BRIEF COMMUNICATION

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# Population Data on the X Chromosome Short Tandem Repeat Locus HumHPRTB in Two Regions of Germany

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**ABSTRACT:** This report contains the results of two population studies on the X chromosome STR HumHPRTB carried out in a Northern and a Southern region of Germany. The numbers of unrelated individuals were 443 and 335, respectively. Eight alleles (alleles 9 to 16) were found. In female individuals 29 different genotypes were encountered. In German populations the HumHPRTB STR was characterized by the following data: PIC = 0.750; HET = 0.769; MEC = 0.556. Allele distribution met the Hardy-Weinberg expectations. The Northern and Southern populations did not show any significant differences.

**KEYWORDS:** forensic science, DNA typing, short tandem repeat, X chromosome, HumHPRTB, population genetics

The short tandem repeat (STR) HumHPRTB is located at Xq 26 in intron 3 of the hypoxanthine guanine phosphoribosyltransferase gene and comprises a variable AGAT repeat (1–3). Two HumHPRTB allele detection protocols for investigating this polymorphism are published in the literature (1,3). Primers designed by Haerne and Todd (3) provide PCR fragments 124 bp shorter than the alternative primers (1). Crouse and Schumm (4) demonstrated that HumHPRTB products are specific for primates (when primers of Edwards et al. are used).

The present report summarizes the results of two studies on the HumHPRTB allele and genotype distribution which were carried out independently in two geographically separated regions of Germany.

X chromosome STRs, such as HumHPRTB, have been proven to be very useful in paternity testing when the disputed child is female. The advantages of this method have become apparent in some cases of disputed parentage, when the alleged father could not be typed. By using the integrated gene map of the X chromosome by Wang et al. (5) it is possible to establish the genetic distance between HPRTB and the loci HumAR (6) and HumDXS7423 (7) which are about 60 cM and 45 cM, respectively. Hence, a linkage disequilibrium between these markers is not likely. Presently, we are conducting a population-genetic study to verify this assumption. Unless the linkage disequilibrium will be definitely excluded, these X chromosome STRs can be used as an alternative in paternity testing, however not as marker set. Mendelian inheritance of HumHPRTB was analyzed by observing the allele transfer in 290 female and 290 male meiotic events.

## Materials and Methods

### *DNA Extraction*

DNA was obtained from mothers, daughters and the alleged fathers for conducting a paternity test. The parents were verified to be unrelated individuals. For calculation the meiosis rate triplets were analyzed when the probability of paternity was computed to be 99.98% or higher. DNA was prepared from fresh blood using the proteinase K/phenol-chloroform extraction method (8).

All reagents used for the DNA extraction procedure were purchased from MERCK KGaA (Darmstadt).

### *Amplification*

The two working groups (Freiburg University and Magdeburg University) which had decided to publish their results together, used different PCR protocols; one group used the protocol of Edwards et al. (1,2) for investigating the Southern population, and the other group examining the Northern population used the protocol of Haerne and Todd (3).

Application of both protocols to the same sample of 50 females confirmed our assumption that the typing results obtained with both methods are identical. Thus, the results of both studies can be combined.

Two blanks were cycled and electrophorized as negative controls with each PCR set up to verify that no contamination had falsified the typing results.

*Northern Population Study*—The PCR mixture contained 5 to 10 ng template DNA, 1x PCR buffer (Serac, Bad Homburg), 0.2 mM each dNTP, 1.5 mM $MgCl_2$, 1.5 pmol each primer and 0.7 U DNA polymerase (Serac, Bad Homburg). Double distilled water of a sufficient quantity was added to produce a total volume of 25 μL.

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The following primers (3) synthesized by Pharmacia Biotech (Freiburg) were used: 5′TCT CTA TTT CCA TCT CTG TCT CC-3′(F); 5′ TCA CCC CTG TCT ATG GTC TCG-3′ (R).

Amplification was carried out in a PTC-100 cycler (MJ Research Inc., Watertown, New York). The cycle conditions were: 94°C-2 min soak, 94°C-1 min, 61°C-1 min, 72°C-1 min, 5 cycles; 94°C-1 min, 60°C-1 min, 72°C-1 min, 5 cycles; 94°C-1 min, 58°C-1 min, 72°C-1 min, 25 cycles; 72°C-5 min.

*Southern Population Study*—The PCR mixture contained about 50 ng template DNA, 1x PCR buffer (Serac, Bad Homburg), 0.2 mM each dNTP, 1.5 mM $MgCl_2$, 1.5 pmol each primer and 0.7 U DNA polymerase (Serac, Bad Homburg). The final volume was brought to 25 μL with double distilled water.

The following primers (1) synthesized by Pharmacia Biotech (Freiburg) were used: 5′ ATG CCACAGATAATACACATC-CCC-3′ (F); 5′ CTCTCCAGAATAGTTAGATGTAGG-3′ (R).

Amplification was carried out in a PTC-100 cycler (MJ Research Inc., Watertown, New York). The cycle conditions were: 94°C 3-min soak, 94°C-45 sec, 56°C-45 s, 72°C-90 s, 30 cycles, 72°C-5 min.

### *Typing*

*Northern Population Study*—Amplified DNA fragments were separated by horizontal electrophoresis through 0.7-mm-thick native gels according to Möller et al. (9).

Polyacrylamide gels: 6.0% acrylamide, 2.15% piperazine di-acrylamide as crosslinker 51 mM TRIS formate (pH 9.0), 0.1% APS (ammonium persulfate), 0.01% TEMED. All electrophoretic reagents were purchased from Bio-Rad Laboratories (Hercules). Bands were visualized by silver staining (10) and alleles were named according to the number of repeats as recommended (11). Typing was performed by side-to-side comparison with an allelic ladder made from alleles 9 to 16, which had been sequenced before as described below. PCR products from amplified K562 DNA (Life Technologies, Gaithersburg, MD) displaying the Hum HPRTB allele 13 were always used as controls to verify the allele typing protocols.

*Southern Population Study*—Amplified DNA fragments were diluted in a double volume of loading buffer denatured at 95°C for 5 min and loaded onto a 1-mm-thick denaturing gel. The loading buffer (pH 8.0) contained 95 mL formamide, 2 μmol EDTA, 50 mg xylene cyanole and 50 mg bromophenol blue per liter distilled water. The gel contained 6% (39:1) acryl:bis-acrylamid gel, 7 M urea, 0.04% TEMED and 0.1% APS. All electrophoretic reagents were purchased from Bio-Rad Laboratories (Hercules). PCR fragments were separated by vertical electrophoresis gels for 6 h at 600 V/60

TABLE 1—*HPRTB alleles structure and fragment sizes in bps. Primers were used according to Edwards et al. (1) and Haerne and Todd (3), respectively.*

| Structure | Allele | Size (1) | Size (3) |
|---|---|---|---|
| $(AGAT)_9$ | 9 | 271 | 147 |
| $(AGAT)_{10}$ | 10 | 275 | 151 |
| $(AGAT)_{11}$ | 11 | 279 | 155 |
| $(AGAT)_{12}$ | 12 | 283 | 159 |
| $(AGAT)_{13}$ | 13 | 287 | 163 |
| $(AGAT)_{14}$ | 14 | 291 | 167 |
| $(AGAT)_{15}$ | 15 | 295 | 171 |
| $(AGAT)_{16}$ | 16 | 299 | 175 |
| $(AGAT)_{17}$ | 17 | 303 | 179 |

TABLE 2—*Allele distribution of the STR HumHPRTB in two regions: North and South Germany.*

| Allele (population) | f ± s.e. Female | f ± s.e. Male | f ± s.e. Total |
|---|---|---|---|
| 9 (South) | 0.005±0.002 | 0.005±0.002 | 0.005±0.002 |
| 9 (North) | 0.009±0.003 | 0.011±0.017 | 0.010±0.001 |
| 10 (South) | 0.016±0.004 | 0.009±0.004 | 0.014±0.003 |
| 10 (North) | 0.021±0.005 | 0.018±0.022 | 0.020±0.012 |
| 11 (South) | 0.121±0.011 | 0.148±0.016 | 0.131±0.009 |
| 11 (North) | 0.127±0.013 | 0.106±0.016 | 0.120±0.010 |
| 12 (South) | 0.293±0.016 | 0.278±0.021 | 0.287±0.013 |
| 12 (North) | 0.275±0.017 | 0.277±0.024 | 0.276±0.014 |
| 13 (South) | 0.318±0.016 | 0.332±0.022 | 0.322±0.013 |
| 13 (North) | 0.294±0.017 | 0.347±0.025 | 0.312±0.014 |
| 14 (South) | 0.157±0.012 | 0.143±0.016 | 0.152±0.010 |
| 14 (North) | 0.186±0.015 | 0.153±0.019 | 0.174±0.012 |
| 15 (South) | 0.076±0.009 | 0.067±0.012 | 0.073±0.007 |
| 15 (North) | 0.076±0.011 | 0.076±0.014 | 0.076±0.008 |
| 16 (South) | 0.014±0.004 | 0.018±0.006 | 0.016±0.003 |
| 16 (North) | 0.012±0.004 | 0.012±0.017 | 0.012±0.003 |
| n Alleles (South) | 420 | 233 | 643 |
| n Alleles (North) | 330 | 170 | 500 |

| South | | | | North | | | |
|---|---|---|---|---|---|---|---|
| PIC | HET | MEC | Hardy-Weinberg Equilibrium* | PIC | HET | MEC | Hardy-Weinberg Equilibrium* |
| 0.750 | 0.769 | 0.556 | $p = 0.981$ | 0.764 | 0.781 | 0.574 | $p = 0.620$ |

* Exact Hardy-Weinberg equilibrium test of Guo and Thomson (12).

TABLE 3—*Genotype frequencies (%) of the STR HumHPRTB in South and North Germany. Figures in bold type refer to the South German sample* (n *= 210 female); figures in italic type refer to the North German sample* (n *= 165 female).*

| Alleles → ↓ (population) | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|---|---|---|---|---|---|---|---|---|
| **9** (South) | **0** | **0** | **0** | **0.48** | **0.48** | **0** | **0** | **0** |
| *9 (North)* | *0* | *0* | *0* | *0* | *0.61* | *0.61* | *0.61* | *0* |
| **10** (South) | | **0** | **1.90** | **1.43** | **0** | **0** | **0** | **0** |
| *10 (North)* | | *0* | *0* | *1.82* | *0.61* | *1.21* | *0.61* | *0* |
| **11** (South) | | | **1.43** | **8.57** | **7.14** | **4.28** | **1.43** | **0** |
| *11 (North)* | | | *1.82* | *7.27* | *8.48* | *4.85* | *0.61* | *0.61* |
| **12** (South) | | | | **7.62** | **18.57** | **9.52** | **8.15** | **0.95** |
| *12 (North)* | | | | *7.27* | *16.97* | *12.12* | *1.82* | *0.61* |
| **13** (South) | | | | | **10.47** | **9.05** | **4.76** | **0.95** |
| *13 (North)* | | | | | *7.88* | *10.91* | *4.85* | *0.61* |
| **14** (South) | | | | | | **2.86** | **2.38** | **0.48** |
| *14 (North)* | | | | | | *1.21* | *4.24* | *0.61* |
| **15** (South) | | | | | | | **1.43** | **0.48** |
| *15 (North)* | | | | | | | *1.21* | *0* |
| **16** (South) | | | | | | | | **0** |
| *16 (North)* | | | | | | | | *0* |

mA/50°C. Bands were visualized by ethidium bromide. Typing was performed by using allelic ladders and control DNA as described for the Northern population study.

*Sequencing*

Direct sequencing was carried out by using PCR products from (hemizygote) male DNA. In cases where rare target alleles could not be obtained in a hemizygote state, PCR fragments were excised from PAG and extracted by elution. This DNA was cleaned and concentrated in 30.000 NMGG Ultrafree MC Filters (Millipore, Bedford). The sequencing primers were the same as those applied to PCR. Sequencing was performed using the Ready Reaction Dyedeoxy Terminator Sequencing Kit and 373A sequencer (PE-Applied-Biosystems, Foster City).

## Results and Discussion

Both the Northern and the Southern populations investigated exhibited eight common alleles including 9 to 16. PCR fragment length ranged from 271 to 303 bp and 147 to 179 bp, respectively (Table 1). Rare variants (e.g., alleles 6 to 8) as have been observed in black Americans (1,2) were not found in the German population. Two samples of each allele repeat were sequenced. The regular repeat motif was AGAT.

Single allele frequencies in both German populations are presented in Table 2. The investigated populations did not show any significant difference.

The number of observed genotypes is shown in Table 3. Statistical tests were carried out separately for each sample following the of Hardy-Weinberg exact proportion test for multiple alleles (12). This test is implemented in the Pater software by C. Brenner (http://www.ccnet.com/~cbrenner/paterbro.htm)

In both studies the allele distribution met the Hardy-Weinberg equilibrium. In total, two mutations (1 male and 1 female) were found in a total of 580 meioses.

Our findings and results reported in the literature (1,2,13) show that there is no significant difference of HumHPRTB allele distribution when compared between Caucasian populations.

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