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Running title: Double heterozygous *HNF1A* and *HNF4A* mutations in Youth

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Title: Digenic heterozygous *HNF1A* and *HNF4A* mutations in two siblings with childhood-onset diabetes.

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Abstract

Monogenic diabetes due to mutations in the transcription factor genes *HNF1A* and *HNF4A* is characterized by islet cell antibody negative, familial diabetes with residual insulin secretion. We report two sisters with childhood onset diabetes who are both heterozygous for the most common mutation in each of two transcription factors, hepatocyte nuclear factor 1A (HNF1A) and hepatocyte nuclear factor 4A (HNF4A). The proband was diagnosed with diabetes at 7 years of age and treated with insulin for 4 years. Her genetic diagnosis resulted in transition to sulfonylureas for one and a half years before insulin therapy was re-initiated due to declining glycemic control. Her sister was diagnosed with diabetes at 14 years of age, treated initially with insulin but has been well controlled on oral sulfonylurea therapy for over two years. Both sisters inherited the *HNF4A* gene mutation R127W from their mother and the *HNF1A* gene mutation P291fsinsC (c.872dup) from their father. The father was diagnosed with diabetes at 45 years of age. Their brother is heterozygous for the *HNF4*A R127W mutation. Both the brother and mother have normal glucose tolerance at the ages of 16 and 46 years, respectively.

Digenic inheritance of *HNF1A* and *HNF4A* mutations is very rare and has only been reported in two families where conclusive evidence for the pathogenicity of their mutations was lacking. Follow-up studies in this family co-segregating the two most commonly reported *HNF1A/HNF4A* mutations will be informative for understanding the effect of digenic inheritance upon phenotypic severity and response to sulfonylurea therapy.

Key words: *HNF1A*, *HNF4A*, MODY, youth

Introduction

Monogenic diabetes including maturity onset diabetes of the young (MODY) results from mutations in single genes that regulate beta cell function and accounts for up to 5% of all diabetes cases in the US(1). Clinicians should consider a diagnosis of monogenic diabetes in lean youth with negative islet cell autoantibodies at diagnosis of diabetes, low insulin requirements and/or a family history of diabetes (2). The most common forms of monogenic diabetes involve mutations in hepatocyte nuclear factor 1A (*HNF1A*), glucokinase (*GCK*) and hepatocyte nuclear factor 4A (*HNF4A*) (2).

The SEARCH for Diabetes in Youth study (SEARCH) is a multicenter, population-based study of youth with diabetes diagnosed under 20 years of age. In the SEARCH monogenic diabetes ancillary study, genomic DNA was sequenced for mutations in *HNF1A* and *HNF4A* in diabetes autoantibody negative participants enrolled in SEARCH between 2001 and 2006, if the fasting C-peptide concentration was >0.8ng/ml and stored DNA was available along with written consent for genetic testing. In one proband we identified a mutation in both the *HNF1A* and *HNF4A* genes.

To date, there are two reports in the literature describing three individuals who have both *HNF1A* and *HNF4A* mutations. The first report describes an adolescent who inherited a different mutation from each parent while the second paper describes a mother and son with both mutations (3, 4). We studied the genotypes and phenotypes within the family of a SEARCH participant with mutations identified in both *HNF1A* and *HNF4A*, in order to investigate the digenic inheritance within this family.

Case Report

The proband is a 14 year old female who presented at 7 years of age with polyuria and polydipsia without ketoacidosis. The diabetes autoantibody panel [glutamic acid decarboxylase-65 (GAD65), insulinoma antigen-2 (IA-2), and insulin autoantibody (IAA)] performed at the Barbara Davis Center (Aurora,CO) at the time of initial presentation was negative. A diagnosis of type 1 diabetes was made and she was treated with NPH and Regular insulin.

On recruitment to SEARCH, at the age of 8 years, she was well controlled on 0.3 units/kg/day of insulin with a hemoglobinA1c (HbA1c) of 5.9% (reference range: 3.9-6.1%). Her fasting C-peptide was 0.86 ng/mL (reference range: 0.5-3 ng/dL) and 60-minute stimulated C-peptide was 1.96 ng/mL. Due to the negative diabetes autoantibody panel, she underwent genetic testing as part of the SEARCH monogenic diabetes ancillary study at 11 years of age demonstrating a heterozygous missense mutation in exon 4 of *HNF4A*, R127W (c.379C>T) and a heterozygous frameshift mutation in exon 4 of *HNF1A*, P291fsinsC (c.872dup). Insulin therapy was completely discontinued and she was started on glipizide (1.25 mg once daily) with the dose titrated to 2.5 mg once daily based on blood sugar checks, with weekly blood sugar reviews and close support from a diabetes specialist nurse practitioner. There was no significant change in her glycemic control (HbA1c was 6.5% before and 6 months after; reference range: 3.5-6.3%). However, 21 months after switching to glipizide, glycemic control worsened (HbA1c: 8.6%) despite increasing doses of glipizide, necessitating re-initiation of insulin therapy. Currently, at 14.5 years of age, she is receiving 0.4 units/kg/day of insulin as NPH and Regular, and glipizide 10 mg/day, with an HbA1c of 6.8%. The choice of NPH and Regular insulin was based on parental request.

Prior to the identification of the gene mutations in the proband, hyperglycemia was detected in the proband's sister at 14 years of age during a routine well-child examination. She was asymptomatic. A diagnosis of type 1 diabetes was made and NPH and Regular insulin therapy (0.3 units/kg/day) was initiated. Due to the findings in the proband, the sister also underwent genetic testing as part of the SEARCH monogenic study and was also found to be heterozygous for both the *HNF1A* and *HNF4A* mutations. Insulin was discontinued and glipizide therapy was initiated and titrated with improvement in glycemic control (HbA1c decreased from 8.7% to 5.8%, 5 months after; reference range: 3.5-6.3%). She has remained on extended release glipizide 2.5 mg/day for the past 27 months with a recent HbA1c of 6.1%.

The proband's father was known to be "borderline diabetic". His oral glucose tolerance test (OGTT) confirmed the presence of diabetes (fasting glucose 92 mg/dL and 2-hour glucose of 215 mg/dL). Genetic testing revealed heterozygosity for the P291fsinsC *HNF1A* mutation. The paternal grandfather was diagnosed with diabetes at age 65 years but was not available for genetic testing. Paternal great-grandfather was reported to have diabetes since the age of 20 years but lived to the age of 82 years (see Figure 1).

The proband's mother was asymptomatic at 45 years of age and an OGTT showed a fasting plasma glucose of 97mg/dl and a 2-hour value of 117 mg/dl, consistent with normal glucose tolerance. Genetic testing revealed the R127W mutation in *HNF4A*. She did not have gestational diabetes mellitus during any of her three pregnancies. Her extended family refused genetic testing. Testing of the proband's brother demonstrated the same *HNF4A* mutation as the mother and a normal OGTT result (fasting plasma glucose 98 mg/dL and 2-hour value 123 mg/dL). All family members were lean with underweight or normal body mass index (BMI).

Discussion

In the family described in this report, we identified two separate transcription factor MODY mutations, both reported in previous studies as pathogenic mutations. The *HNF1A* P291fsinsC (c.872dup) mutation is the most common mutation causing HNF1A-MODY, described in 234 families and the *HNF4A* R127W mutation is the most common mutation causing HNF4A-MODY, described in 15 families throughout the world (5)(Colclough K et al. 2012, submitted to Human Mutation). Digenic cases are likely to be very rare, but may be under ascertained due to sequential gene testing in molecular genetic laboratories meaning that if an *HNF1A* mutation is found, *HNF4A* analysis is not undertaken. Simultaneous mutation testing of multiple genes by next generation sequencing will reveal the true prevalence of digenic inheritance in MODY.

In the previous reports of families with two transcription factor mutations the evidence for the pathogenicity of both mutations is inconclusive. The mother and son reported by Beijers et al.(4) were heterozygous for *HNF1A* G31D and *HNF4A* H214Y, but the G31D substitution has subsequently been identified in 7/4300 European exomes (Exome variant server, NHLBI GO Exome Sequencing Project http://evs.gs.washington.edu/EVS/). It is therefore unlikely to be causative of MODY. The proband described by Forlani et al.(3) was heterozygous for *HNF1A* E508K and *HNF4A* R80Q. Both mutations are novel and whilst a different mutation, R80W, has been reported in *HNF4A (6)*, further evidence to support the pathogenicity of E508K is lacking.

The siblings we describe with the *HNF1A* P291fsinsC and *HNF4A* R127W mutations are the first cases of digenic transcription factor MODY where both mutations have previously been reported as being pathogenic. The *HNF1A* P291fsinsC (c.872dup) mutation is the most common of all MODY mutations: it results in a frameshift and premature termination codon. There is no

doubt over its pathogenicity and both sisters had inherited this mutation from their diabetic father. The *HNF4A* R127W mutation was first described by Furuta et al. in 1997(7) and is the most common *HNF4A* mutation, reported in the literature in 15 families from multiple countries. The *R127W* mutation is a missense mutation which is thought to retain 50% of the wild-type activity without dominant negative effect (8). Initial functional studies failed to demonstrate an effect upon transcriptional activation(9) but other in vitro assays showed decreased DNA binding and reduced transcriptional activation. (8, 10) Interestingly *HNF4A R127W* has recently been identified in 1/4300 European exomes (Exome Variant Server, NHLBI GO Exome Sequencing Project, http://evs.gs.washington.edu/EVS/). This could be a chance finding of an individual predisposed to MODY or a suggestion that the *R127W* mutation may not cause monogenic diabetes. Additional data from exome sequencing in large cohorts is required for conclusive proof of the pathogenicity of this mutation.

The absence of a maternal family history of diabetes may be explained by a *de novo* mutation in the mother, particularly since *de novo* mutations are more common in the *HNF4A* gene compared to *HNF1A(6).* Unfortunately, the mother's family was not available for genetic testing.

The age at diagnosis of diabetes in patients with MODY varies considerably (from 4 to 77 years for patients with *HNF1A* or *HNF4A* mutations in the Exeter cohort) and is influenced by type 2 diabetes risk variants(11), intrauterine hyperglycemia(12) and mutation type or location(13). The proband and her sister described in this study were diagnosed at 7 and 14 years respectively. Whilst we might speculate that digenic inheritance could cause earlier onset of diabetes, the mean age at diagnosis for individuals within the SEARCH study with single gene mutations is 12 years (*HNF1A*) and 11 years (*HNF4A*) (Gilliam LK et al. 2012, submitted to Diabetes Care). A good response to sulfonylurea therapy is a characteristic of HNF1A/4A MODY (14, 15). While

both siblings with digenic mutations responded to sulfonylureas in the short term, one re-started insulin after 18 months due to deterioration in glycemic control. Thus, long term studies are required to assess eventual insulin requirement in patients with digenic diabetes.

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235 Table 1: Clinical Features and Mutations in First-degree Relatives

	Current Age (years)	Age at diagnosis of diabetes (years)	HNF 1A mutation	HNF 4A mutation	Term Birth weights (kg)	BMI (kg/m^2)
Proband	14	7	P291fsinsC/N	R127W/N	3.37	16.5
Sister	16	14	P291fsinsC/N	R127W/N	4.11	17.7
Brother	18	-	N/N	R127W/N	3.26	17.3
Mother	48	-	N/N	R127W/N	2.75	18.9
Father	47	45	P291fsinsC/N	N/N	3.01	21.5

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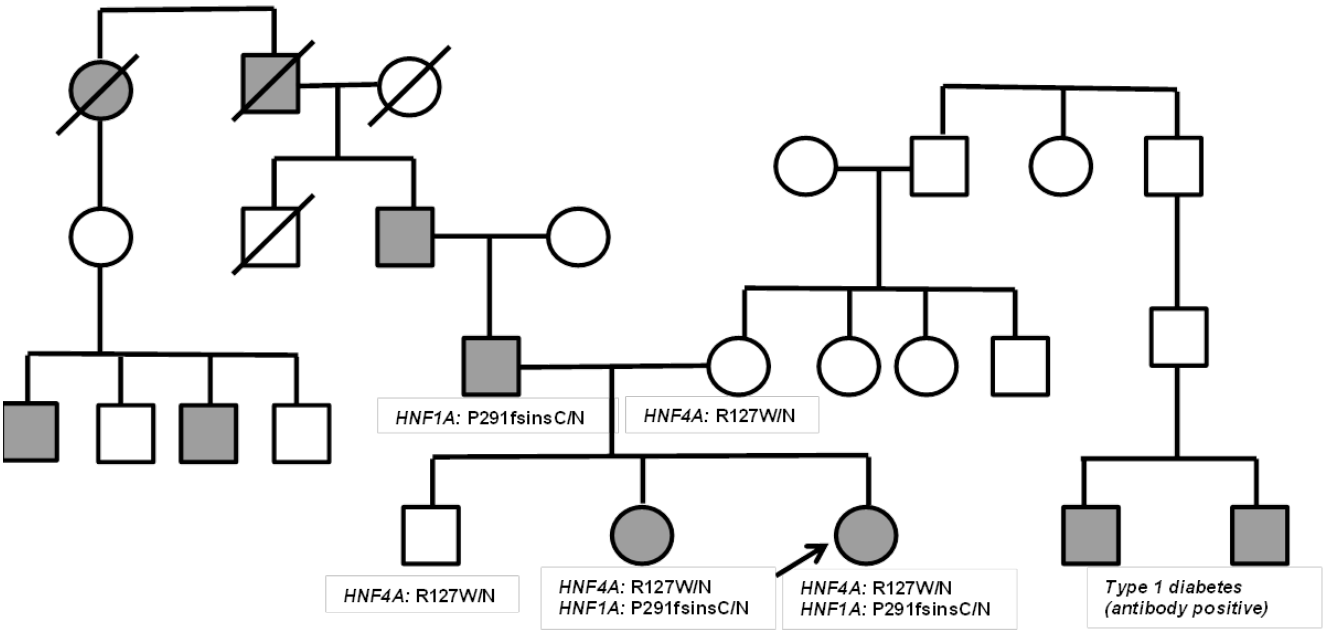
238 Figure1. Pedigree chart of family depicting family history of diabetes



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