Modification Form for Permit BIO-RRI-0006

Permit Holder: Robert Hegele

Approved Personnel
(Please stroke out any personnel to be removed)

Additional Personnel
(Please list additional personnel here)

J Wang, H Cao, R Martins, R Hassell
M Ban, A McIntyre, B Kennedy, J Robinson,
P lahiry, C Johansen, M Lanktree

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	ME DH10B, ME DH5-alpha, ME DH5A T1	
Approved Cells	[Rodent] (established): 3T3-L1, NIH/3T3. [Non-human Primate] (established): COS-7 [Human] (established: HEK293, HEP62 [Human Primary]: Fibroblast- (GM05659, GM08398, , GM03348 GM03513, Fibroblast,	Fibroblast, finite primary cell line human AG16409 Coriell Cell Repository Fibroblast, finite primary cell line human HGADFN167 Progeria Research Foundation
Approved Use of Human Source Material	Blood, serum/plasma	
Approved GMO	SV 40 Large T antigen (expressed in COS7 cells), pCDNA3 plasmid, pcDNA 3.1 Echo Expression Vector Kit. [ADD]: pCMV6, pcDNA3.1, pENTR 11	
Approved use of Animals		
Approved Toxin(s)		

* *PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.*
** *PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.*

As the principal investigator, I have ensured that all of the personnel named on the form have been trained. I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.wph.uwo.ca.

Signature of Permit Holder: _____

Classification: 2

Date of Last Biohazardous Agents Registry Form: May 15, 2009

Date of Last Modification (if applicable): October 22, 2009

BioSafety Officer(s): _____

Chair, Biohazards Subcommittee: _____

2009 Biohazard Agents Registry, Dr. Robert Hegele 22-Oct-09

Name	CAT. NO.	SUPPLIER	MSDS	Comment	Use
3T3-L1	CL-173	ATCC	Y	Murine fibroblast	Mutant LMNA is transfected into these cells and immunofluorescence studies and Western analysis performed
NIH/3T3	CRL-1658	ATCC	Y	Murine	
COS-7: note SV40 promoter	CRL-1651	ATCC	Y	Green monkey (Ceropithecus aethiops)	
HEK293: note Adeno E1A promoter	CRL-1573	ATCC	Y	Human	
HepG2	CRL-11997	ATCC	Y	Human	
Fibroblast, finite primary cell line human	GM05659	Coriell Cell Repository	Y	Human: unaffected	Cells are cultured, morphology and growth curves evaluated and immunofluorescence studies, Western analysis, DNA and RNA isolated and microarray and high throughput sequencing analysis performed.
Fibroblast, finite primary cell line human	GM08398	Coriell Cell Repository	Y	Human: unaffected	
Fibroblast, finite primary cell line human	GM03348	Coriell Cell Repository	Y	Human: unaffected	
Fibroblast, finite primary cell line human	AG03513	Coriell Cell Repository	Y	Human: HGPS proband	
Fibroblast, finite primary cell line human	AG04456	Coriell Cell Repository	Y	Human: unaffected	
Fibroblast, finite primary cell line human	AG16409	Coriell Cell Repository	Y	Human: unaffected	
Fibroblast, finite primary cell line human	30950	Dr. T.C. Rupar	na	Human	Cells are cultured, DNA and RNA extracted and microarray and high throughtput sequencing analysis performed
Fibroblast, finite primary cell line human	40916	Dr. T.C. Rupar	na	Human	
Fibroblast, finite primary cell line human	20750	Dr. T.C. Rupar	na	Human	
Fibroblast, finite primary cell line human	70280	Dr. T.C. Rupar	na	Human	
pCMV6	PS100001	Origene	y		Transfection vectors used in the lab
pcDNA3.1	350492	Invitrogen	Y		
pENTR 11 dual selection vector	A10562	Invitrogen	Y		
Subcloning Efficiency™ DH5a™ Competent Cells	18265-017	Invitrogen	Y	E. Coli	Cells used to as vehicles for transfection of genetic material for overexpression studies and phenotypic characterization
Electromax DH10B competent cells	18290-015	Invitrogen		E. Coli	
ME DH10B competent cells	18297-010	Invitrogen		E. Coli	
ME DH5-alpha competent cells	18258-012	Invitrogen	Y	E. Coli	
ME DH5A T1 page resist comp cells	12034-013	Invitrogen		E. Coli	
Fibroblast, finite primary cell line human	HGADFN167	Progeria Research Foundation	Y	Human: HGPS proband	same use as Coriell Cell Repository

Catalog ID: **AG16409** Product (Source): CELL CULTURE ADD TO CART

Overview

Collection NIA Aging Cell Culture Repository
Subcollection Apparently Healthy Collection
Sample Description APPARENTLY HEALTHY NON-FETAL TISSUE
Biopsy Source Unspecified
Cell Type Fibroblast
Tissue Type Skin
Transformant Untransformed
Species Homo sapiens
Common Name Human
Age 12 YR
Sex Male
Race Caucasian
Family 1936
Family Member 1
Relation to Proband proband
Clinically Affected No
Confirmation Clinical summary/Case history
ISCN 46,XY
Remarks The donor was clinically normal having suffered a cervical spine injury at age 5. He was ventilator-dependent. He died of brain death with cardiorespiratory arrest at age 12. The culture was initiated on 7/12/2000 using explants of minced skin tissue taken post-mortem. The cell morphology is fibroblast-like. The karyotype is 46,XY with 4% of the cells examined showing random chromosome loss and 2% showing random chromosomal aberrations.

Catalog ID AG16409
Product Cell Culture
Pricing Commercial Pricing: $155.00
Academic and not-for-profit pricing: $85.00
NIA Grantees: $0.00
How to Order Online Ordering
Assurance Form (Must have current form on file)
Statement of Research Intent Form (Information will be entered electronically when order is placed. DO NOT fax form to Coriell Customer Service)

Characterizations

Sample Description APPARENTLY HEALTHY NON-FETAL TISSUE
PDL at Freeze 4
Passage Frozen 2

IDENTIFICATION OF SPECIES OF ORIGIN Species of Origin Confirmed by Nucleoside Phosphorylase,Glucose-6-Phosphate Dehydrogenase, and Lactate Dehydrogenase Isoenzyme Electrophoresis

Phenotypic Data

Remark The donor was clinically normal having suffered a cervical spine injury at age 5. He was ventilator-dependent. He died of brain death with cardiorespiratory arrest at age 12. The culture was initiated on 7/12/2000 using explants of minced skin tissue taken post-mortem. The cell morphology is fibroblast-like. The karyotype is 46,XY with 4% of the cells examined showing random chromosome loss and 2% showing random chromosomal aberrations.

Publications

Data are not available

External Links

dbSNP dbSNP ID: 11159

Images

Data are not available

Protocols

PDL at Freeze	4
Passage Frozen	2
Split Ratio	1:4
Temperature	37 C
Percent CO2	5%
Medium	Eagle's Minimum Essential Medium with Earle's salts and non-essential amino acids
Serum	10% fetal bovine serum Not inactivated
Substrate	None specified
Subcultivation Method	trypsin-EDTA

Public Health Agency of Canada / Agence de la santé publique du Canada

Name and/or Organization: **University of Western Ontario**
Robarts Research Institute
Attn: Dr. Robert Hegele

Address: P.O. Box 5015
100 Perth Drive, Rm 4-25
London, ON
N6A 5K8

The following biological material does not require a Public Health Agency of Canada import permit under the HPIR*:

Human fibroblast cell line from healthy donor (AG16409), as provided by Coriell Institute for Medical Research, 403 Haddon Avenue, Camden, NJ, USA 08103.

Marianne Heisz
Chief, Importation and Regulatory Affairs

JULY 16, 2009
Date

NOTICE

***HPIR (HUMAN PATHOGENS IMPORTATION REGULATIONS)**

- We are in receipt of your application for an importation permit for biological materials. The **HPIR** apply **only** to the importation of infectious substances which cause human disease and their subsequent distribution or transfer. Other materials, which are deemed by the importer to be non-infectious for humans, **do not** require a permit under these regulations. It should be noted that the importation of biological materials may also be subject to other federal, provincial and municipal laws.
- For animal or plant pathogens one **must** apply to The Canadian Food Inspection Agency (CFIA) for a permit to import. If this material is of animal or plant origin it may also require a permit from the CFIA. Please contact the CFIA for their consideration. CFIA contact numbers are as follows:
  (613) 221-7068 for information concerning animal pathogens/material
  (613) 225-2342 [ext. 4334] for information concerning plant pathogens/material
- Importation of this material may also be subject to the requirements of the *New Substances Notification Regulations (Organisms)* of the *Canadian Environmental Protection Act, 1999*, administered by Environment Canada and Health Canada. Please contact the New Substances Information Line at 1-800-567-1999 or nsn-infoline@ec.gc.ca.
- You may be required to provide the Canada Border Services Agency (CBSA) customs officers with a declaration that the imported material is non-infectious and non-hazardous.

Should you require further information, please contact:
Office of Laboratory Security
Centre for Emergency Preparedness and Response
(613) 957-1779

Canada

The Progeria Research Foundation
Cell and Tissue Bank

Dear Dr. Robinson,

October 19th, 2009

Blackburn Cardiovascular Genetics Laboratory
Robarts Research Institute
Room 4-28, PO box 5015, 100 Perth Drive
London, On, Canada, N6A 5K8

Please find enclosed one flask each of the following cell lines which you requested from the PRF Cell and Tissue Bank for your research.

Cell Line #	Passage #	Clinically Affected?	Relation to Proband	Age at Donation	Exon 11 mutation Yes or no C→T
HGADFN167	4	yes	proband	8 yrs. 5mos	yes

Please place cells at 37°C and 5% CO_2 for 24 hours upon receipt and then change the culture medium and split (if necessary) as directed. Please see the attached sheet containing specific culture conditions. If you have any further questions do not hesitate to contact me at leslie_gordon@brown.edu or Lorraine Fast (Laboratory Technician) at 401-444-7564 or lfast1@lifespan.org.

Sincerely,

Leslie B. Gordon, MD, PhD
Principal Investigator, The Progeria Research Foundation Cell and Tissue Bank

Email: progeria@netzero.net Website: www.progeriaresearch.org
P. O. Box 3453, Peabody, MA 01961-3453 Tel: (978) 535-2594 Fax: (978) 535-5849

Culture Conditions:
DMEM (Gibco 11960-044), + 2mM L-Glutamine, Pen/Strep and 15% FBS.

Split Conditions:
0.25% Trypsin/ EDTA - 1ml/T25 flask, evenly coating cells. Incubate 2-3 minutes. Gently tip flask to dislodge. Pool cells in culture medium and replate or freeze down.

Freezing Conditions:
10% DMSO in culture media

Email: progeria@netzero.net Website: www.progeriaresearch.org
P. O. Box 3453, Peabody, MA 01961-3453 Tel: (978) 535-2594 Fax: (978) 535-5849

Public Health Agency of Canada / Agence de la santé publique du Canada

Name and/or Organization: **University of Western Ontario**
Robarts Research Institute
Attn: Dr. Robert Hegele

Address: P.O. Box 5015, Rm 4-25
100 Perth Drive
London, ON
N6A 5K8

The following biological material does not require a Public Health Agency of Canada import permit under the HPIR*:

Human fibroblast cell line from donor with Hutchinson-Gilford Progeria Syndrome (HGADFN167), as provided by The Progeria Research Foundation Cell Bank, 532 Lowell Street, Peabody, MA, USA, 01960.

Marianne Heisz
Chief, Importation and Regulatory Affairs

JULY 16, 2009
Date

NOTICE

***HPIR (HUMAN PATHOGENS IMPORTATION REGULATIONS)**

- We are in receipt of your application for an importation permit for biological materials. The **HPIR** apply **only** to the importation of infectious substances which cause human disease and their subsequent distribution or transfer. Other materials, which are deemed by the importer to be non-infectious for humans, **do not** require a permit under these regulations. It should be noted that the importation of biological materials may also be subject to other federal, provincial and municipal laws.
- For animal or plant pathogens one **must** apply to The Canadian Food Inspection Agency (CFIA) for a permit to import. If this material is of animal or plant origin it may also require a permit from the CFIA. Please contact the CFIA for their consideration. CFIA contact numbers are as follows:
  (613) 221-7068 for information concerning animal pathogens/material
  (613) 225-2342 [ext. 4334] for information concerning plant pathogens/material
- Importation of this material may also be subject to the requirements of the *New Substances Notification Regulations (Organisms)* of the *Canadian Environmental Protection Act, 1999*, administered by Environment Canada and Health Canada. Please contact the New Substances Information Line at 1-800-567-1999 or nsn-infoline@ec.gc.ca.
- You may be required to provide the Canada Border Services Agency (CBSA) customs officers with a declaration that the imported material is non-infectious and non-hazardous.

Should you require further information, please contact:
Office of Laboratory Security
Centre for Emergency Preparedness and Response
(613) 957-1779

Canada

The Progeria Research Foundation, Inc

DECLARATION STATEMENT

The contents of this package are as follows:

Cultured Human dermal fibroblasts specimen in medium containing 15% fetal bovine serum (certified free of infectious agents) in a sealed collection tube.

These samples are considered to be non-infectious and are for research purposes only.

These samples are being shipped from Dr. Leslie Gordon and Dr. Douglas Hixson for studies funded by The Progeria Research Foundation. Samples are packed in approved blood mailers and are perishable. Samples ***are not*** known to be infectious. **Please do not delay**.

If you have any questions or concerns, please contact Dr. Leslie Gordon, Principal Investigator, at the following phone number: (508) 889-6655
Sincerely,

Leslie B. Gordon, MD, PhD

P. O. Box 3453, Peabody, MA 01961-3453
Tel: (978) 535-2594, Fax: (978) 535-5849,
Email: info@progeriaresearch.org

www.progeriaresearch.org

THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: March 27, 2009
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biohazardous agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must ~~also~~ be updated at least every 3 years or when there are changes to the biohazards being used.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190) for distribution to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety

PRINCIPAL INVESTIGATOR	Dr. Robert Hegele
SIGNATURE	
DEPARTMENT	Robarts Research Institute, Vascular Biology Research Group
ADDRESS	100 Perth Dr. Room 4-06
PHONE NUMBER	519-931-5271 Lab. 519-931-5777 ext. 34412
EMERGENCY PHONE NUMBER(S)	519-931-5271
EMAIL	hegele@robarts.ca

Location of experimental work to be carried out: Building(s) RRI Room(s) 4288, 4292, 4286

*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 12.0, Approvals).

FUNDING AGENCY/AGENCIES: HSFO, CIHR
GRANT TITLE(S): _____

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.

Names of all personnel working under Principal Investigators supervision in this location:

Jian Wang
Henian Cao
Brooke Kennedy
John Robinson
Reina Hassell
Rebecca Provost
Matthew Ban
Chris Johansen
Piya Cahill
Matthew Lanktree

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

1.0 Microorganisms

1.1 Does your work involve the use of microorganisms or biological agents of plant or animal origin (including but not limited to viruses, prions, parasites, bacteria)? ☒YES O NO
If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? O YES ☒NO
If YES, please give the name of the species. _____
What is the origin of the microorganism(s)? _____
Please describe the risk (if any) of escape and how this will be mitigated:

_____
_____
_____

Please attach the CFIA permit.
Please describe any CFIA permit conditions:

_____
_____
_____

1.2 Please complete the table below:

Name of Biological agent(s)*	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
DH 10B	O Yes ☒No	O Yes ☒No	O Yes ☒No	0.007-0.010	Invitrogen	☒1 O2 O3
DH 5α	O Yes ☒No	O Yes ☒No	O Yes ☒No	0.007-0.010	"	☒1 O2 O3
	O Yes O No	O Yes O No	O Yes O No			O1 O2 O3
	O Yes O No	O Yes O No	O Yes O No			O1 O2 O3

*Please attach a Material Safety Data Sheet or equivalent from the supplier.

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? ☒YES O NO
If no, please proceed to Section 3.0

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture in the table below

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	☒Yes O No	See Attached	Not applicable
Rodent	☒Yes O No	" "	"
Non-human primate	O Yes ☒No		
Other (specify)	O Yes ☒No		

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

2.3 Please indicate the type of established cells that will be grown in culture in the table below.

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	☒ Yes O No	See Attached	See Attached
Rodent	☒ Yes O No	" "	ATCC
Non-human primate	☒ Yes O No	COS-7	ATCC
Other (specify)	O Yes O No		

*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required O 1 ☒ 2 O 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? ☒ YES O NO
If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Known to Be Infected With An Infectious Agent? YES/NO**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid	Research Subjects	O Yes ☒ No		O 1 ☒ 2 O 3
Human Blood (fraction) or other Body Fluid	Research Subjects	O Yes ☒ No		O 1 ☒ 2 O 3
Human Organs or Tissues (unpreserved)		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (preserved)		O Yes O No		O 1 O 2 O 3

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? ☒ YES O NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? ☒ YES, complete table below O NO

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results
DH5α	pcDNA 3.1 CAT	Bacteria (Invitrogen)	APOE	Morphological

* Please attach a Material Data Sheet or equivalent if available.

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

4.3 Will genetic modification(s) involving viral vectors be done? O YES, complete table below ☒NO

Virus Used for Transduction *	Vector(s) *	Source of Vector	Gene Transfected	Describe the change that results

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV O YES, please specify _____ ☒NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ ☒NO
- SV 40 Large T antigen ☒YES O NO
- E1A oncogene ☒YES O NO
- Known oncogenes O YES, please specify _____ ☒NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ ☒NO

4.5 Will virus be replication defective? ☒YES O NO

4.6 Will virus be infectious to humans or animals? O YES ☒NO

4.7 Will this be expected to increase the containment level required? O YES ☒NO

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted using the viral vector in 4.0? O YES ☒NO
If no, please proceed to Section 6.0 If YES attach a full description of the make-up of the virus.

5.2 Will virus be able to replicate in the host? O YES O NO

5.3 How will the virus be administered? _____

5.4 Please give the Health Care Facility where the clinical trial will be conducted:_____

5.5 Has human ethics approval been obtained? O YES, number: _____ O NO O PENDING

6.0 Animal Experiments

6.1 Will live animals be used? O YES ☒NO If no, please proceed to section 7.0

6.2 Name of animal species to be used_____

6.3 AUS protocol # _____

6.4 Will any of the agents listed be used in live animals O YES, specify: _____ O NO

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any of the following animals or their organs, tissues, lavages or other body fluids including blood be used?

- Pound source dogs O YES ⊠NO
- Pound source cats O YES ⊠NO
- Cattle, sheep or goats O YES ⊠NO
- Non-human primates O YES, please specify species _____ ⊠NO
- Wild caught animals O YES, please specify species & colony # _____ ⊠NO
- Birds O YES ⊠NO
- Others (wild or domestic) O YES, please specify _____ ⊠NO

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES ⊠NO If no, please proceed to Section 9.0

8.2 If YES, please name the toxin(s)_____
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the LD_{50} (specify species) of the toxin_____

8.4 How much of the toxin is handled at one time*? _____

8.5 How much of the toxin is stored*? _____

*For information on biosecurity requirements, please see:
http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? O YES ⊠NO
If no, please proceed to Section 10.0

9.2 If YES, please give the name of the species. _____

9.3 What is the origin of the insect? _____

9.4 What is the lifestage of the insect? _____

9.5 What is your intention? O Initiate and maintain colony, give location: _____
O "One-off" use, give location: _____

9.6 Please describe the risk (if any) of escape and how this will be mitigated:
_____
_____
_____

9.7 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:
_____
_____
_____

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? O YES ☒ NO
If no, please proceed to Section 11.0

10.2 If YES, please give the name of the species. _____

10.3 What is the origin of the plant? _____

10.4 What is the form of the plant (seed, seedling, plant, tree...)? _____

10.5 What is your intention? O Grow and maintain a crop O "One-time" use

10.6 Do you do any modifications to the plant? O YES O NO
If yes, please describe: _____
_____

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:
_____
_____
_____

10.8 Is the CFIA permit attached? O YES O NO

10.9 Please describe any CFIA permit conditions:
_____
_____
_____

11.0 Import Requirements

11.1 Will any of the above agents be imported? ☒ YES, please give country of origin USA
If no, please proceed to Section 10.0 O NO

Letter attached Stating NOT Require

11.2 Has an Import Permit been obtained from HC for human pathogens? ☒ YES O NO

11.3 Has an import permit been obtained from CFIA for animal or plant pathogens? O YES ☒ NO

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O NO

12.0 Training Requirements for Personnel Named on Form

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS (Western or equivalent)
- Employee Health and Safety Orientation

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biohazardous agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE _____ May 1/07

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

13.0 Containment Levels

13.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. O 1 ☒ 2 O 3

13.2 Has the facility been certified by OHS for this level of containment?
☒ YES, permit # if on-campus B10-RR1-0006
O NO, please certify
O NOT REQUIRED for Level 1 containment

14.0 Procedures to be Followed

14.1 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.wph.uwo.ca/

SIGNATURE [signature] Date: Apr. 24, 2009

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: [signature]
Date: 15 May 2009

Safety Officer for Institution where experiments will take place: SIGNATURE: [signature]
Date: May 07, 2009

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: [signature]
Date: May 15/09.

Approval Number: B10-RR1-0006 Expiry Date (3 years from Approval): May 14, 2012

Special Conditions of Approval:

* DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED*

2009 Biohazard Agents Registry, Dr. Robert Hegele

Name	CAT. NO.	SUPPLIER	MSDS	Comment	Use
3T3-L1	CL-173	ATCC	Y	Murine fibroblast	Mutant LMNA is transfected into
NIH/3T3	CRL-1658	ATCC	Y	Murine	cells and immunofluorescence st
COS-7: note SV40 promoter	CRL-1651	ATCC	Y	Green monkey (Ceropithecus aethiops)	and Western analysis performed
HEK293: note Adeno E1A promoter	CRL-1573	ATCC	Y	Human	
HepG2	CRL-11997	ATCC	Y	Human	
Fibroblast, finite primary cell line human	GM05659	Coriell Cell Repository	Y	Human: unaffected	Cells are cultured, morphology ar
Fibroblast, finite primary cell line human	GM08398	Coriell Cell Repository	Y	Human: unaffected	growth curves evaluated and immunofluorescence studies, We
Fibroblast, finite primary cell line human	GM03348	Coriell Cell Repository	Y	Human: unaffected	analysis, DNA and RNA isolated a microarray and high throughput
Fibroblast, finite primary cell line human	AG03513	Coriell Cell Repository	Y	Human: HGPS proband	sequencing analysis performed.
Fibroblast, finite primary cell line human	AG04456	Coriell Cell Repository	Y	Human: unaffected	
Fibroblast, finite primary cell line human	AG06234	Coriell Cell Repository	Y	Human: HGPS proband	
Fibroblast, finite primary cell line human	AG06297	Coriell Cell Repository	Y	Human: unaffected	
Fibroblast, finite primary cell line human	30950	Dr. T.C. Rupar	na	Human	Cells are cultured, DNA and RNA
Fibroblast, finite primary cell line human	40916	Dr. T.C. Rupar	na	Human	extracted and microarray and hig
Fibroblast, finite primary cell line human	20750	Dr. T.C. Rupar	na	Human	throughtput sequencing analysis
Fibroblast, finite primary cell line human	70280	Dr. T.C. Rupar	na	Human	performed
pCMV6	PS100001	Origene	y		Transfection vectors used in the l
pcDNA3.1	350492	Invitrogen	Y		
pENTR 11 dual selection vector	A10562	Invitrogen	Y		
Subcloning Efficiency™ DH5a™ Competent Cells	18265-017	Invitrogen	Y	E. Coli	Cells used to as vehicles for trans
Electromax DH10B competent cells	18290-015	Invitrogen		E. Coli	of genetic material for overexpre
ME DH10B competent cells	18297-010	Invitrogen		E. Coli	studies and phenotypic characte
ME DH5-alpha competent cells	18258-012	Invitrogen	Y	E. Coli	
ME DH5A T1 page resist comp cells	12034-013	Invitrogen		E. Coli	

In 2003, we proposed in HSFO #NA5320 to expand understanding of single-gene predisposition to cardiovascular disease (CVD) and since found causative genes and mutations for 3 forms of partial lipodystrophy, namely familial partial lipodystrophies types 2 and 3 (FPLD2, FPLD3) and acquired partial lipodystrophy (APL). Using "phenomics", defined as integrated multidisciplinary research to understand the complex consequences of genomic variation through systematic discovery and cataloguing of standardized phenotypes, we developed standardized, optimized quantitative non-invasive imaging procedures for carotid arteries and adipose stores, all key to this renewal application. The work was recognized by merit awards from several groups, including the American Heart Association in 2004 and the Genetics Society of Canada in 2006. ***We will build on the scientific momentum from the initial funding period with this renewal application.***

• **BACKGROUND AND SIGNIFICANCE:** The constellation of disturbed carbohydrate and insulin metabolism, with central obesity, dyslipidemia (elevated triglycerides [TG] with depressed HDL cholesterol), hypertension, and type 2 DM (T2DM) is called the 'metabolic syndrome' (MetS). Evaluation of patients with extreme monogenic forms of MetS will help us to understand common MetS, just as the study of patients with monogenic dyslipidemias improved understanding and treatment of those diseases. Some monogenic forms of MetS have been molecularly characterized – including those discovered in the Hegele lab - providing important insights and model systems for common MetS.

• **PROPOSED RESEARCH:** We propose to continue to expand our database of lipodystrophy kindreds, to significantly increase our characterization of biochemical, vascular and adipose phenotypes in monogenic MetS and then to assess these new markers in the general population. Specific aims are:

1) To extend our measurements of traditional and non-traditional serum biomarker phenotypes of subjects with FPLD2, FPLD3, APL, other lipodystrophies and familial hypercholesterolemia (FH; positive control for early CVD) and to contrast these according to molecular basis of the disease.
2) To measure baseline non-invasive ultrasound (US) vascular and magnetic resonance imaging (MRI) adipose phenotypes in subjects with FPLD2, FPLD3, APL, other forms of lipodystrophy and FH, and to contrast these according to molecular basis of the disease.
3) To serially measure non-invasive US vascular and MRI adipose phenotypes in subjects with FPLD2, FPLD3, APL, other forms of lipodystrophy and FH in order to evaluate disease progression, and to contrast these according to molecular basis of the disease.
4) To determine association between risk factors, intermediate phenotypes and atherosclerosis read-outs variables in subjects with FPLD2, FPLD3, APL, other forms of lipodystrophy and FH.

We expect to observe between-genotype differences in phenotypes such as: 1) lipoprotein, metabolic, cytokines, chemokines and other serum biomarkers; 2) carotid IMT at baseline and serial progression; 3) association of traditional and non-traditional risk factors with IMT; and 4) distribution of adipose stores in different forms of lipodystrophy. The presence of novel serum and imaging biomarkers identified in the studies of patients with monogenic MetS will subsequently be evaluated in individuals from the general population with MetS. We expect that some biomarkers that we will identify as being part of the monogenic MetS metabolic signature profile will be translated to the "garden variety" form of MetS.

• **RELEVANCE:** Our past record of finding determinants of CVD risk in Canadian families with monogenic MetS will continue with renewed funding of this project. Our success in finding MetS genetic determinants in pilot studies leads us to expect that this expanded set of samples will provide new insights. MetS is an important new prevalent CVD risk factor, with numerous metabolic abnormalities and a very strong relationship with vascular disease. Better characterization of early phenotypes in monogenic MetS – sensitive "phenomics" – may provide new clues to help solve the puzzle of the common metabolic syndrome. Any between-genotype differences in phenotype severity will help to plan future experiments assessing specific diagnosis and/or treatment.

Since 1989, the Hegele lab has identified: 1] the genomic basis of 10 human diseases; and 2] >100 human mutations disease-causing mutations in dyslipidemia and other metabolic disorders. Among other recognitions, this work received the 2004 JM Hoeg Basic Science and Clinical Research Award from the American Heart Association and the 2006 WF Grant & PB Moens Award of Excellence from the Genetics Society of Canada. This new application proposes to take this discovery record in a new direction, with the ***objective*** to ***define genomic factors underlying elevated plasma triglyceride*** [TG].

• **BACKGROUND AND SIGNIFICANCE:** Elevated plasma TG contributes to increased risk of cardiovascular disease [CVD]. Despite >20 years of research, there has been little progress in defining the molecular basis of susceptibility to two genetic disorders with very high TG, namely hyperlipoproteinemia (HLP) types 5 and 3. Furthermore, the role of apo C-I in human TG metabolism remains poorly defined. The time is ripe for re-addressing these issues due to the convergence of several factors, including availability of sufficient numbers of affected subjects, specification of numerous candidate genes, access to robust genomic technologies and the fortuitous discovery of a natural human *APOC1* mutation in a well-characterized population.

• **PROPOSED RESEARCH:** We will advance the understanding of the genetic basis of human dyslipoproteinemia by identifying and classifying new human genomic mutations in candidate genes affecting lipoprotein metabolism. To this end, we will study patients with primary dyslipoproteinemias characterized by elevated plasma TG and we will take advantage of a newly discovered human mutation in *APOC1* as a "probe" to learn more about the normal and pathophysiological function of this protein.

Hypotheses:

1] Multiple rare mutations in multiple candidate genes in lipoprotein metabolism are present in a substantial proportion of subjects with HLP type 5;

2] Multiple rare mutations in multiple candidate genes in lipoprotein metabolism are present in a substantial proportion of subjects with HLP type 3 homozygotes for the *APOE* E2/E2 genotype;

3] The private *APOC1* T45S polymorphism of the Oji-Cree will be associated with metabolic phenotypes, analogous to phenotypes seen in induced-mutant mouse models.

• **Specific aim 1: Advancing the understanding of HLP type 5 through:** a] extending the mutational spectrum in causative genes through large-scale genomic DNA sequence analysis; b] identifying mutations in candidate HLP type 5 genes; c] using array-based detection methods to identify common regions of the genome with large-scale copy number variations [CNVs] that are shared between HLP type 5 subjects; and d] defining *in vitro* mechanism[s] of disease of selected HLP type 5 mutations.

• **Specific aim 2: Advancing the understanding of HLP type 3 through:** a] extending the mutational spectrum in causative genes through large-scale genomic DNA sequence analysis in HLP type 3 subjects who have the *APOE* E2/2 genotype; b] identifying mutations in candidate HLP type 3 genes; c] using array-based detection methods to identify common regions of the genome with large-scale copy number variations [CNVs] that are shared between HLP type 3 subjects; and d] defining *in vitro* mechanism[s] of disease of selected HLP type 3 mutations.

• **Specific aim 3: Advancing the understanding of the physiological and pathophysiological role of** apo C-I by taking advantage of the private *APOC1* T45S polymorphism of the Oji-Cree and intensively studying its association with metabolic phenotypes.

• **RELEVANCE.** Serum TG is an important emerging CVD risk factor, with numerous determinants. Better characterization of the genetic determinants of elevated TG starting with simpler familial forms such as HLP types 5 and 3 will provide new clues to help solve the puzzle of the common hypertriglyceridemia. The results will provide genomic data that will identify potentially important new metabolic pathways for elevated TG and generate hypotheses for future cell biology, biochemical and clinical studies. The *APOC1* studies in the Oji-Cree will help to clarify the normal and pathophysiological role of this protein in humans. Our past record of finding determinants of CVD risk in Canadian patients at risk for CHD will continue with this project.

5. A complete informative summary of the proposal on this page.
Présenter sur cette page un résumé complet du projet de recherche.

Background: The importance of well-defined CVD phenotypes was highlighted by recent coining of the term 'phenomics', defined as integrated multidisciplinary research to understand the complex consequences of genomic variation. Sensitive new phenomic tools can reveal new phenotypic markers, sometimes called 'early' or 'intermediate' phenotypes. Because well-established 'traditional' risk factors do not account for all CVD events, 'non-traditional' risk factors have been proposed, including insulin resistance (IR). Evaluation of monogenic IR syndromes might help understand common IR, just as the study of monogenic forms of dyslipidemia, such as familial hypercholesterolemia (FH) improved understanding of those diseases. Some monogenic forms of IR have been molecularly characterized, providing interesting and sometimes important insights. One of these is Dunnigan-type familial partial lipodystrophy (FPLD), which results from mutations in one of two genes: LMNA or PPARG. FPLD is associated with early atherosclerosis and exists in Canadian families.

PROPOSED RESEARCH

Study objectives

1) To measure traditional and non-traditional biochemical phenotypes of subjects with FPLD-LMNA, FPLD-PPARG, and FH (positive control monogenic disease with atherosclerosis) and to contrast these according to molecular basis of the disease.
2) To measure baseline non invasive ultrasound (US) and magnetic resonance imaging (MRI) carotid phenotypes of FPLD-LMNA, FPLD-PPARG and FH subjects, and to contrast these according to molecular basis of the disease.
3) Over three years, to serially measure non-invasive US and MRI vascular (carotid) phenotypes of FPLD-LMNA, FPLD-PPARG, and FH subjects in order to evaluate disease progression, and to contrast these according to molecular basis of the disease.
4) To determine association between risk factors, intermediate phenotypes and atherosclerosis read-outs variables in FPLD-LMNA, FPLD-PPARG, and FH subjects.

Experimental strategy to test for genotype-phenotype associations

Subjects: Patients with FPLD-LMNA (N=60), FPLD-PPARG (N=20), FH (N=30) and matched controls (N=40)
Genotype: DNA sequence determination of LMNA, PPARG or LDLR as appropriate
Phenotypes: 1) Lipoprotein profile: cholesterol (total, HDL & LDL), TG, apo B & A1, Lp(a); 2) metabolic variables: insulin, glucose, glycosylated hemoglobin (HbA1C), CRP, C-peptide, free fatty acids, adiponectin, leptin, resistin, ASP; 3) Coagulation and inflammatory variables: FBG, PAI-1, TNF-a, IL-6; 4) Carotid US: IMT, TPA, TPV; 5) Carotid MRI: carotid wall volume, plaque composition; 6) Total body MRI: fat redistribution in selected individuals (3 per group)
Hypotheses: We will observe between-genotype differences in: 1) lipoprotein, metabolic coagulation and inflammatory markers corrected for age, sex and body mass; 2) carotid US and carotid MRI variables; 3) carotid US and MRI plaque progression; 4) association of traditional and non-traditional risk factors with non-invasive vascular read-outs; 5) distribution of adipose stores in FPLD-LMNA versus FPLD-PPARG.

Management plan. Years 1 and 2: Baseline DNA, biochemical and imaging analysis for 130 subjects; Years 2 and 3: 1-year and 2-year follow-up US and MRI for 130 subjects.

Relevance. Monogenic illnesses have provided key insights into CVD risk factors such as hypertension and dyslipidemia. IR is an important newer risk factor, with a very strong relationship with vascular disease. Better characterization of subjects with monogenic IR using sensitive methods - 'phenomics' - may provide clues to help solve the puzzle of the common metabolic syndrome.

Executive Summary

The vision of this program is to establish an integrated facility providing Ontario scientists with access to all aspects of disease gene discovery, including collection of family material, full genome scanning, fine genetic and physical mapping, identification of disease genes, mutational spectrum analysis and diagnostic testing applications. By having team members working towards the common goal of disease gene identification and by integrating the team into the genomics community in Ontario, The Facility for Advanced Genetic Analysis will be unique in the world.

The collaborating institutions include the University of Ottawa (Ottawa Hospital Research Institute), The Hospital for Sick Children, The Toronto Hospital and The John P. Robarts Research Institute.

A variety of commercial applications have been identified for the output of this project, including products in the area of pharmacogenomics, gene therapy and diagnostic screening tests.

DESCRIPTION: State the application's broad, long-term objectives and specific aims, making reference to the health relatedness of the project. Describe concisely the research design and methods for achieving these goals. Avoid summaries of past accomplishments and the use of the first person. This abstract is meant to serve as a succinct and accurate description of the proposed work when separated from the application. If the application is funded, this description, as is, will become public information. Therefore, do not include proprietary/confidential information. DO NOT EXCEED THE SPACE PROVIDED.

The overall goal of this proposal is to image inflammation in atherosclerosis by developing and testing novel activatable MR imaging agents that can sense myeloperoxidase (MPO) activity in atheromata. Emerging evidence suggests that activated macrophages secrete various enzymes that mediate inflammation in atherosclerosis, and that MPO in particular may be indicative of high-risk ("vulnerable") lesions. A recent clinical trial has further shown that elevated MPO levels strongly predict adverse cardiovascular outcomes in patients with chest pain. It is therefore hypothesized that imaging of local MPO activity will be useful in identifying vulnerable atherosclerotic lesions.

We have recently described two novel approaches for imaging peroxidases using either low molecular weight amplifiable paramagnetic substrates (Mol Imaging 2002;1:16-23) or superparamagnetic enzyme pseudosubstrates (Nanolett 2004;4:119-122). The latter method is based on magnetic relaxation switching (MRSW) a phenomenon that occurs when magnetic nanoparticles are brought into close contact to each other (R2 increase of 4-6 fold with negligible R1 change). These activatable, "smart" agents harness enzyme mediated amplification strategies and can be used to quantitatively measure enzyme activity and inhibition by MR imaging. The current proposal represents an effort to integrate investigators with different expertises to develop, implement and validate the larger field of *enzyme imaging* in atherosclerosis. Together, the team will translate basic results into new methodologies for *in vivo* MR imaging. The ultimate goal of this research is to develop clinically useful imaging tools for the molecular assessment of atherosclerosis in vivo, which are currently limited.

The overall objective of the Hegele lab is to discover the genetic basis of atherosclerosis susceptibility. This is being achieved through two complementary human genetic strategies: 1) studies of monogenic (mendelian) forms of atherosclerosis in Canadian probands and families; and 2) studies of complex traits related to atherosclerosis in Canadian communities and subpopulations. Recently, the lab's focus has shifted towards understanding the genetic basis of the metabolic syndrome (MetS), type 2 diabetes (T2DM) and obesity. Programs, objectives and operating funding are summarized in Table 1.

Table 1: Research programs for the Hegele lab under HSFO CI award #4380

Program name	Specific objective	Funding source(s)
Monogenic human disease models of atherosclerosis	1) Disease gene discovery:	CIHR MFI-43010 (2000-04)
	a) genes for monogenic MetS	CGDN (1998-2005)
	b) genes for monogenic dyslipidemias	
	c) genes for monogenic premature aging	
	2) Functional studies of LPLD mutations	CIHR MFI-43010 (2000-04)
	3) Laminopathies and atherosclerosis	CIHR MFI-43010 (2000-04)
	4) Human molecular genetics of dyslipidemia	CIHR MFI-43010 (2000-04)
	5) Phenomics of atherosclerosis	HSFO CS320 (2004-06)
	6) OjiCree T2DM and complications	CDA #1620 (2004-06)
	7) Extending mutation spectrum in known human disease genes	CIHR MFI-43010 (2000-04)
Complex human traits related to atherosclerosis	Find genetic determinants of atherosclerosis endpoints and intermediate traits in ethnic groups, including Canadian aboriginal people	HSFO CI-772 (2000-05) CIHR FRN-44087 (2000-05) IHRT CRI-43825 (2000-05)

A) Monogenic human disease models of atherosclerosis and intermediate phenotypes. The relevance to HSFO is that several of these monogenic diseases are associated with atherosclerosis and serve as human model systems of atherosclerosis. Characterizing the mutant gene in these monogenic diseases reveals new pathogenic pathways, establishes the importance in humans of a molecular pathway, and permits molecular stratification of mutant gene carriers. This will be accomplished by: 1) study of the human molecular genetics of LPLD; 2) *in vitro* structure function studies of new LPLD mutations; 3) studies of other laminopathies such as Hutchinson-Gilford progeria syndrome and atherosclerosis; 4) study of the human molecular genetics of selected dyslipidemias, such as familial hypercholesterolemia (FHD); 5) study of the phenomics of atherosclerosis by evaluation of extended families with monogenic forms of MetS and dyslipidemia; 6) continuation of studies of OjiCree diabetes from examination to vascular complications; 7) extending the mutation spectrum in human disease genes, such as abetalipoproteinemia, Tangier Disease, MODY, familial chylomicronemia, hypobetalipoproteinemia, familial amyloidosis, sitosterolemia and congenital complete lipodystrophy.

B) Complex human traits related to atherosclerosis. 1) Genetic determinants of atherosclerosis endpoints and intermediate traits in Canadian ethnic groups. The inheritance of CHD and its intermediate phenotypes does not fit a simple genetic model, so we have used the complementary strategies of association and subpart linkage analysis. We study homogeneous samples, such as Alberta Hutterites, Ontario Keewatin Inuit, Greenland Inuit, Trinidadian neonates, Chinese Canadians, European Canadians and 530 South Asian Canadians and 377 subjects with premature atherosclerosis. Phenotypes to be studied include lipoproteins, apolipoproteins, insulin, C-peptide and adipocytokines. A diverse set of approaches is documented in >100 peer-reviewed publications reporting results from these samples since this laboratory began in 1995. Protocols for genotyping, sequencing and other common assays have been established and experience is documented for the published work.

Relevance. Unveiling the molecular genetic basis of disease has introduced new mechanisms and therapeutic targets for study in MetS and CHD. Continued application of this approach will define new entire pathways, mechanisms and therapeutic targets. The programs will build on discoveries in Canadian populations based on regional, aboriginal health and pharmacogenetic research.

Summary of Research Proposal

The metabolic syndrome (MetS) affects 25% of Canadian adults and is a potent risk factor for atherosclerosis. Our strategy to understand the common MetS and dyslipidemia is to study rare human monogenic forms, such as familial partial lipodystrophy (FPLD) and familial hypercholesterolemia (FH). We will build on the ongoing record of discovery of causative genes for familial partial lipodystrophy types 2 (FPLD2; MIM 151660) and 3 (FPLD3; MIM 604367) and of human disease mutations causing hyperlipidemia, obesity and diabetes funded by CIHR application #MOP13430.

OBJECTIVE: ***To discover and define additional genetic determinants of obesity and plasma lipoproteins by studying rare monogenic human MetS in Canadian families and communities.***

Goals: 1) Advancing the understanding of FPLD by: a) extending the mutation spectrum in FPLD2 and FPLD3; b) identifying mutations in new FPLD genes; c) defining *in vitro* mechanism(s) of disease of selected mutations; d) extending the FPLD phenotype; defining phenotypic-genotype correlations. **2) Advancing the understanding of FH by:** a) extending the mutation spectrum in FH; b) identifying mutations in new FH genes; c) extending the FH phenotype; defining phenotypic-genotype correlations.

Experimental details: 1a) Extending the mutational spectrum in FPLD2 and FPLD3. Conventional direct sequencing of genomic DNA (gDNA) from 83 FPLD probands showed that 51 had no mutations in genes for FPLD2 [*LMNA* encoding lamin A/C] or FPLD3 [*PPARG* encoding peroxisome proliferator activated receptor-γ]. The candidate gene approach showed that two patients had novel rare missense mutations in *EMD* encoding emerin. These families will be expanded and evidence for causation will be obtained from co-segregation with phenotype, absence from normal controls and *in vitro* functional assessment. We will determine whether rare *EMD* missense mutations represent a new molecular form of FPLD – "FPLD4". The remaining 49 subjects with no mutation will have these genes re-screened using a new method to detect deletions called MLPA. Some probands with normal MLPA testing of candidate genes may have mutations in new FPLD genes. **1b) Identifying mutations in new FPLD genes.** We will use a candidate gene approach focusing on direct sequence analysis of gDNA. Candidates include genes involved in nuclear envelope biology, such as *LMNB1, LMNB2, LBR, LAP2, NARF, SENP2, MAN1* and *ZMPSTE24/FACE1*, and genes with a functional link to lipodystrophy, such as *RXRA, SREBP1, AGPAT2* and *BSCL2*. Also, some families may be sufficiently informative to perform linkage analysis. **1c)** ***In vitro*** **studies for selected FPLD mutations.** *LMNA* T496FS is the first splicing mutation in FPLD2. We will express the *LMNA* mutant in model cell lines – liver, adipocyte and fibroblast – and will assess effects on cellular phenotype morphologically, including assessment of fat content and assessment of 3-D nuclear structure, co-immunoprecipitation with other nuclear lamin constituents, such as emerin and with mRNA expression profiling. *PPARG* Y355X will be studied *in vitro* collaboratively with Dr. Todd Leff. **1d) Extending the FPLD phenotype.** We will: i) extend novel FPLD3 kindreds with γ4 promoter -14A>G and E1 FS ΔAATG; ii) perform lipidomic, proteomic and transcriptomic comparison of FPLD2 and FPLD3; iii) define the phenotype of pre-symptomatic FPLD children. **2a) Extending the mutational spectrum in FH.** Conventional direct sequencing of gDNA from 109 FH probands showed that 62 had no mutation in the HCHOLAD1 gene [*LDLR*]. We will re-screen these genes from gDNA of affected subjects using MLPA. We will sequence from gDNA of candidate genes including *APOB*, *NARC1/PCSK9* and *ARH*. **2b) New FH genes.** Some FH families have no mutation in a known gene and may be informative for linkage analysis. **2c) Extending the FH phenotype.** We will: 1) extend novel FH families and compare phenotypes between molecular forms.

Relevance. Demonstrating mutant *LMNA* in FPLD introduced a new mechanism - abnormal nuclear envelope structure and function - to the study of MetS and its metabolic complications. Disease mutations can serve as probes for new pathways or mechanisms. The planned studies will also help determine whether haploinsufficiency is an important disease mechanism in FPLD and whether simple replacement with normal gene product is feasible. Mutations in new will specify new pathways, disease mechanisms and therapeutic drug targets.

HEGELE, Robert A. SUMMARY OF RESEARCH PROPOSAL
"Genetic determinants of blood pressure"

We have submitted a duplicate of this proposal to HSFC, in which we propose to study Canadian genetic isolates to find genetic determinants of blood pressure (BP) primarily by testing for associations with candidate genes, whose products are involved in vascular biology. This approach has proven to be successful with 15 publications since 1997 on the topic of genetic determinants of BP.

OBJECTIVE

To discover new genetic determinants of BP and related traits in genetically isolated Canadian populations

HYPOTHESIS

Genomic variation within selected candidate genes is associated with variation in BP in Alberta Hutterites (AH), Ontario Oji-Cree (OC) and Keewatin Inuit (KI).

SPECIFIC AIMS

1. To evaluate reported candidate genes that contribute to variation in BP
2. To evaluate new candidate gene SNPs already discovered in our lab
3. To characterize new candidate gene SNPs, which can then be evaluated

Component 1: Candidate gene association studies in will be carried out in adults from our three samples, including evaluation of SNPs discovered in the Hegele lab (*CTSS* -256*G/A*; *LMNA* 1908C/T) and evaluation of previously reported SNPs (namely, *AGT* -6G/A, M/T174 & T/M235; *AGTR1* 1166A/C; *CYP11B2* -344C/T; *B3ADR* Y/R64; *B2ADR* R/G16 & Q/E27). Genotype X gender interactions will also be assessed. candidate gene associations will be evaluated in a sample of adolescent OC.

Component 2: Defining promoter sequence variants in *DAX1*. We showed that variation in the *DAX1* coding sequence is associated with hypotension. We will test the hypothesis that common variation in the *DAX1* promoter is associated with BP variation

Component 3: Additional candidate gene association studies. Other candidate genes for BP variation containing SNPs for which *in vitro* loss of function has been demonstrated include the *POMC*, *MTHFR*, *ET1*, *GRL* and *ANP* genes, and these are present priorities for evaluation in our study samples.

Relevance: Characterizing the genomic basis of BP variation is a first step towards developing: 1) diagnostic tests to stratify patients for evidence-based clinical decision making and intervention; and 2) novel targets for classical pharmacologic and/or genetic interventions. Given the success in identifying genetic determinants of BP, there is merit in studying this set of samples to investigate new candidate genes.

Time course, personnel and management plan: *Years 1 and 2:* Component 1 *Year 3:* Component 1 and 2. *Year 4:* Component 2 and 3; *Year 5:* Component 3. *Personnel:* Candidate genotyping, sequence analysis and transient gene expression and most statistical analyses will be performed by Dr. Jian Wang (Research Associate).

V SCIENTIFIC SUMMARY (Maximum one (1) page)

Structural and Functional Annotation of the Human Genome for Disease Study

In this application, we plan integrated and comprehensive computational and laboratory-based experiments to produce a more sophisticated and complete structural and functional annotation of human genomic variation, within four major, integrated themes. These themes are: Theme 1 (Scherer): To completely characterize the recently described phenomenon of large-scale copy variations (LCVs) in the human genome; Theme 2 (Hughes and Frey): To elucidate all gene coding sequences; Theme 3 (Blencowe and Frey) To characterize all splicing isoforms of all genes; and Theme 4 (Hegele): To evaluate the role of these newer genomic variations in mechanisms underlying selected human diseases, specifically atherosclerosis, diabetes and breast cancer. Since a complete understanding of genome biology is the ultimate goal of genomic investigation, analysis of patients will play a focal role in increasing our knowledge of function and regulation of the genes and types of variation involved. Our efforts to construct comprehensive new genomic datasets will enhance the characterization of clinical samples, in particular, for monogenic versions of complex diseases such as atherosclerosis, diabetes and breast cancer. Each of the four integrated and complementary themes will use high-throughput technologies within the OGI/Genome Resource Platform to assist in data gathering and will build additional capacity and new infrastructure. State-of-the-art technologies and advanced computational applications that we have developed in recent collaborations will be extended and applied to support each of our four Themes. Importantly, our activities will also be co-ordinated with the Canadian and International scientific community to enhance their application. Partnerships with the Sanger Institute, the European Bioinformatics Institute, the Human Epigenetics Consortium, Affymetrix, Agilent, Rosetta/Merck, and other USA and European genome scientists, have already been established to facilitate the work.

The annotated databases from this project will be made available to the international community, via links to existing genomic resources. They will serve as an international resource for biomedical researchers to address research inquiry into the spectrum of genomic variation in health and disease. The methods and algorithms used to generate the datasets we will also represent intellectual property. Furthermore, the databases will be maintained and curated in Canadian and European centres, in part from support from by private sector co-funds. The addition of the broad range of phenotype data linked to the unprecedented depth of genomic data will permit sophisticated modelling and hypothesis testing. The new markers of inter-individual genomic and early phenotype variation developed as a result of this project will provide new reagents for individual diagnosis and therapies.

A multi-disciplinary, multi-institutional GE^{3}LS team will address: (i) conceptions of health, disease, illness, normalcy & disability; (ii) socioeconomics of monogenic health services; (iii) informed choice to participate in monogenic disease research; (iv) professional & educational issues in the conduct of monogenic disease research.

Understanding the structure and organization of chromosomes, genes, transcripts and their corresponding variants is the first step towards systematic analysis of the normal function of genes and their regulation in an organism. Furthermore, annotation of the full range of human genomic variation provides a starting point for understanding inter-individual differences underlying various phenotypic conditions, including disease states. Canada's long-standing track record in genetic and genomic discovery, coupled with a comprehensive health care network, creates an unrivalled opportunity to make an international impact in the discovery of all types of human genetic variations and mechanisms of disease. Ultimately, our genomic discoveries will provide both fundamental and applied information contributing to the diagnosis and treatment of illness, spearheading improvements in health care for Canadians and for communities world-wide.

CIHR Team in Circumpolar Health Research:
Averting Emerging Chronic Diseases in Northern Populations:

1. Overview

Northern populations in the circumpolar region have begun to experience the emergence of chronic diseases such as cardiovascular diseases, diabetes, obesity and the metabolic syndrome which have occurred in other populations undergoing rapid social, cultural and economic transition.

Our Team Grant will create, develop and sustain an international, collaborative research program to monitor the burden of emerging chronic diseases among northern peoples; investigate genetic, behavioural and environmental risk factors that may be unique to these populations; and design and evaluate interventions in order to avert future epidemics. It formalizes an existing network across several circumpolar countries, linking academic research centres, regional health authorities, and indigenous peoples' organizations. It leverages funding from other sources and incorporates as integral components knowledge translation, research dissemination and training support. It aims to create a long-lasting legacy of enhanced capacity for robust health research in the North and for the North and improvement in the health of northern residents.

1.1 Importance and relevance

While there are many different pressing health concerns affecting northern populations, strategically the time to conduct intensive research into chronic diseases prevention is now, when the problem is still relatively limited in scope and early in its evolution. Northern populations are in the unique situation where rapid translation of research into policies, programs and practices can have a significant impact on improving health. Northern populations share many characteristics, including small size, remoteness and lack of human resources, and a team approach to research, especially one that adopts a circumpolar perspective, is essential. Our proposal is a direct response to the recommendations of the tri-council *Dialogue on Northern Research* workshop held in Whitehorse in 2004 [www.nserc.gc.ca/about/northern_summ_e.htm].

1.2 Overall Objectives

The proposed research program addresses the following broad scientific and public health questions. Specific objectives relating to individual projects are presented in Section 9.

- What is the current burden and distribution of chronic diseases among northern populations and what genetic, behavioural and environmental factors are responsible for their development?
- Why do northern communities differ in their risk of chronic diseases and in the impact of various health determinants?
- Why do northern peoples fare poorly compared to the general population of the larger nation-states with which they are associated?
- What can be done to reduce the burden and impact of prevalent and emerging chronic diseases affecting northern peoples?

The proposed Team, in executing its research plan, aims to cross traditional academic disciplines, while connecting multiple investigators, institutions, research sites and geographic regions. At the end of the 5-year Team Grant, the following short-term process outcomes or "deliverables" are expected:

3. Objectives of the Program Grant

Cellular Programs and Responses in Atherosclerosis: Linking Genotype to Phenotype

Our Central Theme is: *A key to understanding atherosclerosis is the elucidation of genetic determinants and ascertaining the impact of these determinants on metabolic outcomes and vascular cell responses - the phenomics of atherosclerosis.*

Objectives and Achievements from the Currently Funded HSFO Program

This application is the renewal of an HSFO Program Grant that has been tremendously successful. Our group established 4 important lines of investigation to examine cell responses in atherosclerosis, which has effectively linked our 5 laboratories to a common objective. Our discoveries of gene expression profiles directed us towards novel genes, pathways, and mechanisms of gene regulation that underlie vascular cell responses and function. Through strategic admixture of our complimentary research technologies, approaches and skill sets, the productivity emanating from the 4 objectives has been excellent. This degree of success would not have been possible without the HSFO Program Grant.

Our objectives for 2001-2005 were:

1. To define differential gene expression profiles in smooth muscle cells (SMCs) and macrophages.
2. To determine how gene mutations and single nucleotide polymorphisms (SNPs) associated with dyslipidemia, insulin resistance and the metabolic syndrome influence vascular cells.
3. To elucidate inflammatory cascades in cardiovascular disease.
4. To understand the relationship between alterations in G protein-coupled receptor (GPCR) signaling and vascular cell responses.

Progress:

Over the past 4 years, our HSFO group peer-reviewed publication record has been excellent and each manuscript has benefited from the programmatic approach we have adopted. Of significance, 25 of these manuscripts constituted formal collaborations between group PIs, as documented by shared authorships. Here we provide selected examples of our productivity in which major discoveries were made;, these advances would not have occurred without this integrative program.

Discovery of novel pathways driving diverse SMC phenotypes. We were the first, and to date only, group to successfully clone non-transformed SMCs from the media of the normal human adult artery. In doing so, we discovered distinct SMC subtypes. We have capitalized on this discovery, and the unique differentiation capacity of one SMC subtype, by undertaking microarray analysis and functional studies of putative novel SMC regulators. Of the many noteworthy findings, we discovered that lipoprotein lipase was differentially expressed and that this enzyme imparted to a subpopulation of SMCs a strong predisposition to accumulate lipids and differentiate into foam cells. We also discovered that Pre B-cell colony-enhancing factor regulates histone deacetylation and drives SMCs to a quiescent state, which attenuates the aging process. These findings opened an entirely new paradigm for foam cell formation and a novel cascade regulating SMC function and viability, with important consequences for plaque stabilization and potential therapy. These discoveries arose from collaborations between Drs. Huff, Pickering, and Hegele and have been published in *Circ Res* (2001), *ATVB* (2004), and *Circ Res* (2005).

Influence of gene mutations and variants, associated with dyslipidemia and the metabolic syndrome on vascular cells: We successfully applied genomic technologies to be the first in the world to discover the genetic basis of human monogenic forms of atherosclerosis, including familial partial lipodystrophy (FPLD) types 2 and 3, due respectively to mutations in *LMNA*, encoding nuclear lamin A/C, and *PPARG*, encoding peroxisomal proliferator-activated receptor gamma. These discoveries quickly lead to identification of novel pre-symptomatic metabolic phenotypes of insulin resistance that are predictors of future disease risk, together with novel vascular phenotypes ascertained through *in vivo* carotid ultrasound in affected subjects with familial partial lipodystrophy (FPLD). In other studies, we discovered and characterized at the transcriptional level a functional promoter variant in *PCK1*, encoding phosphoenol-pyruvate carboxykinase-1, the key enzyme in gluconeogenesis, and then

proceeded to show disparate associations between this *PCK1* variant and two vascular phenotypes, 'plaque volume' derived from 3-D ultrasound and conventional intima-media thickness (IMT), in aboriginal Canadians with type 2 diabetes. Furthermore, we elucidated superiority of 'plaque volume' over IMT as a biomarker of atherosclerosis in aboriginal Canadians with diabetes. This has established our key analytical strategies as a validated experimental paradigm for defining genotype-phenotype correlations in atherosclerosis. These discoveries arose from collaborations between Drs. Hegele, Huff and Pickering that were published in *Circulation* (2001), *ATVB* (2003), *JCEM* (2004) and *Stroke* (2005).

Elucidation of novel signaling events in vascular regulation and inflammation. We have made several key advances pertaining to novel pathways through which G-protein-coupled receptors (GPCRs) regulate events central to vascular function. Major discoveries include identification of a nitric oxide-based mechanism by which aldosterone mediates acute vasoconstriction via GPCR signaling. We also established, using microarray analysis, that the phenomenon of blunted ß-adrenergic receptor signaling in SMCs derived from hypertensive animals extends beyond the failure of SMCs to relax, since it also manifests as a global suppression of growth-related gene expression. Our focus on uncoupling of GPCRs from G-protein interactions led to the surprising finding that the GPCR regulatory protein ß-arrestin interacted with RalGDS to reorganize the cell cytoskeleton. Furthermore, a GPCR polymorphism was discovered that is associated with constitutive binding of ß-arrestin to a specific GPCR (fMLP receptor) and to increased plasma C-reactive protein, highlighting a new linkage between GPCR signaling and vascular inflammation. These discoveries arose from collaborations between Drs. Ferguson, Feldman, Pickering and Hegele that have been published in *Nature Cell Biology* (2002), *Circulation* (2003), and *Molecular Pharmacology* (2005) as well as a submission to *J Biol Chem*.

Proposal for Renewal of HSFO Program

Background: Atherosclerosis develops over time as a consequence of hemodynamic stress, abnormal metabolism, and local biological perturbations including monocyte and lymphocyte recruitment to the arterial intima, smooth muscle cell (SMC) migration and proliferation, matrix production, foam cell formation, and thrombus deposition. While several genes responsible for rare single-gene vascular disorders have been described, unravelling the genetic determinants of the complex pathogenesis of common atherosclerosis remains a significant challenge. Our group proposes to combine efforts across a spectrum of model systems and technologies in order to efficiently accumulate new insights into the genomic-phenomic interplay that underlies atherosclerosis.

Hypothesis: *Elucidation of genetic determinants and ascertainment of their impact on metabolic and vascular responses will reveal novel pathways involved in initiation and progression of atherosclerosis and will provide new insights into, and targets for, prevention and treatment.*

Our Experimental Approaches: Each investigator brings unique *in vitro* and *in vivo* models and approaches to understanding the pathogenesis of atherosclerosis. We will utilize a systems approach through which we will integrate discovery science with our hypothesis-driven research. This integrated HSFO Program Grant capitalizes on our now well-developed core resources, including DNA sequencing, high-density microarray analysis, confocal microscopy, metabolic phenotyping, molecular pathology, and vascular imaging. Together with the established expertise of the principal investigators, this approach will add substantial value to each existing component research program. Three integrated objectives have been identified.

Specific Objectives:

1. To link genotype to phenotype in vascular disease.
2. To elucidate novel cellular programs in smooth muscle cells and macrophages that underlie the productive remodeling of the atherosclerotic artery.
3. To define novel signaling pathways that dynamically regulate vascular cell function.

Objective 1: To link genotype to phenotype in vascular disease

Introduction: The genetic basis of atherosclerosis and its constituent risk factors, such as metabolic syndrome (MetS), is exceedingly complex. Over the current term of this Program Grant, we made

substantial strides towards elucidating genomic determinants of atherosclerosis, of its risk factors and of vascular cell behaviour. Our research direction is both to expand the repertoire of genetic/genomic players and then to link these with structural and functional phenotypes in humans, animal models and vascular cells. Our key experimental paradigms are: 1) gene discovery in model systems, including ethnic communities, vulnerable families, animals, organs, tissues and cells; 2) characterization of genotype-phenotype relationships, including intervention studies, in human monogenic disease states and in other model systems; 3) microanalyses of vulnerable plaque heterogeneity, linking morphology, cellular and molecular pathways, and the transcriptome.

One important context in which to examine these paradigms is the MetS, a phenotype found in 25% of Canadians that is a potent risk factor for atherosclerosis. Our strategy to understand MetS is to study rare human monogenic forms, such as FPLD and human genetic variants causing hyperlipidemia, hypertension, obesity, diabetes and vascular traits. Genes discovered using this paradigm provide the springboard for the application of our full spectrum of scientific expertise and core technologies.

Proposed research: In research supported by GIAs 1, 2, 3 and 5, Drs. Hegele, Pickering and Huff will advance understanding of atherosclerosis-related traits by: a) identifying mutations in new genes; b) extending the mutational spectrum in monogenic forms of disease in which the gene is already known; c) using functional assays to define disease mechanisms that are caused by specific human gene variants; and d) extending metabolic and vascular phenotypes in molecularly-characterized patients and disease families. Phenotyping methods include extensive analyses of a wide range of biochemical markers and metabolic indices together with the use of novel imaging modalities (such as 3D-ultrasound and MRI) to expedite whole patient phenotyping with a focus on vascular structure and fat distribution profiles. Additional phenotypes include *in vivo* plaque morphology, plaque compartmentalization using laser capture microscopy and expression analysis of aging genes.

Determinants of human atherosclerosis. We will apply our genomic analysis platforms, including expression arrays, 500K SNP DNA arrays for both large-scale copy number variation and association analyses, together with state-of-the-art bioinformatics methods, in order to identify new gene clusters, genes and/or variants that are associated with metabolic and/or vascular phenotypes. Gene variants so identified can be examined for association with human phenotypes in independent replication samples. Validated novel genes and variants associated with metabolic or vascular phenotypes at the population level can then be extensively studied using biochemical, cellular and molecular biological approaches in order to expand our understanding of new pathways. Conversely, candidate genes arising from unbiased experiments in model systems can be studied for association with MetS, dyslipidemia and accelerated atherosclerosis in families and populations. Examples of functionally characterized genes in which variants were or will be identified and studied for phenotype associations in various populations include *PBEF*, *WTAP*, *HSP47*, *fMLP*, *NPC1L1*, *AC6*, and *GPR30*. We will extend novel disease families and compare phenotypes between different molecular forms of the same gene product, which will allow us to define variability in the phenotype. We will combine this information with strategic use of functional experiments in animal and/or cell models. These approaches used together will elucidate novel pathways in pathogenesis of vascular disease. As we have repeatedly demonstrated, human disease mutations can serve as probes for new pathways or mechanisms.

Determinants of insulin-related phenotypes in atherosclerosis. Insulin resistance is associated with a specific pattern of hepatic gene expression that is associated with overproduction of atherogenic lipoproteins, partly as a consequence of the failure of insulin to activate specific intracellular signaling pathways. We discovered that, like insulin, naturally occurring molecules called flavonoids reverse this pattern of gene expression. Importantly, these flavonoids activate insulin signaling pathways independent of the insulin receptor, potentially via a novel GPCR. Experiments in cultured human hepatocytes and in diet-induced insulin-resistant LDL-receptor knockout mice are proposed to implicate specific components of the cell signaling pathways and to determine whether flavonoids attenuate insulin resistance and atherogenesis.

Determinants of cellular aging in atherosclerosis. Genotype-phenotype relationships will also be studied in the milieu of cellular aging and atherosclerotic plaque stability. Atherosclerotic plaques are exposed to stresses that can accelerate cellular aging (by oxidation and replication) which could, in

turn, have profound implications for plaque stability. We have discovered that the longevity of vascular SMCs can be extended by manipulating the expression of genes that generate or consume NAD. We will define the topographical expression profile of these and other longevity/survival genes in discrete regions of human atherosclerotic tissue dissected by laser capture microscopy. The gene expression fingerprint relevant to aging will be mapped within plaque subregions and according to morphological features such as fibrous cap thickness. These multi-level transcriptome-phenotype linkages will reveal novel molecular patterns intrinsic to plaque aging and stability.

Mutations in new genes, and elucidation of vital gene expression programs, will specify new pathways, disease mechanisms and therapeutic targets. We have a potent collaborative partnership involving all members of this application. Our ready access to leading edge genomic map data, genotypes, sequences, expression profiles and phenomics uniquely positions us to make ground-breaking, multi-faceted discoveries in an accelerated manner, resulting in increased understanding of atherosclerosis.

Objective 2: Elucidate novel cellular programs in smooth muscle cells and macrophages that underlie the productive remodeling of the atherosclerotic artery.

Introduction: Vascular SMCs are normally quiescent, with their primary function being to contract and relax in response to physiological stimuli. In vascular disease however SMCs acquire new attributes that enable them to proliferate, migrate, produce and degrade extracellular matrix (ECM). SMCs that adopt this more assertive synthetic phenotype are critical for repairing diseased arteries and stabilizing atherosclerotic plaques. However, synthetic SMCs often fail to prevent plaque rupture. One reason is that SMCs replicate as the aging process accelerates which creates susceptibility to apoptosis and enfeebles their ability to perform vital functions. We discovered novel genes that regulate the maintenance of SMCs within a maturation-competent, slowly aging state. Our group also discovered that hyperlipidemia profoundly affects SMCs, forcing them into a phenotype akin to macrophage foam cells, which in turn incapacitates the elaboration of extracellular matrix. Macrophage-derived foam cells themselves play a focal role at each stage of lesion development, yet the pathways regulating their survival and governing their ability to efflux cholesterol remain poorly understood.

Proposed Research: ***Molecular determinants of plaque cell viability.*** In research supported by GIAs 3, 4 and S-GIA 1, Drs. Huff, Pickering and Hegele will investigate novel and recently discovered regulatory pathways that direct the performance of SMCs and macrophages. One major advance is our discovery of Pre-B-Cell Colony Enhancing Factor (PBEF), an enzyme that drives the activity of sirtuins, which, through deacetylation of key nuclear proteins, act as guardians against cell senescence. We hypothesize that enhanced PBEF activity will attenuate SMC aging and will sustain their ability to productively remodel the artery wall. This function will be evaluated using virus-mediated gene transfer into human cells. We also propose that PBEF enhances macrophage survival, allowing them to retain their ability to efficiently efflux cholesterol via the reverse cholesterol transport pathway. Expression profiles from microarray analysis of SMCs and macrophages that have been activated by PBEF will be interrogated for genes that program survival and longevity. Comparing transcriptomes and functional responses within and between these 2 cell types will provide a unique integrative picture of novel and potentially powerful determinants of the SMC and macrophage contribution to plaque stability.

Molecular determinants of foam cell formation. A second major discovery is that cholesterol efflux from macrophages can be strikingly enhanced through selective activation of endogenous oxysterol production. Oxysterols activate the liver X receptor which drives the expression of cholesterol efflux transporters. To broaden the implications of this oxysterol-based reprogramming of gene expression, we will define the potential for oxysterol-induced cholesterol efflux in SMC-derived foam cells.

Cellular and extracellular matrix determinants of plaque composition. The assembly and stability of extracellular matrix (ECM) fibrils also determines productive arterial remodeling and plaque stability. However, as SMCs age and accumulate lipids, the assembly and stability of the ECM decline precipitously. We propose that impelling both PBEF-mediated cell survival and cholesterol efflux pathways will attenuate or even reverse this decline. We will test this hypothesis using our novel models of fibrillogenesis and foam cell formation induced by atherogenic human lipoproteins isolated from genetically-defined dyslipidemic patients (see objective 1). ECM synthesis and degradation by SMCs and macrophages, respectively, will be measured using our well established techniques. The impact of

enhanced oxysterol synthesis on atherogenesis and plaque composition will be assessed *in vivo* in LDL-receptor knockout mice. The proposed link to activation of LXR-mediated gene expression will be tested in detail using laser capture microscopy to dissect subpopulations of arterial cells.

Objective 3. Defining novel signaling pathways that dynamically regulate vascular cell function.

Introduction: G protein-coupled receptors (GPCRs) play a central role in regulating vascular SMC responses to circulating and locally released hormones. The regulation of vascular smooth muscle tone represents a balance between endothelial and vascular smooth muscle receptor-mediated mechanisms of regulation, the activation of GPCRs linked to vasodilation and vasoconstriction and GPCR-regulated signaling linked to proliferation. Moreover, the activity of the GPCRs regulating these pathways is modulated by intracellular mechanisms that define receptor G protein-coupling, desensitization and resensitization. We discovered that the steroid hormone aldosterone mediates acute effects on vascular smooth muscle tone by mediating contractile responses potentially through the activation of the orphan GPCR GPR-30. In addition, the activity of different GPCRs linked to vasodilation and vasoconstriction of vascular SMCs can be differentially regulated based on differences in: 1) their intracellular trafficking patterns between various endosomal compartments; 2) their association with different Rab GTPases and 3) their propensity to either resensitize or remain desensitized.

Proposed Research: Research supported by GIAs 6, 7 and 8, Drs. Feldman, Ferguson and Pickering will examine the impact of novel components of GPCR signaling on vascular SMC function.

Determinants of vascular tone. We will exploit our recent discovery that aldosterone acutely regulates vascular tone through GPCR signaling. We will test the hypothesis that aldosterone mediates its acute effects in SMCs via the activation of the orphan GPCR, GPR-30. The biochemical consequences in human SMCs challenged acutely with aldosterone will be examined following the targeted knockdown by short hairpin RNA or overexpression of either GPR-30 or the mineralocorticoid receptor, using viral vectors. Functional consequences will be evaluated in single cells by dynamic time-lapse imaging. The concept of aldosterone-mediated acute activation of GPCR signaling will be extended to endothelial cells and intact vessels in organ culture using our well developed model systems. The role of this aldosterone-induced signaling in the pathogenesis of hypertension will be elucidated *in vivo* using genetic (SHR) and acquired (Dahl salt-sensitive) rat models of hypertension and ultimately in humans with hypertension using gluteal biopsies and perfusion myography.

Cellular determinants of GPCR signaling in the vasculature. We will capitalize on our discovery that specific RabGTPases are critical for GPCR recycling. This has led us to hypothesize that Rab GTPases critially regulate human SMC contractile function by orchestrating the endocytosis, trafficking, recycling and resensitization of the β_2 adrenergic receptor and angiotensin-1 receptor. We will assess whether the resensitization of endogenous β_2AR and AT_1AR in adult human arterial SMCs is altered by the adenoviral-induced expression of dominant-negative or constitutively active GFP-tagged Rab5, Rab4 and Rab11 proteins. Using confocal microscopy and biochemical determinations, we will determine whether these Rab GTPases regulate the endocytosis and trafficking of GPCRs to endosomes (Rab5) as well as the recycling of receptors back to the cell surface via rapid (Rab4) and slow (Rab11) recycling endosomes. Alterations in SMC-mediated vascular responsiveness will be assessed in organ cultures from rat aortic and mesenteric arteries transfected with adenoviral constructs encoding dominant negative and constitutively active Rab GTPases. The long-term goal of these experiments will be to develop mice in which Rab GTPases are over-expressed or knocked out, in a vascular smooth muscle-specific manner, and assess the impact on hypertension and predisposition to atherosclerosis.

Summary

This program will create significant synergies resulting in the establishment of coherent lines of investigation that link our laboratories to a central theme – the phenomics of atherosclerosis. Our proposed studies in humans, *ex vivo* tissues, animal models and cells will capitalize on state-of-the-art technologies to elucidate genetic determinants of atherosclerosis and their impact on metabolism and vascular function. Our results will allow us to identify novel pathways involved in the pathogenesis of this complex disease and provide an enhanced and improved rationale for the diagnosis and treatment of atherosclerosis.

Your file / Votre référence

Our file / Notre référence

Name and/or Organization: **University of Western Ontario**
Robarts Research Institute
Attn: Dr. Robert Hegele

Address: P.O. Box 5015, 100 Perth Drive
London, ON
N6A 5K8

The following biological material does not require a Public Health Agency of Canada import permit under the HPIR*:

Human fibroblast cell lines from healthy donor (AG06234) and from donor with Hutchinson-Gilford progeria syndrome (AG06297), as provided by Coriell Institute for Medical Research, 403 Haddon Avenue, Camden, NJ, USA 08103.

Marianne Heisz
Chief, Importation and Regulatory Affairs

APRIL 20, 2009
Date

NOTICE

***HPIR (HUMAN PATHOGENS IMPORTATION REGULATIONS)**

- We are in receipt of your application for an importation permit for biological materials. The **HPIR** apply **only** to the importation of infectious substances which cause human disease and their subsequent distribution or transfer. Other materials, which are deemed by the importer to be non-infectious for humans, **do not** require a permit under these regulations. It should be noted that the importation of biological materials may also be subject to other federal, provincial and municipal laws.
- For animal or plant pathogens one **must** apply to The Canadian Food Inspection Agency (CFIA) for a permit to import. If this material is of animal or plant origin it may also require a permit from the CFIA. Please contact the CFIA for their consideration. CFIA contact numbers are as follows:
  (613) 221-7068 for information concerning animal pathogens/material
  (613) 225-2342 [ext. 4334] for information concerning plant pathogens/material
- Importation of this material may also be subject to the requirements of the *New Substances Notification Regulations (Organisms)* of the *Canadian Environmental Protection Act, 1999*, administered by Environment Canada and Health Canada. Please contact the New Substances Information Line at 1-800-567-1999 or nsn-infoline@ec.gc.ca.
- You may be required to provide the Canada Border Services Agency (CBSA) customs officers with a declaration that the imported material is non-infectious and non-hazardous.

Should you require further information, please contact:
Office of Laboratory Security
Centre for Emergency Preparedness and Response
(613) 957-1779

Canada

Public Health Agency of Canada | Agence de santé publique du Canada

Your file | Votre référence

Our file | Notre référence

Date issued: October 4, 2006

Name and/or Organization: **Robarts Research Institute**
Attn: Dr. Robert A. Hegele

Address: 100 Perth Drive, Room 4-06
London, ON
N6A 5K8

The following biological material does not require a Public Health Agency of Canada import permit under the HPIR*:

Human fibroblast cell lines from healthy donor (AG04456) and from donor with Hutchinson-Gilford Progeria Syndrome (AG03513), as provided by Coriell Cell Repositories, NJ, USA.

Paul J. Payette, Ph.D.
Director, Office of Laboratory Security

October 4, 2006
Date

NOTICE

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Canada

2009 Biohazard Agents Registry, Dr. Robert Hegele

Name	CAT. NO.	SUPPLIER	MSDS	Comment	Use
3T3-L1	CL-173	ATCC	Y	Murine fibroblast	Mutant LMNA is transfected into t
NIH/3T3	CRL-1658	ATCC	Y	Murine	cells and immunofluorescence stu
COS-7: ~~note SV40 promoter~~	CRL-1651	ATCC	Y	Green monkey (Ceropithecus aethiops)	and Western analysis performed
HEK293: ~~note Adeno E1A promoter~~	CRL-1573	ATCC	Y	Human	
HepG2	CRL-11997	ATCC	Y	Human	
Fibroblast, finite primary cell line human	GM05659	Coriell Cell Repository	Y	Human: unaffected	Cells are cultured, morphology an
Fibroblast, finite primary cell line human	GM08398	Coriell Cell Repository	Y	Human: unaffected	growth curves evaluated and immunofluorescence studies, Wes
Fibroblast, finite primary cell line human	GM03348	Coriell Cell Repository	Y	Human: unaffected	analysis, DNA and RNA isolated an
Fibroblast, finite primary cell line human	AG03513	Coriell Cell Repository	Y	Human: HGPS proband	microarray and high throughput sequencing analysis performed.
Fibroblast, finite primary cell line human	AG04456	Coriell Cell Repository	Y	Human: unaffected	
Fibroblast, finite primary cell line human	AG06234	Coriell Cell Repository	Y	Human: HGPS proband	
Fibroblast, finite primary cell line human	AG06297	Coriell Cell Repository	Y	Human: unaffected	
Fibroblast, finite primary cell line human	30950	Dr. T.C. Rupar	na	Human	Cells are cultured, DNA and RNA
Fibroblast, finite primary cell line human	40916	Dr. T.C. Rupar	na	Human	extracted and microarray and hig
Fibroblast, finite primary cell line human	20750	Dr. T.C. Rupar	na	Human	throughtput sequencing analysis
Fibroblast, finite primary cell line human	70280	Dr. T.C. Rupar	na	Human	performed
pCMV6	PS100001	Origene	y		Transfection vectors used in the l
pcDNA3.1	350492	Invitrogen	Y		
pENTR 11 ~~dual selection vector~~	A10562	Invitrogen	Y		
~~Subcloning Efficiency~~™ DH5a™ ~~Competent Cells~~	18265-017	Invitrogen	Y	E. Coli	Cells used to as vehicles for transf
~~Electromax~~ DH10B ~~competent cells~~	18290-015	Invitrogen		E. Coli	of genetic material for overexpre
ME DH10B ~~competent cells~~	18297-010	Invitrogen		E. Coli	studies and phenotypic character
ME DH5-alpha ~~competent cells~~	18258-012	Invitrogen	Y	E. Coli	
ME DH5A T1 ~~page resist comp cells~~	12034-013	Invitrogen		E. Coli	