DETECTION OF A SILENT GC ALLELE IN A DANISH MOTHER AND CHILD

M.Thymann, H.E.Hansen and J.Dissing

Department of Forensic Genetics
Institute of Forensic Medicine, University of Copenhagen,
11 Frederik V´s Vej, DK-2100 Copenhagen, Denmark

INTRODUCTION

The Group-Specific Component (GC, vitamin-D binding protein, DBP) is a polymorphic plasmaprotein still used for paternity investigations in many laboratories (theoretical chance of exclusion 31%). In a Danish case of disputed paternity inverse homozygosity was observed in a mother and her child by isoelectric focusing. Extended investigations including conventional bloodtyping systems, RFLP- and AMP/FLP-examinations were carried out. PCR/SSCP analysis of a DNA fragment containing two mutation sites specific for the alleles GC 1F, GC 1S and GC 2 in exon 11 of the GC gene was also performed.

MATERIALS AND METHODS

Conventional GC typing was carried out by isoelectric focusing in polyacrylamide gel in a non-linear pH 4.5-5.5 gradient. Visualization was performed by immunoblotting and staining with indolylphosphate/nitrobluetetrazolium using rabbit anti-human GC as the first antibody and phosphatase conjugated swine anti-rabbit immunoglobulin as the second antibody. For GC typing by PCR/SSCP, amplification of exon 11 was performed by the use of 25- and 28-mer primers (Dissing unpublished result) designed from the known DNA sequence of the GC gene (Witke et al.) Electrophoretic separation of the single stranded DNA (SSCP, Orita et al.) was done in 20% polyacrylamide gel at 12°C using the automatic Pharmacia Phast System and silver staining. Conventional bloodgrouping was performed according to the routine procedures of the laboratory. The methods used for D1S80- and RFLP-analysis have been described by Thymann et al. and by Morling & Hansen; Hansen & Morling.

RESULTS AND DISCUSSION

The GC phenotypes of the mother (GC 2) and child (GC 1S) obtained by isoelectric focusing are shown in figure 1. The electrophoretic pattern gave no indication of decreased protein level and the results were confirmed by repeated examination of new samples. GC genotyping at the DNA level is possible due to the existence of allele specific base differences at codons 432 and 436 in exon 11 of the GC gene (Reynolds and Sensabaugh). Using PCR and SSCP a 186 bp-DNA fragment spanning this exon was analyzed. The GC type of the

mother was detected as GC 1S,2 and that of the child as GC 1S (figure 2). The extended investigation comprised nine conventional bloodgrouping systems (ABO, MNS, RH, HP, PGM1, ACP1, GPT, ESD, GLO), seven VNTR single locus system D2S44 *YNH24*, D4S139 *pH30*, D5S43 *MS8*, D7S21 *MS31*, D7S22 *g3*, D12S11 *MS43a*, D16S309 *MS205*) and one AMPFLP system (D1S80 *MCT118*). The results obtained are given in table 1a and 1b. The calculated probability of maternity exeeded 10.000 to 1. The results of the SSCP analysis showed that the silent GC allele in the mother and child is not caused by a major deletion involving the entire GC gene since exon 11 appeared to be intact.

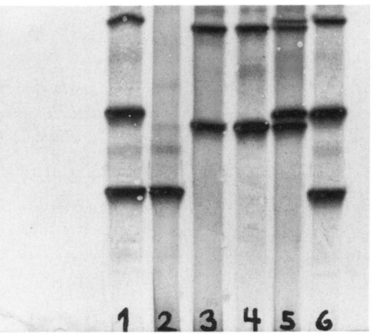

Figure 1
GC phenotypes of mother, child and several controls as obtained by IEF in polyacrylamide gel followed by immunoblotting. Anode at the top. (1) GC 1F,2; (2) mother GC 2; (3) child GC 1S; (4) GC 1S; (5) GC 1F,1S; (6) GC 1F,2.

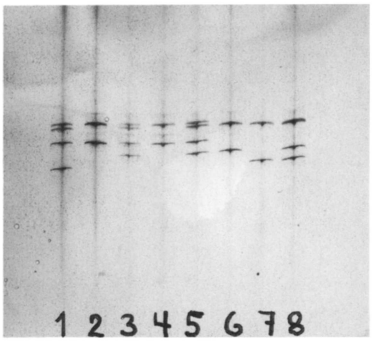

Figure 2
GC genotypes of mother, child and controls as obtained by PCR/SSCP analysis of a 186 bp DNA fragment of exon 11. (1) GC 1F,1S; (2) GC 1S; (3) mother GC 1S,2 (4) child GC 1S (5) GC 1S,2; (6) GC 2; (7) GC 1F; (8) GC 1F,2.

Table 1a. Results obtained by bloodgrouping of a Danish mother and child.

	GC	ABO	MN Ss	RH	HP	PGM1	ACP1	GPT	ESD	GLO
Mother	**2** IEF **1S**PCR **2**SSCP	0	M ss	cc D Ee	2FS	1F	A	1 2	1	1
Child	**1S**	0	M ss	cc D EE	1F 2FS	1F	A	2	1	1 2

Table 1b. Results obtained by DNA-analysis of a Danish mother and child.

	D1 S80	D2 S44	D4 S139	D5 S43	D7 S21	D7 S22	D12 S11	D16 S309
Mother	18	4.26 4.80	4.20 7.23	5.16 8.37	5.38 8.70	6.61 9.01	5.27 8.88	1.59 3.03
Child	18 31	3.28 4.76	7.24 8.13	2.80 5.19	5.36 5.48	7.07 9.07	8.77 10.00	2.78 3.01

CONCLUSION

Inverse homozygosity was observed in a Danish mother and child by GC phenotyping. Using isoelectric focusing the type of the mother was GC 2 and the type of the child GC 1S. GC genotyping was performed by PCR/SSCP analysis of exon 11 of the GC gene. The mother and child were typed as GC 1S,2 and 1S, respectively. After extended investigations the probability of maternity exeeded 10.000 to 1. The silent GC allele is not due to a major deletion of the entire GC gene since exon 11 appeared to be intact.

REFERENCES

Hansen H E and Morling N: Paternity testing with VNTR DNA systems. II.Evaluation of 271 cases of disputed paternity with the VNTR systems D2S44, D5S43, D7S21, D7S22, and D12S11. Int J Leg Med (1993) 105:197-202.

Morling N, and Hansen H E: Paternity testing with VNTR DNA systems. I.Matching criteria and population frequencies of the VNTR systems D2S44, D5S43, D7S21, D7S22, and D12S11 in Danes. Int J Leg Med (1993) 105:189-196.

Orita M, Suzuki Y, Sekiya T, and Hayashi K: Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. Genomics (1989) 5:874-879.

Reynolds R L, and Sensabaugh G F: Use of the polymerase chain reaction for typing GC variants. Adv. Forensic Haemogenet (1990 3: 158-161

Thymann M, Nellemann L J, Masumba G, Irgens-Møller L, and Morling N: Analysis of the locus D1S80 by amplified fragment length polymorphism technique (AMP-FLP). Frequency distribution in Danes. Intra and inter laboratory reproducibility of the technique. Forensic Sci Internat (1993) 60:47-56.

Witke W F, Gibbs P E M, Zielinski R, Yang F, Bowman B H, and Dugaiczyk A: Complete structure of the GC gene: Differences and similarities between members of the Albumin gene family. Genomics (1993) 16: 751-754.