

1 Running Title Page

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

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

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27 **Abstract**

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

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

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

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49

50 **Introduction**

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

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

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

71

72 Case Report

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

78 On recruitment to SEARCH, at the age of 8 years, she was well controlled on 0.3 units/kg/day of
79 insulin with a hemoglobinA1c (HbA1c) of 5.9% (reference range: 3.9-6.1%). Her fasting C-
80 peptide was 0.86 ng/mL (reference range: 0.5-3 ng/dL) and 60-minute stimulated C-peptide was
81 1.96 ng/mL. Due to the negative diabetes autoantibody panel, she underwent genetic testing as
82 part of the SEARCH monogenic diabetes ancillary study at 11 years of age demonstrating a
83 heterozygous missense mutation in exon 4 of *HNF4A*, R127W (c.379C>T) and a heterozygous
84 frameshift mutation in exon 4 of *HNF1A*, P291fsinsC (c.872dup). Insulin therapy was
85 completely discontinued and she was started on glipizide (1.25 mg once daily) with the dose
86 titrated to 2.5 mg once daily based on blood sugar checks, with weekly blood sugar reviews and
87 close support from a diabetes specialist nurse practitioner. There was no significant change in her
88 glycemic control (HbA1c was 6.5% before and 6 months after; reference range: 3.5-6.3%).
89 However, 21 months after switching to glipizide, glycemic control worsened (HbA1c: 8.6%)
90 despite increasing doses of glipizide, necessitating re-initiation of insulin therapy. Currently, at
91 14.5 years of age, she is receiving 0.4 units/kg/day of insulin as NPH and Regular, and glipizide
92 10 mg/day, with an HbA1c of 6.8%. The choice of NPH and Regular insulin was based on
93 parental request.

94 Prior to the identification of the gene mutations in the proband, hyperglycemia was detected in
95 the proband's sister at 14 years of age during a routine well-child examination. She was
96 asymptomatic. A diagnosis of type 1 diabetes was made and NPH and Regular insulin therapy
97 (0.3 units/kg/day) was initiated. Due to the findings in the proband, the sister also underwent
98 genetic testing as part of the SEARCH monogenic study and was also found to be heterozygous
99 for both the *HNF1A* and *HNF4A* mutations. Insulin was discontinued and glipizide therapy was
100 initiated and titrated with improvement in glycemic control (HbA1c decreased from 8.7% to
101 5.8%, 5 months after; reference range: 3.5-6.3%). She has remained on extended release
102 glipizide 2.5 mg/day for the past 27 months with a recent HbA1c of 6.1%.

103 The proband's father was known to be "borderline diabetic". His oral glucose tolerance test
104 (OGTT) confirmed the presence of diabetes (fasting glucose 92 mg/dL and 2-hour glucose of
105 215 mg/dL). Genetic testing revealed heterozygosity for the P291fsinsC *HNF1A* mutation. The
106 paternal grandfather was diagnosed with diabetes at age 65 years but was not available for
107 genetic testing. Paternal great-grandfather was reported to have diabetes since the age of 20 years
108 but lived to the age of 82 years (see Figure 1).

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

116 **Discussion**

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

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

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

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

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

153 The age at diagnosis of diabetes in patients with MODY varies considerably (from 4 to 77 years
154 for patients with *HNF1A* or *HNF4A* mutations in the Exeter cohort) and is influenced by type 2
155 diabetes risk variants(11), intrauterine hyperglycemia(12) and mutation type or location(13). The
156 proband and her sister described in this study were diagnosed at 7 and 14 years respectively.

157 Whilst we might speculate that digenic inheritance could cause earlier onset of diabetes, the
158 mean age at diagnosis for individuals within the SEARCH study with single gene mutations is 12
159 years (*HNF1A*) and 11 years (*HNF4A*) (Gilliam LK et al. 2012, submitted to Diabetes Care). A
160 good response to sulfonylurea therapy is a characteristic of HNF1A/4A MODY (14, 15). While

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

164

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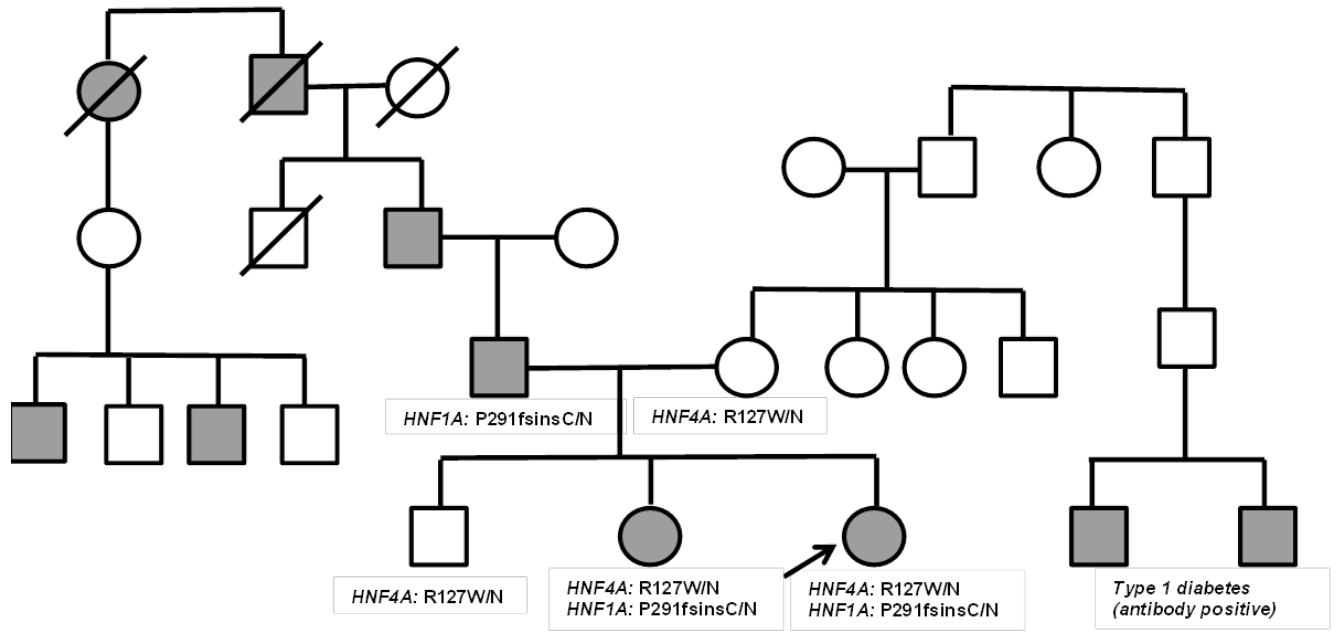
235 Table 1: Clinical Features and Mutations in First-degree Relatives

	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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