Genetic markers in Welsh gypsies

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SUMMARY Data are presented on polymorphic genetic markers of 84 Welsh gypsies, members of an inbred population characterised by a high incidence of phenylketonuria and other recessively inherited diseases. Sixteen polymorphic loci were studied: the ABO blood group distribution was comparable to that of the surrounding population, the B gene frequency being 7.5%, a lower frequency than most earlier studies in gypsies. The haptoglobin 1 gene frequency (23.7%) was very different from that of the British population, resembling the frequency found in India and in some previously reported gypsy groups. The data on Welsh gypsies for the various loci are discussed in relation to the known evidence for a number of European gypsy populations, and in relation to the genetic structure of these populations. It is concluded that genetic divergence has occurred in the European gypsies, and that this may have been accentuated by the dispersal, isolation, and inbreeding which have occurred during the present century.

The gypsies of Europe, despite centuries of dispersal over a wide geographical area, still form a distinctive ethnic group. Though frequently confused by outsiders with other travelling populations, gypsies themselves have a clear concept of their own identity and while intermarriage with host populations and other travelling and migrant groups has occurred, it is still not general. The traditionally ascribed origin of the gypsies is from the Indian subcontinent and this has received strong support from philological studies (Sampson, 1926), which have shown close

resemblance between Sanskrit and the Romany language as spoken until recently in North Wales and other parts of Europe.

The studies of blood groups and other genetic markers on gypsy populations have so far been few; most have been from Continental Europe, in particular France, and the scattered data have recently been assembled by Mourant *et al.* (1976), who make a plea for further studies. The only comprehensive study from Britain has been that of Clarke (1973), whose subjects were English gypsies. Data are presented here on 84 Welsh gypsies, members of a

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Table 1 ABO blood groups in gypsy populations

Country	Author	Number	Phenotypes						Gene frequencies (%)*			
			A ₁	A ₂	B	A ₁ B	A ₂ B	O	p ₁	p ₂	q	r
Wales	Present study	70	25	5	10	0	0	30	20.1	4.7	7.5	67.6
England	Clarke (1973)	109	36	16	9	5	0	43	21.0	9.9	6.6	62.6
France (South)	Cazal <i>et al.</i> (1951)	115	26	3	43	11	5	25	18.7	4.4	31.4	45.9
(North)	Ely (1961)	47		10	9	3	25		14.8	13.6	71.6	
(South)	Ely (1966)	41		17	11	4	9		30.6	20.6	48.8	
(South)	Nicoli and Sermet (1965)	92		37	9	7	39		27.5	9.0	63.5	
Sweden	Beckman <i>et al.</i> (1965)	115	55	13	7	3	1	36	29.6	9.3	4.9	56.2
Jugoslavia	Hočevár (1965); Avčín (1969)	350	117	56	36	13	8	120	20.7	12.5	8.5	58.3
	Schmidt (1930); (two localities)†	299		69	73	23	134		16.7	17.5	65.8	
		126		50	25	9	42		27.2	14.6	58.2	
Hungary	Verzar and Weszeczky (1921)	385		81	150	22	132		14.5	25.8	59.7	
USSR (Uzbekistan)	Libman (1930)†	104		44	30	10	20		31.3	22.0	46.8	
Romania	Hesch†	102		28	38	9	27		20.4	26.8	52.8	
Czechoslovakia	Bernasovsky <i>et al.</i> (1976)	2935	966	71	740	248	25	885	23.6	2.2	19.3	54.9

*All the gene frequencies were calculated or recalculated using the formula given in Mourant (1954).

†Quoted by Hočevár (1965).

socially but not geographically isolated population in South Wales, whose genetic characteristics have been discussed in the preceding paper (Williams and Harper, 1977).

Methods

All individuals studied were gypsies living in and originating from Wales. The sample was drawn from as wide an area of South Wales as possible, extending from Cardiff in the East to Pembroke in the West, and was not confined to the kindred containing phenylketonuric individuals. Travellers of non-Romany origin were not studied, neither were Romany gypsies of English origin, though some individuals included had ancestors of English gypsy or non-Romany descent.

Venous blood samples, both heparinised and clotted, were studied from each patient. The plasma protein polymorphisms, Hp and Tf, were detected in the same starch gel by horizontal electrophoresis

according to the methods of Smithies (1955), using a discontinuous system of buffers (Poulik, 1957). The Hp/Hb complex was visualised using a benzidine stain (Smithies, 1959) and the Tf phenotypes by using a 1% amido-black solution (Smithies, 1957). Red cell lysates were made after the method of Ager and Lehmann (1961) and stored at -20°C . Horizontal starch gel electrophoresis was again employed to detect the following red cell isoenzyme poly-

Table 2 *Rh blood groups in Welsh gypsies*

Phenotype		Chromosome frequency (%)	
CCDee	23	CDE	0
CcDEe	12	cDE	21.1
CcDee	17	CDe	53.6
ccDEE	6	cdE	3.2
ccDEe	9	Cde	0
ccddEe	1	cDe	0
ccdde	2	cde	22.1
Total	70	Total	100.0

Table 3 *Rh D antigen in gypsy populations*

Country	Authors	Number	Phenotypes		Gene frequency (%)	
			D+	D-	D	d
Wales	Present study	70	67	3	79.3	20.7
England	Clarke (1973)	109	93	16	61.7	38.3
France (South)	Cazal <i>et al.</i> (1951)	107	91	16	61.3	38.7
France (North)	Ely (1961)	42	41	1	84.6	15.4
France (South)	Ely (1966)	41	36	5	65.1	34.9
France (South)	Nicoli and Sermet (1965)	81	71	10	64.9	35.1
Hungary	Backhausz <i>et al.</i> (1950)	189	165	24	64.4	35.6
Sweden	Beckman <i>et al.</i> (1965)	115	110	5	79.1	20.9
Jugoslavia (Slovenia)	Hočevár (1965)	350	284	66	56.6	43.4
Czechoslovakia	Bernasovsky <i>et al.</i> (1976)	2935	2628	307	67.7	32.3

Table 4 *MN blood groups in gypsy populations*

Country	Authors	Number	Phenotypes			Gene frequencies (%)	
			MM	MN	NN	M	N
Wales	Present study	70	16	33	21	46.4	53.6
England	Clarke (1973)	109	54	45	10	70.2	29.8
France (South)	Cazal <i>et al.</i> (1951)	86	31	40	15	59.3	40.7
Sweden	Beckman <i>et al.</i> (1965)	115	23	64	28	47.8	52.2
Jugoslavia (Slovenia)	Hočevár (1965); Avčín (1969)	350	67	164	119	42.6	57.4
Czechoslovakia	Bernasovsky <i>et al.</i> (1976)	405	110	209	86	53.0	47.0

Table 5 *Haptoglobin types in gypsy populations*

Country	Authors	Number	Phenotypes			Gene frequencies (%)	
			1-1	2-1	2-2	Hp ¹	Hp ²
Wales	Present study	76	3	30	43	23.7	76.3
England	Clarke (1973)	103	16	54	33	41.7	58.3
Sweden	Beckman <i>et al.</i> (1965)	115	0	27	88	11.7	88.3
Czechoslovakia	Gáliková <i>et al.</i> (1969)	360	6	96	258	15.0	85.0
Jugoslavia (Macedonia)	Fraser <i>et al.</i> (1969)	38	2	9	27	17.1	82.9

Table 6 Various genetic polymorphisms in Welsh gypsies

<i>System</i>	<i>Number</i>	<i>Phenotypes</i>						<i>Gene frequencies (%)</i>							
Blood groups															
MN	70	MM	16	MN	33	NN	21	<i>M</i>	46.4	<i>m</i>	53.6				
Ss	70	S+	45	S-	25			<i>S</i>	40.2	<i>s</i>	59.8				
Fy (Duffy)	69	Fya+	44	Fya-	25			<i>Fy^a</i>	39.8	<i>Fy^b</i>	60.2				
Kk (Kell)	70	K+	0	K-	70			<i>K</i>	0	<i>k</i>	100				
Kp	59	Kp ^a +	0	Kp ^a -	59			<i>Kp^a</i>	0	<i>Kp^b</i>	100				
P	48	P ₁ +	38	P ₂ -	10			<i>P₁</i>	48.7	<i>P₂</i>	51.3				
Jk (Kidd)	18	JkA+	12	JkA-	6										
Cw	35	Cw+	2	Cw-	33										
Serum types															
Hp (haptoglobin)	76	1-1	3	2-1	30	2-2	43	<i>Hp¹</i>	23.7	<i>Hp²</i>	76.3				
Tf (transferrin)	77	C	74	B-C	3			<i>B</i>	1.9	<i>C</i>	98.1				
Enzymes															
AP (acid phosphatase)	64	A	12	AB	19	B	28	BC	5	<i>P^A</i>	33.6	<i>P^B</i>	62.4	<i>P^C</i>	3.9
AK (adenylate kinase)	71	1-1	71	2-1	0	2-2	0			<i>Ak¹</i>	100	<i>Ak²</i>	0		
PGM ₁ (phosphoglucomutase)	69	1-1	45	2-1	21	2-2	3			<i>PGM¹</i>	80.5	<i>PGM²</i>	19.6		
EsD (esterase D)	68	1-1	61	2-1	7	2-2	0			<i>EsD¹</i>	94.9	<i>EsD²</i>	5.1		

Table 7 Various genetic polymorphisms in gypsy populations—published data

System	Country	Authors	Number	Phenotypes			Gene frequencies (%)	
P	France (South)	Cazal <i>et al.</i> (1951)	74	P ₁ 65	P ₂ 9		P ₁ 65.1	P ₂ 34.9
Ss	England	Clarke (1973)	109	S+ 45	S- 64		S 23.5	s 76.5
Lutheran	England	Clarke (1973)	107	LuA+ 5	LuA- 102		Lu ^a 3.3	Lu ^b 97.7
Kell	England	Clarke (1973)	108	K+ 5	K- 103		K 3.4	K 97.6
Duffy	England	Clarke (1973)	106	FyA+ 63	FyA- 43		Fy ^a 36.2	Fy ^b + Fy 63.8
Secretor	Hungary	Backhausz and Deak (1952)	62	Secretor 47	Non-secretor 15		Se 50.8	se 49.2
Transferrin	{ Sweden England	Beckman <i>et al.</i> (1965)	115 103	TfC 115	TfB-C 0		Tf ^C 100	Tf ^B 0
		Clarke (1973)		100	3		98.6	1.4
Gc	Jugoslavia (Macedonia)	Fraser <i>et al.</i> (1969)	18	1-1 11	2-1 7	2-2 0	Gc ¹ 80.5	Gc ² 19.5
Pseudocholinesterase	Jugoslavia	Fraser <i>et al.</i> (1969)	33	U 33	1 0	A 0	E ₁ ^u 100	E ₁ ^a 0
Phenylthiourea tasting	England	Clarke (1973)	36	Taster 25	Non-taster 10		T 46.5	t 53.5
Esterase D	England	Welch and Lee (1974)	153	1-1 122	2-2 30	2-2 1	EsD ¹ 89.5	EsD ² 10.5
G-6PD	Jugoslavia	Fraser <i>et al.</i> (1969).	34	Normal ♂ 32	Deficient ♂ 2	Normal ♀ 6	Intermediate ♀ 2	Deficient ♀ 0

morphisms: acid phosphatase (AP) using the method described by Hopkinson *et al.* (1963), phosphoglucumutase (PGM) using the method described by Spencer *et al.* (1964), adenylate kinase (AK) using the method of Fildes and Harris (1966), and esterase D using the method of Parkin and Adams (1975).

Results

Data on the ABO blood group system from our own and other studies are given in Table 1. The gene frequencies of the Welsh gypsies are close to those found by Clarke (1973) for English gypsies; the low B frequency (7.5%) in particular differs strikingly

from the high B frequency found in some of the earlier studies, as discussed later.

Table 2 gives the full Rh genotyping for our series, while results of other studies testing for the Rh D antigen alone are given in Table 3 to facilitate comparison. Data for the MN blood groups are given in Table 4 while Table 5 summarises the results for the haptoglobins. Our results for other loci where either the degree of polymorphism was low or where data for comparison are few are given in Table 6, while Table 7 gives similar data from published studies.

Discussion

A number of blood group studies of gypsy populations from Continental Europe have been undertaken, but before their results can be compared with those of the present study, the nature of these populations must be considered. The most detailed study is that of the isolated gypsy community of Prekmurje, Yugoslavia, studied by Avčin (1969) and discussed in the previous paper (Williams and Harper, 1977). Blood group studies on this population were reported by Hočevar, (1965) and give comparative data on local non-gypsy inhabitants as well as blood donors for the region. The gypsy population formed a closed isolate of 683 individuals. Extensive data have recently been reported from Czechoslovakia for the ABO, Rh, and MN loci (Bernasovsky *et al.*, 1976), but so far without other genetic data. The French gypsies studied by Ely (1961; 1966) were also members of small isolates, one group in Paris, another in Avignon; the latter group possessed only 5 surnames in the population and showed frequent consanguineous marriages. The Swedish gypsies reported by Beckman and Takman (1955) and Beckman *et al.* (1965) formed an equally small and isolated group, with a total population of under 900, descended from a small group of founders. All these populations are likely to have been subject to random influences such as genetic drift and the founder effect which may have produced wide genotypic deviations from the population of origin, quite apart from any changes resulting from pressure of natural selection. The present series of Welsh gypsies, while not forming a single geographical isolate, shows a degree of social isolation and consanguinity that makes it likely that here too non-selective forces could have exerted a considerable effect.

Considering the various genotypic systems in turn, evidence is most abundant for the ABO blood groups, including several studies done before the disruption of European gypsy populations in the Second World War. Table 1 summarises the data. The early series

agreed in showing a strikingly high B gene frequency, from countries as far apart as Russia, France, Hungary, and Romania. This correlated well with the traditional and linguistic evidence for an Indian origin of the gypsies, since most populations of the Indian subcontinent also show a high B gene frequency of 15 to 40%, maximal in the north west part of the subcontinent (Mourant *et al.*, 1976).

Apart from the recent large study from Czechoslovakia of Bernasovsky *et al.* (1976) with a B gene frequency of 19.3%, most later studies have produced contrary evidence, including those from Sweden (Beckman *et al.*, 1965), Yugoslavia (Hočevar, 1965), and England (Clarke, 1973), all showing B gene frequencies comparable with the surrounding populations. Our own results on Welsh gypsies agree with this, showing a B gene frequency of 7.5%, compared with frequencies of 7.17 and 6.68% for corresponding areas in Wales (Kopeč, 1970).

Complete Rh genotyping was performed on the present sample but not on most of the other series available for comparison. If the d gene frequency is used as a guide (Table 3), most of the gypsy results are within the European range of 30 to 45% and differ considerably from the frequencies in the Indian subcontinent which range from 10 to 30%. Only the present sample (23.4%) and the small sample of Ely (15.4%) approach the Indian range and in neither case is the difference from the overall mean gypsy value significant.

The MN blood group system shows a high M gene frequency (55 to 70%) over most of India, but the MN data on gypsies (Table 4) show only two series with M gene frequencies in this range, these being the English series of Clarke (1973) and the South French series of Cazal *et al.* (1951). In the present study the M gene frequency of 46.4% is similar to those found in Swedish and Slovenian gypsies and is in fact lower than the values of 54 to 58% found in Wales (Mourant *et al.*, 1976), where the M gene frequency is higher than in England.

The Kell and other blood group systems are less informative in relation to the origins of the gypsies, since these gene frequencies are comparable in European and Asiatic populations. Among other genetic markers the haptoglobins give greatest discrimination. Most Indian populations lie within the range of Hp¹ gene frequencies of 10 to 25%, in contrast with that of 30 to 40% seen over Europe. Of the 5 gypsy populations studied, 4, including the Welsh gypsies, show Hp¹ values of less than 25%, the only exception being the series of Clarke (1973). The haptoglobin locus thus provides strong overall support for an Indian genetic affinity.

Among the red cell enzymes general population data are considerably more scanty, but both the acid

phosphatase and PGM₁ loci are highly polymorphic. In each case the values found in the present series are closer to those found in general United Kingdom series than to data available from Indian populations (Mourant *et al.*, 1976).

No specific marker indicative of gypsy origin has yet been identified with certainty, but it is of interest that all those individuals found to carry the rare Gregory blood group GyA-ve have been of gypsy origin, some from Czechoslovakia, others from England (Clarke *et al.*, 1975). Testing of a small number of members of the present series (courtesy of Dr R. M. Barnes, Southampton) proved normal, but it will be of interest to see if this antigen proves to be of general distribution among gypsy populations.

From the results discussed above it is clear that considerable heterogeneity exists between the present-day European gypsy populations, though there are still pronounced genetic differences between gypsies and the surrounding 'host' communities. Several possible explanations for this situation exist, and are not mutually exclusive.

There is substantial evidence in favour of the genetic affinity of gypsy populations and those of India. The high B gene frequency of a number of populations, together with the low Hp¹ gene frequency, supports this view and there is no other European population that shows comparable gene frequencies at either of these loci.

Admixture with local populations, genetic drift, and the founder effect are all possible explanations for the genetic variation among gypsy populations, seen most strikingly at the ABO locus. It is perhaps relevant that the studies showing the highest B gene frequencies were the earlier ones, while those showing lower frequencies were more recent, and were performed on isolated groups of relatively small size, in which genetic drift and related factors would be of particular importance. This might suggest that present day gypsy populations are increasingly diverging in genetic structure both from each other and from the original ancestral pattern, and would explain differences between loci in this respect; for example the Swedish gypsies (Beckman *et al.*, 1965) show a low B gene frequency, unlike many central European gypsy populations, but have retained an extremely low Hp¹ gene frequency. The fact that Czechoslovakia, with its extensive and widely distributed gypsy population, has maintained a high B gene frequency supports this view.

An alternative explanation would be that the gypsies are descended from more than one ancestral group and that present variation reflects the genetic differences in these original populations. While it is likely that successive waves of immigrants travelled westwards over Europe in the first millennium AD,

this hypothesis does not explain the present geographical distribution: those gypsy groups not showing a high B gene frequency are widely scattered across Europe from Sweden to Yugoslavia, while those in neighbouring regions (e.g. Yugoslavia and Romania) show pronounced differences. Nor does a heterogeneous origin agree with the existence of a common language with strong Sanskrit affinity spoken by Romany gypsies over most of Europe until the present time.

Conclusion

Studies of genetic markers in gypsy populations must be interpreted in the light of population structure as well as geographical location. Most present groups are small, show a high rate of consanguinity, and are isolated either geographically or socially from the surrounding community. All these factors favour the operation of non-selective deviations in genotype and it is thus not surprising that a comparison of data from Wales with other European gypsies shows pronounced variations in gene frequencies. Where differences between the gypsies and neighbouring populations are found, they are compatible with the long-standing view that the gypsies originated from the Indian subcontinent, but it is clear that extensive genetic divergence has occurred since that time, so that the European gypsies can no longer be considered a single homogeneous group.

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