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## Permanent Neonatal Diabetes Caused by a Novel Mutation

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Most cases of permanent form of neonatal diabetes mellitus (PNDM) are due to dominant heterozygous gain of function (activating) mutations in either *KCNJ11* or *ABCC8* genes, that code for Kir 6.2 and SUR1 subunits, respectively of the pancreatic  $\beta$ -cell KATP channel. We describe the interesting case of an infant with PNDM, in whom a compound heterozygous activating/ inactivating mutation was found with clinically unaffected parents, each carrying a heterozygous mutation in *ABCC8*, one predicting gain of function (neonatal diabetes) and the other a loss of function (hyperinsulinemia).

**Key words:** *ABCC8, Activating/ inactivating, Compound heterozygous, Neonatal diabetes, Permanent, Sulphonylurea.*

Permanent neonatal diabetes (PNDM), which refers to onset of diabetes before the age of 6 months with persistence through life, is a rare disorder occurring in one in 0.2-0.5 million live births [1]. Almost all cases of neonatal diabetes (NDM) are of monogenic etiology in contrast to the autoimmune etiology of diabetes presenting in children beyond 6 months of age [2]. Activating mutations in *KCNJ11* and *ABCC8* genes, that encode the Kir6.2 and SUR1 subunits, respectively, of the pancreatic  $\beta$ -cell KATP channel together account for approximately 40% of all cases of PNDM [3].

Mutations in *KCNJ11* and *ABCC8* give rise to two opposing phenotypes of congenital hyperinsulinemia (CHI) and NDM. Loss of function (inactivating) mutations abrogate the channel function, causing CHI while gain of function (activating) mutations impair the ability of ATP to close the channel, causing NDM [2]. While CHI is caused by recessively inherited homozygous mutations, most cases of NDM are caused by heterozygous (dominant) *de novo* mutations.

Recessive inheritance of PNDM is rare and has been described in only 8 patients till date, out of which 3 each had homozygous and compound heterozygous activating mutations of *ABCC8*, respectively [4]. Previously described in only 2 patients [4], with our baby representing the third case, a novel mutational mechanism has been observed in which there is compound heterozygous activating/ inactivating mutation of the *ABCC8* gene, i.e., one allele has a mutation with loss of function effect and the other has a mutation that predicts gain of function.

### CASE REPORT

A 4 week old baby boy was diagnosed as diabetes mellitus (with ketoacidosis at onset) at a hospital in Punjab and referred to us at the age of 8 weeks. He was the first born baby of non-consanguineous parents, delivered at term with weight appropriate for dates, with no significant perinatal problems. There was history of diabetes in paternal grandfather and great grandmother. The infant was managed with twice daily injections of NPH insulin

with regular insulin as needed, with frequent blood sugar monitoring.

Blood samples of infant and both parents were sent for molecular genetic analysis to Royal Devon and Exeter NHS Foundation Trust, UK. The infant remained on frequent follow up, with reasonable glycemic control, and no episode of ketoacidosis or severe hypoglycemia. We received the genetic report when the infant was 8 months old. At this time, he had appropriate growth but mild motor delay, was on 0.3 U/kg/day of insulin and HbA1c was 8.1%.

**Molecular genetic analysis:** Sequencing analysis showed that the infant was heterozygous for two missense mutations, R168C and G1256S, in exons 4 and 31 of the *ABCC8* gene. The C>T mutation at nucleotide 502 (c.502C>T) results in substitution of cysteine for arginine at codon 168 (p.Arg168Cys) and has been reported previously in a patient with CHI [5]. The G>A mutation at nucleotide 3766 (c.3766G>A) results in the substitution of serine for glycine at codon 1256 (p.Gly1256Ser), and has been identified in another patient with PNDM (Flanagan and Ellard, unpublished data). Testing of parents showed that the father was heterozygous for the missense mutation G1256S while the mother was heterozygous for the missense mutation R168C, implying that father was a carrier of NDM while mother was a carrier of CHI. In the proband, the mutations were in trans and this was consistent with a diagnosis of recessively inherited NDM due to compound heterozygous activating/ inactivating mutations in *ABCC8* gene.

The infant was admitted for transfer from insulin to oral sulphonylurea. NPH insulin was stopped and glibenclamide was started in gradually increasing dose along with short acting insulin when required. Glycemic control was achieved at 0.4 mg/kg/day of glibenclamide which was decreased further to 0.3 mg/kg/day on follow-up. Subsequently, blood sugar was maintained in the range of 80- 140 mg/dL with no hypoglycemic episodes and no requirement of short acting insulin. HbA1c was 6.8% when checked 4 months after switchover with further decline to 5.2% at 8 months.

## DISCUSSION

Significant advances in elucidating the genetic basis of NDM have been made since the initial description in 1995 [6]. In 1997, the first mutation leading to PNDM with pancreatic agenesis (IPF/PDX1) was identified [7], followed between 2004 and 2008, by identification of mutations in *KCNJ11*, *ABCC8* and *INS* genes as responsible for majority of PNDM [3,4,8]. Sulphonylureas, which bind to SUR1 and close the KATP

channel, triggering insulin secretion, have been found to be effective in most patients with mutations in *KCNJ11* or *ABCC8* genes [9]. Among patients with *KCNJ11* related PNDM, efficacy and safety of sulphonylurea has been documented over follow-up of 34 months [9].

Although, there are many examples of genes in which mutations with opposite effects can result in opposing clinical phenotypes, *e.g.*, activating GCK mutations, cause hypoglycemia while inactivating mutations result in hyperglycemia [10]; to the best of our knowledge, PNDM is the first disease phenotype reported to be a result of compound heterozygosity for both gain of function and loss of function mutations.

Attempting to make a genetic diagnosis in patients with PNDM is important, not only because it can lead to transfer of patients with *KCNJ11* or *ABCC8* mutations to sulphonylureas, but also for genetic counselling. While all *KCNJ11* mutations in patients with NDM reported to date are heterozygous dominant; the *ABCC8* mutations may be heterozygous dominant (commonest), homozygous recessive, or compound heterozygous for another activating mutation or rarely an inactivating mutation of the other allele. The risk that unaffected parents will have a second affected child is considerably higher for recessive mutations than de novo dominant mutations (25% *vs.* the risk of germline mosaicism), but, in the next generation, the offspring of the proband are very unlikely to be affected for a recessive acting mutation, compared with the 50% risk for a dominant heterozygous mutation.

An interesting observation in our family was that although the 28 year old father, who carried the heterozygous activating mutation, was unaffected; his own father and grandmother had diabetes from the age of 51 and 62 years, respectively. We were not able to test the grandparents for the mutation, an interesting theoretical possibility remains that heterozygous missense mutation G1256S in *ABCC8* gene may manifest as type 2 diabetes in late adulthood.

**Contributors:** VJ and SK were involved in the clinical evaluation and management of the child; SF performed the molecular genetic testing which was interpreted by SE. All authors contributed to the review of literature and preparation of the manuscript. VJ will act as guarantor.

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## Novel STXBP2 Mutation Causing Familial Hemophagocytic Lymphohistiocytosis

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Familial Hemophagocytic Lymphohistiocytosis (FHL) is a rare autosomal recessive disorder. Diagnosis is established in presence of genetic mutation or positive family history in one of the siblings. Common genetic mutations associated with FHL are mutations in gene *PRF-1* (also known as *FHL 2*), *UNC13D* (*FHL 3*) and *STX11* (*FHL 4*). Recently mutation in *STXBP2* encoding syntaxin binding protein 2 (Munc 18 -2) has been found to be associated with FHL type 5. Here we describe the first reported Indian patient with homozygous mutation in *STXBP2* gene (c1697 G>A resulting in amino acid change p.G566D) causing FHL 5.

**Key words:** Familial Hemophagocytic Lymphohistiocytosis, FHL 5, STXBP2 mutation.

**F**amilial hemophagocytic lymphohistiocytosis (FHL) is a genetically heterogeneous immune disorder characterized by widespread organ infiltration with activated macrophages and lymphocytes. The clinical course usually starts in infancy and is fatal unless treated with hematopoietic stem cell transplant. According to the guidelines of the Histiocyte Society, diagnosis of Hemophagocytic Lymphohistiocytosis (HLH) requires fulfillment of 5 out of 8 criteria – prolonged fever, splenomegaly, bicytopenia, elevated triglycerides/low fibrinogen, increased ferritin, hemophagocytosis in bone marrow, decreased NK cell cytotoxicity and increased soluble CD25 [1]. Diagnosis of FHL is established in presence of certain genetic mutations. Positive family history of affected siblings strongly suggests the diagnosis of FHL. Disease causing

mutations in gene *PRF-1* encoding perforin, *UNC13d* encoding MUNC 13-4, and *STX11* encoding syntaxin11 have been identified in approximately 80% of cases. Recently mutation in *STXBP2* encoding syntaxin binding protein 2 (Munc 18 – 2) has been reported in a few patients. We report a patient with mutation in *STXBP2* gene from India.

### CASE REPORT

A 28 day-old girl presented with fever, progressive abdominal distension and lethargy for seven days. She was the first child born to third degree consanguineous parents. During antenatal period mother gave history of fever and rash at fifth month of gestation. At 36 weeks of gestation mother had premature rupture of membranes and baby was delivered by caesarian section for