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Subtyping of Hp: Common and rare phenotypes

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INTRODUCTION

Many techniques for Hp subtyping on protein level have hitherto been described (Connell et al. 1962, Pastewka et al. 1973). They either combine a purification step with subsequent electrophoretical separation and protein staining or include Hp identification by peroxidase staining (Linke 1984) or immunoblotting (Teige et al. 1985). Electrophoresis of reduced and alkylated Hp suffered from an unsatisfactory separation of the gene products hpα2. This was greatly improved by Shibata and coworkers (1982), who introduced an IEF technique, that clearly separates the gene products hp 2FS (SF), hp 2SS, hp 2FF, hp 1F and hp 1S. This IEF technique together with a batchwise ion exchange chromatography was used in this work to investigate gene frequencies of Hp subtypes in a Southwestern German population. Additionally separation of Hp chains in hybrid IEF will be presented and discussed. Furthermore a Hpα2-variant and two different Hp Johnson subtypes will be described.

MATERIALS AND METHODS

Purification and subsequent reductive cleavage of Hp was done as described previously (Zischler et al. 1987). 250 x 125 x 0.5 mm acrylamide gels (T5%/C3%) were cast with a final concentration of 0.8% 5-7, 0.8% 3-10 and 0.4% 6-8 Ampholine carrier ampholytes (Shibata et al. 1982). Maximum settings were 1500V, 25 mA and 5W. 1 mol phosphoric acid and 1 mol NaOH were used as electrolytes. Samples were applied cathodally on the prefocused gel and run until the presetted voltage was reached. Staining was done according to Blakesley and Boezi (1977).

RESULTS AND DISCUSSION

Figure 1 shows 10 Hp phenotypes as revealed by IEF of reduced and alkylated Hp. In our family material the phenotypes Hp 2FS-2FF

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and Hp 2FF-1F, that are not represented on this picture, were also observed.

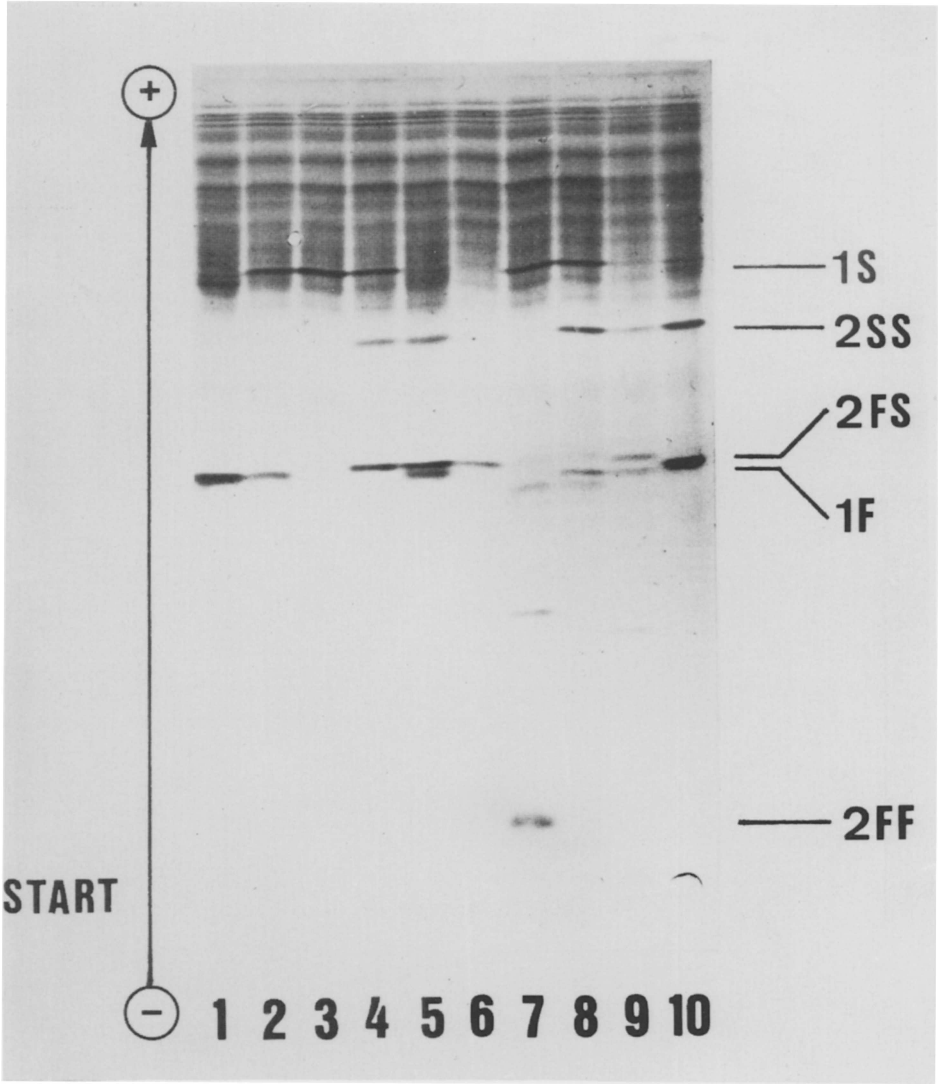

Fig. 1.Hp phenotypes (1) Hp 1F-1F, (2) Hp 1F-1S, (3) Hp 1S-1S, (4) Hp 2FS-1S, (5) Hp 2FS-1F, (6) Hp 2FS-2FS, (7) Hp 2FF-1S, (8) Hp 2SS-1S, (9) Hp 2FS-2SS, (10) Hp 2SS-1F as revealed by IEF of reduced and alkylated Hp

In Fig. 2 the subtype patterns of Fig. 1 are diagrammatically represented. Faint bands indicate the frequently occurring minor bands of gene products hp 2SS and hp 2FS.

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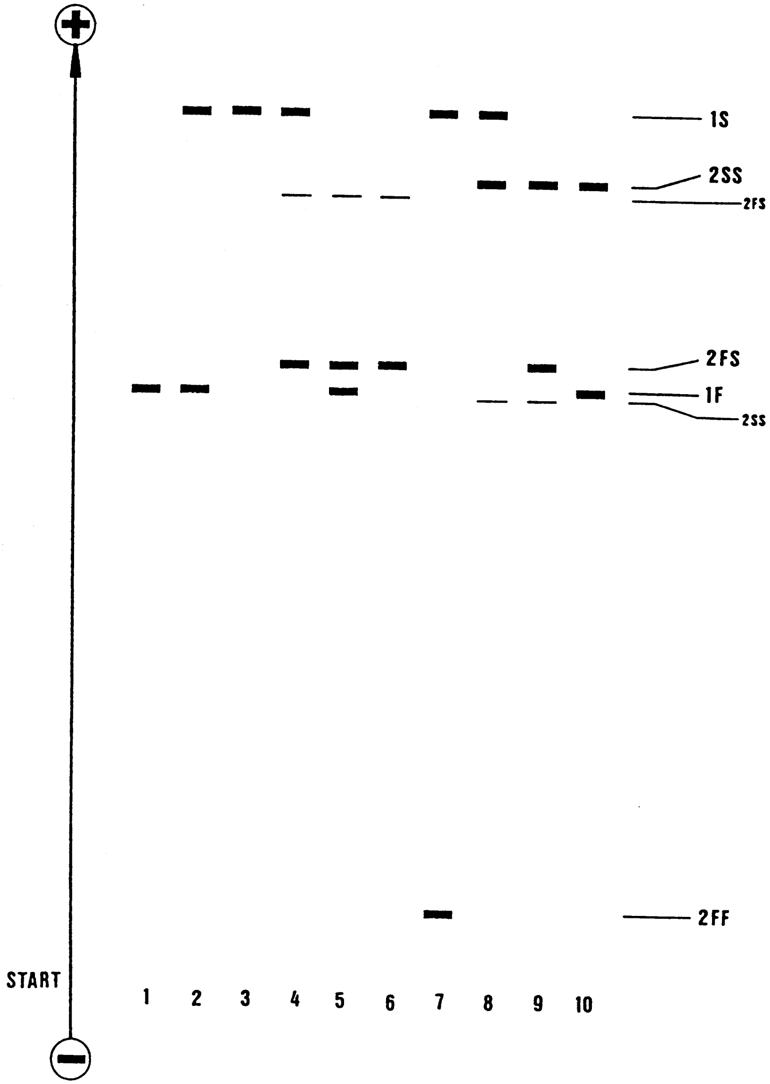

Fig. 2. Diagrammatical representation to Fig. 1. Major gene products hpα are drawn as strong bands

Analyzing 91 families with 268 children from Southwestern Germany the following gene frequencies were obtained: Hp*1F: 0.144, Hp*1S: 0.254, Hp*2FS: 0.574, Hp*2SS: 0.024, Hp*2FF: 0.004. These values are in good agreement with gene frequencies established in other European countries with the IEF method (Shibata et al. 1982, Patzelt and Schröder 1985) and show only slight deviation in Hp*1F-frequency calculated for a Norwegian population sample (Teige et al. 1985). Regarding this study the a priori exclusion chance of the Hp system raises to 28%.

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One technical problem in Hp subtyping using this method is the sometimes unsatisfactory resolution of hp 2FF. Overloading effects near by the application point can lead to smeary and weak hp 2FF bands and therefore favour mistyping. To overcome this a technique of hybrid isoelectric focusing was tried: Polyacrylamide gels of the same size as above were cast with a linear immobilized pH range 5-7 as recommended by the supplier. Carrier ampholytes pH 5-7 (LKB) were added directly to the gel solution to give a final concentration of 0.5%. After polymerisation gels were run for 6 hours without prefocusing applying 12W, 4mA and 3000V. 25 mmol Glu/Asp and 0.1 mol NaOH were used as electrode solutions. Sample treatment, loading and staining were done in the same way as for the carrier ampholyte IEF gels. 9 different phenotypes as revealed by IEF in hybrid Immobiline gels are demonstrated in Fig. 3. Samples were first separated in a carrier ampholyte IEF gel, frozen and thawed next day for focusing in the hybrid Immobiline gel.

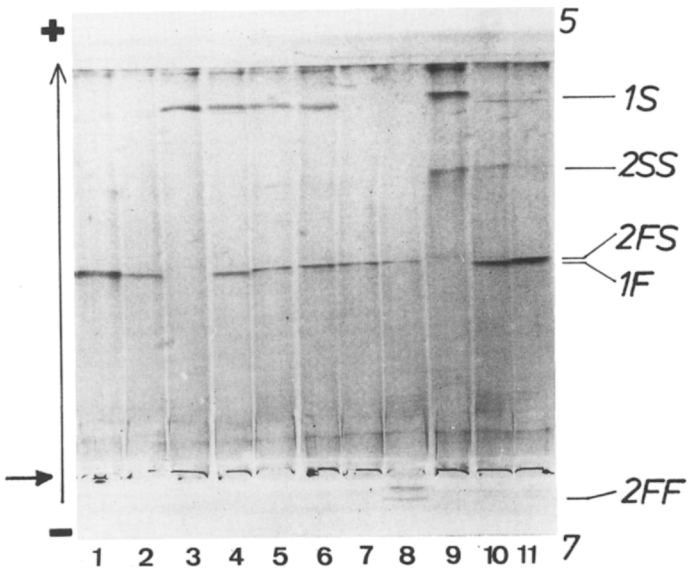

Fig. 3.Hybrid IEF separation of Hp chains. Phenotypes from left to right: (1) Hp 2FS-1F, (2) Hp 1F-1F, (3) Hp 1S-1S, (4) Hp 1F-1S, (5) Hp 2FS-1S, (6) Hp 2FS-1S, (7) Hp 2FS-2FS, (8) Hp 2FS-2FF, (9) Hp 2SS-1S, (10) Hp 2SS-1F, (11) Hp 2FS-1F

Due to the linear pH gradient and the short separation distance of 125 mm, gene products hp 2FS and hp 1F are worse separated in this gel as compared to the carrier ampholyte generated pH gradients. The advantage of this technique is the improvement of hp 2FF-typing. As can be seen in sample (8), hp 2FF is the only gene product that migrates cathodally together with an additional band. Both bands are sharp and clearly recognizable. One freeze-thawing cycle of treated samples does not seem to be detrimental

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to separation quality. The hybrid Immobiline technique is therefore suitable to confirm hp 2FF-diagnosis after carrier ampholyte IEF without need of a second isolation step.

In addition to the main Hp phenotypes many variants both for the α and ß-chain genes were described, most of them recognizable after electrophoresis of native Hp (for review see Ritter et al. 1975, Bowman and Kurosky 1982). In this report an α2-chain variant, only recognizable by the subtyping method, is presented. Figure 4 shows variant Hp phenotypes observed in a Turkish family, as revealed by the IEF method. The father (1) was phenotyped to be Hp 2FS. The mother (2), pretyped Hp 2-1 by horizontal starch gel electrophoresis of native Hp, shows a gene product hp 1S and a second band anodal to the 2FS-position. This variant, which is according to the pretyping result an α2-variant, was transmitted to the first (3) and second (4) child.

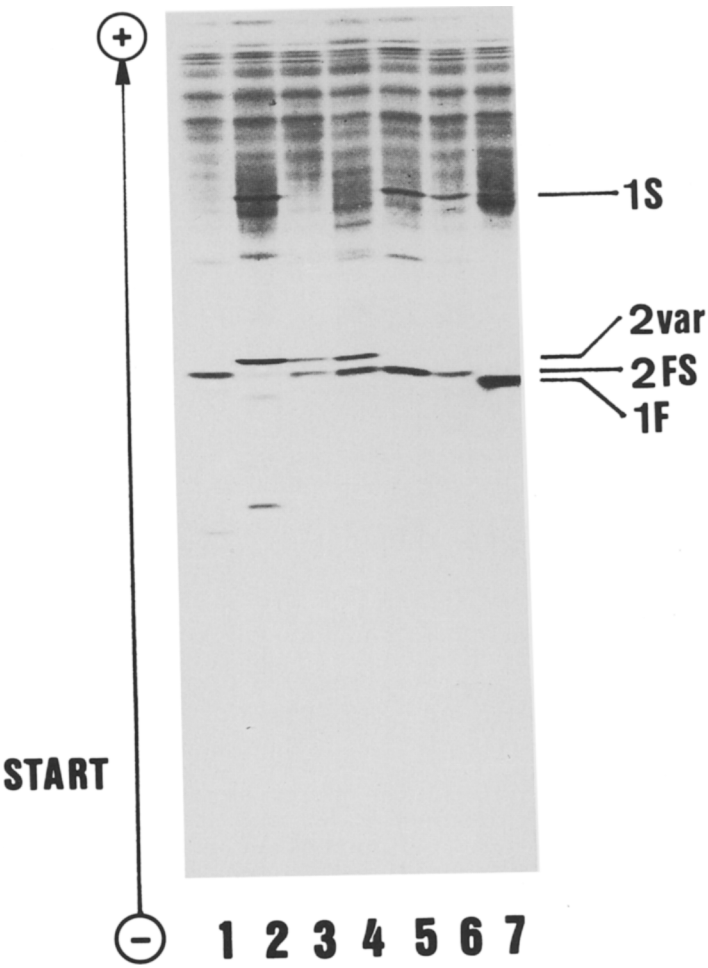

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Fig. 4. Presentation of a hpα 2-variant after IEF. Phenotypes from left to right: Father (1) Hp 2FS-2FS, mother (2) Hp 2var-1S, first child (3) Hp 2FS-2var, second child (4) Hp 2FS-2var, third child (5) Hp 2FS-1S, fourth child (6) Hp 2FS-1S. (7): Reference sample Hp 1F-1F

Unequal but homologous crossing-over involving an intragenic recombination between two alleles Hp*2 gives rise to a triplicated α -chain gene called Hp Johnson. The Johnson phenotype has been observed in various populations in low frequencies and seems to vary slightly in its electrophoretical pattern (for review see Ritter et al. 1975). This phenotypic variation may be due to the 8 possible combinations of S- and F-parts of the α_3-polypeptide (Smithies et al. 1962). In this study four different persons (two of them kindly provided by Dr. Arnold, Augsburg) were typed Hp J-1 using starch gel electrophoresis. These results were confirmed by horizontal SDS-acrylamide gradient electrophoresis of isolated and reduced Hp.

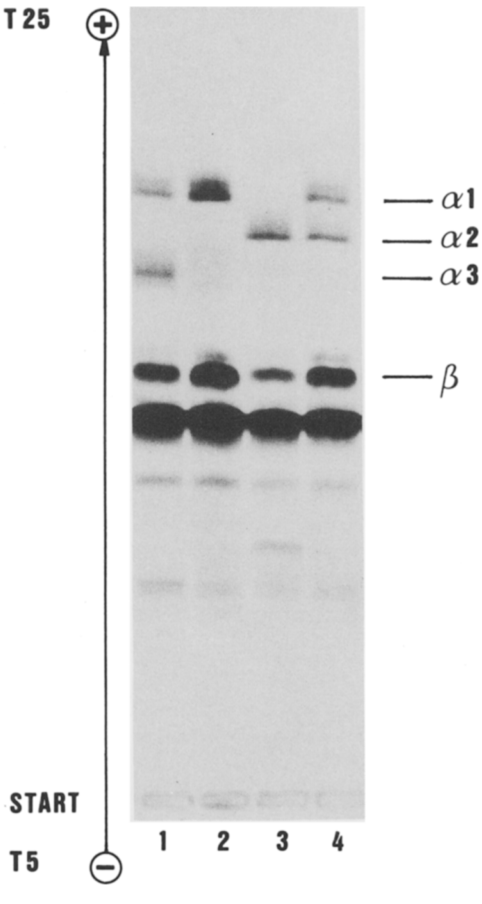

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Fig. 5. SDS electrophoresis of isolated Hp. (1) Hp J-1, (2) Hp 1, (3) Hp 2 and (4) Hp 2-1

0.5 mm thick acrylamide gels were poured with a linear gradient T5%-T25% at constant C=3%. 0.375 mol Tris-HCl pH 8.8 with 0.1% SDS was used as gel buffer. The bridge buffer consisted of 0.025 mml Tris, 0.05 mol glycine and 0.1% SDS. Gels were run at 5^{o}C with maximum settings of 1400V, 50mA and 40W. Hp was isolated from 200ul serum as described and treated with 100ul 1% SDS and 0.05% DTT in 10 mmol Tris-HCl pH 8.8. Figure 5 shows a Hp J-1 together with the main phenotypes Hp 1 (2), 2 (3) and 2-1 (4). From these four persons two different subtype patterns were obtained when reduced and alkylated Hp was subjected to IEF (Fig. 6)

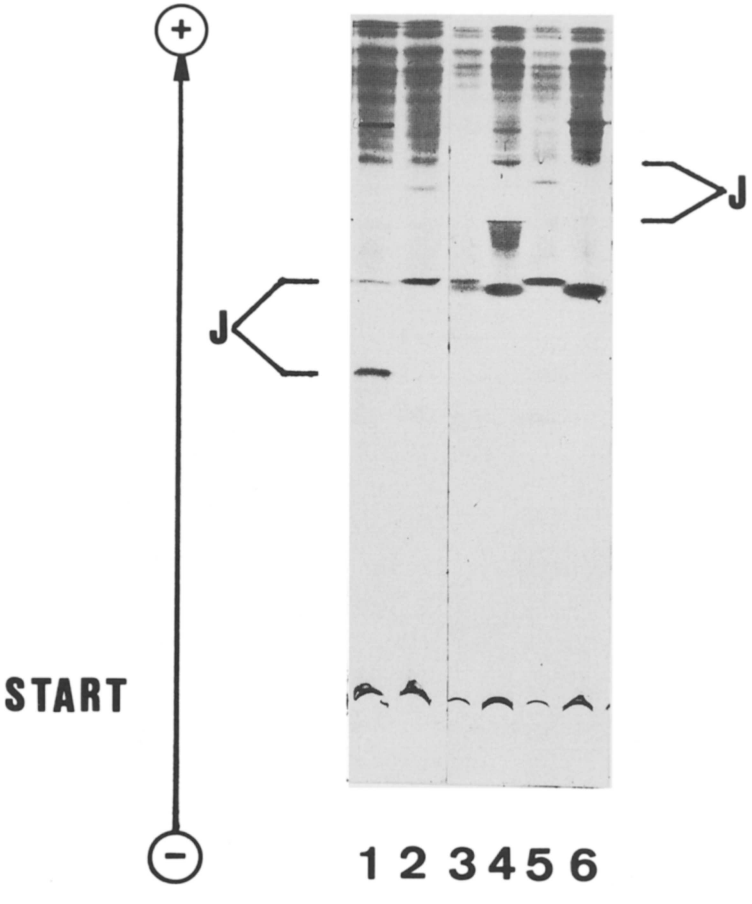

Fig. 6. Two different Johnson subtypes as revealed by IEF. (1) Hp J-1S, (2) Hp 2FS-2FS, (3) Hp 2FS-1F, (4) Hp J-1F, (5) Hp 2FS-2FS,

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(6) Hp 1F-1S.

First Johnson subtype pattern (1) shows beside hp 1S a second intense band cathodal to the 2FS-position and an additional faint band in the 2FS-position. The second Johnson subtype pattern (4) is composed of a clearly visible hp 1F- and two further bands both anodal to the 2FS-position. With respect to staining intensity the more anodal protein seems to represent the main band.

To conclude, Hp subtyping can be done routinely by ion exchange chromatography, reductive cleavage and separation by IEF. Hitherto unknown α-chain variants could be detected by this method. Triplication of α-chains in Johnson phenotypes can be simply verified by SDS-acrylamide electrophoresis. Two different subtypes of Hp Johnson were identified by IEF.

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