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Epidemiological investigation of the UGT2B17 polymorphism in doping control urine samples and its correlation to T/E ratios

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ABSTRACT: The deletion polymorphism of the enzyme UGT2B17 is known to correlate with the level of the testosterone to epitestosterone (T/E) ratio in urine specimen. Due to the importance of the T/E ratio to detect testosterone abuse in doping analysis, a PCR-ELISA system (Genotype® UGT test, AmplexDiagnostics) was established to identify the UGT2B17 phenotype in urine samples. Epidemiological investigations in a set of 674 routine doping controls (in- and out-of-competition) resulted in 22.8% homozygote gene-deleted and 74.5% UGT2B17-positive athletes. The validated test system has shown to be robust and sensitive: in only 18 cases (2.7%) isolation of cell material from urine failed.

Following hydrolysis of glucuronidated conjugates, steroids were analyzed as bis-TMS derivatives by gas chromatography-mass spectrometry (GC-MS), for example, testosterone (T) and epitestosterone (E). Additionally, isotope ration mass spectrometry (IRMS) analysis and luteinizing hormone (LH) measurement were applied. Mean T/E ratios significantly correlated with the UGT2B17 phenotype (del: T/E 0.9; pos: 1.7), however the values did not differ as distinctive as reported in previous studies. Additionally, the T/E ratios in the gene-deleted group did not show a normal curve of distribution (median of T/E 0.5). Obviously, beside the UGT2B17 deletion further influences have to be taken into account, for example, polymorphisms or induction of other metabolizing enzymes. Our results indicate that the UGT2B17 polymorphism might be insufficient when utilized solely as a crucial parameter for individual interpretation of T/E in urine. Nevertheless, the detection of the UGT2B17-gene deletion in urine samples would provide additional information important for gathering evidence in analysis of steroids in doping control.

Keywords: doping analysis; testosterone/epitestosterone ratio; UGT2B17 deletion; glucuronidation; Genotype® UGT test

Introduction

Genetic variations, responsible for several diseases, might be associated with an elevated risk of cancer or influence the activity of metabolising enzymes. An enzyme polymorphism is defined as a variation observed in more than 1% of a population, and can occur in case of phase-I or phase-II enzymes, for example. Alterations in enzyme activities would not necessarily result in perceptible interferences or remarkable adverse effects for health. The polymorphism of the enzyme UDP-glucuronosyltransferase (UGT) 2B17 investigated in the present work was initially related with the risk of prostate cancer, kidney damage, and osteoporosis, the results of which have been controversially discussed.[1–7]

UGT2B17 is one representative of the UGT enzyme superfamily, which catalyzes the transfer of sugar residues to various substrates to improve their renal excretion from the body. Depending on their sequence similarities, UGT enzymes are divided into two enzyme families (UGT1, UGT2) and further subfamilies. Androgens are mainly converted by enzymes belonging to the subfamilies UGT1A and UGT2B, which catalyse the conjugation with glucuronic acid as the predominant phase-II reaction in human.[8] Glucuronidation of steroids commonly occurs at a hydroxyl group, although a significant stereo- and regioselectivity of the involved UGTs has been observed.[9–11] The resulting glucuronides are rapidly excreted in urine, due to their hydrophilic properties.

For doping control purposes, detection of prohibited anabolic androgenic steroids is carried out following hydrolysis of glucuronidated metabolites in urine. Concerning this matter, the relevance of UGT polymorphisms has been shown before, particularly regarding the misuse of testosterone (T).[12,13] T is the main endogenous androgen produced in male, and its administration is prohibited in sports.[14] A decisive factor in doping control is the ratio of the T concentration to the reference epitestosterone (E), calculated after cleavage of glucuronide conjugates in urine. The threshold of T/E 4[15] is utilized to select samples from screening for further investigations. T applications would result in an elevated T/E ratio, caused by an increase of T level in urine and assuming that E is not generated from T. Due to the negative feedback on biosynthesis of androgens, the excretion of E may consequentially be reduced, which moreover contributes to the increase of the T/E ratio. Additional effects are detectable: further characteristic metabolites are excreted to a higher extend in urine, for example, androsterone (A) and etiocholanolone (Etio).[16] To

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deal with broad individual variations, population-based reference ranges have been established and are utilized to determine alterations of the urinary steroid profile. According to the Technical Document,[15] concentrations of several endogenous steroids (dehydroepiandrosterone, A, Etio, T, E) as well as the T/E ratio are observed.

Additionally, the release of luteinizing hormone (LH) might be suppressed in consequence of the negative feedback on the hypothalamic-pituitary-gonadal axis.[16] In connection with the T concentration and the specific gravity of the urine sample, LH level measured in male may provide further advice.

To ascertain an exogenous T uptake or the application of T-related substances, the analyses of $\delta^{13}C$ values by a gas chromatography/online combustion/isotope ratio mass spectrometry system (GC/C/IRMS) is used. Due to influences of the diet, the $\delta^{13}C$ values of diagnostic metabolites (e.g. A, Etio, androstanediols) have to be evaluated in comparison to unaffected endogenous reference compounds (e.g. pregnanediol, pregnanetriol).

In conclusion, IRMS analysis to confirm T misuse is applied only in cases of a suspicious finding in the GC-MS screening. Thus, the limit of the approach is the selection of samples depending on steroid values, which are established on the statistical distribution of the reference population. The T/E threshold of 4.0 has been the most important criterion for the assessment so far, although a broad inter-individual variety exists.

Glucuronosyltransferases relevant for steroid metabolism in human are characterized by significant substrate specificities. T is conjugated with glucuronic acid mainly by the enzyme UGT2B17 and only to a minor part by UGT2B15. In contrast, the glucuronidation of the endogenous reference E is predominantly catalyzed by the enzyme UGT2B7.[11,17,18] Consequentially, the concentration of T-gluc and E-gluc in urine, and therefore the individual basal T/E ratio, depends directly on the activity of the mentioned glucuronosyltransferases. Notably, genetic variations have been reported for UGT2B7, 2B15 as well as 2B17,[12,19] and may result in a reduced or even in an absent enzyme activity.

Regarding the glucuronosyltransferase UGT2B17 a polymorphism has been described, which is characterised by the deletion (del) of the 6 exons in the DNA sequence coding for the enzyme.[20] Following, the enzyme is not expressed in case of the homozygote gene deletion at both alleles. Therefore, two phenotypes can be differentiated – UGT2B17-gene positive (two inserted copies: ins/ins and one deletion: ins/del) and UGT2B17-gene deleted (homozygote: del/del). The deletion of the gene is known to depend on the ethnic background and is most frequently in the asian polulation (Asians 81%, Africans 22%, Caucasians 10%, Hispanics 7%, investigated in 171 individuals[21]).

The strong connection to T/E ratios in urine was demonstrated, whereby individuals devoid of UGT2B17 had significant lower T/E levels (mean 0.14) compared to UGT2B17-positives (ins/ins T/E 2.3; ins/del T/E 1.4) and may not exceed the threshold of 4 following an application of testosterone.[13] Consequentially, variations from the individual steroid profile without exceeding general thresholds would not be noticed by the laboratory, for example, in case of extremely low endogenous T/E ratio. Therefore, the risk of a false-negative doping control is higher in case of the UGT2B17-gene deletion, due to the considerable spread between the basal T/E ratio and the threshold value.

To deal with this problem and to improve sensitivity of the steroid screening, two strategies might be taken into account. One possibility is the evaluation of the individual endogenous steroid profiles and subject-based thresholds. In terms of longitudinal studies, it has been already practised in case of athletes with a physiological elevated T/E ratio. For that purpose, the results of several urine samples from the same athlete have to be stored in a database and managed by the result management authority. This strategy requires statistically reliable data and the extensive evaluation and interpretation of the steroid concentrations.[22,23] WADA has started activities to implement data of the urinary steroid profile in the athlete biological passport, and the submission of values by the laboratories via the result module of ADAMS (Anti-doping administration and management system) is intended. The knowledge of individual values would allow a more sensitive selection of doping control samples for subsequently IRMS analysis and would enhance efficiency of T testing.

Another approach dealing with differentiated cut-off levels based on the UGT2B17 genotype was suggested by Schulze *et al.* In case of the deletion of the gene, a threshold of T/E 1.0 would be more efficient. In contrast, a threshold of 6.0 is proposed as cut-off in ins/del and ins/ins subjects.[13,24] Implementation would require the knowledge of the UGT2B17 genotype of each athlete. Usually, genotyping is carried out in blood samples, but in doping control urine specimens are utilized for steroid detection. Therefore, one of the study's challenges was the establishment of a test kit to identify the UGT2B17 gene in urine specimen. Consequentially, the detection method should be applied under real conditions in the doping control laboratory.

Furthermore, we intended to prove the assumption that the UGT2B17 genotype correlates with the T/E ratio and therefore can be used as an undisputed and unaffected parameter for doping control. Previous studies aimed at investigating the differences between ethnic groups and were done in limited cohorts. The focus of the present work was the epidemiological analysis of the UGT2B17 polymorphism, to evaluate influencing factors, reliability, and effectiveness for detection of testosterone abuse in doping control samples. The data should allow an appraisal regarding the suitability of the method in doping control, for example, to optimize selection of suspicious samples for IRMS analysis or to provide additional information for interpretation of steroid profiles.

Materials and Methods

Reference material, reagents, solvents for steroid analyses

Certified reference materials (testosterone, epitestosterone, d_3-testosterone) were purchased from NMI (Pymble, Australia). Reagents and solvents were of analytical grade: t-butyl methyl ether, KH_2PO_4, Na_2HPO_4 (Sigma-Aldrich, St Louis, MO, USA); β-glucuronidase from *E.coli* (Roche, Mannheim, Germany).

The derivatization reagent consists of 20 ml of N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA) (Macherey-Nagel, Düren, Germany), 100 mg of ammonium iodide and 40 µl of 2-propanethiol (Merck, Darmstadt, Germany).

Urine samples

Analyses were carried out in a set of doping control samples, which were sent to the laboratory within a period of about two months. Given that the athlete had agreed to investigations for research purposes, all samples (in- and out-of competition) were processed. Before starting analyses, urine samples were recoded to ensure anonymity according to WADA ISL.[25] In total 674

doping control samples were selected (421 male, 253 female subjects). The period between collection and analysis of urines was documented.

In accordance to the general procedure, A-samples had been stored in the refrigerator upon arrival, and were kept at −18°C after finishing the screening procedures. B-samples were stored frozen immediately after receipt in the laboratory, and were analyzed for confirmation if required.

An aliquot of 10ml of the A-sample was centrifuged (10min; 3000g), the supernatant was separated for steroid analysis and the pellet utilized for DNA preparation.

Steroid screening

Urine samples were prepared following the validated screening method of the laboratory. After centrifugation, 2ml of the supernatant were processed. Testosterone-d_3 was added as internal standard (IS; 200ng/ml). Hydrolysis was done at pH 6.5 (adjusted by aqueous buffer solution of KH_2PO_4/ Na_2HPO_4, 0.5M each) using β-glucuronidase from *E.coli*, for 1h at 55°C. Extraction of the cleaved steroids was carried out with t-butyl methyl ether by shaking for 10min. After evaporation of the organic layer to dryness, residue was treated with 40µl of the derivatization reagent to form bis-TMS derivatives, for 25min at 55°C. Samples were measured by GC-MS (Agilent GC 6890N coupled to MSD 5975B). A ZB-1ms GC column was applied (10m × 0.18mm ID × 0.18µm; Phenomenex, Aschaffenburg, Germany) with the following parameters: carrier gas helium (constant pressure 9.5 psi), injection volume 1µl (split injection 10:1), injector temperature 260°C, oven temperature program: initial 130°C (0min), 55 deg/min up to 185°C, 5deg/min up to 235°C, 30deg/min up to 325°C (hold for 2min). The following ions were monitored for quantification in selected-ion-monitoring (SIM) mode: *m/z* 432.3 (testosterone and epitestosterone), *m/z* 435.3 (IS).

Isotope ratio mass spectrometry

Analyses were done regarding the laboratory's routine protocol utilizing a GC/C/IRMS instrument (GC HP 6890 coupled via a combustion furnace to a Micromass IsoPrime stable isotope MS). Samples were selected for IRMS in case of a T/E ratio equal or greater than 4.0, as well as in suspicious findings in GC/MS screening according to WADA (2004).[15]

For this purpose, the isotopic ratio $^{13}C/^{12}C$ (delta unit ‰) of relevant testosterone metabolites (e.g. A, Etio, androstanediols) were determined and compared to that of urinary endogenous reference compounds (ERC). Appropriate ERC within the sample have to be unaffected by administration. Usually 11-hydroxyetiocholanolone, 11-hydroxyandrosterone, 11-ketoetiocholanolone, pregnanediol and pregnanetriol, respectively, are analyzed.

When the $^{13}C/^{12}C$ value measured for the metabolite(s) differs significantly i.e. by 3 delta units or more from that of the urinary reference steroid chosen, the results indicate the presence of an exogenous source of testosterone and/or related prohormones.

Quantification of luteinizing hormone

According to the laboratory's standard operating procedure, concentration of LH is measured by a chemiluminescent immunoassay system (IMMULITE). LH values in male are interpreted with respect to the urinary specific gravity as well as in connection to the GC/MS steroid profile, and may point to the exogenous uptake of anabolic androgenic substances. In our study, LH values below 1IU/L (at a specific gravity of 1.020) are chosen to trigger further investigations, for example, scrutiny of steroid profile and IRMS analysis.

Genotype® UGT test

DNA preparation

All pellets were resuspended in 300µl hyplex® lysis buffer (AmplexDiagnostics, Gars, Germany) and mixed well. These solutions were heated to 99°C on a heating block for 10min and centrifuged 2min at 10 000g. DNA isolation and purification was performed with the hyplex® PrepModule (AmplexDiagnostics) according to the manufacturer's instruction of use.

Multiplex Polymerase Chain Reaction (PCR)

The amplification was performed in a thermocycler with heated lid (Mastercycler personal, Eppendorf, Hamburg, Germany). Amplifications were carried out in 0.2-ml tubes (Eppendorf). 2µl of the primer mix and 1µl of nucleotide mix of the Genotype® UGT test system (AmplexDiagnostics) were added for each reaction. One unit of Tth-DNA polymerase (AmplexDiagnostics) was used. Finally, 5µl of the isolated and purified DNA was added to the PCR mix.

The cycling conditions were according to manufacturer's instructions: initial denaturation step at 94°C for 5min, 40 cycles of denaturation at 94°C for 25s, annealing at 52°C for 25s, and elongation at 72°C for 20s with increasing 1s per cycle. After a final elongation step of 3min at 72°C, the amplification products were used in reverse hybridization.

Reverse hybridization

Amplification products were heated at 95°C for 5min, cooled down immediately using an ice block and then 15µl was mixed with 150µl of cool hybridization buffer. 50µl of this mixture were given in the colour-coded microwell plate cavities in which specific capture oligonucleotide probes for the UGT2B17 gene and the human ß-globin gene (used as in internal control) are immobilized.

Hybridization of amplification products with immobilized oligonucleotide capture probes took place during incubation at 50°C for 30min. To remove unspecifically or not bound PCR products, three stringent washing steps with a prewarmed (50°C) washing solution were required. After an additional washing step with a second washing buffer, incubation with a peroxidase (POD) conjugate for 15min at 37°C, three washing steps and addition of tetramethylbenzidin (TMB) substrate as a developer changed into a blue colour in case of positive results during incubation for 15min at room temperature. Stopping the reaction by adding a stop solution results in a change of the blue colour into yellow. The samples were measured using a microwell plate photometer at 450nm. As reference, optical density at 630nm was also measured and the test result was obtained to subtracting A630 from A450. Values greater than OD (optical density)≥0.300 were defined as positive. For each run, a negative control (sterile water) was processed like the original samples.

When performing the Genotype® UGT PCR, an amplification product of the human β-globin gene is also produced that, after reversible hybridization with the specific internal control (IC) probe, should show a positive signal with an OD > 0.300.

If this is not the case, the internal control is negative and the test is, thus, invalid. An invalid test result can arise from an error made during the DNA extraction procedure that results in the failure to isolate DNA or from inappropriate human cell material in the urine sample. Consequently, only a true negative result (that is, the absence of UGT2B17 has been demonstrated in a correctly taken specimen from which DNA was subsequently successfully isolated) is passed on.

Results and Discussion

The Genotype® UGT test system based on the isolation of cells from urine, which naturally originate from the epithelia of the urinary tract and belong to the respective athlete. The main principle of the method is the detection of the unchanged sequence coding for UGT2B17, present either on one or both alleles of the genome. Therefore, the PCR-ELISA system is able to discriminate between the UGT2B17-positive phenotype (corresponds to the genotypes ins/del and ins/ins) and the homozygote UGT2B17-gene deleted subjects (del/del) by the photometric signal.

The detection is characterized by a 2-fold specificity to significantly identify the UGT2B17 gene. First, due to the use of appropriate primer binding within the functional gene, PCR would only result in an amplicon in case of UGT2B17 positives. Second, a purpose-made capture probe fitting in the sequence of exon 5 is used, which would specifically hybridize with the amplicon for photometric detection. A yellow colour indicates then the unaffected UGT2B17 gene in the sample.

The internal check (β-globin, blue coloration) ensures the validity of the test result. In case of a missing globin-signal, no cells have been isolated from urine and the test failed.

The Genotype® UGT kit has been applied to a representative set of doping control samples to provide epidemiological information about the distribution of the gene deletion. The procedure turned out to be a robust and sensitive method. From all 674 tested urines, only 18 samples (2.7%) gave a non-valid test result (Figure 1 'n.v.'). The ability to isolate cells from urine is adversely affected by the time of storage; however, sample characteristics like specific gravity or pH were not correlated.

The UGT2B17-positive phenotype (pos) was detected in 502 (74.5%) and the gene deletion (del) was identified in 154 samples (22.8%) (Figure 1). That means, the frequencies nearly correspond to the theoretically distribution of a dominant-recessive inheritance. In contrast, different occurrence of UGT2B17 deletion polymorphism has been reported before, significantly depending on ethnicity of the investigated subjects.[16] Due to the anonymous character of doping control samples, no information is available regarding ethnicity of the athletes in the present study. Furthermore, the analysis of multiple samples from the same athlete is possible and could also influence the results.

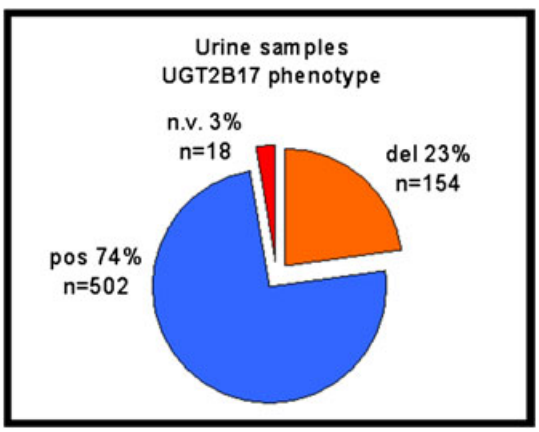

Figure 1. Results of UGT2B17-gene detection in 674 doping control samples utilizing the Genotype® UGT kit. Phenotype pos: genotypes ins/ins and ins/del. Phenotype del: genotype del/del. n.v.: test result not valid (extraction of cells from urine failed).

According to published data, evaluation of T/E ratios and concentrations of T (corrected for specific gravity[15]) resulted in significant differences between the UGT2B17-positive and the UGT2B17-deleted phenotype in urine samples ($p < 0.001$, t-test, Figure 2); however, E values were unaffected by the polymorphism.

The box plot represents the following urinary T/E values: -: T/E range; x: 1st and 99th percentile, resp.; Whisker: 5th and 95th percentile, resp.; Box: 25th and 75th percentile, resp.

Comparing with data from literature, the mean T/E ratio of the gene-deleted individuals in our study was higher then previously reported (mean value 0.9 in contrast to 0.14[13] and 0.29,[26] respectively). However, comparability of the results might be limited due to the diverse composition of the investigated populations, for example, age, ethnicity, number of individuals.

In detail, there were numerous samples with untypical elevated T/E values in our UGT-del group, even above the threshold of 4; and the median T/E of 0.5 does not indicate a normal curve of distribution in case of UGT2B17 deletion.

Further analyses have been carried out to investigate possible interferences and are discussed below, in particular to verify the UGT2B17 phenotype and to exclude testosterone administration.

We considered the following circumstances to deal with the incoherent findings for UGT2B17 phenotype and steroid concentrations:

Exogenous administration of testosterone

The application of testosterone or related substances has been investigated by IRMS analysis as well as by measurement of LH concentration (in male athletes). No suspicious results were detected. However, the application of testosterone preparations

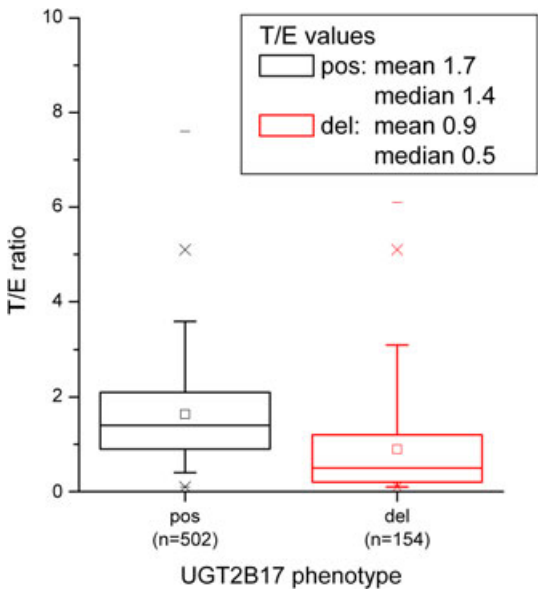

Figure 2. Distribution of T/E values in urine (674 doping control samples), depending on the UGT2B17 phenotype.

with a $^{13}C/^{12}C$ ratio similar to the endogenous values cannot be excluded definitely.

Inaccurate processing

In suspicious samples, identification of UGT2B17 phenotype using the Genotype® UGT kit was reprocessed in A- and B-sample, and additionally carried out in a second laboratory (AmplexDiagnostics). Repeatability of the results showed that blunders during the analytical process can be excluded, for example, interchanges of samples or detection errors due to non-sterile conditions in the doping control laboratory.

External contamination of specimen

One aim of the study was to check the suitability of the procedure in terms of doping control. Therefore, the real circumstances are considered, particularly non-sterile conditions during sampling (non-sterile containers) and during processing of urines. Basically, contamination of the urine specimens by cells from another person than the athlete has to be taken into account as a serious cause of errors. For example, a false phenotype in the urine of a real UGT2B17-positive athlete would be detected if a contamination of external cell material with a present UGT2B17 deletion occurred. Assuming that the urine was devoid of respective cells from the athlete, only external cells were prepared instead. The test system would then show a globin signal of the foreign cells, but no colouration for the UGT2B17 gene of the athlete. In such situations, an additionally independent urine or blood sample may be useful to clarify the UGT2B17 phenotype of the athlete.

Reliability of the test system

In case of nine samples with unexpected UGT2B17-deletion phenotype (Table 1), the functionality of the capture probe was examined by visualization of potential PCR products by standard agarose gel electrophoresis. In all nine cases, a ß-globin gene specific amplification product with a size of 171 base pairs (bp), but no PCR product of the UGT2B17 gene (194 bp) was detectable. Thus, the sensitivity and selectivity of the capture probe was affirmed without any indication of dysfunction.

Furthermore, as a confirmation method, an UGT2B17 exon 6 specific PCR was performed as described by Wilson *et al.*[20] Again, no specific amplification product (size 200 bp) was detected, confirming the UGT2B17-gene deletion in the selected samples. Accordingly, the accurate functionality of the Genotype® UGT kit was verified.

In conclusion, nine samples (UGT2B17-del and T/E greater than 1) have been comprehensively examined. The results are summarized in Table 1. All tests regarding the UGT2B17 polymorphism resulted in the deleted genotype, but the samples showed neither typical steroid characteristics for the gene deletion (concentrations of 5α-androstanediol and dihydrotestosterone, unpublished data) nor evidences for exogenous uptake of testosterone (LH, IRMS analyses). Following, the findings are discussed presuming either the UGT2B17 phenotype has been incorrectly or correctly identified.

Presumption: UGT2B17 deletion incorrectly detected

The phenotype 'UGT2B17-deleted' was detected in the urine sample of the athlete, despite the enzyme is in fact expressed. Reasons for the incorrect phenotyping could be a massive contamination of the specimen with cells from a person carrying the gene-deletion, as described previously. Another explanation could be an unknown modification of the gene sequence (e.g. a single-nucleotide polymorphism), which would prevent binding of PCR primers and formation of the amplicon in both methods (Genotype® UGT kit as well as confirmation method[20]); but would not influence biosynthesis and physiologically functionality of the enzyme. Therefore phase-II metabolism of steroids remains unaffected and steroid concentrations in urine are in the corresponding range.

To exclude external contamination of the specimen, the analysis of additional urine samples from the same athlete or the genotyping in blood samples are indicated.

To deal with the possibility of an interfered primer binding, the whole UGT2B17 gene sequence could be analyzed to enable the direct verification of the exon deletion.[27]

Presumption: UGT2B17 deletion correctly detected

Even though the enzyme UGT2B17 is actually deleted in the athlete, the steroid profile and T/E ratio does not show phenotype-characteristic values in urine. Assumedly, the lack of glucuronidation activity has been compensated by other UGT enzymes capable to conjugate T, for example, UGT2B15,

Table 1. Summarized results of selected urine samples of the study (T/E > 1; UGT2B17-gene deletion). Detection of the UGT2B17 gene was carried out in A and B samples using the Genotype® UGT kit (in two different laboratories), and confirmation method by gel electrophoresis. del: genotype del/del. n.v.: test result not valid.

		Doping analysis (A-sample)				UGT2B17 phenotype (A- and B-sample)		
Sample	Gender	Specific gravity	T/E ratio	LH [IU/L]	IRMS result	Genotype® UGT kit (Lab1)	Genotype® UGT kit (Lab2)	Confirmation method[15]
16	m	1.005	1.9	4.9	neg	del	del	del
49	m	1.008	1.8	4.5	neg	del	del	del
27	m	1.007	3.7	6.5	neg	del	del	del
32	m	1.025	1.5	18.1	neg	del	del	del
68	f	1.026	1.2	—	neg	del	del	del
83	m	1.014	1.2	6.3	neg	del	del	del
55	m	1.016	3.6	12.8	neg	del	del	del
50	m	1.026	4.4	11.0	neg	n.v.	del	del
82	m	1.025	1.6	5.8	neg	del	del	del

2B28, 1A8.[8] Additional polymorphisms are possible, as well as induction of further metabolising enzymes.

Analyses of uncleaved urinary conjugates may be helpful to identify the conformation and/or position of glucuronidation, and therefore get information about the involved glucuronosyltransferases.

Conclusion

A sensitive, reproducible test procedure has been established to identify the UGT2B17 phenotype in urine samples. Due to the easy handling of the UGT test, the method is suitable to implement in the analytical workflow of a doping control laboratory. Caused by the non-sterile sampling of urine, interferences cannot be excluded and therefore genotyping in blood samples was indicated in particular cases.

The deletion polymorphism of the enzyme UGT2B17 is linked with the conjugation of steroids and therefore has a direct influence on the results in doping control. The results of the study confirmed the correlation of the T/E ratio and the UGT2B17 phenotype, but the mean value of the deleted groups did not differ as substantially as reported in previous works. Therefore, it has been assumed that the UGT2B17 genotype is not the exclusive parameter which would allow an individual interpretation of T/E ratios for purposes of doping control. The investigations of nine atypical cases showed that other influences have to be taken into account in addition to the UGT2B17 polymorphism. In particular, further research regarding coexistent modifications of other enzyme activities seems to be necessary.

However, the identification of the UGT2B17 phenotype is important for gathering evidence in analysis of steroids and would provide additional information to improve efficiency of T detection in terms of doping control. Knowledge of the UGT2B17 phenotype of an athlete would complete the subject-based approach and the data registered in the athlete biologic passport.

SUPPORTING INFORMATION

The frequency of the UGT2B17-deletion polymorphism was investigated in German doping control samples by a PCR-ELISA system (Genotype® UGT test) established for urine specimen. dta_332_GTOC.doc]

Acknowledgements

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