Screening of Insulin *(INS)* Gene in Permanent Neonatal Diabetes During The First Year of Life

Thesis

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Abstract

Background: Permanent neonatal diabetes (PND) is a monogenic form of diabetes resulting from mutations in a number of different genes encoding proteins that play a key role in the normal function of the pancreatic beta-cell. A correct genetic diagnosis can affect treatment and clinical outcome. Mutations in the insulin gene (*INS*) itself have been identified as a cause of neonatal diabetes. This study aimed to investigate the genetic variations in the coding region and intronic boundaries of *INS* gene and their genotype phenotype correlation in a group of Egyptian PNDM infants with onset in the first 12 months of age.

Methods: We screened exons 2 and 3 with intronic boundaries of *INS* gene by direct gene sequencing in 30 PND patients (14 males and 16 females) and in 20 healthy control subjects to verify the resulting single nucleotide polymorphisms (SNPs). A detailed clinical phenotyping of the patients was carried out to specify the diabetes features in those found with an *INS* variant.

Results: We identified five variants (four SNPs and one silent mutation), c.187+11T>C, c.-17-6T>A, c.*22A>C, c.*9C>T and c.36G>A (p.A12A) with allelic frequency of 96.7%, 80%, 75%, 5% and 1.7%, respectively. All showed no statistically significance difference compared to the controls except for c.*22A>C.

Conclusion: Genetic screening for the *INS* gene did not reveal any evident role in diagnosis of PNDM among the studied group of Egyptian children.

Keywords: Permanent neonatal diabetes (PND); *INS*; mutations; DNA sequencing

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List of Abbreviations

ABCC8	ATP-binding cassette transporter subfamily C, member 8
AD	Autosomal dominant
APS	Adenosine 5'phosphosulfate
AR	Autosomal recessive
CBP	Creb-binding protein
CCD	Charge coupled device
ddNTP	Dideoxynucleotide
DEND	Developmental delay, Epilepsy and Neonatal Diabetes
DKA	Diabetic ketoacidosis
DMR	Differentially methylated region
dNTP	Deoxynucleotide
EIF2AK3	Eukaryotic translation Initiation Factor 2 Alpha kinase 3
ERAD	Endoplasmic reticulum associated degradation
FOXP3	Forkhead box P3
GCK	Glucokinase gene
GAD	Glutamate decarboxylase
GLIS	Glioma-associated oncogene similar
GlisBS	Glis binding site
GLUT2	Glucose transporter 2
GWAS	Genome wide association studies
HCR	Hydrophobic core region
HGVS	Human Genome Variation Society
HIL	Hypomethylation at imprinted loci
HYMAI	Hydatiform mole-associated and imprinted
IA-2A	Islet antigen-2 antibody
IC	Imprinting center
IDDM2	Insulin dependent diabetes mellitus 2
ILPR	Insulin-linked polymorphic region
INS	Insulin gene
IPEX	Immune dysregulation, Polyendocrinopathy, and Enteropathy, X-linked syndrome
IPF1	Insulin Promoter Factor-1
IRDN	Insulin-related DNA polymorphism
IUGR	Intra-uterine growth retardation
IVS	Intervening sequences

K_{ATP}	ATP-sensitive potassium channel
KCNJ11	Potassium inwardly-rectifing channel, subfamily J, member 11
Kir	Inwardly rectifying potassium channels
MafA	v-maf musculoaponeurotic fibrosarcoma oncogene homolog A
MDI	Monogenic diabetes of infancy
MODY	Maturity onset diabetes of the young
NCBI	National Centre of Biotechnology Information
NDM	Neonatal Diabetes Mellitus
NEUROD1	Neurogenic differentiation 1
NGS	Next-generation sequencing
PCR	Polymerase Chain Reaction
PDX1	Pancreas/ duodenum homeobox protein 1
PGD	Pre-implantation genetic diagnosis
PLAGL1	Pleomorphic adenoma of the salivary gland gene like 1
PNDM	Permanent Neonatal Diabetes Mellitus
PPi	pyrophosphate
PTF1A	Pancreas transcription factor 1A
RFX	Regulatory factor X-box binding family
SGA	Small for gestational age
SLC2A2	Solute carrier family member 2
SLC19A2	Solute carrier family 19 member 2
SNPs	Single nucleotide polymorphisms
SOLiD	Sequencing by Oligo Ligation Detection
SP	Signal peptide
SUR	Sulfonylurea receptor
TNDM	Transient Neonatal Diabetes Mellitus
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
UPD	Uniparental disomy
UTR	Untranslated region
VNTR	Variable number tandem repeat
WRS	Wolcott-Rallison syndrome
ZAC	Zinc finger protein which regulates apoptosis and cell cycle
ZFP57	Zinc finger protein 57
ZMWs	Zero-mode waveguides

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Introduction

Monogenic diabetes is a heterogeneous group of disorders characterized most often by pancreatic beta-cell dysfunction as a result of a single gene mutation. Monogenic subtypes include neonatal diabetes that is either permanent or transient (PNDM/TNDM) and maturity onset diabetes of the young (MODY) ***(Rubio-Cabezas and Argente, 2008; Edghill et al., 2010; and Schwitzgebel, 2014)***.

Over the last decade the insight into the causes of neonatal diabetes has greatly expanded. Neonatal diabetes was once considered a variant of type 1 diabetes that presented early in life. Studies in understanding of this disorder have established that neonatal diabetes is not an autoimmune disease, but rather is a monogenic form of diabetes resulting from mutations in a number of different genes encoding proteins that play a key role in the normal function of the pancreatic beta-cell ***(Stoy et al., 2010; and American Diabetes Association, 2014)***.

Mutations in the genes encoding the ATP-sensitive potassium channel (K_{ATP}) subunits [Potassium inwardly rectifying channel, subfamily J, member 11 *(KCNJ11)* encoding Kir6.2, ATP-binding cassette transporter subfamily C member 8 *(ABCC8)* encoding *SUR1*] and in the gene encoding insulin *(INS)* account for most cases of PNDM/monogenic diabetes of infancy [MDI] ***(Russo et al., 2011; and American Diabetes Association, 2014).***

As in monogenic diabetes a single gene mutation decides about the disease phenotype, it is possible to establish a specific diagnosis based on a DNA analysis in a considered patient. The search for a mutation is typically performed by automated sequencing. Making such a diagnosis usually has significant clinical importance as it may influence diabetes treatment, explain pleiotropic features and define the prognosis in the

examined subject as well as in other family members ***(Ellard et al., 2008; and Rubio-Cabezas et al., 2011)***.

Aim of the work

This study aimed to investigate the genetic variations in the coding region and intronic boundaries of *INS* gene and their genotype phenotype correlation in a group of Egyptian PNDM infants with onset in the first 12 months of age.

Chapter 1

Permanent Neonatal Diabetes Mellitus

Neonatal diabetes (ND) is a monogenic form that is usually defined as overt diabetes diagnosed during the first 6 months of life ***(Hussain et al., 2013)***. The disease can be clinically subdivided into transient (TNDM) and permanent (PNDM) forms depending on whether or not insulin dependence resolves in infancy ***(Garin et al., 2012)***.

Monogenic diabetes is a result of a single gene mutation at different allelic loci and does not involve an environmental component in disease formation as with polygenic forms ***(Rubio-Cabezas and Argente, 2008)***. PNDM is caused by mutations affecting genes that play a critical role in the development, survival and function of pancreatic β-cells ***(Miki et al., 2001; and Rubio-Cabezas et al., 2011)***.

Incidence of PNDM

PNDM is a rare condition with a reported incidence of approximately 1: 200,000 births in Caucasian populations ***(De Franco et al., 2013)***.

Diagnosis of PNDM

Neonatal diabetes mellitus presents as hyperglycemia, failure to thrive and, in some cases, dehydration and ketoacidosis, which may be severe with coma in a child within the first months of life ***(Polak and Cavé, 2007)***.

Almost two-thirds of the infants diagnosed in the first 6 months were born small for gestational age (SGA), in contrast to 15% of those presenting later ***(Iafusco et al., 2002)***. Because insulin exerts potent growth-promoting effects during intrauterine development ***(Gicquel and Le Bouc, 2006)***, a low birth weight might reflect reduced insulin secretion by the fetal pancreas suggesting a more precocious damage to