**Sulfonylurea Treatment of Chromosome 6q24-related Transient Neonatal Diabetes (6q24-TND)**

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**Abstract**

**Context:** 6q24-related transient neonatal diabetes (6q24-TND) is a rare form of diabetes caused by an overexpression of *PLAGL1* and *HYMAI*.Sulfonylurea (SU) therapy is highly effective in the treatment of KATP channelopathies.

**Objective:** We sought to characterize the insulin secreting potential and response to SU therapy in adults with 6q24-TND identified through the University of Chicago Monogenic Diabetes Registry (<http://monogenicdiabetes.uchicago.edu>).

**Design:** Three patients had a mixed meal test (MMT) and arginine stimulation test (AST), with all insulin products withheld on the morning of testing. A five-day course of glyburide (glibenclamide) was then initiated and insulin steadily withdrawn. A maximum target dose of 1mg/kg of glyburide was used to achieve euglycemia. A repeat MMTs and AST were performed on day five.

**Results:** C-peptide values were significantly higher in patients follow a course of SU (p<0.05 by repeated measures ANOVA) when both MMT were compared. The C-peptide response was again higher following a course of SU (p<0.05) during the AST. Patients were discharged on glyburide therapy alone or in combination with other oral agents. A fourth patient was also successfully transitioned off insulin in lieu of oral agents. All patients remained off insulin therapy with good diabetes control at re-evaluation at least 3 months after transition.

**Conclusions:** The best treatment for 6q24-TND following recurrence of hyperglycemia later in life is uncertain. Our data demonstrates that SU therapy can be effective as monotherapy or in combination with other oral medications.

**Introduction**

6q24-related transient neonatal diabetes (6q24-TND) is a rare form of diabetes with an estimated incidence of 1 in 200,000-500,000 live births (1-3). It is caused by overexpression of the maternally imprinted genes *PLAGL1* and *HYMAI*. It has been suggested that defects of the 6q24 locus are responsible for 70% of all cases of transient neonatal diabetes mellitus (4,5) and that 50-60% of neonatal diabetes is transient (6). The term transient neonatal diabetes can be misleading as many patients develop permanent hyperglycemia later in life (7).

In the neonatal period patients are typically treated with insulin therapy. While treatment options of 6q24-TND in later life is less certain, insulin therapy is often needed (1,7). There is a paucity of data on the use of other glucose lowering agents in the treatment of this condition. We sought to characterize the beta cell function and glucose homeostasis in patients with 6q24-TND and assess their response to sulfonylurea (SU) therapy. We also aimed to safely transition patients off insulin therapy if possible.

**Subjects**

Individuals with suspected or established monogenic forms of diabetes were consented for participation through the University of Chicago Monogenic Diabetes Registry (http://monogenicdiabetes.uchicago.edu/registry/) through which longitudinal information regarding the diagnosis and treatment of diabetes, diabetes complications, other medical problems, family history and genetic testing results, is collected through surveys and medical records (4). Those adults with 6q24-TND leading to transient neonatal diabetes and recurrence of hyperglycemia requiring insulin therapy later in life were invited to participate in a trial of SU therapy.

6q24-TND was diagnosed using methylation sensitive PCR (8). Duplication was confirmed using microsatellite analysis using standard methods and published microsatellite markers (9). Diagnostic genetic testing was performed by the division of human genetics, University of Southampton (UK) or department of human genetics, University of Chicago.

**Methods**

All subjects were consented for participation through protocols approved by the institutional review board at the University of Chicago. Subjects over 18 years of age with 6q24-TND treated with insulin therapy and agreeable to a trial of SU were invited to participate. We sought to characterize the insulin secreting potential and also the response to SU therapy in adults with 6q24-TND. A mixed meal test (MMT) and arginine stimulation test (AST) were performed on day one and day five. All insulin products were withheld on the morning of day one. Metabolic testing was performed in the morning (between 7 and 10 A.M.) after an eight-hour overnight fast, without food or drink (with the exception of water). Subjects ingested 12 oz. of BOOST® High Protein (calories 360, total fat 9g, total carbohydrate 49.5g, total protein 22.5g) over one minute. Samples for glucose and C-peptide were obtained at 0, 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 120, 150 and 180 minutes.

Each AST was performed following the MMT. Arginine was infused 185 minutes after commencing the MMT. A single dose of 5g of arginine hydrochloride was infused over thirty seconds. Glucose and C-peptide samples will be obtained at 184, 187, 188, 189, 190, 192, and 195 minutes. A five-day course of glyburide (glibenclamide) was initiated aiming to achieve euglycemia with a maximum target dose of 1 mg/kg using a published protocol used successfully for those with mutations affecting the KATP channel (10). All insulin was steadily withdrawn before a repeat MMT and AST performed on day five. Plasma glucose concentrations were determined by the hexokinase method. Plasma C-peptide was measured by double antibody radioimmunoassay.

Patients were discharged on glyburide therapy alone or in combination with other oral agents. HbA1c was recorded prior to metabolic testing and at least 3 months following cessation of insulin therapy. HbA1c was measured by high performance liquid chromatography.

**Statistical analysis**

Data was analyzed using Graphpad Prism 6 (GraphPad Software Inc. Ca, USA). Repeated measures analysis of variance (ANOVA) was used to identify differences in metabolic variables between treatment conditions. Statistical significance was considered at p < 0.05.

**Results**

166 individuals with an established monogenic cause of neonatal diabetes were identified within the monogenic diabetes registry. The majority of patients had mutations within the *KCNJ11* (48.8%), *ABCC8* (13.3%)or *INS* (15.7%) gene.Of 21 (12.7%) subjects with 6q24-TND, all 6 who are currently over 18 years of age reported recurrence of hyperglycemia requiring insulin therapy. 3 adults (UC153A, UC90A and UC277A) were available for dynamic studies while a fourth patient (UC702A) was transitioned to oral SU therapy as an outpatient. Their clinical details are outlined in table 1. None of the available patients were on SU therapy in the six-month period prior to the study and all had uniparental disomy of chromosome 6 (UPD6) as the underlying genetic cause of 6q24-TND.

When comparing MMT off SU with those after a five-day course of SU, there was no difference in the serum glucose. C-peptide values were significantly higher in patients following a course of SU (p<0.05 by repeated measures ANOVA). When comparing AST off SU with those after a five-day course of SU, there was no significant difference in the serum glucose. The C-peptide response was again higher following a course of SU (p<0.01 by repeated measures ANOVA). The results of the MMT and AST are displayed in figure 1.

All four subjects successfully transition off insulin therapy in lieu of oral medications. Patients had their glycemic control assessed at varying times after transitioning. Subjects’ insulin doses immediately prior to transition medications and medications at reassessment are outlined in table 1. Two patients required metformin and sitagliptin combination therapy in addition to an SU to achieve adequate glucose control. Another patient required metformin in addition to SU therapy.

**Discussion**

6q24-TND can be caused by a number of genetic and epigenetic changes at the 6q24 locus. Overexpression of the *PLAGL1* (also known as *ZAC*) and *HYMAI* can result from uniparental disomy of chromosome 6, submicroscopic duplication of the paternal 6q24 allele or isolated loss of maternal methylation at the differentially methylated region at 6q24. Uniparental disomy of chromosome 6 is a sporadic event in embryonic development without an increased risk of recurrence in future offspring. In patients with submicroscopic duplication of the paternal 6q24 allele, there is a 50% chance of offspring inheriting the duplication. Isolated loss of maternal methylation is a poorly understood phenomenon with an unknown etiology and uncertain recurrence and inheritance risk. There are also rare autosomal recessive conditions which give rise to multiple loci with loss of methylation (8,11).

The pathogenesis of hyperglycemia 6q24-TND is uncertain with a variable course of DM in the neonatal period and later life. The true incidence of relapse is also unclear. The TNDM29 transgenic mouse uses the human *PLAGL1* and *HYMAI* gene locus to mimic the genotype seen in humans. This model suggests decreased levels of transcription factors (PDX1, NGN3, PAX6) and a reduction in beta cell mass at birth (12). There appears to be a compensatory increase in beta cell number but is inadequate to maintain euglycemia later in life in the mouse model (13). Recent data in humans would also suggest that some patients may be at risk of significant hypoglycemia during the remission period (14) and point toward some element of beta cell dysfunction rather than simply an overall reduction in cell number.

Other groups have demonstrated abnormal insulin response to hyperglycemia but a normal response to glucagon use (15) in patients with 6q24 methylation defects. Our data suggests that agents that promote non-glucose dependent insulin release may represent treatment alternatives to insulin therapy. All four of our patients were successfully transitioned off insulin therapy in lieu of SU therapy alone or a combination of multiple oral agents. There are reports of successful treatment with SU therapy for over 10 years following relapse (16).

SU therapy has been successfully used for the treatment of KATP channelopathies, which make up the majority of permanent neonatal DM. While the pathogenesis of hyperglycemia in 6q24-TND is less well characterized, there are some anecdotal reports of SU use in the neonatal period. SU therapy in type 2 diabetes can result in hastening of beta cell failure (17). However our data and other reports would suggest that insulin therapy may not be required for many years after relapse of diabetes in these patients (18).

All participants diagnosed with 6q24-TND who are currently over 18 years of age reported recurrence of hyperglycemia and were treated with insulin. Each received a genetic diagnosis in adulthood despite the classical features seen in the neonatal period. Reports suggesting that 6q24-TND is responsible for 40-50% of all cases of neonatal diabetes (6). 6q24-TND is under represented in our registry (12.7% of cases) and remains an infrequently recognized form of diabetes despite the 6q24 locus being associated transient neonatal diabetes for almost 20 years (19). Our data suggests that 6q24-TND may be mistaken for other forms of diabetes when hyperglycemia recurs outside the neonatal period. Failure to correctly identify the underlying genetic etiology results in a missed opportunity to offer individualized pharmacotherapy.

**Conclusions**

6q24-TND may be mistaken for other forms of diabetes when hyperglycemia recurs. A careful birth and neonatal history is important when assessing patients newly diagnosed with diabetes. The exact pathogenic mechanisms underlying this unusual type of diabetes remains incompletely understood. Correct identification of the underlying genetic diagnosis may impact clinical and therapeutic strategies. The role of non-insulin based therapies in 6q24-TND warrants further study and should be considered.

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**Table 1. Subject clinical details.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **UC90A** | **UC153A** | **UC277A** | **UC702A** |
| **Genetic defect** | UPD6 | UPD6 | UPD6 | UPD6 |
| **Sex** | Female | Male | Female | Female |
| **Gestational age (weeks)** | 33 | 34 | 40 | 38 |
| **Birth weight (grams)** | 1280 | 2470 | 2240 | 1810 |
| **Age at initial DM diagnosis (days)** | 1 | 1 | 1 | 1 |
| **Age at DM remission (months)** | 4 | 7 | 6 | 3 |
| **Age at DM recurrence (years)** | 13 | 12  | 27 | 12  |
| **Age at cessation of insulin (years)**  | 20 | 23 | 29 | 28 |
| **BMI at cessation of insulin (kg/m2)** | 26.28 | 31.38 | 21.66 | 29.44 |
| **Insulin dose at transition (units/kg)** | 0.59 | 0.73 | 0.41 | 0.76 |
| **Medications at reassessment** |   |   |   |  |
| **-Glyburide**  | 0.59 mg/kg | 0.53 mg/kg | 0.13 mg/kg | 0.23 mg/kg  |
| **-Sitagliptin** | 100 mg | 100mg | - | - |
| **-Metformin** | 2g | 2g | - | 1.5g |
| **HbA1c at cessation of insulin** | 8.2% | 7.8% | 7.2%  | 9.9%  |
| **HbA1c at Reassessment****-Months off insulin therapy** | 7.1%5 | 6.6%8 | 7.3%17 | 7.5%6 |

UPD6- Uniparental disomy of chromosome 6

|  |  |
| --- | --- |
| **A** | **B** |
|  |  |
| **C** | **D** |
|  |  |

**Figure 1. Metabolic testing.** A-B) Mixed meal test glucose (A) and C-peptide (B) levels. C-D) Arginine stimulation test glucose (C) and C-peptide (D) levels. Values are expressed as mean ± SEM. \* Repeated measures ANOVA used to identify differences in metabolic variables between treatment conditions.