# STUDIES ON THE HUMTH01 AND HUMVWA POLYMORPHISMS IN A SOUTH WEST GERMAN POPULATION

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**System and locus:** HUMTH01 (11p15.5-p15)

**Population and sample size**: South West Germany. N = 305

**Methods:**

DNA was extracted from EDTA-blood using 5 % Chelex 100 [1]

Primers: 5' GTG GGC TGA AAA GCT CCC GAT TAT 3'
5' GTG ATT CCC ATT GGC CTG TTC CTC 3' [2]

PCR amplification conditions: The amplification mix contained 10-30 ng template DNA, 1 U Taq-polymerase (Pharmacia), 5 µl 10 x buffer (Pharmacia), 0,2 mM dNTP mix (Pharmacia), 0,25 µM of each primer (Biometra), 0,16 µg/µl BSA (Boehringer) and was diluted to a final volume of 50 µl with distilled water. The reaction mixture was overlayed with 20 µl mineral oil.

After a first denaturation for 5 min at 94°C a total of 30 cycles for 45 sec at 94°C, 30 sec at 60°C, 30 sec at 72°C was carried out, followed by a last extension for 7 min at 72°C (Thermocycler: Biometra TRIO-Thermoblock)

Electrophoretic methods: Electrophoretical separation of the PCR products was performed in polyacrylamid gels (7,2% T, 3% C, 750 µm thick, horizontal) with piperazin diacrylamid as cross-linker using a discontinuos buffer system [4]. Gels were run at
1000 V, 40 mA, 5 W for 90 min
1000 V, 40 mA, 10 W for 60 min
1000 V, 40 mA, 15 W until the bromphenol blue front had reached the anode.
The seperationdistance was 20 cm. Gels were silver stained using a standard protocol [5].
The allels were typed by side to side comparison with an allele ladder (Serac), composed of the allels 5,6,7,8,9,9.3 and 10.

**Results:**

**Observed genotypes**

| Gen. | Obs. | Gen. | Obs. | Gen. | Obs. | Gen. | Obs. |
|---|---|---|---|---|---|---|---|
| **5-5** | 0 | **6-6** | 13 | **7-8** | 12 | **8-10** | 1 |
| **5-6** | 3 | **6-7** | 16 | **7-9** | 15 | **9-9** | 13 |
| **5-7** | 0 | **6-8** | 14 | **7-9.3** | 36 | **9-9.3** | 38 |
| **5-8** | 0 | **6-9** | 22 | **7-10** | 4 | **9-10** | 2 |
| **5-9** | 0 | **6-9.3** | 47 | **8-8** | 2 | **9.3-9.3** | 21 |
| **5-9.3** | 0 | **6-10** | 2 | **8-9** | 11 | **9.3-10** | 2 |
| **5-10** | 0 | **7-7** | 9 | **8-9.3** | 21 | **10-10** | 1 |

Chi-square = 8.22, d.f. = 14, $0.70 < p < 0.90$
Cells with expected values of less than five were calculated together

**Allele frequencies**

| Allele | Frequency | Allele | Frequency |
|---|---|---|---|
| **5** | 0.0049 | **9** | 0.1869 |
| **6** | 0.2131 | **9.3** | 0.3049 |
| **7** | 0.1656 | **10** | 0.0213 |
| **8** | 0.1033 | | |

**System and locus:** HUMVWA (12p12-12pter)

**Population and sample size:** South West Germany. N = 206

**Methods:**

Primers: 5' CCC TAG TGG ATG ATA AGA ATA ATC AGT ATG 3'
5' GGA CAG ATG ATA AAT ACA TAG GAT GGA TGG 3' [3]

PCR amplifation conditions were the same as for HUMTH01 with the exception that a total of 35 cycles was carried out.

Electrophoretic methods were identical with that of HUMTH01.

Typing was performed by side to side comparison with an allele ladder (Serac), composed of the alleles 13 - 21.

**Results:**
**Observed genotypes**

| Gen. | Obs. | Gen. | Obs. | Gen. | Obs. | Gen. | Obs. |
|---|---|---|---|---|---|---|---|
| **13-13** | 0 | **14-15** | 11 | **15-18** | 5 | **17-18** | 24 |
| **13-14** | 0 | **14-16** | 6 | **15-19** | 4 | **17-19** | 6 |
| **13-15** | 0 | **14-17** | 16 | **15-20** | 0 | **17-20** | 2 |
| **13-16** | 0 | **14-18** | 9 | **16-16** | 9 | **18-18** | 10 |
| **13-17** | 2 | **14-19** | 0 | **16-17** | 26 | **18-19** | 9 |
| **13-18** | 0 | **14-20** | 0 | **16-18** | 24 | **18-20** | 1 |
| **13-19** | 0 | **15-15** | 0 | **16-19** | 13 | **19-19** | 0 |
| **13-20** | 0 | **15-16** | 8 | **16-20** | 1 | **19-20** | 1 |
| **14-14** | 2 | **15-17** | 10 | **17-17** | 7 | **20-20** | 0 |

Chi-square = 13.78, d.f. = 15, $0.50 < p < 0.70$
Cells with expected values of less than five were calculated together

**Allele frequencies**

| Allele | Frequency | Allele | Frequency |
|---|---|---|---|
| **13** | 0.0049 | **17** | 0.2427 |
| **14** | 0.1117 | **18** | 0.2233 |
| **15** | 0.0922 | **19** | 0.0801 |
| **16** | 0.2330 | **20** | 0.0121 |

**Comments:**

The oberved HUMTH01 and HUMVWA genotype frequencies are in a good agreement with the expected distribution under the Hardy-Weinberg law. Heterozygosity : HUMTH01 80.6% (81.73 expected), HUMVWA 86.4% (83.21% expected). Discrimination power: HUMTH01 72.5%, HUMVWA 75.5%.
With the nondenaturating polyacralamid gels used in our experiments a clear-cut decision between allele HUMTH01 9.3 and 10 could not be made on each gel. A small sample of 40 true family trios showed for both systems normal segregation with no examples of mutation or non-Mendelian inheritance.

**References:**

[1] Walsh P S, Metzger D A, Higuchi R, Bio Tech 1991; 10: 506-513
[2] Gill P, Kimpton C, D'Aloja E, Andersen J F, Bär W, Brinkmann B, Holgersson S, Johnsson V, Kloostermann A D, Lareu M V, Nellemann L, Pfitzinger H, Phillips C P, Schneider P M, Stenersen M, Forensic Sci Int 1994; 65: 51-59
[3] Kimpton C, Walton A, Gill P, Hum Molecular Genet 1992; 1: 287
[4] Budowle B, Chakraborty R, Giusti A M,Esenberg A J, Allan R C, Am J Hum Genet 1991; 48: 137-144
[5] Allen RC, Graves G, Budowle B, Bio Tech; 7: 736-744
[6] Nei M, Roychoudhury A K, Genetics 1974; 76: 379-390