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Molecular Genetics of Idiopathic Hyperphosphatasia

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This thesis is submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

JUNE, 2004
This thesis is dedicated to my loving parents
David and Christina
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Finally, a very special thanks you to my husband Bruce and my parents, for believing in me and for helping me through everything.
ABSTRACT

The subject of this thesis is the molecular genetic study of idiopathic hyperphosphatasia. The work in this thesis describes linkage analysis, mutation screening of candidate genes and the functional analysis of mutant proteins expressed in patients with a clinical diagnosis of idiopathic hyperphosphatasia.

Idiopathic hyperphosphatasia is an autosomal recessive bone disease characterized by excessive bone resorption and bone formation. Affected children are normal at birth but develop deformities of long bones, kyphosis and acetabular protrusion with increasing severity as they pass through adolescence. There is considerable variability in phenotype, with some cases diagnosed in infancy and others in later childhood. A genome-wide search of a New Zealand family affected by idiopathic hyperphosphatasia suggested linkage to a locus on the long arm of chromosome 8 (8q24). The gene TNFRSF11B encoding osteoprotegerin (OPG), which lies within 8q24, was an obvious candidate gene given the critical role of OPG in regulating osteoclast development. Mutation screening of this gene indicated an apparent disease-causing mutation in exon 3 in affected individuals of the New Zealand family. Subsequently eight families, recruited by the members of the Idiopathic Hyperphosphatasia Collaborative Group in Turkey, Germany, Argentina and the United Kingdom were also screened for mutations in the TNFRSF11B gene. Recombinant wild-type and mutant OPG cDNAs were expressed in human epithelial kidney cells, and secreted OPG was collected from the conditioned medium. In vitro measurements of osteoclastic bone resorption showed that wild type OPG suppressed bone
resorption, whereas the mutant forms did not, confirming them to be inactivating mutations.
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ABBREVIATIONS AND SYMBOLS

SI (Système Internationale d’Unités) abbreviations for units and standard notations for chemical elements, formulae, and chemical abbreviations are used throughout this work. Other abbreviations commonly used in the text are listed below.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>aa</td>
<td>amino acid</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>bp</td>
<td>base pairs</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>cDNA</td>
<td>complementary DNA</td>
</tr>
<tr>
<td>CDTA</td>
<td>trans-1,2-diaminocyclohexane-n,n,n',n'-tetraacetic acid</td>
</tr>
<tr>
<td>cm</td>
<td>centimetre</td>
</tr>
<tr>
<td>dATP</td>
<td>2'-deoxyadenosine 5'-triphosphate</td>
</tr>
<tr>
<td>dCTP</td>
<td>2'-deoxycytosine 5'-triphosphate</td>
</tr>
<tr>
<td>dGTP</td>
<td>2'-deoxyguanosine 5'-triphosphate</td>
</tr>
<tr>
<td>dTTP</td>
<td>2'-deoxythymine 5'-triphosphate</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>DNase</td>
<td>deoxyribonuclease</td>
</tr>
<tr>
<td>dNTPs</td>
<td>2'-deoxynucleoside 5'-triphosphates</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediamine-tetra-acetic acid (disodium salt)</td>
</tr>
<tr>
<td>EtBr</td>
<td>ethidium bromide</td>
</tr>
<tr>
<td>g</td>
<td>specific gravity</td>
</tr>
<tr>
<td>kb</td>
<td>kilobase pairs</td>
</tr>
<tr>
<td>kDa</td>
<td>kilodalton</td>
</tr>
<tr>
<td>M</td>
<td>moles per litre</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger ribonucleic acid</td>
</tr>
<tr>
<td>MW</td>
<td>molecular weight</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate buffered saline</td>
</tr>
</tbody>
</table>
PCR  polymerase chain reaction
®  Registered
RNA  ribonucleic acid
RNase  ribonuclease
rpm  revolutions per minute
SDS  sodium dodecyl sulphate
TAE  Tris-acetate-EDTA buffer
TE  Tris-EDTA buffer
Tm  melting temperature of DNA
™  Trademark
Tris  [2-amino-2-(hydroxymethyl)-propane-1, 3 diol (tris)]
Tris HCl  Tris solution, pH adjusted with HCl
U  units of enzyme (as defined by the manufacturer)
UV  ultraviolet light
v/v  volume per volume
w/v  weight per volume
COMPANY ABBREVIATIONS

The following list contains the abbreviations of suppliers of chemicals, enzymes, and equipment used during the course of this work.

Applied Biosystems  ABI, Foster City, CA, USA
Amersham  Amersham International, Buckinghamshire, UK
BDH  BDH Chemicals NZ Ltd, Palmerston North, NZ
Bio-Rad  Bio-Rad Laboratories, Hercules, CA, USA
Roche Biochemicals Ltd  Roche Biochemicals Ltd, Indianapolis, IN, USA
Genta Systems  Genta Systems Inc, Minneapolis, MN, USA
Life Technologies  Bethesda Research Laboratories, Life Technologies Inc, MD, USA
Perkin Elmer  Perkin Elmer Cetus, CT, USA
Promega  Promega Corporation, Madison, WI, USA
Qiagen  Qiagen GmbH, Hilden, Germany
Riedel-de Haën  Riedel-de Haën, Seelze, Germany
Sigma  Sigma Chemical Company, St Louis, MO, USA
Stratagene  Stratagene Cloning Systems, San Diego, CA, USA
<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetabular protrusion</td>
<td>Protrusion of the cup-shaped cavity at the base of the hipbone into which the ball-shaped head of the femur fits.</td>
</tr>
<tr>
<td>Calcified</td>
<td>To make calcareous by deposit of calcium salts</td>
</tr>
<tr>
<td>Cancellous</td>
<td>Having a porous structure made up of intersecting plates and bars that form small cavities or cells</td>
</tr>
<tr>
<td>Cartilage</td>
<td>A usually translucent somewhat elastic tissue that composes most of the skeleton of vertebrate embryos and except for a small number of structures (as some joints, respiratory passages, and the external ear) is replaced by bone during ossification in the higher vertebrates</td>
</tr>
<tr>
<td>Chondrocytes</td>
<td>A cartilage cell</td>
</tr>
<tr>
<td>Collagen</td>
<td>An insoluble fibrous protein of vertebrates that is the chief constituent of the fibrils of connective tissue (as in skin and tendons) and of the organic substance of bones and yields gelatin and glue on prolonged heating with water</td>
</tr>
<tr>
<td>Connective tissue</td>
<td>A tissue of mesodermal origin rich in intercellular substance or interlacing processes with little tendency for the cells to come together in sheets or masses</td>
</tr>
<tr>
<td>Endocrine system</td>
<td>The glands and parts of glands that produce endocrine secretions, help to integrate and control bodily metabolic activity, and include especially the pituitary, thyroid, parathyroids, adrenals, islets of Langerhans, ovaries, and testes</td>
</tr>
<tr>
<td>Endocrinopathy</td>
<td>A disease marked by dysfunction of an endocrine gland</td>
</tr>
<tr>
<td>Euchromatic</td>
<td>The genetically active portion of chromatin that is largely composed of genes</td>
</tr>
<tr>
<td>Fusiform</td>
<td>Tapering toward each end</td>
</tr>
<tr>
<td>Granulocyte</td>
<td>A polymorphonuclear white blood cell (as a basophil, eosinophil, or neutrophil) with granule-containing cytoplasm</td>
</tr>
<tr>
<td>Hematopoiesis</td>
<td>The formation of blood or of blood cells in the living body -- called also hemopoiesis</td>
</tr>
<tr>
<td>Interferon</td>
<td>Any of a group of heat-stable soluble basic antiviral glycoproteins of low molecular mass</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>weight that are produced usually by cells exposed to the action of a virus, sometimes to the action of another intracellular parasite (as a bacterium), or experimentally to the action of some chemicals, and that include some used medically as antiviral or antineoplastic agents</td>
<td><strong>Interleukin</strong> Any of various compounds of low molecular weight that are produced by lymphocytes, macrophages, and monocytes and that function especially in regulation of the immune system and especially cell-mediated immunity</td>
</tr>
<tr>
<td>Kyphoscoliosis</td>
<td>Backward and lateral curvature of the spine</td>
</tr>
<tr>
<td>Lymphotoxin</td>
<td>A lymphokine that lyses various cells and especially tumor cell</td>
</tr>
<tr>
<td>Macrophages</td>
<td>A phagocytic tissue cell of the mononuclear phagocyte system that may be fixed or freely motile, is derived from a monocyte, and functions in the protection of the body against infection and noxious substances</td>
</tr>
<tr>
<td>Monocytes</td>
<td>A large white blood cell with finely granulated chromatin dispersed throughout the nucleus that is formed in the bone marrow, enters the blood, and migrates into the connective tissue where it differentiates into a macrophage</td>
</tr>
<tr>
<td>Nervous system</td>
<td>The bodily system that in vertebrates is made up of the brain and spinal cord, nerves, ganglia, and parts of the receptor organs and that receives and interprets stimuli and transmits impulses to the effector organs</td>
</tr>
<tr>
<td>Ossified</td>
<td>To form or be transformed into bone</td>
</tr>
<tr>
<td>Perichondrium</td>
<td>The membrane of fibrous connective tissue that invests cartilage except at joints</td>
</tr>
<tr>
<td>Pleiotropic</td>
<td>Producing more than one genetic effect; specifically: having multiple phenotypic expressions</td>
</tr>
<tr>
<td>Proliferation</td>
<td>Rapid and repeated production of new parts (as in a mass of cells by a rapid succession of cell divisions)</td>
</tr>
<tr>
<td>Skeletal system</td>
<td>The bone and cartilages of the body.</td>
</tr>
<tr>
<td>Skeleton</td>
<td>A usually rigid supportive or protective structure or framework of an organism; especially: the bony or more or less cartilaginous framework supporting the soft tissues and protecting the internal organs of</td>
</tr>
<tr>
<td><strong>Paracrine</strong></td>
<td>Of, relating to, promoted by, or being a substance secreted by a cell and acting on adjacent cells</td>
</tr>
<tr>
<td><strong>Pseudoxanthoma elasticum</strong></td>
<td>Pseudoxanthoma elasticum (PXE) is the name given to a group of connective tissue disorders that affects the elastic tissue of the skin, blood vessels, and the eyes. It is also known as Gronblad-Strandberg syndrome.</td>
</tr>
</tbody>
</table>