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ORIGINAL ARTICLE

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Changes in the phenotype of polymorphic plasma proteins after liver transplantation – new data and medico-legal consequences

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Abstract The genetically inherited polymorphic plasma protein types have always been considered stable for lifetime in humans. Most of these proteins are synthetised in the liver. Phenotypes for 14 plasma proteins in donors and recipients of liver transplants prior to and after transplantation were determined in 15 patients who had undergone liver transplantation at the university hospitals Charité and Rudolf Virchow in Berlin. The plasma proteins investigated were HP, TF, GC, PI, ORM1, ITI, A2HS, PLG, FXIIIB, BF, C3, C6, C8 and FH. Evidence was provided of irreversible change from the recipient type to the donor type in at least one patient for all the systems investigated. This is the first time such data have been obtained for ITI, A2HS, C8 and FH. These results clearly support the point that the dogma of life-long stability of genetically determined protein phenotypes is merely of limited validity. Against the background of good long-term results of liver transplantation, there are consequences for the practice of legal medicine in the particular context of certification of parentage, identification and stain analysis.

Key words Plasma proteins · Genetic polymorphism · Liver transplantation · Stain analysis · Paternity testing

Introduction

The effects of liver transplantations on the phenotype pattern of polymorphic plasma proteins are described and discussed in this paper.

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Postoperative change from the recipient type to the donor type must be recordable from cases in which the phenotypes of the protein concerned differ between donor and recipient and if the liver is the main site of protein synthesis.

Liver transplantation used to be restricted to a few research projects up to 1980 [25] but has now found widespread clinical application owing to the advent of the immunosuppressive cyclosporine A, the availability of improved surgical procedures, the use of University of Wisconsin (UW) solution for organ preservation and thus considerable increase of long-time survival rates [21]. Ever since the first liver transplantation was performed by Starzl et al. [24] in Denver 48,967 patients have received liver transplants worldwide up to 1995, among them 19,435 in Europe and 3,349 in Germany [4]. The all-European 5-year patient survival rate was 66 % between 1988 and 1995 [5]. In this context, what are the implications for legal medicine of growing numbers of liver transplantations and increasing long-time survival rates?

Materials and methods

Phenotypes of donors and recipients were determined prior to transplantation and in the postoperative course in 15 patients for a broad spectrum of plasma proteins [3, 15, 17]. In addition to seven patients on whom detailed reports have been published elsewhere [23], we typed eight patients who had received liver transplants at the Rudolf Virchow Medical Center in Berlin in April and May 1996. All investigations were approved by the Ethics Commission of the Faculty of Medicine.

The plasma proteins investigated were haptoglobin (HP), transferrin (TF), glycoprotein GC (group-specific component), alpha-1-antitrypsin (PI), orosomucoid 1 (ORM1), inter-alpha-1-antitrypsin-inhibitor (ITI), alpha-2-HS glycoprotein (A2HS), plasminogen (PLG), factor B of coagulation factor XIII (FXIIIB), factor B of complement (BF), complement factor 3 (C3), complement factor 6 (C6), complement factor 8 (C8) and factor H (FH). Typing was carried out by isoelectric focusing as described previously [14, 22, 23].

Table 1 Phenotype change of the protein systems after live transplantation

System	Patient	Donor	Rec.b.t.	Recipient after transplantation						
				Day 0	Day 1	Day 2	Day 4	Day 5	Day 7	From day 10
HP	2	1S2FS	2FS	–	1S 2FS	–	–	–	1S 2FS	1S 2FS
	7	1S2FS	2FS	–	2FS	–	–	2FS	1S 2FS	1S 2FS
	8	1S2FS	2FS	–	1S 2FS	1S 2FS	1S 2FS	1S 2FS	1S 2FS	1S 2FS
	9	2FS1F	1S1F	–	2FS 1F	2FS 1F	2FS 1F	2FS 1F	2FS 1F	2FS 1F
	10	1S	2FS1F	1S 2FS (1F)	1S 2FS (1F)	1S (2FS)	1S (2FS)	1S (2FS)	–	1S
	11	1S1F	2FS1F	–	1S (2FS) 1F	1S 2FS 1F	1S (2FS) 1F	–	1S 1F	1S 1F
	13	1S1F	2FS	–	1S 2FS 1F	1S 1F	1S 1F	1S 1F	–	1S (2FS)1F*
	14	2FS	1S2FS	–	1S 2FS	(1S) 2FS	(1S) 2FS	(1S) 2FS	2FS	2FS
	15	2FS	2FS1F	2FS 1F	2FS 1F	–	2FS	–	2FS	2FS
TF	2	1	1–2	–	1	–	–	–	1	1
	4	1–2	1	–	–	–	1	–	1	1
	5	1	1–2	–	–	–	1	–	–	1
	9	1–2	1	–	1	1 (2)	1 (2)	1 2	1 2	1 2
	10	1	1–2	1 2	1 2	1 (2)	1 (2)	1 (2)	–	1
	11	1–2	1–3	–	1 (3)	1 (2) (3)	1 (2)	–	1 2	1 2
	12	1–2	1	–	1	1	1 2	–	1 2	1 2
GC	4	2–1S	1F-1S	–	2 1S	–	–	–	2 1S	2 1S
	5	1S	2–1F	–	–	–	(2) 1S	–	–	2 1S
	7	1S	2–1S	2 1S	2 1S	1S	–	1S	1S	1S
	9	2–1S	1S	–	2 1S	2 1S	2 1S	2 1S	2 1S	2 1S
	11	2–1S	2	–	2 1S (1F)	2 1S (1F)	2 1S	–	2 1S	2 1S
	13	2	1F-1S	–	2 1F 1S	2 1F 1S	2 1F 1S	2 (1F) (1S)	–	2
	15	1S	2–1F	2 1F 1S	(2) 1F 1S	–	(1F) 1S	1S	1S	1S
PI	1	M1	M1M2	–	–	M1	–	–	–	M1
	4	M1M2	M2	M1	M1 M2	M1 M2	M1 M2	–	M1 M2	M1 M2
	5	M1	M1M2	–	M1	M1	M1	M1	M1	–
	6	M1M2	M1	M1	M1	M1	M1	M1	M1	–
	7	M1M2	M1	M1	M1 M2	M1 M2	–	M1 M2	–	M1 M2
	9	M1Z	M1	–	M1 Z	M1 Z	M1 Z	M1 Z	M1 Z	M1 Z
	11	M1M2	M2Z	–	M1 M2 Z	M1 M2	M1 M2	–	M1 M2	M1 M2
	13	M1M2	M1S	–	M1 S	M1 M2 S	M1 M2 S	M12 M2 (S)	–	M1 M2
	14	M1M3	M1	–	M1	M1 M3	M1 M3	M1 M3	M1 M3	M1 M3
	15	M3M2	M1M2	M1 M2	M1 M3 M2	–	M1 M3 M2	–	M3 M2	M3 M2
ORM1	4	F1S	F1	–	F1 S	–	–	–	F1 S	F1 S
	7	F1S	F2S	F1 F2 S	F1 S	–	–	F1 S	–	F1 S
	13	F1	F1S	–	F1 S	F1 S	F1 (S)	F1 (S)	–	F1
	14	S	F1	–	F1 S	(F1) S	(F1) S	(F1) S	(F1) S	S
ITI	5	1–2	1	–	–	–	1 2	–	1	1 2
	7	1	1–2	1 2	1 2	1	–	1 2	–	1
A2HS	4	1–2	1	–	1 2	–	–	–	–	1 2
	5	1	1–2	1 2	–	–	1 2	–	–	1 2
	6	1–2	1	1 2	–	1 2	–	–	1 2	–
	8	1	2	–	1 2	1 2	1 2	1 2	1 (2)	1
	9	1	1–2	–	1 2	1 2	1 2	1 (2)	1 (2)	1
	10	1	1–2	1 2	1 2	1 2	1 2	1 2	–	1
	12	1	2	–	1 2	1 2	1 2	–	1 (2)	–
	15	2	1–2	1 2	1 2	–	1 2	1 2	–	(1) 2**
PLG	7	A	AB	A B	A B	–	–	A B	A B	A
	9	AB	A	–	A B	A B	A B	A B	A B	A B
	10	AB	A	A B	A B	A B	A B	A B	–	A B
	11	AvarA	A	–	A	Avar A	Avar A	–	Avar A	Avar A
	12	A	AB	–	A B	A	A	–	A	A
	14	AB	A	–	A B	A B	A B	A B	A B	A B

Table 1 (continued)

System	Patient	Donor	Rec.b.t.	Recipient after transplantation						
				Day 0	Day 1	Day 2	Day 4	Day 5	Day 7	From day 10
FXIIIB	6	1	1–3	–	–	1 3	–	–	1	–
	7	1	3	1 3	1 3	1	–	1	–	1
	8	1	3	–	1 3	1 3	1	1	1	1
	10	1–2	1	1	1	1	1 2	1 2	–	1 2
	12	1–3	1	–	1	1	1 3	–	1 3	1 3
	13	1	1–2	–	1 2	1 2	1	1	–	1
BF	1	S	FBS	–	–	S	–	–	–	S
	2	S	FBS	–	S	–	–	–	S	S
	8	FBS	S	–	S	FB S	FB S	FB S	FB S	FB S
C3	8	FvarS	S	–	S	S	Fvar S	Fvar S	Fvar S	Fvar S
	12	FS	S	–	F S	F S	F S	–	F (S)	–
	13	S	FS	–	F S	F S	(F) S	S	–	S
C6	7	AB	A	A or AB	–	–	–	A B	–	A B
	9	A	AB	–	–	A	A	A	A	A
	10	A	AB	A	–	A	A	A	–	A
	12	A	AB	–	A B	A (B)	A	–	A	A
	13	A	AB	–	A B	A B	A	A	–	A
	14	A	AB	–	A B	A	A	A	A	A
C8	9	AB	B	–	A B	A B	A B	A B	A B	A B
	13	AB	MvarB	–	Mvar B	Mvar B	A B	A B	–	A B
	14	AB	A	–	A	A B	A B	A B	A B	A B
	15	AM	A	A	A M	–	A M	A M	A M	A M
FH	8	1–2	2	–	1 2	1 2	1 2	1 2	1 2	1 2
	9	2	1	–	1 2	2	2	2	1	2
	10	2	1	1 2	1 2	1 2	1 2	2	–	2
	11	2	1–2	–	1 2	2	2	–	2	2
	12	2	1	–	1 2	1 2	2	–	2	2
	14	1	1–2	–	1 2	1 2	1	1	1	1

Rec.b.t. = Recipient before transplantation
( ) = Gene products of weak expression
– = not detected
* day 16: type 1S 1F
** days 14, 15, 16: type 2

Results

An informative selection of results obtained from the protein polymorphism analyses is given in Table 1. For each of the systems involved, results are given only of patients with donor-recipient incompatibility. Summarised in the last column of Table 1 are results obtained as of the tenth day, since with two exceptions, no more changes were noticeable in the further postoperative course. Patient No. 7 was exposed to the longest observation, with all results being confirmed on the 103rd postoperative day.

The usual mode of designation was used for pre-transplantation phenotypes of donors and recipients. Symbols are given in isolation and unhyphenated, to clearly show that the postoperative data may be mixtures of allotypes of different individuals (liver donors and recipients, blood donors).

Discussion

We examined a wide range of plasma protein systems with relevance to certification of parentage [13, 19], identification and stain analyses [6].

For at least one patient, evidence was provided of an irreversible change from recipient type to donor type in the systems HP, TF, GC, PI, ORM1, PLG, FXIIIB, BF, C3 and C6, which was in agreement with the international literature [1, 2, 7, 9–12, 16, 18, 20, 26]. The same evidence was produced for the first time for ITI, A2HS, C8 and FH.

With the exception of Alper et al. [2], previous publications in this field were restricted to 1 or 2 systems or a small number of patients. The present study is probably the most extensive analysis regarding both the number of protein systems involved and the number of follow-up controls.

The point in time of phenotype change from recipient to donor type within the postoperative course is given in

Table 1. Since the half-life of a protein is determined by synthesis, distribution and degradation, simple conclusions regarding half-life and synthesis rate based on the occurrence of the new phenotype in the plasma are not possible [8].

Allele products, different in intensity, were repeatedly observed in an intermediate phase, from the first to the tenth day post-transplantation. Also detected was the simultaneous presence of three allele products.

Blood transfusions, extrahepatic sites of synthesis or defective transplant function may be the causes for the non-occurrence of phenotype changes (recorded for the TF system in patient No. 4 and for the A2HS system in patient No. 5) or the emergence of new types (recorded for the GC system in patient No. 11, day 1 and 2 after transplantation) (see Table 1). These unexpected findings were discussed in greater detail in previous publications [22, 23].

Positive detection of phenotype change from recipient to donor type for a wide range of polymorphic plasma proteins after liver transplantation has clearly supported the assumption that the dogma of life-long stability of hereditary plasma protein characteristics is not of unlimited validity. The growing number of transplantations and survival time, in this context, are developments that lead to consequences for forensic protein typing, especially with regard to certification of parentage. Previous blood transfusions are already considered for the purposes of case history. However, individuals under review should be additionally interrogated for a previous liver transplantation, a demand that should be added as an amendment to the "Guidelines for parentage testing" [19]. In individuals with a record of liver transplantation, plasma protein systems cannot be used for haemogenetic appraisal. DNA analysis would be an alternative in such cases, since the synthesis of DNA in lymphocytes or other body cells is not affected by liver transplantation.

Liver transplantation should also be ruled out from the history of cases in which plasma protein characteristics are to be used for stain analysis or identification. If unambiguous exclusion is not possible, the possibility of liver transplantation should be borne in mind when evaluating of test results.

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