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Identification of Athletic Human Growth Hormone Gene Polymorphisms

Mohammed H Awwad* and Hany A. Eldeeb**
* Department of Zoology, Faculty of Science, Benha University.
** Department of Training sports, Faculty of Physical Education, Monofia University.

Abstract

Background: PCR/RFLP of Human growth hormone gene was used to differentiate among ten athletes.

Results: *Aat*I, *Avi*II, *Bsa*I, *Hind*II, and *Sac*I restriction endonucleases gave high levels of intraspecific profile for all the studied gene of the ten athletes. Other enzymes including *Ava*I, *Age* and *Dra*I were tested for their ability to differentiate these individuals.

Conclusion: The study demonstrated that the HGH gene contains useful genetic markers for the identification of athletes.

Key words: ***Polymorphisms, Athletes, PCR/RFLPs, HGH gene*.**

Introduction

Human growth hormone (GH) affects a wide variety of physiological parameters such as growth performance, carcass composition, and milk production (Chung *et al*., 1983; Bauman *et al*., 1985 and Etherton *et al*., 1986). The genomic structure of the GH gene has been studied in different animals, including rat (Barta *et al*., 1981), bovine (Woychik *et al*., 1982), sheep (Byrne *et al*., 1987), pig (Vize and Wells 1987), human (Fiddes *et al*, 1979; Roskam *et al*., 1979 and DeNoto *et al*., 1981), goat (Kioka *et al*., 1989), chicken (Tanaka *et al*., 1992), and mice (Das *et al*., 1996). These animals share a similar gene structure containing five exons and four introns.

HGH is a polypeptide hormone suspected of being used by elite athletes to enhance sporting performance. Discovery of recombinant human GH (rhGH) in the possessions of Chinese swimmers bound for the 1998 World Swimming Championships and similar problems at the Tour de France cycling event in 1998 strongly suggest the abuse of GH at an elite level. This problem may affect the broader community, as shown by a report of GH use in high-school students in the U.S. (Rickert *et al*., 1992).

The rationale for the use of exogenous GH to enhance athletic performance is multifactorial. GH administration during childhood may augment adult height. GH's anabolic and lipolytic actions (Press, 1988) are demonstrated in studies in adults with GH deficiency, where modest doses of rhGH have been shown to increase lean body mass, skeletal muscle mass, proximal muscle force, and maximal and submaximal aerobic performance and to reduce fat mass (Cuneo *et al*., 1992). Other potentially beneficial effects of GH administration in adults with GH deficiency include increased cardiac output during exercise, increased sweating rates and improved thermal homeostasis, lipolysis to provide fuel for endurance sports, and possibly enhanced ligamentous strength and wound-healing rates. The side-effects of supraphysiological doses of rhGH include sodium and water retention (acute onset) and accelerated osteoarthritis, hypertension, cardiac failure, and an increased incidence of malignancies (delayed onset). Such concerns do not appear to deter abuse.

The International Olympic Committee and major sporting bodies ban administration of GH by athletes to enhance performance, but there is currently no approved means of detection. Measurement of serum or urinary total GH itself is unlikely to represent an optimal detection method because: 1) exogenous rhGH and endogenous GH have identical amino acid sequences, making chemical or immunological distinction difficult; 2) normal serum GH concentrations fluctuate widely, reflecting endogenous pulsatility and responses to stress and exercise (Cuneo

and Wallace, 1994); 3) measurement of urinary GH is relatively insensitive as a marker of GH administration or acromegaly (Saugy *et al*., 1996 and Flanagan *et al*., 1997); and 4) chemical tagging of rhGH by pharmaceutical manufacturers would not solve the problem of unlicensed manufacture. Therefore, markers of GH action are being investigated as a potential test for GH abuse.

GH exerts major regulatory influences over several components of the GH/insulin-like growth factor (IGF) system. For example, GH modulates its own receptor expression and the circulating form of the extracellular component of the receptor, the high affinity GH-binding protein (GHBP) (Leung *et al*., 1987). GH exerts powerful stimulatory regulation over IGF-I, a protein produced in many tissues, where it exerts anabolic and mitogenic actions, and to a lesser extent over IGF-II. The IGFs are transported in serum bound to a number of IGF-binding proteins (IGFBPs), the predominant form comprising the ternary complex of IGF-I, IGFBP-3, and acid labile subunit (ALS) (Baxter, 1994). Responses of the GH/IGF system to rhGH administration are well described in GH-deficient adults (Rahkila, 1991), but data in normal adults and athletes are limited (Crist *et al*., 1988; Yarasheski *et al*., 1992; 1993 and Deyssig *et al*., 1993). The ratio of IGF-I/IGFBP-2 at rest has been proposed as a test for GH abuse (Kicman *et al*., 1997). Acute exercise, however, may also influence markers of GH action. For example, serum IGF-I (Bang *et al*., 1990; Cappon *et al*., 1994 and Schwarz *et al*., 1996) and IGFBP-1 (Suikarri *et al*., 1989 and Koistinen *et al*., 1996) both increase transiently after acute exercise. In developing a GH detection system, the rate of disappearance of changes on markers of GH action must be described, but data in athletes either before or after exercise are unknown.

Height (Ht) is determined by genetic, as well as environmental factors. The involvement of genetic factors in Ht is supported by several reports about the significant correlation between final Ht and parental Ht and about the growth of twins (Tanner *et al*., 1970; Smith, 1977; Martheny, 1990; Phillips and Matheny, 1990; Tsutomu *et al*., 1990 and Pai *et al*., 1994).

The effect of GH secretion on Ht during childhood is well established. Human with a deletion in the *GH-1* gene are extremely short (Illig *et al*., 1971 and Phillips *et al*., 1981). Ht during childhood correlates with GH secretion determined by measurements of either GH levels integrated over 24 h or serum GH-dependent parameters, such as insulin-like growth factor (IGF) I and IGF binding protein-3 (Albertsson-Wikland and Rosberg, 1988 and Blum *et al*., 1993). Recently, in many endocrine disorders, polymorphisms of relevant human genes have been reported to be associated with polygenic disease. Examples of this includes the b-*3 adrenergic receptor* gene in noninsulin-dependent diabetes mellitus and obesity (Clement *et al*., 1995; Walston *et al*.; 1995 and Widen *et al*., 1995) and the *Vitamin D receptor* gene in osteoporosis (Morrison *et al*., 1994).

The present study attempted to identify polymorphisms in the *GH* gene. Also, The aim of the study is to investigate the use of restriction map resulting from digestion of the HGH genes of athletes for diagnosis and selection at molecular level.

Material and Methods

Subjects: Ten healthy, adult athletes between the ages of 16 and 18 years were recruited for this study. DNA was extracted from whole blood using standard techniques (Higuchi 1989). Obtain 65-100 µl of blood with a heparinized tube. The blood expel immediately into a 1.5 ml microfuge tube containing 20 µl of 10 mM EDTA. Mix immediately to prevent clot formation. Store on ice until processing. Add 200 µl Lysis Buffer (0.32 M Sucrose; 10 mM Tris-HCl (pH 7.5); 5 mM MgCl2; 1% v/v Triton X-100) to each tube and vortex to suspend evenly. Microfuge 60 seconds at 5000 xg to pellet nuclei. Remove and discard supernatant and repeat the steps two more times, or until no hemoglobin remains. Resuspend nuclear pellet in 100 µl PBND (50 mM KCl; 10 mM Tris-HCl (pH 8.3); 2.5 mM MgCl2; 0.1 mg/ml gelatin; 0.45% (v/v) Nonidet P40 and 0.45% (v/v)

Tween 20) with 60 µg/ml proteinase K and incubate at 55 ^{o}C for 60 minutes (or overnight, if convenient). Heat samples to 97 ^{o}C for 10 minutes to inactivate proteinase K. One µl of the resuspended pellet was checked by agarose gel electrophoresis for the presence of DNA, as in figure 1.

Polymerase Chain Reaction Amplification: To amplify the complete GH gene, one-5 µl DNA of whole-cell DNA template was used plus oligonucleotide primers complementary either to the 5` and 3` ends of the gene (1F1: 5´-GAATTCAGGACTGAATCGTGC-3´, and 1R2631: 5´-TCAACAGGCATCTACT-GAGTGGA- 3´). The standard PCR reaction mixture was used (Kessing *et al.*, 1989). The standard polymerase chain reaction program for amplification of GH gene was: 30-35 cycles; one minute, 94^{o}C; two to three minutes, 45^{o}C; and three minutes, 72^{o}C. Deoxynucleotide triphosphates (dNTP; dATP, dGTP, dTTP, and dCTP) were from Perkin Elmer Cetus, USA. The taq DNA polymerase used for HG gene amplification was from Boehringer Mannheim Biochemica (Germany) and Gibco/BRL (Gaithersburg, Md., USA). One µl of the PCR products was checked by gel electrophoresis for the presence of HG gene size (~2650 bp), as in figure 2.

Restriction fragment length polymorphisms(RFLPs): RFLPs of the PCR products by the primers 1F1 and 1R2631, which covered the entire gene, were checked in 10 samples of the subjects. The enzymes used were *Aat*I, *Age*I, *Ava*I, *Avi*II, *Bsa*I, *Dra*I, *Hind*II, and *Sac*I.

Results

DNA genome was extracted from ten training physical human between the ages of 16 – 18 years (Fig. 1). The full-length Human Growth Hormone gene PCR products resulted in a product of approximately 2650 bp (Fig. 2).

*Age*I, *Ava*I and *Dra*I restriction endonucleases did not differentiate between the ten samples of the Human Growth Hormone gene. *Age*I restriction enzyme digested the human Growth Hormone gene of the ten samples into two restriction fragments (~100 and ~2550 bp; Fig. 3 and Table 1). *Ava*I restriction endonuclease cut the gene of the ten samples into four restriction bands (~300, ~320, ~660 and ~1370 bp; Fig. 4 and Table 2). Also, *Dra*I restriction enzyme fragmented the gene of the ten individuals into two fragments (~150 and ~2500 bp; Fig. 5 Table 3).

*Avi*II, *Bsa*I, *Aat*I, *Hind*II and *Sac*I restriction endonucleases clustered the ten individuals into two clusters with some differences. *Avi*II restriction enzyme digested the gene of the samples (1-4 and 7-9) into three restriction fragments (~750, ~900 and ~1000 bp) and the samples (5, 6 and 10) into four fragments (~200, ~550, ~900 and ~1000 bp) (Fig. 6 and Table 4). *Bsa*I restriction endonuclease grouped the ten individuals into two groups when cut the gene samples (1-6 and 9) into six restriction bands (~150, ~200, ~250, ~350, ~650 and ~1050 bp) and did not digest the gene of samples 7, 8 and 10 (Fig. 7 and Table 5). *Aat*I enzyme differentiated the ten individuals into two clusters when digested the gene of samples 1-5 and 9 into two restriction fragments (~950 and ~1700 bp) and the samples 6-8 and 10 into three bands (~650, ~950 and ~1050 bp); Fig. 8 and Table 10. *Hind*II restriction endonuclease grouped the individuals into two groups when digested the gene of samples 1-5 and 9 into two restriction bands (~450 and ~2200 bp) and the gene of samples 6-8 and 10 into four bands (~100, ~350, ~650 and ~1550 bp); Fig. 9 and Table 7. Also, *Sac*I restriction enzyme clustered the ten individuals into two clusters when the enzyme digested the gene of samples 1-6 into two bands (~1050 and ~1600 bp) and did not react with the gene of 7-10 samples (Fig. 10 and table 8).

Table (1): The length of HGH genes fragments, resulted from digestion with *Age*I enzyme in the investigated athletes. (see Fig. 3)

	Band #1	Band # 2	Band # 3	Band # 4	Band # 5	Band # 6
Sample # 1	~100	~2550	…….	…….	…….	…….
Sample # 2	~100	~2550	…….	…….	…….	…….
Sample # 3	~100	~2550	…….	…….	…….	…….
Sample # 4	~100	~2550	…….	…….	…….	…….
Sample # 5	~100	~2550	…….	…….	…….	…….
Sample # 6	~100	~2550	…….	…….	…….	…….
Sample # 7	~100	~2550	…….	…….	…….	…….
Sample # 8	~100	~2550	…….	…….	…….	…….
Sample # 9	~100	~2550	…….	…….	…….	…….
Sample # 10	~100	~2550	…….	…….	…….	…….

Table (2): The length of HGH genes fragments, resulted from digestion with *Ava*I enzyme in the investigated athletes. (see Fig. 4)

	Band #1	Band # 2	Band # 3	Band # 4	Band # 5	Band # 6
Sample # 1	~300	~320	~660	~1370	…….	…….
Sample # 2	~300	~320	~660	~1370	…….	…….
Sample # 3	~300	~320	~660	~1370	…….	…….
Sample # 4	~300	~320	~660	~1370	…….	…….
Sample # 5	~300	~320	~660	~1370	…….	…….
Sample # 6	~300	~320	~660	~1370	…….	…….
Sample # 7	~300	~320	~660	~1370	…….	…….
Sample # 8	~300	~320	~660	~1370	…….	…….
Sample # 9	~300	~320	~660	~1370	…….	…….
Sample # 10	~300	~320	~660	~1370	…….	…….

Table (3): The length of HGH genes fragments, resulted from digestion with *Dra*I enzyme in the investigated athletes. (see Fig. 5)

	Band #1	Band # 2	Band # 3	Band # 4	Band # 5	Band # 6
Sample # 1	~150	~2500	…….	…….	…….	…….
Sample # 2	~150	~2500	…….	…….	…….	…….
Sample # 3	~150	~2500	…….	…….	…….	…….
Sample # 4	~150	~2500	…….	…….	…….	…….
Sample # 5	~150	~2500	…….	…….	…….	…….
Sample # 6	~150	~2500	…….	…….	…….	…….
Sample # 7	~150	~2500	…….	…….	…….	…….
Sample # 8	~150	~2500	…….	…….	…….	…….
Sample # 9	~150	~2500	…….	…….	…….	…….
Sample # 10	~150	~2500	…….	…….	…….	…….

Table (4): The length of HGH genes fragments, resulted from digestion with *Avi*II enzyme in the investigated athletes. (see Fig. 6)

	Band #1	Band # 2	Band # 3	Band # 4	Band # 5	Band # 6
Sample # 1	~750	~900	~1000	…….	…….	…….
Sample # 2	~750	~900	~1000	…….	…….	…….
Sample # 3	~750	~900	~1000	…….	…….	…….
Sample # 4	~750	~900	~1000	…….	…….	…….
Sample # 5	~200	~550	~900	~1000	…….	…….
Sample # 6	~200	~550	~900	~1000	…….	…….
Sample # 7	~750	~900	~1000	…….	…….	…….
Sample # 8	~750	~900	~1000	…….	…….	…….
Sample # 9	~750	~900	~1000	…….	…….	…….
Sample # 10	~200	~550	~900	~1000	…….	…….

Table (5): The length of HGH genes fragments, resulted from digestion with *Bsa*I enzyme in the investigated athletes. (see Fig. 7)

	Band #1	Band # 2	Band # 3	Band # 4	Band # 5	Band # 6
Sample # 1	~150	~200	~250	~350	~650	~1050
Sample # 2	~150	~200	~250	~350	~650	~1050
Sample # 3	~150	~200	~250	~350	~650	~1050
Sample # 4	~150	~200	~250	~350	~650	~1050
Sample # 5	~150	~200	~250	~350	~650	~1050
Sample # 6	~150	~200	~250	~350	~650	~1050
Sample # 7	~2650	…….	…….	…….	…….	…….
Sample # 8	~2650	…….	…….	…….	…….	…….
Sample # 9	~150	~200	~250	~350	~650	~1050
Sample # 10	~2650	…….	…….	…….	…….	…….

Table (6): The length of HGH genes fragments, resulted from digestion with *Aat*I enzyme in the investigated athletes. (see Fig. 8)

	Band #1	Band # 2	Band # 3	Band # 4	Band # 5	Band # 6
Sample # 1	~950	~1700	…….	…….	…….	…….
Sample # 2	~950	~1700	…….	…….	…….	…….
Sample # 3	~950	~1700	…….	…….	…….	…….
Sample # 4	~950	~1700	…….	…….	…….	…….
Sample # 5	~950	~1700	…….	…….	…….	…….
Sample # 6	~650	~950	~1050	…….	…….	…….
Sample # 7	~650	~950	~1050	…….	…….	…….
Sample # 8	~650	~950	~1050	…….	…….	…….
Sample # 9	~950	~1700	…….	…….	…….	…….
Sample # 10	~650	~950	~1050	…….	…….	…….

Table (7): The length of HGHgenes fragments, resulted from digestion with *Hind*II enzyme in the investigated athletes. (see Fig. 9)

	Band #1	Band # 2	Band # 3	Band # 4	Band # 5	Band # 6
Sample # 1	~450	~2200	…….	…….	…….	…….
Sample # 2	~450	~2200	…….	…….	…….	…….
Sample # 3	~450	~2200	…….	…….	…….	…….
Sample # 4	~450	~2200	…….	…….	…….	…….
Sample # 5	~450	~2200	…….	…….	…….	…….
Sample # 6	~100	~350	~650	~1550	…….	…….
Sample # 7	~100	~350	~650	~1550	…….	…….
Sample # 8	~100	~350	~650	~1550	…….	…….
Sample # 9	~450	~2200	…….	…….	…….	…….
Sample # 10	~100	~350	~650	~1550	…….	…….

Table (8): The length of HGHgenes fragments, resulted from digestion with *Sac*I enzyme in the investigated athletes. (see Fig. 10)

	Band #1	Band # 2	Band # 3	Band # 4	Band # 5	Band # 6
Sample # 1	~1050	~1600	…….	…….	…….	…….
Sample # 2	~1050	~1600	…….	…….	…….	…….
Sample # 3	~1050	~1600	…….	…….	…….	…….
Sample # 4	~1050	~1600	…….	…….	…….	…….
Sample # 5	~1050	~1600	…….	…….	…….	…….
Sample # 6	~1050	~1600	…….	…….	…….	…….
Sample # 7	~2650	…….	…….	…….	…….	…….
Sample # 8	~2650	…….	…….	…….	…….	…….
Sample # 9	~2650	…….	…….	…….	…….	…….
Sample # 10	~2650	…….	…….	…….	…….	…….

Figure 1: Total DNA genome from ten athletes.

Figure 2: Full-length of HGH gene of the ten athletes.

Figure 3: The length of HGH genes fragments, resulted from digestion with *Age*I enzyme in the investigated athletes.

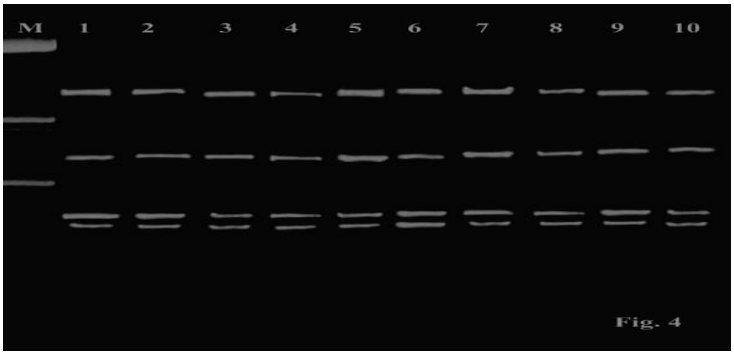

Figure 4: The length of HGH genes fragments, resulted from digestion with *Ava*I enzyme in the investigated athletes.

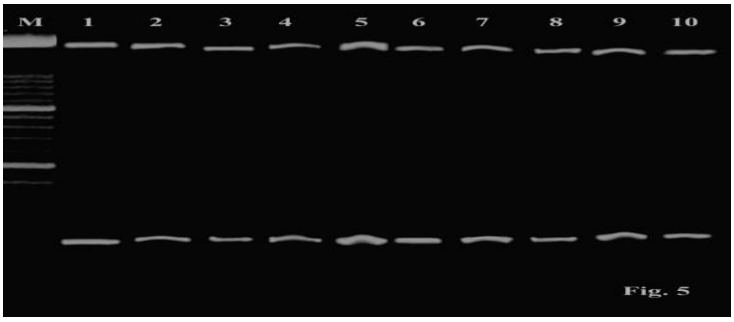

Figure 5: The length of HGH genes fragments, resulted from digestion with *Dra*I enzyme in the investigated athletes.

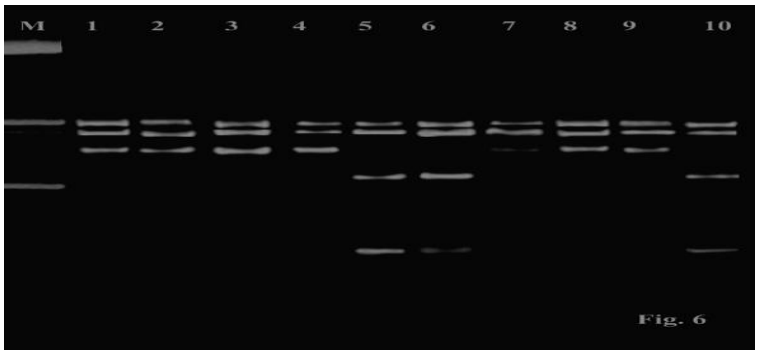

Figure 6: The length of HGH genes fragments, resulted from digestion with *Avi*II enzyme in the investigated athletes.

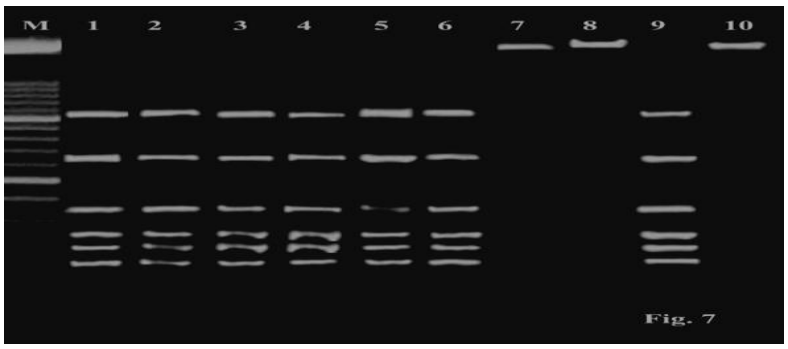

Figure 7: The length of HGH genes fragments, resulted from digestion with *Bsa*I enzyme in the investigated athletes.

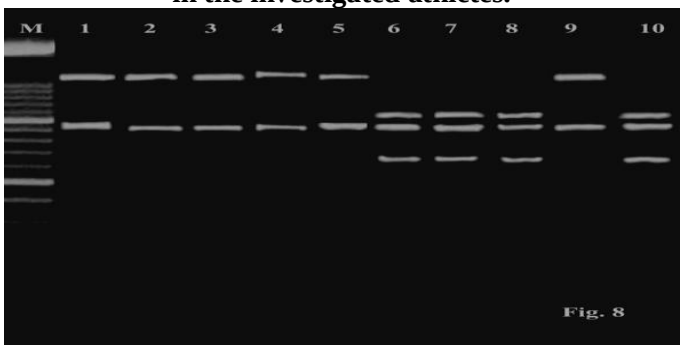

Figure 8: The length of HGH genes fragments, resulted from digestion with *Aat*I enzyme in the investigated athletes.

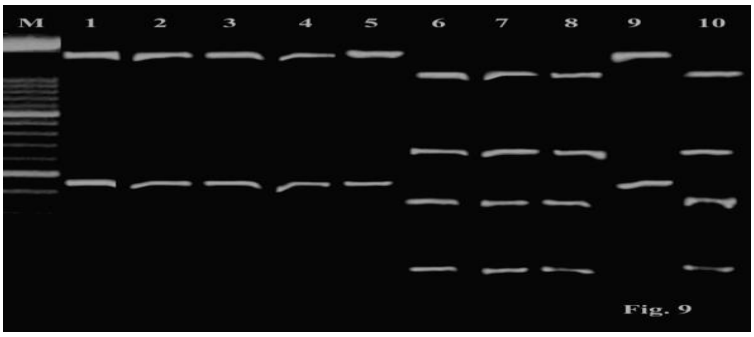

Figure 9: The length of HGH genes fragments, resulted from digestion with *Hind*II enzyme in the investigated athletes.

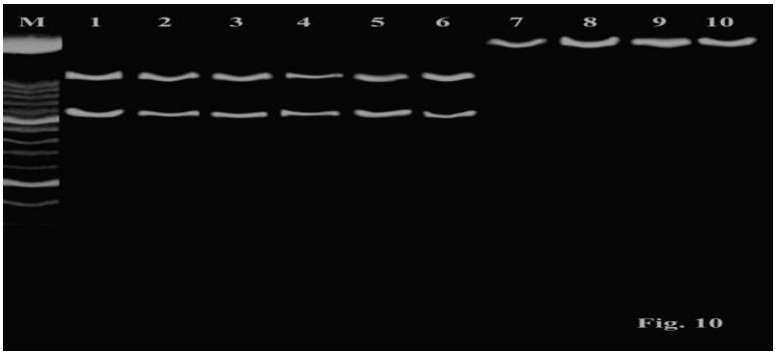

Figure 10: The length of HGH genes fragments, resulted from digestion with *Sac*I enzyme in the investigated athletes.

Discussion

In recent years, DNA polymorphisms have been widely studied in the GH gene of various animals (Stephen *et al*., 2001). Some polymorphisms in the *GH* gene were identified in this study, which may explain part of the genetic determination of GH at the molecular level. Polymorphisms in the *GH* gene may also be associated with idiopathic GH deficiency. The molecular mechanism of variation in GH has not yet been fully analyzed. Although more than 10 polymorphic sites of the *GH* gene were reported (DeNote *et al*., 1981, Chen *et al*., 1989, Giordano *et al*., 1997 and Wagner *et al*., 1997).

A point mutation of the coding or noncoding *GH* gene was reported to cause the absence or decrease in GH secretion (Duquesnoy *et al*., 1990; Cogan *et al*., 1993; Binder *et al*., 1996; Cogan *et al*., 1996 and Missarelli *et al*., 1997), which allows for the hypothesis that the polymorphisms of the *GH* gene may be considered as part of the continuous spectrum of mutations in this gene. The study of the molecular mechanisms of this phenomenon would be worth pursuing.

The PCR/RFLP analysis of the HG gene has proven to be effective in diagnosis and selection studies of athletes to refute the classical identification and to estimate genetic similarities and differences among them. The molecular data emerged as a useful tool for studying athletes with great phenotypic plasticity (Oriola *et al*., 1991 and Parry *et al*., 1991 and Hasegawa *et al*., 2006).

With these genotypically defined groups as a basis, a PCR-linked restriction fragment length polymorphism (RFLP) was devised for rapid identification of the ideal athletic individuals. The research results indicated that the first four specimens of the ten athletic individuals are polyphylogenic

when compared to the rest of the ten individuals. Also, the results proved that the first four individuals were the most suitable for athletic selection.

Inexpensive way to resolve this dilemma was to use an existing method of studying relationships among the ten athletes in which phylogeny is reconstructed on the basis of HGH gene RFLPs. RFLPs are dissimilarities in electrophoretic profile patterns. The contribution was to find the sites in the HGH genes where some restriction endonucleases act. Many investigators (Rivera *et al*., 1998; Wolfforth *et al*., 2000; Nakamura *et al*, 2002 and Shneider *et al*., 2004**)** have used restriction fragment length polymorphisms (RFLPs) of some genes as a basis for examining relationships among fitness, height and weigh of the athletes. Using restriction enzyme analysis of the HGH gene as an experimental tool to examine athletes proved to be suitable to find specific enzymes for selection of individuals.

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التعرف على تعدد أشكال جين هرمون النمو البشرى للرياضيين

محمد حسين عواد * و هانى الديب**

*- قسم علم الحيوان – كلية العلوم – جامعة بنها

**- قسم التدريب الرياضى – كلية التربية الرياضية – جامعة المنوفية

أصبحت متطلبات عمليات التدريب الرياضي وتطوير القدرات الوظيفية في الآونة الأخيرة مصدر ضروري يعتمد على الأساليب العلمية والطرق التدريبية المقننة التي تعتمد على علم البيولوجى بالإضافة إلى العلوم الأخرى. وقد أصبح من الأهمية أن يتعرف ويتفهم العاملون في مجال التدريب الرياضي على ما يحدث داخل أجسامنا من وظائف وعمليات تقوم بها أجهزة الجسم المختلفة حتى يستطيع أن يتكيف مع العمل الرياضي الذي يقوم به ويواجه التعب.

إن عملية الانتقاء الرياضي تعتبر المشكلة العصرية والرئيسية للعلماء المهتمين بشئون التربية البدنية والرياضية، وهذا يرجع إلى التقدم السريع والمتلاحق للمستوى الرقمي والإنجاز الرياضي في المنافسات والمسابقات الرياضية المختلفة.

على الرغم من أن التدريب والإعداد يؤديان إلى الارتقاء بمستوى مواصفات وقدرات الرياضيين إلا أن الجينات تلعب الدور الأكبر في تحقيق الرياضيين للمستويات العالمية، حيث أتضح أن العديد من القدرات من التحمل العام والتي كان يعتقد في الماضي أن التدريب هو الأساس في التطوير فقد ثبت أنه لا يمكن الارتقاء إلا بنسبة لا تتجاوز 25% من المستوى الموجود عند الرياضي من الوراثة، كما أن هناك مواصفات أخرى (مورفولوجية) لا تتغير كثيراً تحت تأثير العملية التدريبية.

وعليه تم استخلاص جين هرمون النمو من الحمض النووى اللاأوكسى ريبوزى لعشرة من الرياضيين وذلك عن طريق استخدام بادئين ذات تتابع نيوكليوتيدى محدد كالاتى:

1F1: 5′-GAATTCAGGACTGAATCGTGC-3′

1R2631: 5′-TCAACAGGCATCTACTGAGTGGA- 3′

واستخدمت فى هذه الدراسة ثمانية أنواع من إنزيمات القصر لهضم جين هرمون النمو للاعبين العشرة وهى:

*Aat*I, *Age*I, *Ava*I, *Avi*II, *Bsa*I, *Dra*I, *Hind*II, and *Sac*I

وذلك للحصول على أطوال القطع المحددة والمتغايرة لجين هرمون النمو موضع الدراسة.

وكانت أبرز النتائج كالتالى:

1- لم تظهر فروق واضحة بين العينات المدروسة عند استخدام إنزيم القصر (*Age*I, *Ava*I و*Dra*I) بينما بينت الإنزيمات الأخرى اختلافات واضحة بين باقى العينات.

2- وجود درجات متفاوتة من الاختلاف بين العينات المدروسة عند استخدام باقى إنزيمات القصر *(AatI, BsaI AviII, HindII SacI)*

3- وجد من خلال الدراسة البيولوجية الجزيئية لهرمون النمو للعينات أن هناك أربع لاعبين يعتبروا من أفضل ما يمكن أن ينتقوا ويليهم باقى اللاعبين وبمستويات متفاوتة.

لذلك فانه من المعتقد أن استخدام طرق البيولوجيا الجزيئية تعطى صورة انتقائية تصنيفية أكثر دقة عن استخدام طرق التصنيف التقليدية. وبالرغم من أن الدراسة على جين واحد ليست كافية إلا انه يمكن استخدام بعض إنزيمات القصر المستخدمة فى هذه الدراسة, خاصة تلك التى أظهرت فروقاً بين العينات, كمجسات للتعرف والتمييز والانتقاء للاعبين.