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

ACKNOWLEDGEMENTS

I would like to thank my supervisors, Dr Don Love and Dr. Tim Cundy. I would like to acknowledge LabPlus, Auckland Hospital the Research Committee of the University of Auckland and the Health Research Council of New Zealand for financial support. Thank you to Graduate Research Fund for providing my travel fund.

I would like to thank the International Hyperphosphatasia Collaborative Group: Madhuri Hegde, Matthew Fawkner, Scott Simonet, Hamilton Cassinelli, Mahmut Coker, John Kanis, Joerg Seidel, Cristina Tau, Beyhan Tüysüz, Bilgin Yüksel, Jill Cornish, Don Love and Tim Cundy for their help with this study. Thanks to Catherin Middleton-Hardie for her help with the NZ family.

I would also like to thank the following people, who at some stage during the three years helped me with practical details, equipment, or materials: To all the people in the Virology and Immunology Department and Cytogenetics Department, Auckland Hospital. I would like to thank Matthew Fawkner for his help and for being so understanding especially during the last few months. Thank you to Dennis Dixon-McIver, Dr Kitty Croxson and John Scott for their help and support.

A very special thanks to Madhuri and her family, Madhuri thanks for all your help and for showing me what true friendship is.

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 (**S**ysteme **I**nternationale 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.

aa	amino acid
ATP	adenosine triphosphate
bp	base pairs
BSA	bovine serum albumin
cDNA	complementary DNA
CDTA	trans-1,2-diaminocyclohexane-n,n,n',n'- tetraacetic acid
cm	centimetre
dATP	2'-deoxyadenosine 5'-triphosphate
dCTP	2'-deoxycytosine 5'-triphosphate
dGTP	2'-deoxyguanosine 5'-triphosphate
dTTP	2'-deoxythymine 5'-triphosphate
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
dNTPs	2'-deoxynucleoside 5'-triphosphates
EDTA	ethylenediamine-tetra-acetic acid (disodium salt)
EtBr	ethidium bromide
g	specific gravity
kb	kilobase pairs
kDa	kilodalton
M	moles per litre
mRNA	messenger ribonucleic acid
MW	molecular weight
PBS	phosphate buffered saline

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
Gentra Systems	Gentra 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

MEDICAL TERMINOLOGY

Acetabular protrusion	Protrusion of the cup-shaped cavity at the base of the hipbone into which the ball-shaped head of the femur fits.
Calcified	To make calcareous by deposit of calcium salts
Cancellous	Having a porous structure made up of intersecting plates and bars that form small cavities or cells
Cartilage	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
Chondrocytes	A cartilage cell
Collagen	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
Connective tissue	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
Endocrine system	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
Endocrinopathy	A disease marked by dysfunction of an endocrine gland
Euchromatic	The genetically active portion of chromatin that is largely composed of genes
Fusiform	Tapering toward each end
Granulocyte	A polymorphonuclear white blood cell (as a basophil, eosinophil, or neutrophil) with granule-containing cytoplasm
Hematopoiesis	The formation of blood or of blood cells in the living body -- called also *hemopoiesis*
Interferon	Any of a group of heat-stable soluble basic antiviral glycoproteins of low molecular

	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
Interleukin	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
Kyphoscoliosis	Backward and lateral curvature of the spine
Lymphotoxin	A lymphokine that lyses various cells and especially tumor cell
Macrophages	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
Monocytes	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
Nervous system	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
Ossified	To form or be transformed into bone
Perichondrium	The membrane of fibrous connective tissue that invests cartilage except at joints
Pleiotropic	Producing more than one genetic effect ; *specifically* : having multiple phenotypic expressions
Proliferation	Rapid and repeated production of new parts (as in a mass of cells by a rapid succession of cell divisions)
Skeletal system	The bone and cartilages of the body.
Skeleton	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

	a vertebrate
Paracrine	Of, relating to, promoted by, or being a substance secreted by a cell and acting on adjacent cells
Pseudoxanthoma elasticum	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.