Hepatocyte nuclear factor 4 alfa (HNF4A) mutations associated to hyperinsulinaemic hypoglycaemia and atypical Fanconi syndrome: Expanding the clinical phenotype

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Abstract

Context: A mutation-specific phenotype is associated with the p.R63W mutation in the Hepatocyte nuclear factor (HNF)-4A gene. This includes macrosomia and atypical renal Fanconi syndrome, in addition to hyperinsulinaemic hypoglycaemia (HI) progressing into maturity-onset diabetes of the young (MODY). We describe 2 infants with additional features of this syndrome.

Cases description: Patient 1, a male born at term with a birth weight of 3.99kg (95^{th} centile, SDS 1.7), was diagnosed with HI on day two of life. He responded to 3mg/kg/day of Diazoxide, which was then discontinued 2 months later. Persistently raised serum creatinine led to investigation of renal tubular function showing leaking of calcium, magnesium, phosphate and protein. He had transient conjugated hyperbilirubinaemia with patchy liver steatosis on ultrasound. Molecular analysis revealed a de novo p.R63W mutation in *HNF4A*.

Patient 2 is a male born at 30^{+2} weeks with a weight of 1.53kg (64^{th} centile, SDS 0.36). His mother had renal Fanconi syndrome. He received parenteral nutrition and first presented with hypoglycaemia at one month of age, while establishing enteral feeds. Biochemistry work-up showed low serum calcium, sodium, and phosphate concentrations, as a result of renal tubular leaking. Hypoglycaemia screen performed at 3 months of age documented HI and he was commenced on 2mg/kg/day of Diazoxide with good response. He was heterozygote for a maternally inherited p.R63W mutation in *HNF4A*. A continue glucose monitoring performed in his mother revealed overnight hypoglycaemia.

Conclusion: Renal Fanconi syndrome represents the only *HNF4A* feature showing complete penetrance. Our cases suggest that p.R63W *HNF4A* mutation must also be considered in

subjects with normal birth weight and postulate the possibility of liver involvement as part of the condition.

Context

Congenital hyperinsulinism (CHI) is characterized by hypoglycaemia caused by unregulated insulin secretion from pancreatic beta-cells. So far, mutations in nine genes which control the insulin secretion (*ABCC8, KCNJ11, GLUD1, GCK, HNF4A, HNF1A, SLC16A1, UCP2* and *HADH*) have been identified as a cause of CHI (1). However, mutations in a single gene may be responsible for different phenotypes. Recent studies demonstrate that mutations in the hepatocyte nuclear factor (HNF)-4A, encoded by the *HNF4A* gene), initially reported in patients with maturity-onset diabetes of the young (MODY), may conversely cause CHI, with variable degree of severity (2,3). Recent reports (4,5) indicate a mutation-specific phenotype associated with the p.R63W mutation in the *HNF4A* gene, with macrosomic CHI and atypical Fanconi syndrome. We now extend the previous observations and present two patients with p.R63W mutation, one of them having a birth weight appropriate for his gestational age, the other having signs of liver involvement.

Cases Description

Case 1, a male was born at 38^{+2} weeks, with a weight of 3.99Kg (95^{th} centile, SDS 1.7). On day two he presented with hypoglycaemic seizures (undetectable blood glucose). Initial investigations showed polycythaemia (Hb 26.2g/dl, Hct 76%), deranged renal function (creatinine 119umol/L (nr 37-93), Potassium 5.9mmol/L), conjugated hyperbilirubinaemia (146umol/L), metabolic acidosis (ph 7.3, PCO2 4.73kPa, HCO_3 21.3mmol/L, BE 4.5mmol/L, Lactate 3.09mmol/L) and Staphylococcus Aureus sepsis. Despite response to antibiotics and

resolution of polycythaemia, he continued having hypoglycaemia episodes, with a glucose requirement up to 18.9 mg/kg/min. Hypoglycaemia screen confirmed hyperinsulinism (Table 1). He achieved stable blood glucose concentrations on 3mg/kg/day of Diazoxide. As a consequence of the persistently raised serum creatinine and high urine amino acids, he had a renal tubular screen done, showing features of renal Fanconi syndrome (Table 1). Abdomen ultrasound revealed normal kidneys and signs of patchy liver steatosis. Conjugated bilirubin normalized with ursodeoxycholic acid treatment. He was heterozygote for the de novo c.187C>T (p.R63W) mutation in *HNF4A*. He is currently on 10mg/kg/day of Diazoxide and three-to-four hourly oral feeds, with stable blood glucose concentrations.

Case 2, a male was born at 30^{+2} weeks by emergency caesarean section. Birth weight was 1.53kg (64^{th} centile, SDS 0.36). His mother had renal Fanconi syndrome, which was diagnosed at the age of two years, when she presented with rickets. On day one of life he was commenced on parenteral nutrition because of suspected necrotizing enterocolitis. While establishing enteral feeds, at the corrected age of 34 weeks, he presented with hypoglycaemia episodes (minimum blood glucose 1.6mmol/L). Echo showed small ventricular septal defect with left-to-right shunt. Biochemistry work-up at the time of hypoglycaemia confirmed hyperinsulinism and revealed low calcium, sodium, and phosphate concentrations. Renal tubular screen showed features of renal Fanconi syndrome, (Table 1). Ultrasound of the kidneys was normal. He has achieved good glycaemic control on 2mg/kg/day of Diazoxide and 3 hourly feeds. Molecular analysis showed a maternally inherited p.R63W mutation. Subsequent continue glucose monitoring performed on his mother (Figure 2) revealed prolonged overnight hypoglycaemia.

Discussion

Our clinical observations in these two patients expand the clinical phenotypes associated to *HNF4A* mutations. *HNF4*A mutation is the third commonest cause of Diazoxide-responsive CHI (1), usually associated with early presentation and macrosomia and MODY (3, 6). These phenotypes are not mutation-specific and a single mutation can cause opposite phenotypes in the same subject, according to the age. Furthermore, the penetrance of each phenotype is incomplete and thus, the absent history of neonatal hypoglycaemia in a subject with MODY or, on the other hand, the absence of any family history of diabetes in patients with CHI, does not exclude the diagnosis. A similar biphasic phenotype (hypoglycaemia – MODY) was initially described in patients carrying mutations in *HNF1A* (1), which is involved in the transcription of HNF4A through the pancreatic-specific promoter P2, and is in turn regulated by HNF4A (7).

The *HNF4A* phenotypic spectrum has recently been expanded to include atypical renal Fanconi syndrome, specifically linked to the p.R63W mutation (4,5). Our cases provide further evidence of a mutation-specific renal phenotype and extend the previous observations, arising important clinical considerations.

The molecular basis of renal Fanconi syndrome due to *HNF4A* mutations are far from clear, but seem to involve an impaired DNA binding. In fact, the R76 residue is in the DNA binding domain and its mutation affects charge and hydrophobicity in an area of intimate DNA contact. It has been hypothesized that this specific residue must be crucial in the renal proximal tubule (4, 5).

Another possible explanation is to be sought into the intimate relationship between *HNF4A*, *HNF1A* and other regulatory proteins. Heterozygote knockout mice for either *HNF4A* or *HNF1A* have no distinct phenotype. On the contrary, although the homozygous knockout of *HNF4A* is lethal, mice with homozygous knockout of *HNF1A* (8) exhibit hepatic dysfunction, phenylketonuria and early onset renal Fanconi syndrome associated with raised urine calcium/creatinine ratio and reduced creatinine clearance.

Given that *HNF4A* and *HNF1A* are involved in a feed-forward loop, it could be speculated that haploinsufficiency of *HNF4A* can impact on *HNF1A*, resulting in specific organ damage. Other genetic factors within the wide regulatory network of *HNF4A* are probably involved. Both *HNF4A* and *HNF1A* can regulate the expression of GLUT2 (*SLC2A2)*, which is responsible, if mutated, for Fanconi-Bickel syndrome (9). Stanescu et al first reported a macrosomic female patient with Diazoxide-responsive CHI and atypical Fanconi syndrome due to a de novo p.R63W *HNF4A* mutation (described as p.R76W) (5), who also presented with liver involvement, thus mimicking Fanconi-Bickel syndrome. In this regard, it is worth noting that in case 1 from our case series, we observed prolonged conjugated hyperbilirubinaemia, which normalized over the first three months of life on ursodeoxycholic acid with patchy liver steatosis. In order to explain the clinical overlap between HNF4A mutation-specific phenotype and Fanconi-Bickel syndrome, a decreased expression of *SLC2A2* either as a direct consequence of *HNF4A* impaired activity, or as a result of the impaired interaction between the mutant *HNF4A* with *HNF1A*, can be hypothesized (5).

The absence of nephrocalcinosis in our cases compared to the others could be explained by the short follow-up. Similar to the other cases, our patients had high serum and urine magnesium concentrations, thus suggesting overflow rather than leaking as underlying cause of the high urine magnesium excretion (4).

Although all the cases previously reported with this specific mutation were born large for gestational age (10), interestingly case 2 from our cohort had a birth weight appropriate to his gestational age. Given that his mother has the same mutation and abnormal glycaemic profile, it is possible that in utero the fetus was exposed to blood glucose concentrations appropriate for his own set-point for insulin secretion, resulting in lower insulin concentrations. In addition prematurity and/or placental factors might have also have contributed.

Conclusion

Knowledge of the genetics of CHI allows prediction of disease progression and comorbidities. Atypical renal Fanconi syndrome represents the only *HNF4A* feature with complete penetrance, being present in all the patients reported carrying the p.R63W mutation. The underlying mechanisms are unclear and probably involve an impaired *HNF4A* regulatory network. Our cases suggest that *HNF4A* mutations must be considered also in the presence of a normal birth weight. Transient or permanent liver involvement may represent part of this condition and therefore awareness about the possibility of associated diseases is needed during follow-up of *HNF4A*–mutation carriers.

Legends

Table 1. Clinical and biochemical features of our patients

Figure 1. Continuous glucose monitoring performed in the mother of Case 2.

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Table 1. Clinical and biochemical features of our patients.

	Case 1	Case 2	
Gender	Male	Male	
Gestational age (weeks)	38+2	30+2	
Birth weight kg/SDS	3.99/1.7	1.530/0.36	
			Normal range
Hypoglycaemia screen			
- Blood glucose mmol/L	2.5	2.2	3.5-5.5
- Insulin mU/L	4.5	6.3	
- C-Peptide pmol/L	233	Not done	
- NEFA mmol/L	0.2	0.36	
- Ketones mmol/L	0.06	<0.05	
- Ammonia umol/L	25	20	<40
Urea, electrolytes and ALP			
- Urea mmol/L	1.1	1.4	0.7-5
- Creatinine umol/L	48	27	13-32
- Sodium mmol/L	143	140	133-146
- Potassium mmol/L	3.9	3.6	3.2-6
- Calcium mmol/L	2.6	2.27	2.17-2.44
- Magnesium	1.09	1.13	0.66-1
- Phosphate mmol/L	1.82	1.39	1.2-2.1
- Alkaline phosphatase U/L	1069	2730	80-425
Renal tubular screen (Urine)			
- Albumin/creatinine	82.5	144	1.7-12.2
- RBP/creatinine	13647	44417	1.5-448

-	NAG/creatinine	549	1088	2-27
-	Calcium/creatinine	5.07	5.6	0.09-2.2
-	Magnesium/creatinine	2.5	5.03	0.4-2.2
-	Phosphate/creatinine	21.5	32.12	1.2-19
-	TRP (%)	52	39	70-100

ALP alkaline phosphatase; RBP retinoid binding protein; NAG N-acetylglucosamine; TRP tubular reabsorption of phosphates

Figure 1. Continuous glucose monitoring performed in the patient's 2 mother (age 34.9 years), showing wide variation of the blood glucose concentrations ranging from 2.2 to 14.3 mmol/L (mean glucose 5.0 mmol/L). Prolonged overnight hypoglycaemia and post-prandial hyperglycaemia indicate a dysregulation in insulin secretion and action.