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Strategy for detecting testosterone doping in Japan – The 1st year

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Abstract

The World Anti-Doping Agency (WADA) criteria of testosterone to epitestosterone (T/E) ratio for implementing isotope ratio mass spectrometry (IRMS) is not said to be enough to detect the testosterone doping in Japan where the prevalence of UGT2B17 deletion genotype is extremely high, even if IRMS is a sensitive method to detect exogenous testosterone. Therefore, Japan Anti-Doping Agency (JADA) and WADA accredited Tokyo Laboratory started the collaborative approach, longitudinal follow-up with individual steroid profiles and the target IRMS testing. We have performed the individual steroid profiling with subject to the 4,823 doping control samples collected from 3,509 Japanese athletes in the official doping control test in 2012 and their testing history during 2010 to 2012 provided by JADA. Only 13 specimens from 9 athletes showed T/E >4 and 9 specimens among these samples tested IRMS confirmatory analysis by the WADA's criteria, but 1,108 samples with T/E ≤4 were considered as negative by longitudinal follow-up with the individual steroid profiling without requiring IRMS confirmatory analysis to judge the samples as negative. In the first year of the study, the target tests with IRMS analysis were also implemented against 458 athletes in high risk sports for doping, and the IRMS results of them were not consistent with the administration of exogenous testosterone related steroids. We realized the necessity of intelligent unannounced testing based on the athlete's passport information such as athlete biological passport.

Introduction

We reported the prevalence of UGT2B17 deletion genotype was extremely high in Japanese athletes (male: 74.5%, female: 60.2%) [1]. Isotope ratio mass spectrometry (IRMS) is a sensitive method to detect the testosterone misuse, but there is some truth in suggestions that the testosterone doping had been missed in Japan by the confirmatory analysis based on the World Anti-Doping Agency (WADA) criteria of testosterone to epitestosterone (T/E) ratio above 4 [2]. Donike *et al.* proposed the longitudinal studies and establishment of subject based reference ranges for parameters of the steroid profile, especially for the T/E ratio [3,4]. The IRMS technique is labour-intensive and costly, thereby making it impractical for analyzing multiple urine samples. Against these backgrounds, Japan Anti-Doping Agency (JADA) and WADA Tokyo laboratory started collaborative approach with a view to establish new testing strategies for detecting testosterone doping in Japan.

Experimental

Analysis of steroid profiles and carbon isotope ratios
Urine samples were prepared according to the common steroid analytical procedure validated for sports doping tests [1,2].

Testing Strategy
During January 2012 to January 2013, a total of 4,823 urine samples collected from 3,509 Japanese athletes (male: 2,300, female: 1,209) by JADA were analyzed in WADA Tokyo laboratory as in-competition test (ICT) and out-of-competition test (OOCT).

Steroid profiling with longitudinal follow-up
In all Japanese athletes received official doping control test during January 2012 to January 2013, WADA Tokyo laboratory

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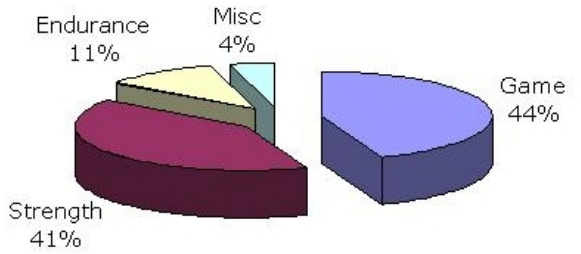
has performed the individual steroid profiling with comparing to the past steroid data provided from JADA. JADA informed the athlete's testing histories to the laboratory as a new Scheme. All the samples were anonymous and don't include the athlete's individual data except for gender and sports (Table 1).

Target IRMS test
Target IRMS tests to 458 (male: 382, female: 76) athletes of 3,509 athletes in high-risk sports were also conducted for investigating the testosterone abuse among Japanese athletes. JADA have selected sports as shown in Figure 1, with the reason listed as below;
(1) The sports that use power, use power in repetition, or use inner muscle to stabilize body;
(2) The sports that athletes are tending to take supplements, especially some kinds of protein;
(3) For ICT, we selected the sports that we do not or we have only few athletes in our Registered Testing Pool (RTP). In OOCT, 26 athletes are selected from RTP. In ICT, we targeted the events that are the national championships or the highest league. Therefore, 432 athletes are high level athletes in the sports in Japan.

Game: Football, Handball, Baseball, American Football, Volleyball
Strength: Wrestling, Bodybuilding, Sumo, Powerlifting, Weightlifting, Judo, Karate
Endurance: Rowing, Cycling, Skating, Swimming
Misc: Dance Sports, Athletics, Golf, Gymnastics



IRMS: Negative	
male athletes (n=382)	female athletes (n=76)
T/E > 4: n=1	T/E > 4: n=0
1<T/E ≤4: n=62	1<T/E ≤4: n=12
T/E ≤1: n= 319	T/E ≤1: n= 64

Figure 1: Target IRMS testing in Japan (n =458, May 2012 to Jan. 2013)

Results and Discussion

4,823 doping control samples collected from 3,509 Japanese athletes (male: 2,300, female: 1,209) were analyzed in WADA Tokyo laboratory and compiled their steroid profiles as shown in Figure 2. Only 13 specimens (0.3%) from 9 athletes showed T/E >4 as shown in Table 1, 9 specimens among these samples tested negative by IRMS. However, the fact remains that the specimens with T/E ≤4 were simply considered as "negative" only based on the current WADA criteria. We realized the necessity of new scheme to prevent missed testosterone doping. Then, in the official doping control test, Tokyo laboratory has performed the individual steroid profiling of all Japanese athletes, based on their testing history during 2010 to 2012.
With the exception that T/E value showed to change from basal value of 1.5 to 2.4 and the results of the IRMS confirmation analysis was negative, the 1,108 samples with T/E≤4 were considered as negative because their T/E values showed to vary from individual basal values by less than 30% for male and 60% for female [2], as shown in Table 1. Thus, 23% samples with T/E ≤4 were verified based on the individual testing history and there were no need to IRMS tests to them. Also in the 2 cases which androsterone (A) or dehydroepiandrosterone (DHEA) concentrations were greater than 10,000 and 100 ng/ml respectively, the IRMS results were negative.

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Target tests were implemented against 458 (13%) athletes in high risk sports for doping, but the IRMS results of them were not consistent with the administration of exogenous testosterone related steroids, the mean of T/E ratios for male and female were 0.49 and 0.44, respectively and the sample with T/E>4 was from only one male, as shown in Figure 2 and Table 1.

	Testing history 2010 to 2012	Compiled data of 2012				
Number of Athletes	Number of tests	Number of sample	IRMS based on WADA criteria	Number of avoided IRMS by longitudinal variation	Target IRMS	Results
T/E > 4						
A	6	2	1	1	0	negative
B	4	3	1	2	0	negative
C	2	2	1	0	0	negative
D	1	1	0	0	1	negative
E	1	1	1	0	0	negative
F	1	1	1	0	0	negative
G	1	1	1	0	0	negative
H	1	1	1	0	0	negative
I	1	1	1	0	0	negative
9		13	8	3	1	
T/E ≤ 4						
316	≥3	1,109	0	1,108	44	negative
517	2	1,034	1[*1]	0	85	negative
2,667	1	2,667	1[*2]	0	328	negative
3,500		4,810	2	1,108	458	
Total						
3,509		4,823	10	1,111	459	

*1: DHEA>100ng/ml
*2: A>10,000ng/ml
Table 1: Summary of steroid profiling for Japanese athletes in 2012

Conclusions

This project started one year prior to implementing Athlete Passport Management Unit, established in WADA Tokyo laboratory, against the risk of false negative of testosterone doping in Japan. Accordingly, profiling with athlete's testing history were carried out for 23% of Japanese athlete and IRMS tests were conducted to 13% of subjects in high risk sports for doping. Although there was no adverse analytical finding of testosterone related substances, we intend to continue the profiling with longitudinal follow-up and the targeted IRMS tests to more various sports. We realized the necessity of intelligent unannounced testing based on the passport information [5,6]. Ultimately the ideal testing structure will be completed when the steroid profiling module in ADAMS is really utilized [7].

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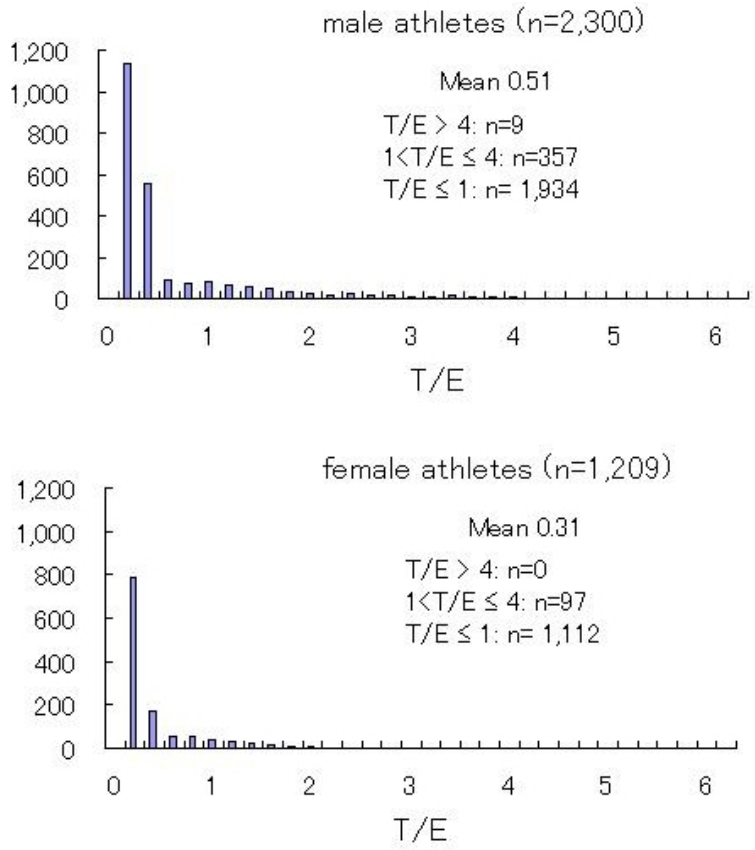

Figure 2: Distribution of T/E ratio in Japanese athletes (Jan. 2012 to Jan. 2013)

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