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Case report

Sulfonylurea-responsive neonatal diabetes mellitus diagnosed through molecular genetics in two children and in one adult after a long period of insulin treatment

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Abstract. A permanent neonatal diabetes mellitus has finally been diagnosed through molecular genetics in two children and one adult after 9 to 35 years of uninterrupted insulin treatment. These patients developed diabetes before 6 months of age and were autoantibody negative. In one boy, a mutation in the KCNJ11 gene was identified at 9 years of age. In the other two patients (daughter and father, 12.6 and 25 years old respectively) the new gene variant (ABCC8/L213P) was found. Switching from insulin to sulfonylurea treatment leads to the definitive discontinuance of insulin therapy, improving metabolic control as well as the amelioration of the associated neurodevelopmental disabilities in the young girl in which an intermediate Development Delay, Epilepsy, Neonatal Diabetes syndrome was diagnosed. (www.actabiomedica.it)

Key words: neonatal diabetes mellitus, PNDM, Molecular genetics, DEND, Sulfonylurea

Introduction

Neonatal diabetes mellitus is a rare condition occurring within the first 6 months of life in 1:300,000-1:400,000 live births, associated with defects in genes that play a role in pancreatic beta-cell development and function. Patients carrying these defects have negative tests for type-1 diabetes autoantibodies (1, 2).

Ninety percent of patients with Permanent Neonatal Diabetes Mellitus (PNDM) show mutations in the genes encoding the ATP-sensitive potassium (KATP) channel subunits: KCNJ11 (Potassium Inwardly Rectifying Channel, Subfamily J, Member 11) also known as Kir6.2 (Potassium-Channel Inwardly Rectifying Subunit), and ABCC8 (ATP-Binding Cassette, Subfamily C, Member 8) also known as SUR1 (Sufanylurea Receptor 1). The mutations of the genes encoding these subunits keep the potassium channels open and inhibit exocytosis of insulin from the pancreas beta cells (2-5). As a consequence, many misdiagnosed patients are initially treated with insulin.

Treatment with oral sulfonylureas has been recently recommended for patients with PNDM, in an attempt to discontinue insulin administration. These drugs binding to the sulfonylurea receptors subunit, expressed at the surface of beta cells, are able to close the potassium channels and to promote insulin exocitosis, which leads to a diabetes remission (6-9).

We have investigated the genetic basis of three Caucasian subjects who developed diabetes before 6 months of age and were treated with insulin over several years, up to the recent genetic diagnosis of PNDM.

Case presentations

Patient n. 1 - Valerio is a boy born at 40 weeks of gestation with a birth weight of 2,470 g (- 2 SD). The father had a MODY 2 due a mutation in glucokinase gene, diagnosed at 15 years of age and treated with a glycemic-controlled diet. Two grandparents have been diagnosed with type 2 diabetes mellitus (Fig. 1). At the age of 40 days, he developed polyuria and poor growth. He was admitted to hospital with a moderate ketoacidosis: blood glucose levels 50,11 mmol/L, pH 7.23, B.E. - 12.3 mmol/L, and HCO3- 10,5 mmol/L. C-peptide was undetectable. HbA1c level was 6.8%. ICA, GAD e IA2 were negative. HLA: DQB1*0202-Non Asp DQB1*0301-Asp. He was treated with 4 daily insulin injections (0.50-0.90 UI/Kg body weight) up to the age of 9 years. HbA1c levels ranged from 6.7 to 7.8%. During insulin treatment the child experienced 6 episodes of severe hypoglycemia with altered mental status. Electroencephalogram tests never showed any abnormalities. At 9 years of age, genomic DNA was isolated from a blood sample. The coding region and the intron-exon boundaries of KCNJ11 were amplified by the polymerase chain reaction, and sequenced in order to identify possible mutations (Laboratory of Mendelian Diabetes, Ospedale pediatrico Bambino Gesù, IRCCS, Rome, Italy). Heterozygous mutation in the KCNJ11 gene was identified, confirming a diagnosis of PNDM due to a mutation in Kir6.2 subunit of the KATP channel. The child was progressively transferred to oral sulphonylurea (Glipizide); the initial dose was 0.17 mg/Kg/d divided into two doses and was gradually increased up to 0.6 mg/Kg/d. Insulin injections were discontinued 12 months after the first oral sulphonylurea dose. C-peptide level increased up to 2.0 ng/ml, and a good diabetes control was obtained: HbA1C levels fell from 7.8% when on insulin to 6.9% on the current regimen. The child is growing regularly in terms of weight and height. Psychomotor development is normal. No hypoglycemia was reported. No adverse drug reactions were observed.

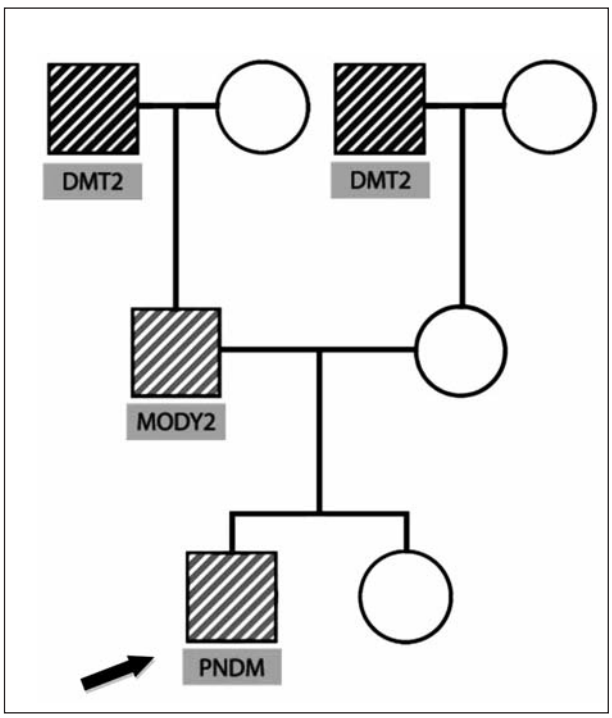

Figure 1. Family pedigree of patient n. 1 (black symbol) indicated by an arrow affected by a mutation in KCNJ11 gene; father affected by a mutation in glucokinase gene (MODY2) and grandparents with Type 2 Diabetes (T2D)

Patient n. 2 - Adriana is a girl born after a gestation of 39 weeks, by cesarean section delivery. At birth she weighed 2,100 g (- 2 SD). Polyuria, poor growth and hypo-reactivity were observed 43 days after birth. Admitted into the intensive care unit, severe dehydration, Kussmaul breathing and a severe ketoacidosis were diagnosed: blood glucose levels 44.72 mmol/L, pH 7.02, B.E. - 23 mmol/L, HCO3- 5.7 mmol/L, HbA1c 6.9%. C peptide was undetectable. ICA, GAD and IA2 were negative. HLA: DQB1*0502-Non Asp DQB1*0602-Asp. Insulin therapy (0.45 to 1.1 UI/Kg/d) lasted 12 years. During this period HbA1c levels ranged from 5.9 to 7.6%, and neither hypoglycemia nor ketoacidosis have been reported. A delayed psychomotor developmental with a defect in motor coordination, difficulty in sustaining attention and language delay was progressively observed by both pediatricians and teachers. At 12 years and 6 months of age, ABCC8 gene sequencing (Laboratory of Mendelian Diabetes, Ospedale pediatrico Bambino Gesù, IRCCS, Rome, Italy) led to the identification of the new mutation ABCC8/L213P, and the diagnosis of PNDM was done. Results showed that Adriana had inherited the same mutation from the father who also presented with iDEND (Fig. 2). After PNDM

diagnosis (performed at 12 years and 6 months of age) switching from insulin therapy to sulphonylurea therapy (Glyburide) was started. The initial total dose was 0.07 mg/Kg/d divided into three doses. It was gradually increased up to 1.2 mg/Kg/d. Insulin was discontinued 1 month after the first Glyburide dose administration and a good blood glucose control was obtained (HbA1C levels ranged from 5.9 to 6.5%). C-peptide level increased up to 1.4 ng/mL. Before starting Glyburide treatment, a neuro-psychological examination, using the WISC-R scale, showed this profile: total I.Q.=71, verbal I.Q.=85, performance I.Q.=60 with a significant difference between verbal and performance tests. A drop in "Pictures arrangement" (weighted score of 2), "Block design" (weighted score of 1) and "Object assembly" (weighted score of 4) was also noted (Fig. 3). Patient's ability to copy some geometrical drawings was assessed using the Bender test before switching. It showed a score of - 2 SD. Six months after starting Glyburide treatment, a further neuro-psychological examination was performed: WISC-R scale showed an improvement in total I.Q.=72 and performance I.Q.=70, but a slight decline in the verbal I.Q.=78. The Bender test also demonstrated an increased score from −2 to − 1. A closer analysis of the Bender test results after switching shows an improvement in the reproduction of the original image, particularly for the angles in the figures: the reproduction was made without distortion errors or image rotations and the trembling, present before the analysis, disappeared (Fig. 4).

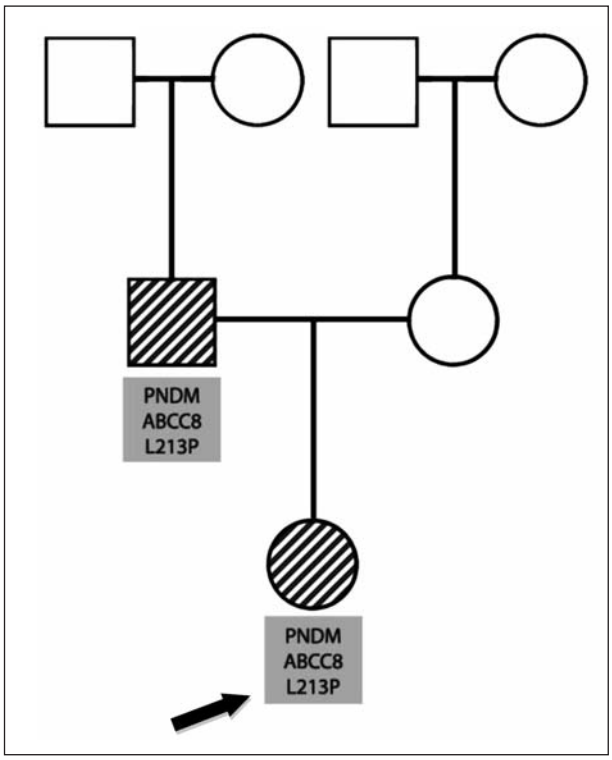

Figure 2. Family pedigree of patient n. 2 with the new mutation in ABCC8/L213P gene associated with an iDEND inherited from the father. Open symbols: unaffected family members

Patient n. 3 - Paolo was born at 40 weeks of gestation with a birth weight of 2,400g (-2SD). Polyuria and poor growth and hypo-reactivity were observed 40 days after birth. He was admitted to hospital with

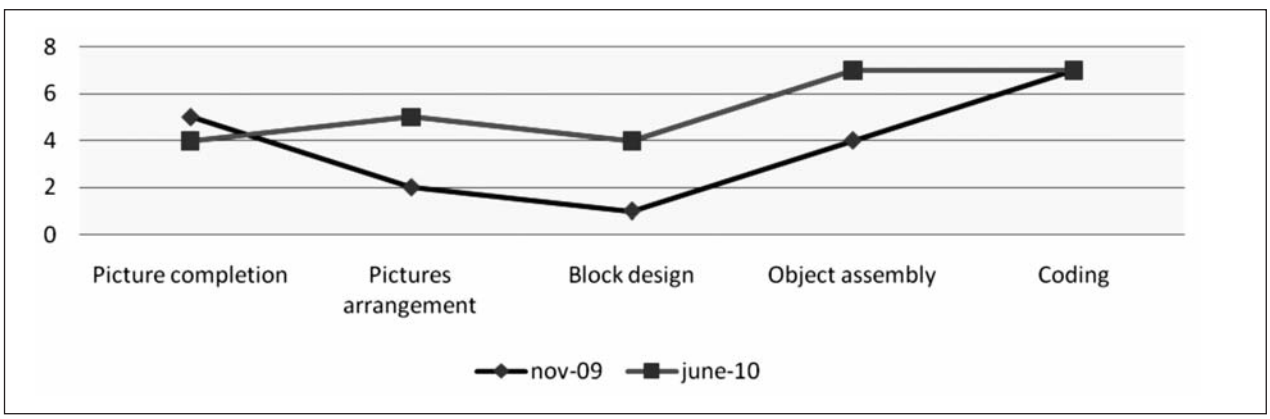

Figure 3. WISC-R case 2: Performance test showed a drop in "Pictures arrangement" (weighted score of 2), "Block design" (weighted score of 1) and "Object assembly" (weighted score of 4)

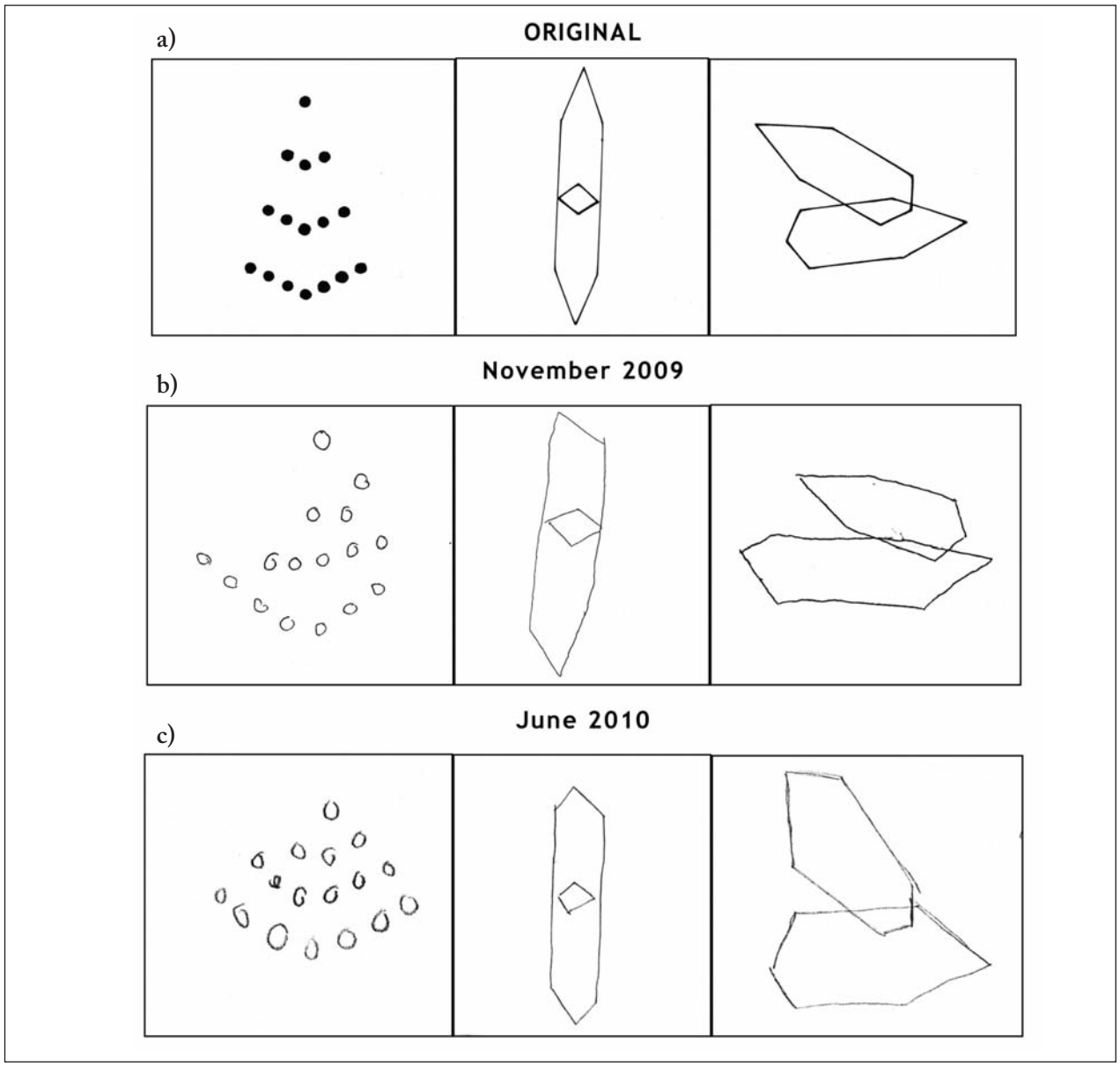

Figure 4. A closer analysis of the Bender test results before and after the switching

a severe dehydration and ketoacidosis (data unavailable). Insulin therapy lasted 46 years (0.75 to 1.5 UI/Kg/d). During this period HbA1c levels ranged from 10.1 to 6.5%. At 34 years of age, ABCC8 gene sequencing (Laboratory of Mendelian Diabetes, Ospedale pediatrico Bambino Gesù, IRCCS, Rome, Italy) led to the identification of a new mutation ABCC8/L213P, and the diagnosis of PNDM was done. Also his daughter Adriana (Patient 2) turned out to have inherited the same mutation from the father and both of them presented with iDEND (Fig. 1/B). Paolo decided to start the switching from insulin therapy to sulphonylurea therapy (Glyburide) 2 years after PNDM diagnosis (performed at 34 years of age), encouraged by the improvements recorded in his daughter's conditions, who had started the same therapy 18 months before . The initial total dose was 0.07 mg/Kg/d divided into two doses. It was gradually in-

creased up to 0.45 mg/Kg/d. Insulin was discontinued 9 days after the first Glyburide dose administration, and a good blood glucose control was obtained (HbA1C levels ranged from 6.1 to 5.8%). C-peptide level increased up to 1.6 ng/mL. Paolo denied his permission for neuro-psychological evaluation.

Discussion

The majority of children with genetically proven PNDM are initially incorrectly diagnosed as Type 1 diabetes, although there is an absence of type 1 diabetes auto-antibodies. A high percentage of them usually have the clinical features of insulin dependency such as hyperglycemia, polyuria, poor growth, ketoacidosis, lethargy and undetectable C peptide. It is therefore not surprising that these children are, for a long time, treated with insulin (10).

This is the case of the three patients above mentioned who met their definitive diagnosis of PNDM, after 9 to 34 years of uninterrupted insulin treatment. Patients n. 1 and 2 were included in two previous studies (11, 12); Patient n. 3 has not been previously reported. All patients had developed permanent hyperglycemia-related symptoms within the first 6 months of life, were autoantibody negative and have HLA types usually uncommon for type 1 diabetes. PNDM diagnosis has been confirmed in the Patient n. 1 after a mutation in the KCNJ11 gene was identified. This mutation is the one most commonly reported in literature (10, 12). In the Patients n. 2 and 3 (daughter and father respectively), a new gene variant (ABCC8/L213P) was found.

Mutations in KCNJ11 and ABCC8 genes are well known to encode the Kir6.2 and SUR1 subunits of sulfonylurea receptors in beta-cells (13,14). These variants keep KATP channels open and inhibit exocytosis of insulin from the pancreas beta cells secretion (3,4,5). Sulfonylureas are known to stimulate insulin secretion in type 2 diabetes by binding to SUR1 and closing KATP channels by an ATP-independent mechanism (13). Studies in vitro, subsequently confirmed in case reports, showed the possibility of blocking KATP channels with oral sulfonylurea in PNDM and promoting insulin discharge from the beta-cells (6-15). Channel closure leads to membrane depolarization, which subsequently activates voltage dependent calcium (Ca^{2+}) channels, leading in turn to an increase in intracellular Ca^{2+}, which triggers insulin exocytosis (13).

This discovery has radically revolutionized PNDM treatment, offering a therapeutic approach which is easily implemented and effective in maintaining normal or near normal HbA1c levels in lieu of insulin (16, 17). The present case reports confirm the efficacy of oral sulfonylureas. The improvement of metabolic control profile observed in our patients is consistent with the international data. The two children of our series continue to grow regularly. To date, no side effects associated with sulfonylurea treatment have been observed in these patients.

KATP channels are expressed not only in beta-cells but also in brain, muscle and in other tissues where their function is still not completely understood (18). It is not therefore surprising that same patients with PNDM may exhibit a neurodevelopmental disability, termed DEND (Development Delay, Epilepsy, Neonatal Diabetes) syndrome (19, 20) of which a severe (DEND) and an intermediate (iDEND) form has been described. The Patient n. 2 of our serie had an iDEND syndrome, having a motor, speech and cognitive delay, but not epilepsy and muscle weakness. An iDEND syndrome has been clinically observed also in the Patient n. 3, father of the Patient n. 2, but he did not assent to investigation into his disability.

Several case reports have demonstrated an improvement of the disabilities in patients with iDEND within months of sulfonylurea treatment, notably with the Glyburide, a sulfonylurea which seems to be able to cross the blood-brain barrier (6, 9). In Patient n. 2, neurodevelopmental outcome early appeared. After 6 months of therapy, notable improvements were recorded in muscle tone, motor coordination and praxia. Distortion and rotation errors that penalized the results of the Bender test before treatment were finally not committed. Figure 4 (a, b, c) showed well defined corners and better maintained proportions between the dimensions of the two original hexagons. The previously observed tremors disappeared. School teachers also reported improvements in the speech defects, in memorizing and in the attention level.

In conclusion, children with a history of a permanent neonatal diabetes diagnosed within the first 6 months of life and with autoantibody negative, have to be investigated for activating mutations in the genes encoding the two subunits of the ATP-sensitive potassium channel, even if they have received a long term treatment with insulin. In these patients, switching from insulin to sulfonylurea treatment leads to the definitive discontinuance of the insulin therapy, improved metabolic control, as well as the amelioration of the occasional associated neurodevelopmental disabilities.

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