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Haptoglobin Subtypes in Berlin, GDR

A Simple Procedure for Haptoglobin Purification and Subtyping

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Summary. Sera were obtained from 1,275 blood donors in Berlin, probands involved in paternity tests, and from 119 families with 235 children; the sera were subtyped by isoelectric focusing, following preparation and reductive molecular cleavage of haptoglobin. In this paper, an uninvolved preparation technique is described for routine testing. Allelic frequencies are: Hp *1F = 0.1471; *1S = 0.2502; *2FF = 0.0020; *2FS = 0.5753; *2SS = 0.0251. Only one deviation from autosomal codominant inheritance was recorded in the family examinations, with illegitimacy considered possible. In the region of Berlin, the changes of ruling out uninvolved individuals in paternity suits have gone up from 18% (conventional technique recording two frequent alleles) to 33% (subtyping).

Key words: Haptoglobin subtypes – Blood groups, Hp

Zusammenfassung. Seren von 1275 Berliner Blutspendern und Probanden aus der Paternitätsbegutachtung sowie von 119 Familien mit 235 Kindern wurden im Hp-System mittels isoelektrischer Fokussierung nach Präparation und reduktiver Molekülspaltung des Haptoglobin subtypisiert. Es wird ein einfaches Präparationsverfahren vorgestellt, das eine routinemäßige Untersuchung gestattet. Die Allelfrequenzen betragen: Hp *1F = 0,1471; *1S = 0,2502; *2FF = 0,0020; *2FS = 0,5753; *2SS = 0,0251. Die Familienuntersuchungen ergaben nur eine Ausnahme vom angenommenen autosomal-kodominanten Erbgang, Illegitimität ist möglich. Die isolierte Ausschlußchance mit Hp für Nichtväter steigt im Berliner Raum von 18% (herkömmliche Technik mit Erfassung zweier häufiger Allele) auf 33% (Subtypisierung).

Schüsselwörter: Haptoglobin, Subtypen – Blutgruppen, Hp

Subtyping has proved to be helpful in increasing the number of recordable frequent alleles in the Hp system from two to five, accompanied by an un-

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ambiguous improvement in the chances of ruling out uninvolved persons in paternity suits. Impressive evidence regarding this achievement has been produced by means of data recorded by Shibata et al. (1982), based on a defined population in southern France.

Large-scale application of the Hp system had been prevented for many years by the need for expensive and tedious, but indispensable purification of the haptoglobin. Several more or less practicable techniques for haptoglobin preparation have become available in the meantime (Santoro et al. 1982; Shibata et al. 1982; Teige et al. 1983) but none of them has proved to be completely satisfactory in terms of economy of time and equipment or reliability of typing.

The authors of this paper, inspired by the work of Shibata et al. (1982), have devised a very simple, time-saving, and low-cost method by which dependable, unambiguous, and safely reproducible findings can be obtained, following isoelectric focusing of haptoglobin cleavage products. Hp preparation from 40 serum samples takes less than 1 h, provided an automatic pipette and centrifuge are available that can hold a sufficient number of samples.

Material and Methods

Absolutely nonhemolytic sera were obtained from 1,275 blood donors in Berlin, probands involved in paternity tests, and from 119 families with 235 children. The sera were Hp-typed either when fresh or after a period of deep-freeze storage. Electrophoresis in starch or polyacrylamide gel was used fo conventional determination (Prokop and Bundschuh 1963; Pastewka et al. 1973), while subtyping was accomplished by means of isoelectric focusing. Haptoglobin was purified by the principle of ion exchange on DEAE cellulose (Schößler et al. 1979). A batch of 300 mg dry exchange substance (e.g., Servacel DEAE 23 SS) was prepared in distilled water to prepare 40 samples. The supernatant was reduced to 30 ml before 0.01 *M* sodium acetate buffer, pH 4.7, was added to make up to 90 ml. This was thoroughly mixed and 2-ml portions of the suspension were poured into tipped centrifuge tubes. An automatic pipette was used to add 50 µl serum, followed by brief shaking. After 3-min centrifugation at 3,000*g* the supernatant was removed and the sediment stirred with 0.01 *M* initial buffer. Centrifugation was repeated and the supernatant discarded. A 0.125 *M* ammonium acetate solution was used for elution, according to Smithies et al. (1962), with 50 µl being added to the sediment and the mixture stirred with a glass rod. Short centrifugation was sufficient to obtain easily 40 µl of haptoglobin-containing supernatant which held about 15% of the starting quantity of haptoglobin.

Molecule cleavage was undertaken, as proposed by Constans and Viau (1975) or Pastewka et al. (1973): 1 g urea was dissolved in 1.2 ml borate buffer (pH 8.8; 0.1 *M* boric acid, 0.04 *M* NaOH), and 20 µl β-mercaptoethanol was added. The same volume of urea-mercaptoethanol solution was added to the 40 µl of Hp eluate. Thirty minutes were allowed for reaction before 8 µl iodoacetamide solution (45 mg/0.5 ml) was added.

Isoelectric focusing was carried out in polyacrylamide flat gels, 260 × 125 × 0.5 mm in dimension, on silanized glass plates, using an LKB Multiphor set. The gels (T = 5%, C = 3% were mixed with 2.2% carrier ampholytes (LKB ampholine), with 16 ml gel volume, with ratios of: 0.6 ml of pH 5–7, 0.2 ml pH 6–8, 0.2 ml pH 3.5–5, and 0.1 ml of pH 3–10. After 45 min of prefocusing (with concomitant Hp cleavage), the samples were placed onto the gel surface, using 0.5 × 1.0 cm strips of filter paper, and allowed to stay there 30 min.

Focusing took 2 h 45 min, with maximum electric values of 1,600 V, 10 mA, 10 W. Electrode solutions were 1 *M* H_3PO_4 and 1 *M* NaOH.

The gels were than fixed, stained, and decolored according to Steck et al. (1980), with formaldehyde being added to the solutions.

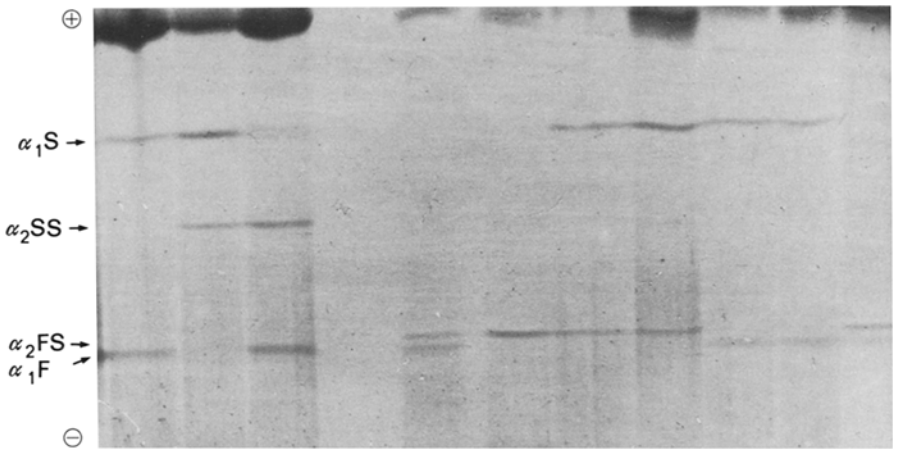

Fig. 1. Representation of Hp subtypes, following haptoglobin preparation and reductive molecule cleavage by means of isoelectric focusing. Types, from left to right, are: Hp 1S–1F, 1S–2SS, 1F–2SS, 1F–2FS, 2FS, 1S–2FS, 1S–2FS, 1S–1F, 1S–1F, 1F–2FS

Table 1. Haptoglobin system subtyping: phenotypes with expected values and allelic frequencies in random sample of Berlin population

Phenotype	Observed		Expected	Allelic frequencies
	n	(%)		
Hp 1F	28	2.20	27.59	Hp *1F = 0.1471
1F–1S	86	6.75	93.85	*1S = 0.2502
1S	83	6.51	79.82	*2FF = 0.0020
1F–2FF	—	—	0.75	*2FS = 0.5753
1F–2FS	220	17.25	215.80	*2SS = 0.0251
1F–2SS	13	1.02	9.42	*J = 0.0004
1S–2FF	—	—	1.28	1.0001
1S–2FS	371	29.10	367.05	
1S–2SS	14	1.10	16.01	$\Sigma\chi^2 = 2.6577$
2FF	—	—	0.01	$df = 5$
2FF–2FS	5	0.39	2.93	$0.8 > P > 0.7$
2FF–2SS	—	—	0.13	
2FS	418	32.78	421.99	
2FS–2SS	35	2.75	36.82	
2SS	1	0.08	0.80	
1S–J	1	0.08	0.26	
Total	1,275	100.01	1,274.51	

Table 2. Hp phenotypes recorded from Berlin region

Parent couples		Phenotypes of children									
Hp	*n*	*n*	1F	1F–1S	1S	1F–2FS	1F–2SS	1S–2FS	1S–2SS	2FS	2FS–2SS
1F × 1F–1S	1	2	—	2	—	—	—	—	—	—	—
1F × 2FS	2	9	—	—	—	9	—	—	—	—	—
1F–1S × 1F–1S	1	1	—	—	1	—	—	—	—	—	—
1F–1S × 1F–2FS	4	7	1	1	—	2	—	3	—	—	—
1F–1S × 1S–2FS	4	6	—	2	1	1	—	2	—	—	—
1F–1S × 2FS	8	9	—	—	—	6	—	3	—	—	—
1S × 1S	2	9	—	—	9	—	—	—	—	—	—
1S × 1F–2FS	9	16	—	8	—	—	—	8	—	—	—
1S × 1S–2FS	3	7	—	—	3	—	—	4	—	—	—
1S × 2FS	4	6	—	—	—	—	—	6	—	—	—
1F–2FS × 1F–2FS	4	7	—	—	—	5	—	—	—	2	—
1F–2FS × 1F–2SS	2	3	2	—	—	—	1	—	—	—	—
1F–2FS × 1S–2FS	11	21	—	7	—	4	—	6	—	4	—
1F–2FS × 2FS	15	32	—	1[a]	—	21	—	—	—	10	—
1F–2FS × 2FS–2SS	1	1	—	—	—	—	—	—	—	1	—
1F–2SS × 2FS	1	1	—	—	—	—	—	—	—	—	1
1S–2FS × 1S–2FS	10	27	—	—	8	1[b]	—	14	—	4	—
1S–2FS × 2FS	16	35	—	—	—	—	—	19	—	16	–
1S–2FS × 2FS–2SS	4	5	—	—	—	—	—	1	2	2	—
1S–2SS × 1S–2SS	1	1	—	—	—	—	—	—	1	—	—
2FS × 2FS	14	27	—	—	—	—	—	—	—	27	—
2FS 2FS–2SS	2	3	—	—	—	—	—	—	—	3	—
Total	119	235	3	21	22	49	1	66	3	69	1

[a] Under the Gc system, this child was also considered to not match its parent couple

[b] Hereditary situation was not even elucidated by serum group testing of persons involved

Results

The Hp-subtype patterns obtained by the technique described are given in Fig. 1. Band localization is in keeping with the isoelectric spots of cleavage products given by Shibata et al. (1982).

Unambiguous separation of α_1F-polypeptide from α_2FS-polypeptide is achieved, provided the conditions we have specified for isoelectric focusing are observed. There will be no problems in coordinating the remaining bands, as no other proteins are focused at these pH levels.

Table 1 presents the distribution of Hp subtypes in an unselected sample obtained from clinically intact blood donors in Berlin, as well as the allelic frequencies derived from that distribution, and the distribution type is obviously Hardy-Weinberg.

The gene frequencies thus determined resulted in a potential paternity exclusion rate of 33%. Parent couples examined by the authors are listed with their children in Table 2. Two of these children were found to disagree with the assumption of autosomal-codominant inheritance. One of them was shown also by the Gc system as not matching with its parent couple, while of the other child could not be typed by serum group testing.

Following subtyping, one type, which was originally defined as Hp J–1 (Johnson) by the conventional technique, exhibited the α_1S-peptide as well as another peptide located between α_1F and α_2FF and with an isoelectric point of about pH 6.

Discussion

The Hp system is an informative instrument which has been used to assess parentage for nearly 30 years. Today its suitability for this purpose is beyond any doubt (Bowman and Kurosky 1982; Prokop and Bundschuh 1963; Smithies 1955).

Although subtypes were discovered 20 years ago (Smithies et al. 1962), their differentiation as a routine practice is closely associated with isoelectric focusing. It is only by this highly sensitive and high-resolution technique that the various Hp*2-gene products can be safely broken up and satisfactory pherograms can still be obtained, despite the relatively low protein concentration in the Hp eluates. Little time is now required for haptoglobin preparation, and no expensive hardware is needed. This has enabled subtyping to be used to determine paternity. The moderate surplus costs involved in the preparation procedure are balanced by the increase in paternity exclusion rates (18% in the past to 33% now).

Although the α_2-peptides, α_2FF, α_2FS, α_2SF, and α_2SS, are all theoretically possible, so far, the α_2SF-peptide is the only one we have not observed. We have also not yet observed, any subtype variants of the frequently occurring "major types". It is not necessary to determine "major types" as a prerequisite for subtype differentiation, as Type Hp 2–1, for example, can be easily determined from 1F–2FS, 1S–2FS, 1S–2SS, 1S-2FF, etc. There have also been no dis-

crepancies between the results obtained from conventional techniques and those recorded from subtyping in the material described in this paper.

When there is little haptoglobin in the serum, an attempt should still be made to obtain the proper proportion by adding three portions of serum to the washed exchange substance. However, in general, subtyping is not feasible if Hp cannot be represented by means of the conventional technique (polyacrylamide-gel electrophoresis).

The technique described in this paper depends on the availability of non-hemolytic sera. Substantial Hp linkage would result even with minor hemoglobin additives. The resulting complexes would not be capable of linking with the exchange substance of pH 4.7 for their higher isoelectric point, as compared to free haptoglobin (4.9–5.3 instead of 3.9–4.2) Yang and Przybylska 1973). This situation would, consequently, rule out the practicability of Hp subtyping from dried traces of blood by means of the procedure herein described.

The excellent applicability of the "complete" Hp system to paternity assessment can be clearly seen from all data so far obtained and presented (see also Constans and Viau 1975; Shibata et al. 1982; Teige et al. 1983; Thymann 1977). This simple preparation procedure has eliminated the greatest obstacle to large-scale field application.

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