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The Common p.R114W *HNF4A* Mutation Causes a Distinct Clinical Subtype of Monogenic Diabetes

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***HNF4A* mutations cause increased birth weight, transient neonatal hypoglycemia, and maturity onset diabetes of the young (MODY). The most frequently reported *HNF4A* mutation is p.R114W (previously p.R127W), but functional studies have shown inconsistent results; there is a lack of cosegregation in some pedigrees and an unexpectedly high frequency in public variant databases. We confirm that p.R114W is a pathogenic mutation with an odds ratio of 30.4 (95% CI 9.79–125, $P = 2 \times 10^{-21}$) for diabetes in our MODY cohort compared with control subjects. p.R114W heterozygotes did not have the increased birth weight of patients with other *HNF4A* mutations (3,476 g vs. 4,147 g, $P = 0.0004$), and fewer patients responded to sulfonylurea treatment (48% vs. 73%, $P = 0.038$). p.R114W has reduced penetrance; only 54% of heterozygotes developed diabetes by age 30 years compared with 71% for other *HNF4A* mutations. We redefine p.R114W as a pathogenic mutation that causes a distinct clinical subtype of *HNF4A* MODY with reduced penetrance, reduced sensitivity to sulfonylurea treatment, and no effect on birth weight. This has implications for diabetes treatment, management of pregnancy, and predictive testing of at-risk relatives. The increasing availability of large-scale sequence data is likely to reveal similar examples of rare, low-penetrance MODY mutations.**

Heterozygous loss-of-function *HNF4A* mutations cause maturity onset diabetes of the young (MODY) (1). *HNF4A* is a transcription factor important in the function of the pancreatic β-cell. Patients with *HNF4A* mutations present with a common phenotype of increased birth weight (median increase of 790 g) and occasional neonatal hypoglycemia (15%) (2). *HNF4A* MODY patients are typically sulfonylurea sensitive, and a genetic diagnosis is important because it determines the best treatment. Mutations in *HNF4A* account for 10% of genetically confirmed MODY cases (3). The most commonly reported *HNF4A* mutation is p.R114W (NM_175914.4:c.340C>T p.Arg114Trp, rs137853336, previously described as p.R127W [4]). In our MODY cohort it accounts for 30/176 (17.1%) *HNF4A* cases, and in two Italian cohorts 5/6 (83.3%) *HNF4A* cases were caused by p.R114W (5,6).

Several pieces of evidence made us question whether p.R114W truly causes MODY. p.R114W is present in the Exome Aggregation Consortium (ExAC) database (7) in 7/32,198 European (non-Finnish) subjects, but we would expect only 0.35 *HNF4A* MODY cases based on a population frequency of MODY of 108 per million and *HNF4A* mutations accounting for only 10% of MODY cases (3). Another source of doubt over the pathogenicity of p.R114W comes from published families. The original p.R114W family (4) contains two phenocopies diagnosed at 11 and 36 years of age and a p.R114W heterozygote who was not diagnosed with diabetes until age 90 years. Shankar et al. (8) presented the case of a digenic

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pedigree of *HNF1A* p.G292fs and p.R114W; however, p.R114W was inherited from a clinically unaffected mother (at age 46 years) who did not have a dominant family history of diabetes. There is also controversy over the functional effect of the mutation. Navas et al. (9) assessed the transcriptional transactivation of p.R114W to be the same as wild type, concluding that it was a polymorphism. Two later studies suggested a 30% and 50% reduction in activity compared with wild type (10,11).

In this study we show that p.R114W is a pathogenic mutation causing MODY-like diabetes but with reduced penetrance and a distinct clinical phenotype.

RESEARCH DESIGN AND METHODS

MODY Cohort

Between 1996 and 2016, 2,289 probands with a clinical suspicion of MODY were referred for genetic testing to the Molecular Genetics Laboratory at the Royal Devon and Exeter Hospital. Clinical information was provided on a standardized referral form by the clinician at the time of referral for genetic testing; this included a question on sulfonylurea sensitivity. The latest version of the referral form can be found at http://diabetesgenes.org/content/mody.

A total of 30 probands and 51 family members with p.R114W were identified by Sanger sequencing of *HNF4A*. One additional proband was heterozygous for p.R114W and a pathogenic *HNF1A* mutation, p.R159Q; this proband was excluded from analysis to avoid confounding results. A total of 100 probands and 224 family members with other pathogenic *HNF4A* mutations were identified. Pedigrees demonstrating coinheritance were defined as those where p.R114W was inherited from a mutation-positive parent with diabetes.

Variants were classified as pathogenic if they were nonsense, frameshift, or essential splice site mutations. Missense mutations had to be reported in the literature as pathogenic in three or more families.

Type 2 Diabetes Case and Control Subjects

The type 2 diabetes case (N = 9,185) and control (N = 12,890) subjects were all of white European ancestry and came from three sources. Clinical characteristics are provided in Supplementary Table 1.

Of these, 6,763 case and 7,073 control subjects were from the Genetics of Diabetes Audit and Research in Tayside Scotland (GoDARTS), described previously (12).

A total of 2,100 were case subjects from the Diabetes UK Warren 2 Repository (13), and 1,519 control subjects were from the UK Blood Services Collection of Common Controls. The case subjects include probands from the Warren 2 sib-pair, trios, and duos resources and additional case subjects diagnosed between 35 and 65 years of age, described previously (14). The control subjects were part of the Wellcome Trust Case Control Consortium, described previously (15).

A total of 770 case and 4,849 control subjects came from the Exeter 10,000 study (EXTEND) (www.exeter10000.org), which recruited volunteers over the age of 18 years living within 25 miles of Exeter, U.K., and the Exeter Family Study of Childhood Health (EFSOCH), described previously (16).

Genotyping

Genotyping p.R114W in the type 2 diabetes case and control study subjects was performed by LGC genomics (Middlesex, U.K.) using a KASP assay. We included five positive control subjects to ensure adequate genotype clustering. The genotyping success rate was >95% in all cohorts, and there were no discrepancies among 758 duplicate pairs.

Haplotype Analysis

The haplotype context of p.R114W was assessed in a subset of 34 patients by genotyping single nucleotide polymorphisms (SNPs) flanking the mutation, based on methods described previously (17). See Supplementary Table 2 for the list of SNPs.

Computational Analyses

Birth weights were corrected for gestational age using the 1990 British child growth reference data (18). Statistical analysis was performed in Stata (version 14). Student t tests assumed unequal variances. The Kaplan-Meier plot records the proportion of heterozygotes with diabetes at each age where data are available; for those patients who did not develop diabetes, age was censored at age of referral. The effect of p.R114W was assessed using in silico tools run using ANNOVAR (19) and Alamut Batch (Interactive Biosoftware, Rouen, France).

RESULTS

p.R114W Is Enriched in the MODY Cohort Compared With Control Subjects and Patients With Type 2 Diabetes

In our MODY cohort we identified 30/2,289 (1.3%) probands with p.R114W, of which 26/1,696 (1.5%) are white European, compared with 4/12,890 (0.03%) control subjects (odds ratio [OR] 49.4 [95% CI 17.1–194], $P = 3.7 \times 10^{-37}$). p.R114W is present in 7/32,198 (0.02%) European (non-Finnish) subjects in ExAC (7), comparable to our control subjects (OR 1.43 [95% CI 0.301–5.62], P = 0.52). To assess whether p.R114W predisposes to type 2 diabetes rather than MODY, we genotyped 9,185 individuals with type 2 diabetes. There are 26/1,696 p.R114W heterozygotes among the white Europeans in our MODY cohort compared with 9/9,185 (0.1%) patients with type 2 diabetes, which gives an OR of 15.6 (95% CI 7.08–38.0, $P = 2 \times 10^{-21}$). The OR of prevalence in the subjects with type 2 diabetes versus control subjects is 3.16 (95% CI 0.88–14.0, P = 0.05) (Supplementary Table 3). Overall these results suggest that p.R114W predominantly predisposes to MODY-like rather than type 2 diabetes.

Coinheritance of p.R114W and Diabetes Is Not Consistent With High Penetrance

Coinheritance studies were possible in 14/30 pedigrees, in which p.R114W showed coinheritance with diabetes in 10/14 (Supplementary Fig. 1). In the four families where p.R114W did not demonstrate coinheritance with diabetes, there are four p.R114W heterozygotes without diabetes aged 36–53 years. A total of 82% of individuals heterozygous for other *HNF4A* mutations are expected to have diabetes by age 36 years (Fig. 1*A*). In addition to the previously reported p.R114W/*HNF1A* digenic pedigree (8), we identified a proband with p.R114W and a pathogenic *HNF1A* mutation, p.R159Q. Although the majority of families tested demonstrate coinheritance consistent with a pathogenic mutation, the presence of multiple family members without diabetes with p.R114W aged >30 years is not consistent with the high penetrance typical of *HNF4A* mutations. A total of 46% of p.R114W heterozygotes did not have diabetes at age 30 years compared with 29% for other *HNF4A* mutations (Fig. 1*A*). Patients with p.R114W are 34.2% less likely to develop diabetes compared with those with other *HNF4A* mutations across the age range in our cohort (Cox proportional hazards model, $P = 0.013$).

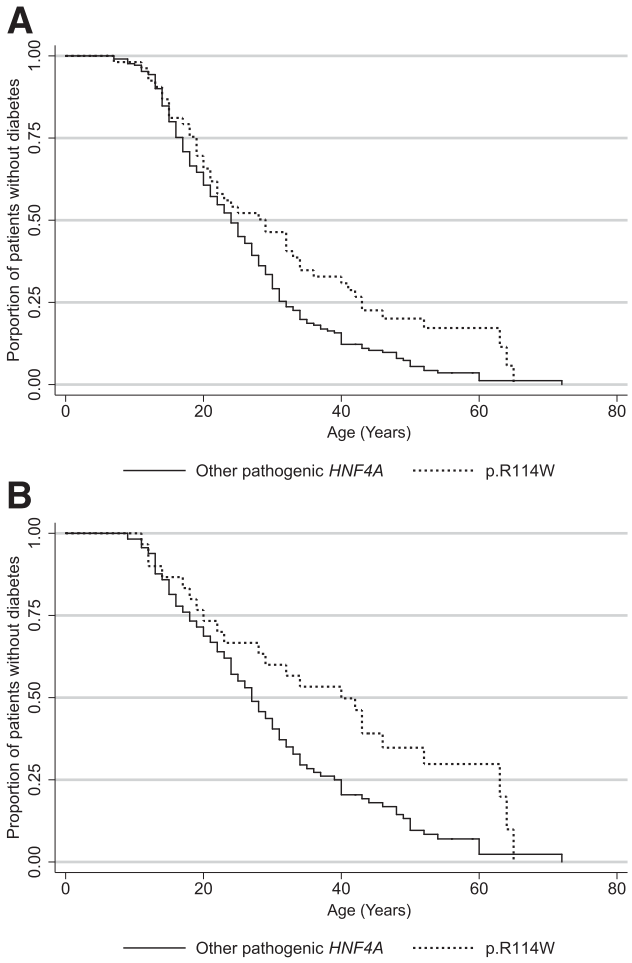

Figure 1—Kaplan-Meier plot comparing the proportion of p.R114W heterozygotes with diabetes to patients with other pathogenic *HNF4A* mutations (at each age where data are available). *A*: Includes all probands and family members. The hazard ratio for diabetes is 0.659 ($P = 0.013$) comparing p.R114W heterozygotes to patients with other *HNF4A* mutations. *B*: Includes only family members to remove the effect of ascertainment bias in the probands. The hazard ratio for diabetes is 0.553 ($P = 0.013$) comparing p.R114W heterozygotes to patients with other *HNF4A* mutations.

Multiple Independent Origins of the p.R114W Mutation

Identifying a variant on multiple haplotype backgrounds provides evidence for pathogenicity by excluding the presence of a separate, unobserved pathogenic mutation on the same haplotype. Analysis of flanking SNPs suggests that p.R114W arose on at least two haplotype backgrounds (maximum shared haplotype <368 kb) (Supplementary Table 2).

p.R114W Heterozygotes Have a Distinct Phenotype Compared With Patients With Other *HNF4A* Mutations

Raised birth weight is a clinically important feature of *HNF4A* MODY as it impacts on pregnancy management (2). p.R114W heterozygotes show no increase in birth weight compared with the general population (mean SD score −0.032 [95% CI −0.67 to 0.61]) and a significant decrease compared with patients with other *HNF4A* mutations (Student *t* test, $P = 0.0003$) (Table 1). The age of diagnosis of diabetes is later, at a median of 34 years of age compared with 24 years for other *HNF4A* mutations (Student *t* test, $P = 0.018$), which is consistent with p.R114W having a lower penetrance. Clinician-reported sensitivity to sulfonylureas is also lower than for other *HNF4A* mutations (47.6% compared with 72.9%, Student *t* test, $P = 0.038$). An analysis of just the white European subset gave similar results (Supplementary Table 4).

DISCUSSION

p.R114W Is a Pathogenic Mutation Causing MODY-Like Diabetes but With Reduced Penetrance

p.R114W is enriched in our MODY cohort and demonstrates partial coinheritance with diabetes. However, individuals with the mutation do not always present with the disease—the mutation therefore has reduced penetrance. It is possible that secondary genetic or environmental factors may be necessary to cause disease. p.R114W affects a conserved base according to its Genomic Evolutionary Rate Profiling score, is a conserved amino acid (down to Fruit fly), and is predicted to be deleterious by in silico tools including Sorting Intolerant From Tolerant and Polyphen (Supplementary Table 5). This supports the case that it is a pathogenic variant.

There is some evidence that p.R114W is a hypomorphic mutation with transactivation activity reduced but not absent in vitro (10,11). p.R114W is in the DNA binding domain of *HNF4A*. Chandra et al. (20) demonstrated that it had reduced DNA binding affinity compared with wild type but with a smaller effect than that observed for other *HNF4A* mutations. The previously reported *HNF4A*

Table 1—Characteristics of carriers of p.R114W compared with carriers of other pathogenic *HNF4A* mutations

	p.R114W mutation	Other pathogenic *HNF4A* mutations	*P*
Probands, *n*	30	100	NA
Heterozygous family members, *n*	27	134	NA
Birth weight corrected to 40 weeks' gestation (g), mean (95% CI)	3,476 (3,160–3,792) (*n* = 23)	4,147 (3,985–4,309) (*n* = 84)	0.0004*
SD scores of birth weight, mean (95% CI)	−0.032 (−0.67 to 0.61) (*n* = 23)	1.36 (1.03–1.68) (*n* = 84)	0.0003*
Birth weight corrected to 40 weeks' gestation >4,000 g	5 (*n* = 23)	45 (*n* = 84)	0.009†
Age of diagnosis of diabetes in probands (years), median (Q1, Q3)	21 (15, 32) (*n* = 30)	21 (16, 28) (*n* = 96)	0.63*
Age of diagnosis of diabetes in family members (years), median (Q1, Q3)	34 (20, 50) (*n* = 21)	24 (16, 34) (*n* = 95)	0.018*
Duration of diabetes (years), median (Q1, Q3)	5 (0, 14.5) (*n* = 51)	7 (1, 19) (*n* = 191)	0.025*
Initial treatment of patients with diabetes, Diet/OHA/Insulin	10/19/16 (*n* = 45)	47/50/59 (*n* = 156)	NA
Patients with diabetes treated with sulfonylureas at some time during diabetes, *n*	21	70	NA
Patients reported as being sulfonylurea sensitive, *n* (%)	10 (47.6%)	51 (72.9%)	0.038†
BMI of patients with diabetes, median (Q1, Q3)	22.65 (22.975, 29.2) (*n* = 40)	23.9 (21.945, 27) (*n* = 128)	0.97*
Median HbA_{1c} of patients with diabetes, mmol/mol (%)	65.0 (8.1) (*n* = 31)	57.4 (7.4) (*n* = 151)	0.97*

NA, not applicable; OHA, oral hypoglycemic agents. *Student *t* test; †Fisher's exact test.

p.R76W is also in the DNA binding domain and causes renal features in addition to the classical *HNF4A* symptoms (21). This suggests that the difference in DNA binding affinity may be the cause of the atypical sets of phenotypes presented by these two mutations, by dictating which target genes the *HNF4A* transcription factor is able to interact with.

Other hypomorphic rare MODY gene variants include the *HNF1A* p.G319S missense/splicing variant in the Oji-Cree population (22,23) and the hypomorphic p.E508K variant in Latinos (24). There is also some evidence that p.T130I is a low-frequency variant predisposing to type 2 diabetes in Europeans (25). These variants are associated with an increased risk of type 2 diabetes, whereas p.R114W appears to predominantly cause MODY-like diabetes in both Caucasian and Japanese pedigrees.

p.R114W Has No Effect on Birth Weight

p.R114W heterozygotes have a distinct clinical presentation of *HNF4A* MODY-like diabetes with normal birth weight. The p.R76W *HNF4A* mutation causes a unique phenotype of renal Fanconi syndrome in addition to the common *HNF4A* diabetic phenotype (21) but still causes increased birth weight. p.R114W appears to have an effect in the adult but not the fetus. One speculative mechanism for this would be a lower threshold of *HNF4A* activity required in the fetus for healthy function.

Reclassifying the Clinical Features of p.R114W Will Impact Patient Treatment and Family Management

p.R114W has a distinct profile of clinical features. This means that specific recommendations for patient management are required. In contrast to offspring who inherit other *HNF4A* mutations, fetal inheritance of p.R114W will not confer a high risk of macrosomia. Although sulfonylurea treatment is successful for some patients, overall response is lower compared with patients with other *HNF4A* mutations. The reduced penetrance of this mutation is important for genetic counseling of at-risk relatives undergoing predictive tests because the chance of presenting with diabetes is no longer based simply on the odds of inheriting the mutation; not all heterozygotes will develop diabetes, and those that do are likely to have a later onset.

Ascertainment Bias Affects Estimates of Penetrance

This study is based on the largest cohort of families with p.R114W included in a single study, giving us the clearest view of the characteristics of the mutation. However, there is a risk of ascertainment bias due to the referral criteria for MODY genetic testing. Probands were selected based on diabetes symptoms, age of diagnosis, and, importantly, a family history of diabetes. This means we are at risk for overestimating the penetrance of p.R114W. However, this will be the case for all MODY genes, making comparisons of penetrance fair. Ascertainment bias is one of the reasons it is hard to establish accurate estimates of penetrance for rare diseases. Publically available sequencing data (such as ExAC [7] and the 1000 Genomes Project [26]) can be used as controls to help generate unbiased estimates of penetrance. However, in the case of MODY, using ExAC as a control has the limitation that it contains some individuals with type 2 diabetes, and a subset of these could have misdiagnosed MODY. Removing these would only strengthen our conclusions.

Identifying p.R114W on two distinct haplotypes demonstrates that although it is possible for there to be a

founder effect in Europeans, it cannot explain all cases of p.R114W. The fact that the majority of p.R114W patients are European could also be attributed to ascertainment bias as the majority of our cohort was recruited in the U.K.

The Pathogenicity of MODY Mutations Found in ExAC Should Be Questioned

The estimated prevalence of MODY in the U.K. is 108 per million population (3). The most frequently reported individual MODY mutation accounts for 15% of MODY cases (27). On the basis of this frequency, we would expect to see the most common individual MODY mutation in the ExAC European (non-Finnish) population at a frequency of only 0.52; thus, any MODY mutation seen multiple times in ExAC is unexpectedly common and should have its pathogenicity and penetrance reevaluated.

Conclusion

The most frequently reported *HNF4A* mutation, p.R114W, causes a distinct clinical phenotype of monogenic diabetes with reduced penetrance, no increase in birth weight, and a lower likelihood of response to sulfonylureas. The increasing availability of large-scale sequence data is likely to reveal similar examples of rare, low-penetrance MODY mutations.

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