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ORIGINAL ARTICLE

S. S. Mastana · P. Fisher

# Haptoglobin subtypes in the East Midlands (United Kingdom)

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**Abstract** Haptoglobin subtypes were analysed by isoelectric focusing (IEF) in 5 regional populations from the East Midlands (United Kingdom). The allele frequencies showed a considerable variation at regional level. Allelic frequency ranges were HP*1S = 24–28%, HP*1F = 11–15%, HP*2FS 54–63%, HP*2SS = 0.5–6% and HP*2FF 0–1%. The data presented here suggest that HP subtypes provide a useful genetic marker for population studies and paternity testing.

**Key words** Haptoglobin subtypes · IEF · East Midlands 5 regions

**Zusammenfassung** Die Haptoglobin-Subtyen wurden mit Hilfe der isoelektrischen Fokussierung (IEF) in 5 regionalen Populationen der East Midlands (United Kingdom) untersucht. Die Allel-Frequenzen zeigen eine beträchtliche Variation auf der regionalen Ebene. Die Streubreiten für die Allel-Frequenzen waren für HP*1S = 24–28%, für HP*1F = 11–15%, HP*2FS 54–63%, HP*2SS = 0,5–6% und HP*2FF 0–1%. Die hier vorgestellten Daten lassen daran denken, daß die HP-Subtypen einen nützlichen genetischen Marker für Populationsstudien und Vaterschaftstests darstellen.

**Schlüsselwörter** Haptoglobin-Subtypen · IEF · East Midlands · 5 Regionen

## Introduction

Haptoglobin was among the first genetic polymorphism demonstrated by the technique of starch gel electrophoresis [1]. Structurally, the haptoglobin molecule consist of an alpha and a beta chain. The alpha chain occurs in 2 forms of different molecular size: A1 and the nearly complete duplicated A2. While the beta chain variants are extremely rare, 5 allelic beta chain variants have been revealed by using the technique of isoelectric focusing. There are 2 different A1 alleles (HP*1F and HP*1S) and 3 different A2 alleles (HP*2FF, HP*2FS and HP*2SS). The molecular structure, genetics and the relationship of different haptoglobin types have already been reviewed [2–6]. The distribution of haptoglobin genes among human populations has mainly been studied by conventional electrophoresis. By the subtyping procedure, initially described by Smithies et al. [7], some populations were studied for A1 subtypes [8]. A recent review by Teige and colleagues [6] showed that the data regarding both A1 and A2 allelic variants are still fragmentary and limited mainly to the populations of Continental Europe and Southeast Asia [6]. In this paper, we present the distribution of haptoglobin subtypes (A1 and A2) and allele frequencies found in the 5 regional populations from the East Midlands in comparison with other populations from Continental Europe and United Kingdom. The analysis further describes how the sub-alleles at the haptoglobin locus makes it a useful marker for population genetic studies and paternity testing.

S. S. Mastana ✉ · P. Fisher
Human Genetics Laboratory, Department of Human Sciences,
Loughborough University of Technology,
Loughborough LE11 3TU, UK

## Material and methods

Blood samples (513) were collected as part of ongoing genetic surveys amongst the populations of East Midlands. The samples tested include unrelated native blood donors from Northwest Derbyshire (103), Northeast Derbyshire (101), South Derbyshire (104), Leicester (103) and Loughborough (102). The first 3 regions belong to the county of Derbyshire while the latter 2 are part of Leicestershire county. The serum samples were analysed using the isoelectric focusing (IEF) method of described by Scherz et al. [9]. In this method the haptoglobin molecule is partially isolated by immunoprecipitation using haptoglobin-specific antiserum before isoelectric focusing. The gene frequencies were calculated by gene counting and the Hardy-Weinberg equilibrium was analysed by the $\chi^2$ test. Phenotype numbers smaller than five were appropriately pooled. The genetic heterogeneity between populations was tested by $\chi^2$ using phenotype numbers. Average power of exclusion and probability of match was computed using the methods of Garber and Morris [10] and Jones [11] respectively.

**Table 1** HP subtypes and allele frequencies along with PE, PM and DP estimates for five regional populations of East Midands

| Phenotype | South Derbyshire | | NW-Derbyshire | | NE-Derbyshire | | Leicester | | Loughborough | | Total Derbyshire | | Total Leicestershire | | Total East Midlands | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Observed | Expected | Observed | Expected | Observed | Expected | Observed | Expected | Observed | Expected | Observed | Expected | Observed | Expected | Observed | Expected |
| 1S-1S | 9 | 6.25 | 13 | 7.89 | 10 | 5.94 | 10 | 6.82 | 10 | 7.69 | 32 | 20.00 | 20 | 14.49 | 52 | 34.48 |
| 1S-1F | 4 | 5.64 | 5 | 6.64 | 8 | 7.52 | 6 | 6.95 | 6 | 6.04 | 17 | 19.88 | 12 | 13.03 | 29 | 32.93 |
| 1S-2FS | 28 | 32.37 | 24 | 30.99 | 20 | 27.89 | 27 | 29.07 | 26 | 32.12 | 72 | 91.50 | 53 | 61.15 | 125 | 152.70 |
| 1S-2SS | 1 | 0.25 | 2 | 3.60 | 1 | 1.45 | 0 | 3.09 | 3 | 1.92 | 4 | 5.10 | 3 | 5.05 | 7 | 10.11 |
| 1S-2FF | 0 | 0.25 | 0 | 0.00 | 0 | 0.24 | 0 | 0.26 | 1 | 0.55 | 0 | 0.51 | 1 | 0.80 | 1 | 1.30 |
| 1F-1F | 4 | 1.27 | 4 | 1.40 | 4 | 2.38 | 4 | 1.77 | 5 | 1.19 | 12 | 4.93 | 9 | 2.93 | 21 | 7.86 |
| 1F-2FS | 11 | 14.60 | 9 | 13.05 | 14 | 17.65 | 13 | 14.81 | 6 | 12.62 | 34 | 45.46 | 19 | 27.49 | 53 | 72.91 |
| 1F-2SS | 0 | 0.11 | 2 | 1.51 | 1 | 0.92 | 0 | 1.57 | 0 | 0.75 | 3 | 2.53 | 0 | 2.27 | 3 | 4.83 |
| 1F-2FF | 0 | 0.11 | 0 | 0.00 | 0 | 0.15 | 0 | 0.13 | 0 | 0.22 | 0 | 0.25 | 0 | 0.36 | 0 | 0.62 |
| 2FS-2FS | 46 | 41.88 | 36 | 30.45 | 38 | 32.73 | 33 | 30.99 | 40 | 33.55 | 120 | 104.61 | 73 | 64.51 | 193 | 169.06 |
| 2FS-2SS | 0 | 0.63 | 7 | 7.07 | 4 | 3.42 | 6 | 6.58 | 4 | 4.01 | 11 | 11.66 | 10 | 10.66 | 21 | 22.39 |
| 2FS-2FF | 1 | 0.63 | 0 | 0.00 | 1 | 0.57 | 1 | 0.55 | 1 | 1.15 | 2 | 1.17 | 2 | 1.68 | 4 | 2.87 |
| 2SS-2SS | 0 | 0.00 | 1 | 0.41 | 0 | 0.09 | 3 | 0.35 | 0 | 0.12 | 1 | 0.33 | 3 | 0.44 | 4 | 0.74 |
| 2SS-2FF | 0 | 0.00 | 0 | 0.00 | 0 | 0.03 | 0 | 0.06 | 0 | 0.07 | 0 | 0.07 | 0 | 0.14 | 0 | 0.19 |
| 2FF-2FF | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 0.01 | 0 | 0.00 | 0 | 0.01 | 0 | 0.01 |
| Total | 104 | | 103 | | 101 | | 103 | | 102 | | 308 | | 205 | | 513 | |
| $\chi^2$ | 2.55 | | 4.16* | | 7.17* | | 1.31 | | 3.53 | | 27.21** | | 6.53* | | 48.57** | |
| DF | 1 | | 1 | | 1 | | 1 | | 1 | | 3 | | 2 | | 4 | |
| *Allele frequencies* | | | | | | | | | | | | | | | | |
| 1S | 0.2452 ± 0.0298 | | 0.2767 ± 0.0312 | | 0.2426 ± 0.0302 | | 0.2573 ± 0.0305 | | 0.2745 ± 0.0312 | | 0.2549 ± 0.0176 | | 0.2659 ± 0.0218 | | 0.2593 ± 0.0137 | |
| 1F | 0.1106 ± 0.0217 | | 0.1165 ± 0.0224 | | 0.1535 ± 0.0254 | | 0.1311 ± 0.0235 | | 0.1078 ± 0.0217 | | 0.1266 ± 0.0134 | | 0.1195 ± 0.0160 | | 0.1238 ± 0.0102 | |
| 2FS | 0.6346 ± 0.0334 | | 0.5437 ± 0.0347 | | 0.5693 ± 0.0348 | | 0.5485 ± 0.0347 | | 0.5735 ± 0.0346 | | 0.5828 ± 0.0199 | | 0.5610 ± 0.0245 | | 0.5741 ± 0.0154 | |
| 2SS | 0.0048 ± 0.0048 | | 0.0631 ± 0.0169 | | 0.0297 ± 0.0119 | | 0.0583 ± 0.0163 | | 0.0343 ± 0.0127 | | 0.0325 ± 0.0071 | | 0.0463 ± 0.0104 | | 0.0380 ± 0.0060 | |
| 2FF | 0.0048 ± 0.0048 | | – | | 0.0050 ± 0.0049 | | 0.0049 ± 0.0048 | | 0.0098 ± 0.0069 | | 0.0032 ± 0.0023 | | 0.0073 ± 0.0042 | | 0.0049 ± 0.0022 | |
| *Power of exclusion* | | | | | | | | | | | | | | | | |
| IEF | 0.2776 | | 0.3548 | | 0.3391 | | 0.3612 | | 0.3289 | | 0.3261 | | 0.3458 | | 0.3342 | |
| CON. | 0.1766 | | 0.1783 | | 0.1819 | | 0.1812 | | 0.1804 | | 0.1803 | | 0.1807 | | 0.1805 | |
| *Probability of match (PM) and discriminant probability (DP)* | | | | | | | | | | | | | | | | |
| PM | 0.2899 | | 0.2094 | | 0.2195 | | 0.2060 | | 0.2403 | | 0.2356 | | 0.2200 | | 0.2288 | |
| DP | 0.7101 | | 0.7906 | | 0.7805 | | 0.7940 | | 0.7597 | | 0.7644 | | 0.7800 | | 0.7712 | |

* Significant at 5% level, ** significant at 1% level

## Results and discussion

The observed numbers and allele frequencies of HP A1 and A2 subtypes in 5 regional sub-populations are given in Table 1. Also in this table are given the phenotypes and allele frequencies in English county levels and total East Midlands region. The technique used in our laboratory has the potential to discriminate 15 different phenotype combinations of 5 common alleles, however only 8–10 phenotypes were observed in different populations. No rare variants were observed in this investigation. Departure from Hardy-Weinberg equilibrium (HWE) was observed for Northwest Derbyshire ($\chi^2 = 4.16$, df 1, $P < 0.05$) and Northeast Derbyshire ($\chi^2 = 7.17$, df 1, $P < 0.05$). Significant deviation from HWE was also observed when the samples were pooled according to county affiliation (total Derbyshire $\chi^2 = 27.21$ df 3, $P < 0.001$ and total Leicestershire $\chi^2 = 6.53$, df 2, $P < 0.05$). Similar deviation from the Hardy-Weinberg equilibrium was observed for the total East Midlands ($\chi^2 = 48.57$, df 4, $P < 0.001$). This deviation suggests limits to random mating and the existence of possible substructure in the East Midlands population. Similar deviation and substructure effects have been observed in a number of genetic systems studied from the region [12].

In all populations studied, 3 phenotypes 2FS-1S, 2FS-1F and 2FS-2FS were most common and showed a very similar range of variation in their phenotype frequencies. The phenotype 2FS-1S varied from 9% in South Derbyshire to 13% in Northwest Derbyshire. The frequency of phenotype 2FS-2FS was lowest in Leicester (32%) and highest in the South Derbyshire sample (44%). The frequencies of the 2FS-1F phenotype ranged from 13% in Leicester to 6% in Loughborough. Other phenotypes also showed a range of variation. The allele frequencies calculated showed a narrow range in the 5 populations, 1S (25–28%), 1F (11–14%), and 2FS (54–63%). Intra and inter-regional comparisons using chi square analysis showed no significant differences amongst the East Midlands populations.

There is only one previous study available on Haptoglobin subtypes from United Kingdom [13]. Comparison of the present data with North-east England showed no significant differences. Only a slight decrease in HP*1F frequency was observed (18% in NE England compared to 11 to 15% in East Midlands). The overall pattern of variation observed in the East Midlands is very comparable to many continental European populations [6].

To judge the suitablility of the Haptoglobin subtypes for parentage testing and forensic analyses we calculated the power of exclusion and probabilities of match and discrimination and these are also presented in Table 1. We found an increase in the exclusion chance in paternity cases from around 18% by conventional starch gel electrophoresis to 28–36% by IEF in the East Midlands populations. There are some statistically non-significant population differences in the PE values. The probabilities of match and discrimination also showed an interesting variation. Haptoglobin subtypes showed the highest discrimination power in the Leicester (79%) population and the lowest in South Derbyshire (71%).

In conclusion, the present analysis suggests that the heterogeneity demonstrated by the haptoglobin subtypes provides useful markers for studies of population structure and are useful in parentage testing and forensic analyses.

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