 58.3
BC	11 6.6	1 2.2	2 4.5	0 —	0 —
CA	5 3.0	2 4.3	3 6.8	1 16.7	0 —
Total	167	46	44	6	12
Phosphoglucomutase					
1-1	84 56.4	22 52.4	32 72.7	3 50.0	6 50.0
2-1	58 38.9	17 40.5	10 22.7	3 50.0	4 33.3
2-2	7 4.7	3 7.1	2 4.5	0 —	2 16.7
Total	149	42	44	6	12
Adenylate kinase					
1-1	149 94.3	38 90.5	39 88.6	5 83.3	11 91.7
2-1	9 5.7	4 9.5	5 11.4	1 16.7	1 8.3
2-2	0 —	0 —	0 —	0 —	0 —
Total	158	42	44	6	12
Esterase D					
1-1	129 81.6	32 76.2	42 95.5	5 83.3	8 60.7
2-1	29 18.4	9 21.4	1 2.3	1 16.7	4 33.3
2-2	0 —	1 2.4	1 2.3	0 —	0 —
Total	158	42	44	6	12
Adenosine deaminase					
1-1	138 87.9	36 85.7	36 85.7	6 100.0	11 91.7
2-1	18 11.5	6 14.3	6 14.3	0 —	1 8.3
2-2	1 0.6	0 —	0 —	0 —	0 —
Total	157	42	42	6	12

results are quite weak, however, and the 95% confidence limits indicate that it is possible that no true relationship exists given the sample sizes involved.

Table 4 gives the phenotypes of the blood group

systems, haptoglobin, and isoenzyme types by stage at presentation. The Kidd and Penney blood groups are omitted owing to small sample sizes. Table 5 summarises the results from the previous table in the form of relative incidences, using, in each case, the Durham control groups as a comparison. The brackets indicate results not significant at the 5% level of probability. The T4 stage was combined with the T3 because of small numbers. The majority of these results are not statistically different from unity. Carcinoma in situ was not included because of the small numbers.

The acid phosphatase and adenosine deaminase systems, which showed a significantly lowered relative incidence in table 3, give similar results at all the stages of presentation. The low relative incidence of phosphoglucomutase, however, is the result of a deficit of the 1-1 phenotype, but this is largely confined to stage 1 and 2 disease.

No particular trends are discernible in the Duffy and Kell blood group systems. A non-significant trend exists in the adenylate kinase system with a progressive deficit of the 1-1 phenotype with increasing stage. There are two other unexpected results; the haptoglobin phenotypes have a gross deficit of the 1-1 phenotypes in stage 2 disease, while there is a statistically significant excess of the 1-1 esterase D phenotype in later stage disease.

TABLE 5 *Relative incidence of phenotypes by stage at presentation of bladder cancer.*

Phenotype	Comparison	Stage and relative incidence		
		T1	T2	T3/T4
Duffy	Fyaa/ab+bb	(0.86)*	(1.22)	(1.23)
Kell	KK+Kk/kk	(1.25)	(0.64)	(1.91)
Haptoglobin	11/21+22	(1.12)	0.23	(1.46)
Acid phosphatase	A+BA/B	(0.65)	(0.75)	(0.71)
Phosphoglucomutase	11/21+22	0.59	(0.50)	(1.06)
Adenylate kinase	11/21+22	(1.46)	(0.84)	(0.65)
Esterase D	11/21+22	(1.26)	(0.91)	4.43
Adenosine deaminase	11/21+22	(0.45)	(0.38)	(0.44)

*Not significant at 95% in brackets.

Discussion

Many of the results presented here have not previously been reported and will need confirmation in future studies. The discussion is concerned with the relationship between these genetic blood factors and aetiology. The significance of stage at diagnosis in relation to phenotypic biases is the second major theme.

There is a general point, however, to discuss before a detailed examination of the results, namely, the appropriateness of the control group. The ages of the control group are different from those of the cases, being younger by an average of 10 years. Subdivisions by age strata within cases and controls, however, revealed no statistical differences by age and it is thought unlikely that any significant bias arises from this source.

The controls originate in County Durham rather than the neighbouring area to the south, Yorkshire. There are two factors in the decision to use these particular controls. Control data, analysed at Durham, are available only from 60 hospital patients in Yorkshire. Their results have not been included in this paper, but they are of similar age to the control subjects from Durham and in no system do the results from this small number conflict with the Durham results. Secondly, no larger series for these polymorphisms exists from Yorkshire from any other source.

The biases in the relative incidence of AP, PGM, and ADA types are all new observations which, if confirmed, could be most useful in defining a high risk group susceptible to bladder cancer in the population. They must be considered in relation to our previous report on the bias to the ABO blood group A gene and the S gene of the MNSs system.¹⁴ For example, there will be relatively few people in the general population who have the A phenotype of the ABO system, the SS phenotype of the MNSs system, and the B phenotype of the AP system, perhaps about 1.5% if the systems are distributed independently, while a case group would have these three gene combinations three times more frequently.

The natural history of different cases of bladder cancer, despite broadly similar histopathological appearances, is highly unpredictable and ranges from a well defined fronded tumour which does not invade or return after treatment to a most aggressive disease which invades the bladder wall and spreads rapidly, causing death within months of diagnosis.

There are various ways in which natural histories of bladder cancer may be examined and recorded; these include a subjective assessment of 'degree of aggressiveness' of the tumour based on hospital records of cystoscopies. This was attempted in an

early paper¹¹ but is poorly reproducible. Alternatively, differential length of survival might be measured, but in the case of the present study several more years of observation would be needed to begin to examine the problem in this fashion. A third approach, which is also useful in a clinical predictive role, is to examine the depth of invasiveness of the tumour at diagnosis. One advantage of this staging procedure is that it is an internationally agreed classification.¹⁷

The carcinoma in situ lesions represent a special case with respect to this classification, however, in that they are potentially highly invasive tumours^{27 28} which are rare but, where recognised, are regarded as lesions needing aggressive treatment in an attempt to stop deep invasion.

As a measure of natural history, the T system is imperfect but has enough advantages to be considered at length. The T1 and T2 types are a mixture of two types: aggressive lesions and the majority of slow growing lesions, which will rarely cause death. Coupled with this hypothesis are the observations that cigarette smoking is only related as a risk factor to T1 and T2 type tumours.²⁹ However, another genetic factor, the phenotypic variants of the liver bound enzyme N-acetyltransferase, has an excess of the slower types associated with T3, T4, and carcinoma in situ.³⁰

These facts rather suggest that despite its imperfection the T types are useful in subdividing bladder cancer into two general types: the broadly invasive group and the broadly non-invasive.

Returning to the new results presented here, the fact that some gene excesses in PGM, haptoglobin, and esterase are confined to either the T1 and T2 or the T3/T4 types is a further confirmation that epidemiological differences probably exist between different T types of bladder cancer.

With respect to the results, it should be remembered that multiple comparisons are inherently dangerous, as some results might be expected to be significant just by chance. A more stringent level of significance of 1% will still result in the statistically significant excess of the 1 gene in ESD and the PGM and haptoglobin results found in table 5.

The significance of these results is not readily apparent although it is clear that the cell surface antigenic structure of cells is related to the invasiveness or otherwise of bladder tumours³¹ with respect to the ABO blood groups, and this might also be the case with other red cell surface markers. In addition, a person's enzymic structure is undoubtedly important in bladder carcinogenesis with respect to N-acetyltransferase³⁰ and this could also be the case with regard to other enzyme systems,

the enzymes in the red blood cell being a reflection of their presence in other tissues.

These observations and those on the ABO and MNSs systems¹⁴ form the basis of a general approach to the identification of high risk subgroups of the general population. Other studies have paralleled these to find risk occupations and other environmental factors.^{15 16} It is the aim of this group of studies in genetics-epidemiology to link all aspects of tumour natural history and environmental exposure in future work with a view to disease control.

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