FORMAL GENETIC DATA ON ORM1 SUBTYPES

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INTRODUCTION

Human plasma orosomucoid has been demonstrated to be coded by two autosomal structural loci, ORM1 and ORM2 (Dente et al., 1985; Yuasa et al., 1986; Escallon et al., 1987). At protein level ORM2 is not polymorphic in European populations while ORM1 reveals two common gene products (Johnson et al., 1969). Later, by isoelectric focusing, 'subtypes' were reported for the anodal gene product (Thymann and Eiberg, 1986; Eap et al., 1988; Krüger et al., 1989; Luckenbach et al., 1989)

Evidence for the duplication of the ORM1 locus was presented by Yuasa et al. (1987, 1988). In this work we present formal and population genetics results on the subtypes of ORM1 obtained in families from SW Germany and NW Portugal which are compatible with this duplication hypothesis.

MATERIAL AND METHODS

Blood samples were obtained by venipuncture and sera or EDTA plasmas were stored at -20°C until use. Sample treatment and phenotyping were performed as previously described (Luckenbach et al., 1989).

RESULTS AND DISCUSSION

Description of phenotypes

Common ORM1 phenotypes present either a single band - interpreted as homozygous - or two (presumable heterozygotes). However some three-banded patterns were also found (Fig.1), showing in the same individual gene products corresponding to ORM1*S, ORM1*F1 and ORM1*F2 or to ORM1*S, ORM1*F1 and ORM1*F3.

Formal genetics and segregation analysis

As stated above, common phenotypes are consistent with the hypothesis that orosomucoid is coded by two autosomal structural loci, ORM1 (with two common alleles) and ORM2.

The appearance of individuals with three gene products suggests, on the contrary, that - at least in some cases - the ORM1 locus can be duplicated, in accordance with the interpretation of Yuasa et al. (1987, 1988). Therefore, a duplication was postulated for segregation analysis. Using the evidence available in both our samples two 'haplotypes' are required: F1F2 and F1F3.

Advances in Forensic Haemogenetics 4
Edited by Ch. Rittner and P. M. Schneider

TABLE 1. ORM1 Mating type distribution in SW Germany and NW Portugal

	SW Germany		NW Portugal		Allele/haplotype
Type	observed	expected	observed	expected	frequencies
F1 x F1	17	15.74	15	16.12	SW Germany:
F1 x F1F2	3	2.10	3	2.02	F1 0.578
F1 x F1F4	2	0.55	0	-	F1F2 0.019
F1 x F1F5	1	0.44	0	-	F1F3 0.004
F1 x F1S	36	42.47	40	39.60	F4 0.005
F1 x S	13	14.33	11	12.14	F5 0.004
F1F2 x F1S	3	2.84	4	2.52	S 0.390
F1F2 x F1F2S	1	0.09	0	0.08	
F1F2 x S	0	0.96	1	0.77	NW Portugal:
F1F3 x F1S	1	0.59	0	-	F1 0.608
F1F4 x F1S	1	0.73	0	-	F1F2 0.019
F1F5 x F1S	1	0.59	0	-	S 0.373
F1S x F1S	34	28.66	24	24.28	
F1S x F1F2S	2	1.88	2	1.52	
F1S x S	23	19.34	13	14.89	
F1F2Sx S	1	0.64	3	0.47	
F1F3Sx S	1	0.13	0	-	
S x S	1	3.26	2	2.28	
Others	0	5.66	0	1.31	
Total	141		118		

In Table 1 we show the mating type distribution found in SW Germany and NW Portugal. It is worth to mention that the research in the two labs, although independent, was carried out using the same techniques and produced homogeneous results, and we underline the fact that in all individuals with F2, F1 was also present.

Both distributions agree well with Hardy-Weinberg expectations; in the first case expected values were calculated according to a 6-allele/haplotype model: F1, S, F1F2, F4, F1F3 and F5, while in the Portuguese sample only F1, S and F1F2 are needed to explain the results; *S, *F4 and *F5 frequencies were estimated by gene counting; while F1F2 and F1F3 'haplotypes' were estimated using F1F2S and F1F3S heterozygotes.

We analysed the accordance of offspring phenotype distributions with mendelian expectations in both samples (totalling 407 and 315 for German and Portuguese, respectively) and we found no significant segregation distortions or unexpected phenotypes (except when F1F2 or F1F3 were involved). In Table 2, for brevity reasons, only the results concerning these matings are shown. Expected segregation ratios were calculated according to the previous hypothesis; for instance, in the first mating type, F1xF1F2, we assumed that the last phenotype can be genotypically F1F2/F1F2 or F1/F1F2.

TABLE 2. Segregation analysis of informative mating types giving evidence for duplicated ORM1 genes; families from SW Germany (G) and NW Portugal (P).

mating type	sample	offspring phenotypes F1	F1F2	F1F3	F1S	F1F2S	F1F3S	S
	G	4	3					
F1 x F1F2		3.45	3.55					
	P	10	4					
		6.89	7.12					
	G	0	3		3	2		
F1F2 x F1S		1.97	2.03		1.97	2.03		
	P	7	2		2	5		
		3.93	4.07		3.93	4.07		
F1F2 x F1F2S	G		0		1	2		
			1.50		0.74	0.76		
F1F2 x S	P				0	3		
					1.48	1.52		
F1F3 x F1S	G	0		1	1		0	
		0.5		0.5	0.5		0.5	
	G					2		1
F1F2Sx S						1.5		1.5
	P					3		4
						3.5		3.5
	G		2		0	5		2
F1S x F1F2S			2.25		2.25	2.25		2.25
	P		3		4	2		3
			3.0		3.0	3.0		3.0
F1F3Sx S	G			2				1
				1.5				1.5

In conclusion, the results clearly support both qualitatively and quantitatively the formal hypothesis for ORM1 locus: two common alleles (*F1, *S) and rarer ones (*F1F2, *F1F3, *F4, *F5) of which two (*F1F2, *F1F3) behave like tandem duplications.

Although we have performed experiments with increasing amounts of alkylating reagents, to confirm that the appearance of three-banded patterns was not resulting from an incomplete reaction (Fig.2), an alternative hypothesis can not be ruled out: *F2 and *F3 are true, non-duplicated, alleles for which the corresponding gene products behave in a similar manner as the ORM2 product fixed in our population, i.e., producing always two bands. More formal, biochemical and molecular results are needed to clarify this issue.

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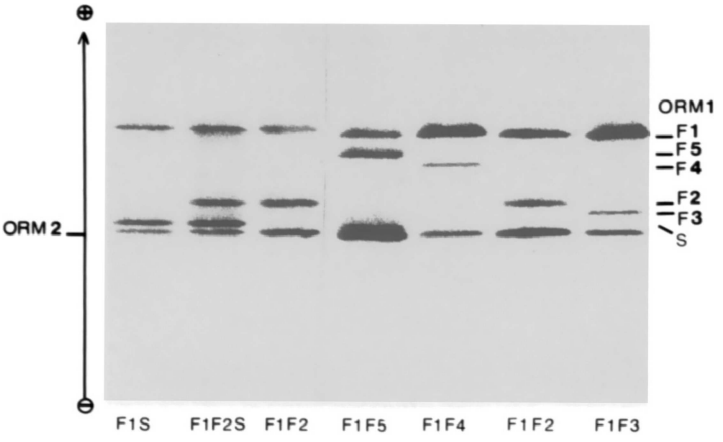

Fig. 1. ORM1 phenotypes. F1F2 sample is a pretyped reference (S Weidinger); for the other variants the nomenclature is tentative, due to the different methods used by previous reports

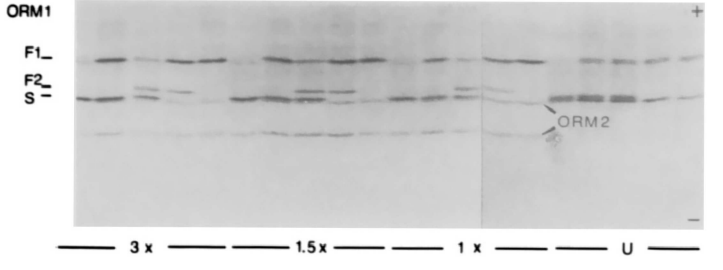

Fig. 2. ORM1 phenotypes after treatment with various amounts of alkylating reagent (iodacetamide). The same set of five samples, by the same order, was untreated (U), treated as described previously (1x) and with increasing amounts of the reagent (1.5x and 3x)

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ACKNOWLEDGEMENTS: This research was partially supported by DAAD.