Molecular genetics of type 2 diabetes in New Zealand Polynesians

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In memory of my grandfather

Leopold Pritchard

Abstract

The risk of developing type 2 diabetes is four fold higher in New Zealand (NZ) Polynesians compared to Caucasians. Hence diabetes is more prevalent in Maori (16.5% of the general population) and Pacific Island people (10.1%) compared to NZ Caucasians (9.3%). It is generally accepted that type 2 diabetes has major genetic determinants and heterozygous mutations in a number of genes have previously been identified in some subsets of type 2 diabetes and certain ethnic groups. The high prevalence of diabetes in NZ Polynesians, when compared with NZ Caucasians, after controlling for age, income and body mass index (BMI), suggest that genes may be important in this population. Therefore, the prevalence of allelic variations in the genes encoding amylin and insulin promoter factor-1 (IPF-1), and exon 2 of the hepatocyte nuclear factor-1α (HNF-1α) gene in NZ Polynesians with type 2 diabetes was determined. These genes are known to produce type 2 diabetes in other populations. The genes investigated were screened for mutations by PCR amplification and direct sequencing of promoter regions, exons and adjacent intronic sequences from genomic DNA. DNA was obtained from 146 NZ Polynesians (131 Maori and 15 Pacific Island) with type 2 diabetes and 387 NZ Polynesian non-diabetic control subjects (258 Maori and 129 Pacific Island). Sequences were compared to previously published sequences in the National Centre for Biotechnology Information database. Allelic variations in IPF-1 and exon 2 of the HNF-1α gene were not associated with type 2 diabetes in NZ Polynesians. However, in the amylin gene, two new and one previously described allele was identified in the Maori population including: two alleles in the promoter region (–132G>A and –215T>G), and a missense mutation in exon 3 (Q10R). The –215T>G allele was observed in 5.4% and 1% of type 2 diabetic and non-diabetic Maori respectively, and predisposed the carrier to diabetes with a relative risk of 7.23. The –215T>G allele was inherited with a previously described amylin promoter polymorphism (-230A>C) in 3% of Maori with type 2 diabetes, which suggests linkage equilibrium exists between these two alleles. Both Q10R and –132G>A were observed in 0.76% of type 2 diabetic patients and were absent in non-diabetic subjects. Together these allelic variations may account for approximately 7% of type 2 diabetes in Maori. These results suggest that the amylin gene maybe an important candidate marker gene for type 2 diabetes in Maori.

Preface

This work was carried out between January 1998 and January 2001 in the School of Biological Sciences, University of Auckland. This thesis is submitted for examination purposes only.

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I declare that this thesis is the result of my own work, includes nothing which is the outcome of this work done in collaboration and has not been submitted in whole or part to any other university.

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Abbreviations

Abbreviations used in the text are described below. Unit abbreviations are described in S.I. [System Internationale (d'Unites)] form, and standard notations are used for chemical formulae.

A	adenine
Aβ	beta amyloid
ABI	Applied Biosystems Incorporated
AD	alzheimer's disease
AP-1	activated protein –1
ApoE	apolipoprotein E
ATP	adenine triphosphate
BMI	body mass index
β3AR	β-3-adrenergic receptor
bp	base pairs
C	cytosine
cAMP	cyclic adenosine monophosphate
CAT	chloroamphenicol acetyltransferase
C/EBP	CAAT/enhancer binding protein
CPE	carboxypeptidase E
CRE	cAMP-response element
CREB	CRE-binding protein
dATP	deoxyadenosine triphosphate
dCTP	deoxycytosine triphosphate
dDTP	deoxythymidine triphosphate
del	deletion
dGTP	deoxyguanosine triphosphate
DNA	deoxyribonucleic acid

dNTP	deoxynucleotide triphosphate
EDTA	ethylenediaminetetra-acetic acid
EMSA	electrophoretic mobility shift assay
ESRF	end stage renal failure
FABP	fatty acid binding protein
fsdel	frame shift deletion
fsins	frame shift insertion
Grb2	growth factor receptor binding protein-2
GLUT	glucose transporter
GTP	guanosine triphosphate
HbA_{1c}	glycosylated haemoglobin
HDL	high density lipoprotein
HLA	histocompatibility leucocyte antigen
HLH	helix loop helix
HNF	hepatocyte nuclear factor
HOMA	homeostasis model assessment
HPLC	high pressure liquid chromatography
IAPP	islet amyloid polypeptide
IB-1	islet brain-1
IDDM	insulin dependent diabetes mellitus
IGF	insulin growth factor
IGT	impaired glucose tolerance
IFG	impaired fasting glucose
IL-6	interleukin-6
ins	insertion
IPF-1	insulin promoter factor-1
IRS	insulin receptor substrate
Isl-1	Lim/homeodomain gene islet-1
JNK	c-Jun N-terminal kinase
Kb	kilobase
kDa	kilo daltons
LDL	low density lipoprotein

LPK	L-type pyruvate kinase
M	molar
MAPK	mitogen activated protein kinase
MELAS	mitochondrial myopathy
MIDD	maternally inherited diabetes and deafness
MMP	matrix metalloelastase
ug	microgram
ul	microlitre
mg	milligram
ml	millilitre
mmol	millimole
min	minute
MODY	maturity-onset diabetes of youth
mtDNA	mitochondrial DNA
MW	molecular weight
NADH	reduced form of nicotinamide adenine dinucleotide
NFAT	nuclear factor of activated T cells
NFκB	nuclear factor κ B
NIDDM	non-insulin dependent diabetes mellitus
ntd	nucleotide
NZ	New Zealand
NCBI	National Centre for Biotechnology Information
NDDG	National Diabetes Data Group
NZHI	New Zealand Health Information Service
OB	obesity
OB-R	obesity receptor
OGTT	oral glucose tolerance test
OHA	oral hypoglycaemic agents
PBS	phosphate buffered saline
PC	prohormone convertase
PCR	polymerase chain reaction
PEPCK	phosphoenolpyruvate carboxykinase

PI3-K	phosphotidylinositol 3-kinase
WHO	World Health Organisation
Rab	ras related protein
Rad	Ras associated with diabetes
RFLP	restriction fragment length polymorphism
RIN	rat insulinoma
RNA	ribonucleic acid
rpm	revolutions per minute
RT	room temperature
SAPK	stress activated protein kinase pathway
SDS	sodium dodecyl sulphate
SH2	src homology domain 2
SNP	single nucleotide polymorphism
T	thymidine
TCA	tricarboxylic acid
TE	tris-EDTA buffer
TGFβ	transforming growth factor-β
thT	thioflavin T
TNF	tumour necrosis factor
tRNA	transfer ribonucleic acid
U	units
UTR	untranslated region
UV	ultra violet
V	volts
VLDL	very low density lipoprotein
VNTR	variable number of tandem repeats
vol	volume
w/v	weight per volume