Short Report

Clinical and molecular characterization of neonatal diabetes and monogenic syndromic diabetes in Asian Indian children


Mutations in the pancreatic ATP sensitive K+ channel proteins [sulfonyluea receptor 1 (SUR1) and inward rectifier K+ channel Kir6.2 (Kir6.2), encoded by ATP-binding cassette transporter subfamily C member 8 (ABCC8) and potassium channel J11 (KCNJ11), respectively], are the most common cause of neonatal diabetes. We describe the clinical presentation and molecular characterization of Asian Indian children with neonatal diabetes mellitus and monogenic syndromes of diabetes. We sequenced KCNJ11, ABCC8 and insulin (INS) genes in 33 unrelated Indian probands with onset of diabetes below one year of age. A total of 12 mutations were identified which included ABCC8 mutations in seven, KCNJ11 mutations in three and INS mutations in two children. The Asp212Tyr mutation in ABCC8 was novel. We also detected two novel mutations (Val67Met and Leu19Arg) in children with syndromic forms of diabetes like Berardinelli Seip syndrome [1-acyl-sn-glycerol-3-phosphate acyltransferase beta (AGPAT2)] and Fanconi Bickel syndrome [solute carrier family 2A2 (SLC2A2)]. Children carrying the KCNJ11 (Cys42Arg, Arg201Cys) and ABCC8 (Val86Ala, Asp212Tyr) mutations have been successfully switched over from insulin therapy to oral sulfonylurea. Our study is the first large genetic screening study of neonatal diabetes in India.

Conflict of interest
The authors declare no conflict of interest.
Neonatal diabetes mellitus (NDM) is a form of monogenic disorder with an incidence of 1 in 90,000 live births in Italy (1). NDM can be either permanent (PNDM) or transient (TNDM). In PNDM, the condition persists throughout the patient’s life and comprises 50% of all neonatal diabetes. In TNDM, diabetes remits within a year, but may relapse in adolescence or early adulthood. Several genetic syndromes associated with diabetes also have their onset during the neonatal period.

Molecular analyses have identified over a dozen genes/loci associated with neonatal diabetes namely potassium channel J11 (KCNJ11) (accession number: NM_000525.3), ATP-binding cassette transporter subfamily C member 8 (ABCC8) (accession number: NM_000352.3), insulin (INS) (accession number: NM_000207.2), 6q24, solute carrier family 2A2 (SLC2A2) (accession number: NM_000340), SLC19A2, eukaryotic translation initiation factor 2-alpha kinase 3 (EIF2AK3), glucokinase (GCK), insulin promoter factor 1 (IPF1), pancreas transcription factor 1 subunit alpha (PTF1A), hepatocyte nuclear factor 1 homeobox B (HNF1B), forkhead box P3 (FOXP3), zinc finger protein 57 (ZFP57), GLIS3, GATA6 and regulatory factor (RFX6) (2–6). Recent developments have highlighted the importance of ion channel mutations in the etiology of both PNDM and TNDM (5, 6). Hetero-octameric ATP-sensitive potassium channels (KATP channels) (7) link metabolism with membrane electrical activity by responding to changes in the adenine nucleotide levels that reflect the energy status of the cell consequent to changes in blood glucose level.

India currently has 62 million people with diabetes (8, 9), 95% of whom have type 2 diabetes, which is a complex genetic disorder. Monogenic forms of diabetes like maturity onset diabetes of young (MODY) and neonatal diabetes are relatively uncommon. However, they are the only forms of diabetes where genetic testing currently has clinical application. We have earlier reported on the genetics of MODY in India (10, 11). The molecular basis of neonatal diabetes has not been systematically studied in India except in four isolated case reports. In this study, we examined KCNJ11, ABCC8 and INS genes in children with neonatal and infantile onset diabetes. We also examined Berardinelli-Seip congenital lipodystrophy 2 (BSCL2) (accession number: NM_001122955.3), AGPAT2 (accession number: NM_001012727.1), SLC2A2 and EIF2AK3 (accession number: NM_004836.5) genes in neonates with syndromic forms of diabetes.

**Methods**

The study group comprised of children with neonatal diabetes and syndromic forms of diabetes referred to Dr. Mohan’s Diabetes Specialties Centre, a large diabetes hospital in south India. For all cases, informed consent was obtained from the parents. All probands included in the study were negative for pancreatic auto-immune antibodies [glutamic acid decarboxylase auto antibodies (GAD) and islet antigen 2 auto antibodies (IA2)]. A total of 33 children who had diabetes as defined by random plasma glucose greater than 200 mg/dl on more than one occasion were included in the study of whom, in 24 children, the onset of diabetes was on or before 6 months of age and we classified them as ‘neonatal diabetes’. This included 22 with PNDM and 2 with TNDM. In six children, diabetes was diagnosed between 6 months and 1 year of age and they were labeled as ‘infantile onset’ diabetes. Three children had syndromic forms of diabetes. Children with delayed milestones (motor and mental) were considered to have developmental delay. We also studied 100 normal glucose tolerant subjects (fasting value <100 mg/dl and 2h value <140 mg/dl) and 100 children with type 1 diabetes with onset of diabetes above 1 year of age and with GAD antibody positive. Parents blood samples were collected wherever possible to check the cosegregation of the mutations identified. We performed
sequencing on control and parents’ DNA wherever possible for confirmation.

DNA was isolated from whole blood by phenol-chloroform method. Direct sequencing was carried out on an ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA) using the Big Dye terminator V3.1 chemistry, and the sequences were compared with the public data bases. Published primers were used to amplify the DNA for KCNJ11, ABCC8 and INS (12, 13). Primers were designed using ‘PRIMER 3’ software for, BSCL2, AGPAT2, SLC2A2 and EIF2AK3. Primer sequences are available upon request.

The intron-less KCNJ11 was amplified in six overlapping fragments. For the other genes, primers were designed to amplify exons and conserved splice sites. In silico predictions of pathogenicity were carried out using the online tools SIFT and POLYPHEN-2.

Results

A total of 33 children were screened for genetic mutations. Mutations were detected in 36.3% of PNDM (8 of 22), 50% of infantile onset DM (3 of 6), and 100% of syndromic forms of diabetes (3 of 3). None of these novel mutations were seen in the chromosomes of NGT subjects or those with type 1 diabetes.

Permanent neonatal diabetes

A total of 22 children had PNDM of whom 8 had mutations, 4 in ABCC8 (50%), 3 in KCNJ11 (37.5%), and 1 in INS (12.5%). In the ABCC8, we identified one novel mutation (Asp212Tyr), one known mutation (Val86 Ala) and two novel intronic variants (IVS22+71C>A and IVS28+46A>C). In KCNJ11, we identified two known mutations namely, the Cys42Arg (14) (family no. 3) and Arg201Cys (6) (family nos. 4 and 5). In INS gene, we identified a known mutation, Gly32Ser in one patient (family no. 7).

Of the five exonic mutations, Asp212Tyr and Val86Ala in ABCC8, Cys42Arg in KCNJ11 and Gly32Ser in INS were de novo in origin (Fig. 1).

Infantile onset diabetes

In ABCC8, we identified two novel synonymous variants, Pro1413Pro (c.4242G>A) and Ile1456Ile (c.4368C>T) (family nos. 11 and 12) and in the INS, we identified Gly32Ser (family no. 13) mutation which was of de novo origin.

Syndromic forms of diabetes

In the case of a child with Berardinelli Seip syndrome, we found a novel homozygous mutation Val67Met and a novel variant Gly137Gly (c.411C>A) in the AGPAT2 upon screening BSCL2 and AGPAT2 genes. Previous studies have shown homozygous mutations in AGPAT2 as causative of this syndrome (15). This child died around 1 year of age because of cardiac failure. In family no. 15, as the child’s features were suggestive of Fanconi Bickel syndrome, we screened SLC2A2 and detected a novel homozygous Leu19Arg mutation. In the case of the child with Wolcott Rallison syndrome (family no. 15), we identified a known mutation Arg1065X (homozygous) in the EIF2AK3. It is of interest that the elder sibling of this proband also died of features suggestive of Wolcott Rallison syndrome. Both the parents are heterozygous for Arg1065X (16). Consanguinity is present in the families of children with syndromic forms of diabetes.

Transient neonatal diabetes

We found variants Val285Ile in KCNJ11 and Arg653Gln in ABCC8 in the two children. The children are 2 and 5 years old, respectively, now and diabetes has not relapsed so far.

Onset of diabetes was below 6 months age in all children with KCNJ11 mutations and all of them presented with ketoacidosis. Developmental delay was seen in 13 of the 33 children (39.3%). Of these, three children had mutations in ABCC8, one had KCNJ11 mutation, three had syndromic forms of diabetes and the remaining six patients did not harbor any mutation in any of the three genes studied. Clinical characteristics of patients included in the study are given in Tables 1 and S1. Summary of all the mutations is given in Table 2.

Transfer to sulfonylurea therapy

Kir2.2 and SUR1 mutations have important therapeutic implications as sulfonylurea therapy is effective in treating patients with mutations in the potassium channel subunits (5, 6, 17). We successfully shifted the children with KCNJ11 (Cys42Arg and Arg201Cys) and ABCC8 (Val86Ala and Asp212Tyr) mutations from insulin therapy to sulfonylurea treatment (Table 2) using an inpatient-based short transfer protocol (18, 19).

Discussion

To our knowledge, this is the first large genetic screening study on children with neonatal diabetes and syndromic forms of diabetes from India and the first to report on ABCC8 gene mutations.

The children with PNDM showed mutations in all the three genes studied. Previous studies have shown the residue 201 in KCNJ11 to be a hotspot for mutations (6). The Arg201Cys (KCNJ11) mutation displayed different clinical characteristics in the two patients (family nos. 4 and 5). Remarkably, the onset of diabetes was different for the two patients (Table 1) and also there was heterogeneity in clinical and neurological manifestations. The two KCNJ11 mutations implicated with NDM are located within the critical functional domains of the gene: Residues associated with neonatal diabetes lie within the putative ATP binding site (Arg201Cys) or are located at the interfaces between Kir6.2 subunits (Cys42Arg) (6) which can suppress...
Fig. 1. Pedigrees of neonates with mutations. Pedigrees of the families showing diabetes status of each member, as well as genetic status: M/M (homozygous mutant), M/N (heterozygous), N/N (homozygous normal). Phenotypic status: NT (not tested), ND (non-diabetic), T2D (type 2 diabetes).

the electrical activity and the insulin secretion by decreasing the ability of ATP molecule to block the KATP channel. It is known that the de novo KCNJ11 mutations arise either during gametogenesis or embryogenesis leading to germ line mosaicism which suggests increased risk not only for subsequent generations, but also for the future siblings of the proband (20).

The Val86Ala mutation of ABCC8 in the PNDM child is positioned in the second transmembrane helix of the transmembrane domain 0 which plays an important role in the trafficking (21) and is a critical site for potassium channel, and thus appears to be functionally important. With respect to the novel mutation, Asp 212 Tyr, mutations have previously been identified at this residue (Asp212N and Asp212Ile) in PNDM patients who had been successfully transferred to sulfonylurea therapy (22). This mutation is located in CL3 domain of ABCC8, which modulates gating of the channel and these changes may affect highly conserved residues (21).

In INS gene, the Gly32Ser mutation was found both in PNDM group and in the infantile onset diabetes group. Previous studies showed that the mutations at residue 32 in INS were found in patients with different monogenic conditions (13). This mutation is located in the β-chain of INS which plays a key role in the formation of preproinsulin molecule. Earlier studies have also reported on the pathogenic changes of mutations in β-chain of insulin molecule (13). A study by Rubio Cabezas et al. has shown INS gene mutations to be common in infants diagnosed between 6 and 12 months (23). In the present study also, INS gene mutations were seen in a small percentage of infantile diabetes with onset between 6 and 12 months of age. The other two children in the infantile onset diabetes group had synonymous variants Pro1413Pro and Ile1456Ile which may not have any pathogenic effect.

The prevalence of TNDM patients appears to be lower in Asian Indian children which is in contrast to other studies although this may well reflect referral bias to the clinic. It is of interest that the occurrence of two heterozygous variants (F = family no. 1 – Table S1) in people with different phenotypic conditions may be explained by opposite functional effects of compound heterozygous mutations (12). Prediction analysis (POLYPHEN-2) showed that the mutations in Val285Ile (KCNJ11), Arg653Gln (ABCC8) and Val67Met (AGPAT2) could cause less, and the mutations Asp212Tyr (ABCC8) and Leu19Arg (SLC2A2) more damage to the protein. The two intronic variants found in ABCC8 did not show any damaging effect on splicing. Further studies are necessary to understand the consequences of these variations and also of the synonymous variants.
Table 1. Clinical characteristics of study patients harboring mutations in the genes studied

<table>
<thead>
<tr>
<th>Family no.</th>
<th>Sex</th>
<th>Diabetes sub type</th>
<th>Mutation identified in</th>
<th>Mutation identified</th>
<th>Mutation status</th>
<th>Gestational age (weeks)</th>
<th>Age at onset of symptoms</th>
<th>Birth weight (kgs)</th>
<th>Birth weight (centiles)</th>
<th>Developmental delay</th>
<th>Other clinical findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Male</td>
<td>PNDM</td>
<td>KCNJ11</td>
<td>Cys42Arg (14)</td>
<td>Known</td>
<td>35</td>
<td>82 days</td>
<td>2.3</td>
<td>2</td>
<td>No</td>
<td>Ketoacidosis</td>
</tr>
<tr>
<td>2</td>
<td>Female</td>
<td>PNDM</td>
<td>KCNJ11</td>
<td>Arg201 Cys (6)</td>
<td>Known</td>
<td>34</td>
<td>5 months</td>
<td>2.7</td>
<td>10</td>
<td>No</td>
<td>Ketoacidosis, sepsis, ketoacidosis</td>
</tr>
<tr>
<td>3</td>
<td>Male</td>
<td>PNDM</td>
<td>KCNJ11</td>
<td>Arg201 Cys (6)</td>
<td>Known</td>
<td>29</td>
<td>48 days</td>
<td>1.4</td>
<td>&lt;2</td>
<td>Yes</td>
<td>Neonatal hepatitis, sepsis, ketoacidosis</td>
</tr>
<tr>
<td>4</td>
<td>Female</td>
<td>PNDM</td>
<td>ABCC8</td>
<td>Val86Ala (19)</td>
<td>Known</td>
<td>34</td>
<td>6 months</td>
<td>2.8</td>
<td>10</td>
<td>No</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>Male</td>
<td>PNDM</td>
<td>ABCC8</td>
<td>Asp212Tyr</td>
<td>Novel</td>
<td>33</td>
<td>2 months</td>
<td>2.5</td>
<td>2</td>
<td>No</td>
<td>Neonatal hepatitis, sepsis</td>
</tr>
<tr>
<td>6</td>
<td>Male</td>
<td>PNDM</td>
<td>INS</td>
<td>Gly32Ser (13)</td>
<td>Known</td>
<td>34</td>
<td>4 months</td>
<td>2.8</td>
<td>10</td>
<td>No</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>Male</td>
<td>Infantile onset DM</td>
<td>INS</td>
<td>Gly32Ser (13)</td>
<td>33</td>
<td>Known</td>
<td>10 months</td>
<td>3.5</td>
<td>50</td>
<td>No</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>Male</td>
<td>Berardinelli Seip syndrome</td>
<td>AGPAT2</td>
<td>Val67Met</td>
<td>Novel</td>
<td>1 month</td>
<td>2.0</td>
<td>&lt;2</td>
<td>Yes</td>
<td>Hirsutism, loss of subcutaneous fat</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Female</td>
<td>Fanconi Bickel syndrome</td>
<td>SLC2A2</td>
<td>Leu19Arg</td>
<td>Novel</td>
<td>1 month</td>
<td>2.5</td>
<td>5</td>
<td>Yes</td>
<td>Doll like facies, deformed legs</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Male</td>
<td>Wolcott Rallision syndrome</td>
<td>EIF2AK3</td>
<td>Arg1065X (16)</td>
<td>Known</td>
<td>33</td>
<td>3 months</td>
<td>2.1</td>
<td>&lt;2</td>
<td>Yes</td>
<td>Acute hepatitis</td>
</tr>
</tbody>
</table>
We found ABCC8 mutations in patients with multiple phenotypes namely PNDM and infantile onset diabetes, but the KCNJ11 mutations in PNDM patients only. As we did not perform screening of 6q chromosomal abnormalities and other genes associated with neonatal diabetes, we cannot rule out additional mutations in children in whom we did not find any mutations (2–6, 24).

Although we did not perform functional studies for any of these mutations, it is possible that some of these are pathogenic as they are located in the important domains of the gene that are conserved among other species. Moreover, none of these mutations were found in normal subjects or in children with type 1 diabetes.

In summary, we report a genetic analysis of 33 children with neonatal and syndromic forms of diabetes. Investigating the genetics of neonatal diabetes could be useful not only for estimating the prognosis but also for enabling therapeutic changes such as switching over from insulin injections to oral sulfonylurea which is a great boon to the patient and the family.

Supporting Information

The following Supporting information is available for this article:
Table S1. Clinical characteristics of study patients who do not harbor mutations in the genes studied

Additional Supporting information may be found in the online version of this article.

Acknowledgements

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References


