CLINICAL STUDY

Amino acid properties may be useful in predicting clinical outcome in patients with Kir6.2 neonatal diabetes

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Abstract

Background: Mutations in the *KCNJ11* gene, which encodes the Kir6.2 subunit of the β -cell K_{ATP} channel, are a common cause of neonatal diabetes. The diabetes may be permanent neonatal diabetes mellitus (PNDM) or transient neonatal diabetes mellitus (TNDM), and in ~20% of patients, neurological features are observed. A correlation between the position of the mutation in the protein and the clinical phenotype has previously been described; however, recently, this association has become less distinct with different mutations at the same residues now reported in patients with different diabetic and/or neurological phenotypes.

Methods: We identified from the literature, and our unpublished series, *KCNJ11* mutations that affected residues harbouring various amino acid substitutions (AAS) causing differences in diabetic or neurological status. Using the Grantham amino acid scoring system, we investigated whether the difference in properties between the wild-type and the different AAS at the same residue could predict phenotypic severity.

Results: Pair-wise analysis demonstrated higher Grantham scores for mutations causing PNDM or diabetes with neurological features when compared with mutations affecting the same residue that causes TNDM ($P=0.013$) or diabetes without neurological features ($P=0.016$) respectively. In just five of the 25 pair-wise analyses, a lower Grantham score was observed for the more severe phenotype. In each case, the wild-type residue was glycine, the simplest amino acid.

Conclusion: This study demonstrates the importance of the specific AAS in determining phenotype and highlights the potential utility of the Grantham score for predicting phenotypic severity for novel *KCNJ11* mutations affecting previously mutated residues.

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Introduction

Neonatal diabetes mellitus (NDM) is a monogenic condition characterized by the onset of hyperglycaemia before 6 months of age. There are two main forms: transient NDM (TNDM), where the diabetes remits, and permanent NDM (PNDM), which is a chronic condition (1).

Activating mutations in the *KCNJ11* and *ABCC8* genes, which encode the Kir6.2 and SUR1 subunits of the pancreatic β -cell ATP-sensitive potassium (K_{ATP}) channel, are the most common cause of NDM accounting for ~50% of all cases (2, 3, 4, 5, 6, 7). In the pancreatic β -cell, glucose metabolism results in an increase in the concentration of intracellular ATP. ATP binds to the Kir6.2 subunit of the K_{ATP} channel initiating channel closure, membrane depolarization and ultimately insulin secretion. Activating mutations in *KCNJ11* and *ABCC8* cause NDM by reducing the sensitivity of the K_{ATP} channel to ATP (2, 3).

The identification of a K_{ATP} channel mutation is clinically important as most patients can be treated with high-dose sulphonylureas, which close the channel independently of ATP resulting in improved glycaemic control (8, 9).

Approximately 20% of patients with *KCNJ11* mutations have neurological features. These range from severe developmental delay and epilepsy diagnosed before 1 year (DEND syndrome) to a milder phenotype known as intermediate DEND syndrome (iDEND) (10). The neurological features are consistent with the expression of mutated K_{ATP} channels in neurons (11). In a minority of cases, the neurological features may be incidental or secondary to complications of diabetes such as ketoacidosis and severe hypoglycaemia (12).

Over 40 different activating mutations in the *KCNJ11* gene have been reported (13). Previous studies have demonstrated a relationship between the position of the mutation and the incidence of neurological features,

with mutations causing isolated diabetes more likely to occur in the ATP binding site and iDEND/DEND mutations often residing in the pore or the slide helix region of the Kir6.2 protein (14, 15, 16). Recently, however, a few exceptions to this genotype/phenotype relationship have been described, and consequently, it is not possible to use the position of a novel *KCNJ11* mutation within the protein to predict phenotype (17, 18). In addition, phenotypic variability associated with different mutations at the same residues has been reported. For example, a substitution of glutamine for arginine at codon 50 (R50Q) causes TNDM whereas a substitution to proline at the same residue (R50P) causes PNDM (17, 19). A second example is seen with mutations affecting codon 53 where a substitution of glycine to asparagine (G53N) causes isolated diabetes while a substitution to an aspartic acid at the same residue (G53D) results in DEND syndrome (19, 20, 21).

As the specific amino acid substitution (AAS) is crucial in determining the phenotype, we have investigated whether there is a correlation between the phenotypic severity and the difference in biochemical properties of the various substituted amino acids at a given residue. In order to quantify differences in the amino acid properties, we used the Grantham matrix (22). This amino acid scoring system has traditionally been used to predict pathogenicity of novel missense variants, with high scores, which reflect large differences

in the biochemical properties of the wild-type and the substituted amino acid, supporting causality (22).

Materials and methods

Cohort

Clinical characteristics of patients with an activating *KCNJ11* mutation were collated from the literature and our unpublished series (13). All mutations affecting residues harbouring two or more different AAS, which resulted in a different diabetic or neurological status, were included in this study. A diabetic and neurological status was assigned to each mutation according to the majority phenotype.

Clinical characterization

Diabetic status was classified as i) TNDM if the diabetes had remitted or ii) PNDM if there was no remission of diabetes. Neurological status was classified as: i) isolated diabetes – no neurological involvement; ii) iDEND – developmental delay without generalized epilepsy diagnosed before 1 year; and iii) DEND – developmental delay with generalized epilepsy diagnosed before 1 year.

To ensure robust classification, all patients under the age of 1 year at the time of the study were excluded

Table 1 All those patients with mutations at residues with multiple mutations from the Exeter data set and the literature are shown. The table displays the number of patients in each mutation group when the exclusion criteria is applied. Diabetic phenotype – the number and percentage of patients with 'TNDM' or 'PNDM' is shown for each mutation. Neurological phenotype – the number and percentage of patients with neurological features is shown for each mutation, this is then characterized into 'DEND' and 'iDEND'. Finally the Grantham score is displayed for each mutation.

Mutation	Grantham score	Diabetic status		Neurological status		
		Patients studied (n)	TNDM or PNDM majority phenotype	Patients studied (n)	Isolated or neurological features (NF) majority phenotype	Severity of neurological features (iDEND or DEND) majority phenotype
R50G	125	1	PNDM (100%)	1	NF (100%)	DEND (100%)
R50P	103	3	PNDM (100%)	4	NF (75%)	iDEND (66%)
R50Q	43	7	TNDM (100%)	7	Isolated (100%)	–
G53D	94	2	PNDM (100%)	3	NF (100%)	DEND (100%)
G53N	80	1	PNDM (100%)	1	Isolated (100%)	–
G53R	125	2	PNDM (100%)	2	NF (100%)	iDEND (100%)
G53S	56	3	TNDM (67%)	3	Isolated (66%)	–
G53V	109	3	TNDM (100%)	3	Isolated (100%)	–
V59G	109	1	PNDM (100%)	1	NF (100%)	DEND (100%)
V59M	21	29	PNDM (100%)	29	NF (93%)	iDEND (82%)
K170N	94	3	PNDM (100%)	3	NF (67%)	iDEND (100%)
K170R	26	2	PNDM (100%)	2	Isolated (100%)	–
K170T	78	1	PNDM (100%)	1	Isolated (100%)	–
I182T	89	1	PNDM (100%)	1	Isolated (100%)	–
I182V	29	1	TNDM (100%)	1	Isolated (100%)	–
V252A	64	4	PNDM (75%)	2	Isolated (100%)	–
V252G	109	1	PNDM (100%)	1	NF (100%)	iDEND (100%)
V252M	21	2	PNDM (50%)	2	Isolated (100%)	–
G334C	159	2	PNDM (100%)	2	Isolated (100%)	–
G334D	94	2	PNDM (100%)	2	NF (100%)	DEND (100%)

from the dataset when i) the diabetes had not remitted or ii) neurological features had not been reported. Individuals were also excluded when their age was not known.

Scoring differences in physicochemical properties of AAS

The Grantham matrix was used to obtain a score for each AAS (range 5–215). The Grantham scores for each of the different AAS affecting the same residue were compared using the Wilcoxon test for pair-wise analysis.

Results

Seven residues (R50, G53, V59, K170, I182, V252 and G334) in the Kir6.2 protein were identified, which harboured different mutations in 71 patients with varying phenotypes (Table 1).

Diabetic status

Three residues (R50, G53 and I182) harboured ten different mutations causing TNDM ($n=4$) or PNDM ($n=6$) (Table 1). Pair-wise comparisons between TNDM and PNDM mutations at the same residue revealed a higher Grantham score for the PNDM mutation when compared with the TNDM mutation at the same residue in 77% (7/9) ($P=0.013$; Table 1, Fig. 1a).

Neurological features

Five residues (R50, G53, K170, V252 and G334) harboured 16 different mutations causing isolated diabetes and diabetes with neurological features (Table 1). Pair-wise comparisons between the mutations revealed a higher Grantham score for the mutation associated with neurological features when compared with the mutation causing isolated diabetes at the same residue in 85% (11/13) ($P=0.016$; Table 1, Fig. 1b).

Three residues (R50, G53 and V59) were identified, which harboured both iDEND and DEND mutations. In two of the three pair-wise comparisons, the Grantham score was higher for the mutations causing DEND syndrome (Fig. 1c).

Discussion

We have used the Grantham matrix to assess whether differences in the biochemical properties between wild-type and various substituted amino acids within Kir6.2 correlate with phenotype. Pair-wise analysis demonstrated higher Grantham scores for mutations causing PNDM ($P=0.013$) or diabetes with neurological features ($P=0.016$) when compared with

Figure 1 Graphs showing the difference in Grantham score for mutations at the same residue with differing phenotypes (pair-wise analysis). Graph A shows the pair-wise analysis of TNDM and PNDM mutations. Graph B shows the pair-wise analysis for mutations causing isolated diabetes and mutations associated with neurological features, and graph C shows the pair-wise analysis of mutations causing iDEND vs DEND syndrome.

mutations affecting the same residue associated with the milder phenotypes of TNDM or isolated diabetes respectively. The Grantham score was higher for DEND mutations when compared with iDEND mutations occurring at the same residue in two of the three cases.

In all five pair-wise analyses where lower Grantham scores were observed for the more severe phenotype, the wild-type residue in each case was glycine. As glycine is the simplest amino acid, any substitution will constitute a large change in biochemical properties and will therefore result in a high Grantham score. This result is in keeping with the study by Koster *et al.* (21) who demonstrated that all substitutions examined at residue

G53 resulted in a decrease in affinity of the channel to ATP, thus supporting a crucial role for the glycine residue at this position. The Grantham scoring system should therefore be used with caution when trying to predict likely pathogenicity or disease severity for any mutations involving the substitution to or from a glycine residue.

The Grantham score has limited use in predicting severity of a single pathogenic mutation at any position within the gene as scores are variable between different residues and phenotypes. For example, the V59M mutation, which is associated with diabetes and neurological features, has a score of 21, while the G53V mutation, which causes isolated diabetes, has a score of 109. Evidence from well-established sources, such as co-segregation within the family, the degree of conservation of the affected residue, and functional studies, when available, should therefore be sought before reporting the likely pathogenicity of novel Kir6.2 variants.

This study has highlighted the potential utility of the Grantham scoring system for predicting disease severity associated with different Kir6.2 mutations at the same residue. Our results support the findings of Zuberi *et al.* (23) who demonstrated that Grantham scores could predict disease severity in patients with epilepsy resulting from missense mutations at different residues in the *SCN1A* gene. Although further studies will be required on larger datasets to assess the robustness of this tool, when taken together the results from these two studies highlight the possible utility of the Grantham scoring system for predicting disease severity in the diagnostic setting. Analysis of Grantham scores may be particularly useful in the context of neonatal diabetes as the finding that patients with a *KCNJ11* or *ABCC8* mutation can be treated with sulphonylureas has resulted in a sharp increase in demand for genetic testing over recent years with many patients now referred for mutation analysis at the time of diagnosis. For patients in whom a previously reported mutation is identified, information on whether the diabetes is likely to be permanent or transient and whether neurological involvement is likely can be gleaned from case reports. However, for patients with a novel *de novo* missense mutation, it is not possible, in the absence of *in vitro* functional studies, to predict phenotype. Therefore, a tool such as the Grantham score may offer a useful insight into the likely clinical course of the diabetes in these patients.

Our study has a number of limitations. First, although information on the neurological phenotype was obtained from the referring clinician or from the literature, there is no globally accepted assessment for neurodevelopment. In addition, neurological features such as mild developmental delay are difficult to diagnose in younger children, and although we tried to minimize this influence by excluding those under 1 year in whom no neurological features had been

reported, it is possible that the neurological status was misclassified in some of the patients. Finally, errors in the classification of diabetic status are possible with remissions being concealed by on-going low-dose treatment. It should also be noted that the genotype/phenotype relationships may not be absolute as other genetic and environmental modifiers may influence disease severity.

In conclusion, this study demonstrates the importance of the biochemical properties of a substituted amino acid in determining phenotype. These results highlight the potential utility of an amino acid scoring system for predicting both diabetic and neurological phenotype by direct comparisons of Grantham scores for novel non-synonymous *KCNJ11* mutations affecting previously mutated residues. Further studies investigating its use in predicting other aspects of phenotype, for example treatment response, or its use in other monogenic diseases, such as *ABCC8* neonatal diabetes or Crouzon syndrome (13, 24, 25), where different mutations at the same residues cause variable phenotypes, are warranted.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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