Genetic and cellular approaches to doping and doping detection

CHINADA/WADA
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Genetics in Sport – a two-edged sword
I. GENETIC DOPING
Improving athletic performance

- Modify environmental factors: opportunity, access to coaching, training.
- "Traditional" drug-based to modify organ function
- Modify organ function by genetic modification (gene doping)
Human genetic modification

to cure disease – “gene therapy”

to enhance normal traits – gene doping
CLASSICAL APPROACH TO THERAPY

A → B → C → disease
disease
How is gene therapy done?
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• Remove pathogenic genes from an infectious virus
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- Replace with a normal therapeutic gene copy (i.e., hemophilia gene).
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- Replace with a normal therapeutic gene copy (i.e., hemophilia gene).
- Introduce modified virus into diseased tissue – modified virus can express the therapeutic gene but cannot produce disease
Clinically effective gene therapy

- Cancer – leukemia, lymphoma, neuroblastoma
- Severe combined immunodeficiency (ADA-SCID, X-SCID)
- Degenerative retinal disease (Leber’s congenital blindness)
- Neurodegenerative disease – adrenoleukodystrophy (ALD)
- Metabolic disease – hemophilia, lipoprotein lipase deficiency (LPL)
Promising diseases

- Forms of cancer
- Thalassemias, other blood diseases
- Many retinal eye diseases, macular degeneration
- Neurological disease (Parkinson’s, Huntington’s, Alzheimer’s)
- Mendelian metabolic diseases
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- Introduce modified virus into normal tissue (i.e., muscle, skin). Modified virus contains no viral genes, cannot produce disease
- Effective in animal models (Epo, IGF-1)
Targets for genetic enhancement in Sport

- Muscle - force and speed of muscle growth, contraction, recovery (growth hormones, IGF-1, myostatin inhibitor)
- Endurance performance, energy utilization (Epo, PPAR-delta)
- Pain perception (endorphins)
Mutated or inhibited myostatin expression
Small enhancements make a big difference

Virus expressing Epo gene

Increase production of erythropoietin, red blood cell oxygen-carrying capacity

Thomas Springsteen
II. CELLULAR APPROACH TO DOPING

Stem cells for tissue regeneration and repair

PROF. DARYL D’LIMA
III. GENETIC TECHNIQUES TO DETECT DOPING
Classical detection of doping

- Chemical or physical detection of drug – steroids, Epo, etc.
- Physiological effects of drugs – hematocrit for Epo, etc.
A new approach to detection – “genetic signatures”

- Hypothesis - exposure to drugs and biological agents changes expression of many of the 25,000 known human genes.
- Therefore, do not search for the doping agent itself - search for specific biological response to the agent.
- The genetic changes constitute a unique genetic “signature” of a specific drug
Expression of all 25,000 human genes on single microarray chip
WADA Research program

- Major effort to develop tools and techniques for traditional chemical detection
- Genetic signatures – HGH and Epo
Human Growth Hormone signature
Human Growth Hormone Signature

<table>
<thead>
<tr>
<th>Gene_ID</th>
<th>Symbol</th>
<th>Description</th>
<th>G1-R</th>
<th>G1-PV</th>
<th>G2-R</th>
<th>G2-PV</th>
<th>G3-R</th>
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<td>0.02</td>
<td>up</td>
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</table>
# Human Growth Hormone Signature

![Venn diagram showing overlap of gene sets G1, G2, and G3 with numbers indicating the counts in each set.]

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Gene_ID</th>
<th>G1 Regulati</th>
<th>pG1 P-value</th>
<th>G2 Regulati</th>
<th>pG2 P-value</th>
<th>G3 Regulati</th>
<th>pG3 P-value</th>
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<tbody>
<tr>
<td>MME</td>
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</tbody>
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<th>G3 Regulati</th>
<th>pG3 P-value</th>
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<td>CUL4B</td>
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<td>0.07</td>
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</tr>
</tbody>
</table>
Why so few diagnostic signature markers for HGH?

• HGH causes only very few genetic changes throughout the genome – unlikely

• Those pathways are protected by rapidly correcting, redundant mechanisms.

• Prof. Bon Gray, Bond University, Australia
Erythropoietin – a different story

Prof. Yannis Pitsiladis
University of Glasgow
Erythropoietin
**Erythropoietin**

<table>
<thead>
<tr>
<th>Probe</th>
<th>Gene</th>
<th>KEN (n=6)</th>
<th>SCO (n=18)</th>
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<td>ILMN_2367126</td>
<td>ALAS2</td>
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<td>ILMN_1701933</td>
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<tr>
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<tr>
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<td>ADIPOR1</td>
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<td>ILMN_1651358</td>
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<td>$-2.9 \pm 1.6 (-6.1 ; 1)$</td>
</tr>
</tbody>
</table>
Summary – powerful new tools in anti-doping

- Diagnostic genetic signatures for doping are within reach
  - Epo or Epo gene (Pitsiladis)
  - HGH or HGH gene (Friedmann, Gray)
- Advanced methods for detecting virus vectors used for gene transfer (Snyder)
Summary - Genetics and Sport

1. New gene-based approaches to doping and doping detection are imminent

2. Genetics is challenging concept of Sport and its value in society

3. Sport is forerunner of broader genetic enhancement in society
The beauty of Sport