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Phenotypic heterogeneity in monogenic diabetes: The clinical and diagnostic utility of a gene panel-based next-generation sequencing approach

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ABSTRACT

Single gene mutations that primarily affect pancreatic β-cell function account for approximately 1–2% of all cases of diabetes. Overlapping clinical features with common forms of diabetes makes diagnosis of monogenic diabetes challenging. A genetic diagnosis often leads to significant alterations in treatment, allows better prediction of disease prognosis and progression, and has implications for family members. Currently, genetic testing for monogenic diabetes relies on selection of appropriate individual genes for analysis based on the availability of often-limited phenotypic information, decreasing the likelihood of making a genetic diagnosis. We thus developed a targeted next-generation sequencing (NGS) assay for the detection of mutations in 36 genes known to cause monogenic forms of diabetes, including transient or permanent neonatal diabetes mellitus (TNDM or PNDM), maturity-onset diabetes of the young (MODY) and rare syndromic forms of diabetes. A total of 95 patient samples were analyzed: 19 with known causal mutations and 76 with a clinically suggestive phenotype but lacking a genetic diagnosis. All previously identified mutations were detected, validating our assay. Pathogenic sequence changes were identified in 19 out of 76 (25%) patients: 7 of 32 (22%) NDM cases, and 12 of 44 (27%) MODY cases. In 2 NDM patients the causal mutation was not expected as consanguinity was not reported and there were no clinical features aside from diabetes. A 3 year old patient with NDM diagnosed at 3 months of age, who previously tested negative for *INS*, *KCNJ11* and *ABCC8* mutations, was found to carry a novel homozygous mutation in *EIF2AK3* (associated with Wolcott–Rallison syndrome), a gene not previously suspected because consanguinity, delayed growth, abnormal bone development and hepatic complications had not been reported. Similarly, another infant without a history of consanguinity was found to have a homozygous *GCK* mutation causing PNDM at birth. This study demonstrates the effectiveness of multi-gene panel analysis in uncovering molecular diagnoses in patients with monogenic forms of diabetes.

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1. Introduction

Monogenic diabetes mellitus includes a heterogeneous group of diabetes types that are caused by mutations in one of an expanding list of genes [1]. It can be familial or sporadic and if familial, the inheritance can be dominant, recessive or X-linked. It is estimated that the monogenic forms of diabetes together could represent as much as 1–2% of all cases of diabetes mellitus [2]. The main phenotypes suggestive of an underlying monogenic cause include transient or permanent neonatal diabetes mellitus (TNDM or PNDM), maturity-onset diabetes of the young (MODY) and rare diabetes-associated syndromes. More than twenty genes highly expressed in the pancreatic beta-cell have been identified in these monogenic subtypes, and many other genes have been implicated in syndromes that often include diabetes. Several etiological mechanisms of dysfunction are involved including impairment of pancreatic beta-cell development and/or gene expression, failure of glucose sensing, disruption of insulin synthesis, disorders of ion channels and increased endoplasmic reticulum stress leading to destruction of the beta-cell [3–5].

Abbreviations: NGS, Next-Generation Sequencing; TNDM, Transient Neonatal Diabetes; PNDM, Permanent Neonatal Diabetes; MODY, Maturity-Onset Diabetes of the Young; NDM, Neonatal Diabetes; GATK, Genome Analysis Tool Kit; ESP, Exome Sequencing Project, GERP, Genomic Evolutionary Rate Profiling; PolyPhen-2, Polymorphism Phenotyping v2; SIFT, Sorting Intolerant from Tolerant; HGMD, Human Gene Mutation Database; VOUS, Variant of Unknown Clinical Significance; WES, Whole Exome Sequencing; WGS, Whole Genome Sequencing.

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It is likely that the majority of patients with monogenic diabetes go unrecognized [6] and continue to be misdiagnosed as type 1 or type 2 diabetes [7–9]. In addition to elucidating the etiology of the patient's diabetes and explaining other associated clinical features, establishing the underlying monogenic cause can provide important prognostic and therapeutic information. Attention has recently focused on the most common forms of PNDM caused by heterozygous activating mutations in the *KCNJ11* and *ABCC8* genes, which encode the protein subunits (Kir6.2 and SUR1) of the ATP-sensitive potassium (K_{ATP}) channel [10–12]. The majority of patients with K_{ATP} channel mutations may be treated successfully with oral sulfonylureas alone in lieu of multiple daily insulin injections. This transition results in improved glycemic control and supports a crucial role for genetic testing in all neonatal diabetes patients [13–15]. In addition, *HNF1A* and *HNF4A* mutations cause forms of MODY that are often sensitive to low-dose sulfonylurea therapy [16,17], while heterozygous mutations in the *GCK* gene generally lead to a mild fasting hyperglycemia that seldom needs treatment and is not associated with significant complications [18,19]. Thus, uncovering a genetic basis by making an accurate molecular diagnosis is extremely important for optimal treatment of these patients and may lead to dramatic improvement in their quality of life. Moreover, once a mutation is established, at-risk family members can be screened and predictive genetic testing can be offered to relatives after appropriate genetic counseling. As monogenic diabetes is a genetically heterogeneous group of disorders, the ability to use next-generation sequencing (NGS) technology to sequence several genes simultaneously is a potentially cost-effective means of increasing the rate of molecular diagnosis in affected probands [20–22]. In this study, we describe the development of the first panel-based NGS assay for monogenic diabetes available in the United States.

2. Methods

2.1. Study subjects

Subjects with neonatal diabetes or with a clinical and/or family history suggestive of MODY or syndromic forms of diabetes were consented for participation through the University of Chicago Monogenic Diabetes Registry (http://monogenicdiabetes.uchicago.edu/registry/) through which longitudinal information regarding the diagnosis and treatment of diabetes, other medical problems or complications, family history and genetic testing results, was collected through surveys and medical records [23]. A neonatal diabetes phenotype was defined as persistent hyperglycemia requiring treatment diagnosed under 1 year of age. A MODY phenotype was defined as diabetes diagnosed under 35 years of age in a non-obese individual with either a linear family history of diabetes or the absence of diabetes-related autoantibodies. All subjects were consented for participation through protocols approved by the Institutional Review Board at the University of Chicago. Genomic DNA was isolated from patients' saliva or blood samples using the Oragene OG-300 non-invasive saliva sampling kit (DNA Genotek Inc., Ottawa, ON, Canada) or the PureGene DNA isolation kit (Qiagen Inc., Valencia, CA) according to the manufacturer's instructions. DNA integrity was verified using a Qubit Fluorometer (Life technologies, Darmstadt, Germany). A total of 95 families were chosen to examine the utility of a gene panel-based next-generation sequencing approach: 19 with known mutations and 76 with a clinically suggestive phenotype but no known genetic etiology.

2.1.1. Gene selection

The monogenic diabetes panel included the most common genes causing NDM/MODY (*ABCC8*, *GCK*, *HNF1A*, *HNF4A*, *HNF1B*, *INS*, *KCNJ11*), genes involved in less common known causes of NDM/MODY and congenital hyperinsulinism (*AKT2*, *BLK*, *CEL*, *CISD2*, *CP*, *EIF2AK3*, *FOXP3*, *GATA6*, *GLIS3*, *GLUD1*, *HADH*, *KLF11*, *IER3IP1*, *INSR*, *NEUROD1*, *NEUROG3*, *PAX4*, *PDX1*, *PTF1A*, *RFX6*, *SLC2A2*, *TBC1D4*, *WFS1*, *ZFP57)* and genes involved in extremely rare syndromic forms of diabetes mellitus (*ALMS1*, *DCAF17*, *SLC19A2*, *SLC29A3*, *PAX6*) (Supplementary Table 1). Only genes that had been proven to cause disease were included in the panel.

2.1.2. Next-generation sequencing

The targeted NGS approach was based on HaloPlex enrichment (Agilent Technologies, Santa Clara, CA, USA) followed by MiSeq Illumina NGS. HaloPlex probes were designed following Agilent's recommendations [24,25] to enrich all exons, plus 10-bp at each end, of the 36 selected genes previously associated with NDM, MODY and very rare syndromic forms of diabetes mellitus (Table 1). HaloPlex enriched Illumina libraries were obtained following Agilent's recommendations with the exception that the gDNA-probe hybrids were amplified using the KAPA HiFi HotStart PCR kit (KAPA Biosystem) (1X KAPA HiFi Fidelity GC buffer, 0.8 mM dNTPs, 1 μM PCR primers, 20 μM Acetic Acid and 1U of KAPA HiFi HotStart polymerase), and the following program in the Applied Biosystems 2720 thermocycler: 98 °C for 2 min; 21 cycles of 98 °C for 30 s, 60 °C for 30 s and 72 °C for 1 min; followed by 72 °C for 10 min then hold on 8 °C. HaloPlex enriched Illumina libraries were quantified using the Agilent 2100 Bioanalyzer, multiplexed (8 samples) and 150PE sequenced on the Illumina MiSeq system following Agilent's recommendations. Data from each sequence run was de-multiplexed and reads aligned to the reference human genome (hg18) using the Burrows–Wheeler Aligner (version 0.6.2). Reads in the regions of the genome that are susceptible to alignment artifacts due to the presence of repetitive sequences were locally realigned using GATK (version 1.0.5506). The NGS data analysis and variant calling were performed using an in-house custom-developed bioinformatics pipeline and a commercial software package (NextGENe, Softgenetics, State College, PA). Raw variant calls were filtered based on various quality metrics such as depth, quality by depth score and directional bias. Variants were then annotated in regard to their positions in transcripts of interest, position relative to the coding sequence, consequence for the protein or mRNA and a collection of direct and indirect evidentiary tools and databases including NCBI dbSNP, 1000 Genomes Project, Exome Sequencing Project (ESP), GERP, Conseq, PolyPhen-2, SIFT and the Human Gene Mutation Database (HGMD).

2.1.3. Sanger sequencing

All sequence variants with putatively deleterious effects were confirmed by conventional Sanger sequencing in both forward and reverse directions on a 3730xl DNA Analyzer (Life Technologies, Grand Island,

Table 1.
Subjects with known mutations used to validate the gene panel.

Patient	Gender	Gene	Nucleotide	Amino acid	Zygosity
1	M	*ABCC8*	c.3440T>G, c.4135C>T	p.Leu1147Arg, p.Arg1379Cys	Comp HET
2	M	*EIF2AK3*	c.1267dup	p.Ile423Asnfs*26	HOMO
3	M	*GATA6*	c.1088_1098del	p.Gln363Argfs*96	HET
4	F	*KCNJ11*	c.602G>A	p.Arg201His	HET
5	F	*KCNJ11*	c.5T>C	p.Leu2Pro	HET
6	M	*RFX6*	c.779A>C	p.Lys260Thr	HOMO
7	F	*FOXP3*	c.1044 + 5G>A	p.?	HET
8	M	*FOXP3*	c.340C>T	p.Arg114Trp	HEMI
9	M	*FOXP3*	c.1227_1235del	p.Asp409_Leu411del	HOMO
10	M	*GCK*	c.449 T>A	p.Phe150Tyr	HET
11	F	*GCK*	c.617C>T	p.Thr206Met	HET
12	M	*GCK*	c.766G>A	p.Glu256Lys	HET
13	M	*HNF1A*	c.476G>A	p.Arg159Gln	HET
14	M	*HNF1A*	c.872dup	p.Gly292Argfs*25	HET
15	F	*HNF1A*	c.862G>T	p.Gly288Trp	HET
16	F	*HNF1A*	c.872del	p.Pro291Glnfs*51	HET
17	M	*HNF4A*	c.416C>T	p.Arg134Gln	HET
18	F	*INSR*	c.1268 + 2 T>C	p.?	HOM
19	M	*PDX1*	c.188del	p.Pro63Argfs*60	HET