Published in final edited form as:
Pediatr Diabetes. 2014 May ; 15(3): 252–256. doi:10.1111/pedi.12086.

Microcephaly, Epilepsy and Neonatal Diabetes Due to Compound Heterozygous Mutations in *IER3IP1*: Insights into the Natural History of a Rare Disorder

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Abstract

Neonatal diabetes mellitus is known to have over 20 different monogenic causes. A syndrome of permanent neonatal diabetes along with primary microcephaly with simplified gyral pattern, associated with severe infantile epileptic encephalopathy was recently described in two independent reports in which disease-causing homozygous mutations were identified in the immediate early response-3 interacting protein-1 (*IER3IP1*) gene. We report here an affected male born to a non-consanguineous couple who was noted to have insulin-requiring permanent neonatal diabetes, microcephaly, and generalized seizures. He was also found to have cortical blindness, severe developmental delay and numerous dysmorphic features. He experienced a slow improvement but not abrogation of seizure frequency and severity on numerous anti-epileptic agents. His clinical course was further complicated by recurrent respiratory tract infections and he died at 8 years of age.

Whole exome sequencing was performed on DNA from the proband and parents. He was found to be a compound heterozygote with two different mutations in *IER3IP1*: p.Val21Gly (V21G) and a novel frameshift mutation p.Phe27fsSer*25. IER3IP1 is a highly conserved protein with marked

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expression in the cerebral cortex and in beta cells. This is the first reported case of compound heterozygous mutations within *IER3IP1* resulting in neonatal diabetes. The triad of microcephaly, generalized seizures and permanent neonatal diabetes should prompt screening for mutations in *IER3IP1*. Since mutations in genes such as *NEUROD1* and *PTF1A* could cause a similar phenotype, next-generation sequencing approaches – such as exome sequencing reported here – may be an efficient means of uncovering a diagnosis in future cases.

Introduction

Neonatal diabetes mellitus is known to have over 20 different monogenic causes (1). A syndrome of neonatal diabetes along with primary microcephaly with simplified gyral pattern, associated with severe infantile epileptic encephalopathy was recently described in two reports as being caused by mutations in the immediate early response-3 interacting protein-1 (*IER3IP1*) gene (2,3). The function of this protein has not been well characterized but is thought to be important in regulating apoptosis in tissues of both neuronal and pancreatic origin. In the first report, affected individuals from two unrelated consanguineous families from Morocco and Argentina were homozygous for two different missense mutations, p.Val21Gly and p.Leu78Pro, respectively. The second report found the same homozygous p.Leu78Pro mutation in four affected individuals from two different consanguineous Egyptian families (3). Here, we report an affected male, with similar clinical presentation, but born to non-consanguineous parents, who is a compound heterozygote with two different mutations in *IER3IP1*, p.Val21Gly and a novel frameshift mutation p.Phe27fsSer*25.

Case report

The proband was the first-born child to a non-consanguineous couple of Jewish origin. The mother was born in Colombia and is of mixed Ashkenazi-Spanish-French (possibly North African) origin. The father is of mixed Libyan-Tangier origin. The parents are healthy and have normal head circumference. During the pregnancy, ultrasound examinations at 15 and 23 weeks of gestation were summarized as normal, although at the latter time point the head circumference (HC) and bi-parietal diameter (BPD) were slightly below the 10th centile. At 35 weeks, ultrasound calculated head circumference was 28.1 cm (−3 SDS), and the BPD was 7.7 cm (−4 SDS). Abdominal circumference and femur length were within the normal range. At 36 weeks, the mother developed toxemia, and an emergency Cesarean section was performed. The proband (UC0207) had a birth weight of 2640 grams (−0.4 SDS) and birth length of 46.5 cm (−0.6 SDS) that were normal but his HC of 29.5 cm (−4 SDS) was markedly reduced. Around the age of two months, he developed intractable generalized seizures that were resistant to therapy. The seizures were defined as tonic-clonic and myoclonic in type and the EEG was described as consistent with hypsarrythmia. Visual evoked potential testing indicated central blindness. Brain MRI at age five months revealed microcephaly with simplified gyral pattern. At this age, he exhibited no social smiling, no eye contact, and no achievement of motor milestones. His weight and length were normal for his age, but he had severe microcephaly (HC was −4 SDS), along with a sloping forehead and mild tapering fingers. He had retractile testes with otherwise normal genitalia.

Elevated blood glucose levels around 300–400 mg/dL were measured during his hospitalization and insulin therapy was initiated. A serum insulin level prior to therapy was 13.9 IU/mL and a C-peptide level was 0.55 nmol/L (reference range 0.3–1.3), indicating some preserved beta-cell function. Anti-glutamic decarboxylase (GAD) antibodies were negative. At age two years, the clinical picture included severe epilepsy, continued diabetes, microcephaly and significant developmental delay. Recurrent pneumonia was documented. His diabetes control was excellent based on HbA1c of 7.5% (58 mmol/mol), with an insulin requirement between 0.6–0.7 units/kg/day using continuous subcutaneous insulin infusion (CSII or “insulin pump”) with insulin lispro. A repeat C-peptide level of 0.71 nmol/L demonstrated continued preservation of at least a low level of endogenous insulin production. At age four years, he exhibited no speech or voluntary motor activity but his diabetes continued to be well-controlled. Despite multi-drug therapy, seizures were witnessed occasionally, mostly as jittering. He slept most of the time, with noisy breathing. The face appeared somewhat coarse, with thick eyebrows, long dense eyelashes, large cheeks, and very long palpebral-fissures (3.45 cm). Similar to other reported cases (3), he had teeth worn down by grinding, thickened lips, and a small mouth with a short philtrum. The auricles were coarse and long at 6.5 cm (above 97th centile), with over-folded helices. He was hypertrichotic, mainly on the back, buttocks and genitalia, and his fingers were tapered with clubbing. At his last visit aged seven years, his weight was 22 kg (−0.4 SDS.). His diabetes continued to be well-controlled (HbA1c 8.3% or 67 mmol/mol) on CSII with a stable insulin dose of 0.65 units/kg/day. There had never been any reports of severe hypoglycemic episodes. Severe convulsions, lasting sometimes 15–20 minutes, were last witnessed around the age of five years and since that time both their frequency and intensity had decreased while continuing on a wide regimen of anti-convulsive agents, most recently including phenobarbital, clonazepam, topiramate and vigabatrin. His only deliberate movement was opening his eyes, with no other motor, communicative or social activity. He thus also had significant tendon shortening. Recurrent infections of lower respiratory tract had continued at a reduced frequency. His death at home was reported at the age of 8 years.

Molecular genetic analysis

Genomic DNA was extracted from peripheral blood from the proband and parents using standard procedures. Whole exome sequencing was performed before publication of the *IER3IP1* reports, as follows. The VCHrome capture reagent (Roche NimbleGen; http://www.nimblegen.com) was used to target the exome that was then sequenced using a HiSeq 2000 instrument (Illumina; http://www.illumina.com) using standard chemistry recommended by the manufacturer. Read mapping was done using Burrows-Wheeler aligner (4), and variants were called using Atlas2 (5). By design, at least 80% of the exome was covered 20x. Variant analysis was performed using SNP and Variation Suite v7.6.9 (Golden Helix, Inc.; http://www.goldenhelix.com). Variants with a minor allele frequency >0.01 in 1000 Genomes phase 1 data released April 4, 2012 v3 or NHLBI ESP6500 data released June 2012 were removed. We further considered variants that are either nonsynonymous or annotated to a splice junction. Variants remaining after initial filtering were further filtered for three potential modes of inheritance: autosomal recessive (102 variants), possible *de novo* (31 variants), and compound heterozygous (36 genes). Upon review of these genes for

potential to cause permanent neonatal diabetes, we prioritized validation of three variants mapped to hg19: one in *ALMS1* and two in *IER3IP1*. The *ALMS1* variant was a three base pair insertion (chr2:73675227_73675228ins.CTC) for which both parents were heterozygous and the proband was homozygous; however, this variant was not confirmed by Sanger sequencing. Two variants were found in *IER3IP1*: a single nucleotide deletion (chr18:44702569delA) leading to a frameshift for which the mother and proband were heterozygous and a missense mutation (chr18:44702587A>C) for which the father and proband were heterozygous. Sanger sequencing validated these mutations in the proband and parents. The single nucleotide deletion causes a frameshift designated as c.79delT, causing alteration of the protein sequence designated as p.Phe27fsSer*25. The missense mutation of the nucleotide sequence c.62T>G causes a valine to glycine change at position 21 of the protein designated as p.Val21Gly (or V21G) and has been reported previously in the homozygous state in a case of Moroccan origin (2).

Discussion

The patient we describe represents the seventh individual from the fifth family presently known to have microcephaly, epilepsy and neonatal diabetes mellitus due to mutations in *IER3IP1* (Table 1). Ours is the first report in a non-consanguineous family and the first with compound heterozygous mutations. Unlike most affected individuals reported to date, our patient survived to his mid-childhood. The present accumulated data enable some understanding of the natural history of the *IER3IP1* associated disorder. This condition has an obvious intra-uterine course, and it seems that microcephaly evolves around the mid-second trimester of pregnancy, becoming more significant during the 3rd trimester of the pregnancy. Similar to the other cases, intractable convulsions and insulin-requiring diabetes mellitus developed in the neonatal-infantile period. Whereas the seizures appear resistant to therapy, the diabetes in our patient was successfully controlled with an insulin pump. Lower respiratory tract infection seems a serious medical life-threatening complication. The natural history presented by our patient indicates that the neurodevelopmental prognosis in mid-childhood is grave. The relevance of the physical features at age 4 years (e.g. tapering fingers, course facial appearance) to the mutated gene, rather than being attributed to the ethnic familial background or to medical care (e.g. hypertrichosis), is yet to be determined as more affected children are described. Another possible feature was noted in one boy in “family 2” reported by de Wit et al. (later described in Poulton et al. as “family 1”) as having “small genitalia” (2,6). Our patient was described as having normal genitalia but did exhibit retractile testes, which can sometimes be seen in hypogonadal patients; however, since he and the other cases died at an age well before expected puberty, it remains unclear whether hypogonadism might be a feature of the syndrome.

Poulton et al. reported a patient of Moroccan origin who was homozygous for the missense mutation p.Val21Gly (2). Our patient was found to be compound heterozygote with the p.Val21Gly mutation inherited from his father who was of Libyan-Tangier ancestry. Further studies are needed in order to determine if this is a mutational hot spot or reflects a common origin for this mutation, which may be present at a low frequency in certain populations.

IER3IP1 is a highly conserved protein that is found in human, mice, zebrafish and yeast, and is highly expressed in the developing brain cortex and in the beta cells of the pancreas (2). The proposed pathogenetic explanation is that the congenital microcephaly and diabetes are caused by the presence of abnormal levels of apoptosis in brain and pancreas. Evidence for this mechanism was shown by extensive neuronal apoptosis in post-mortem brain specimen, as well as increased apoptosis in fibroblasts in vitro (2). In addition, there was evidence for insufficient myelination, which suggests either that early common progenitors of both neural and glial lineage are depleted or that maturation of the oligodendrocyte progenitors is blocked. Pancreatic histology revealed the presence of few insulin-staining beta cells that appeared reduced compared to normal pancreas (2). Given that our patient exhibited stable insulin requirements and diabetes control over several years of follow-up, in addition to preserved C-peptide production on two occasions, it is tempting to speculate that IER3IP1 may be important in preventing apoptosis primarily in the developing pancreas, whereas mature beta cells may not rely on expression of functional IER3IP1 protein for survival.

The differential diagnosis is clinically challenging, particularly in an era with growing database of genetic causes for neonatal diabetes, epilepsy and microcephaly. The group of de Wit et al. sequenced their patient for mutations in *EIF2AK3*, the gene responsible for Wolcott-Rallison syndrome, and testing was negative (6). The main features of this syndrome are skeletal features including multiple epiphyseal/spondyloepiphyseal dysplasia, diabetes mellitus, and other variable clinical features (7). We screened our proband for mutations in *EIF2AK3* as well as other genes including *KCNJ11* and *ABCC8*, since mutations in these genes are among the most common causes of permanent neonatal diabetes (8) and some patients also have severe developmental delay and muscle weakness, epilepsy and mild dysmorphic features (9,10). We also tested *PTF1A* and *NEUROD1* as possible candidates based on the microcephaly, as well as other relatively common gene causes *INS*, *FOXP3* and *PDX1*, even if they do not typically have the multiple syndromic features exhibited by our case.

The laborious and time-consuming process of sequencing multiple possible candidate genes in this case exemplifies the strength of next generation sequencing technology in elucidating the underlying monogenic cause of his condition. An isolated case of early onset diabetes could have any of now at least 20 known causes, many of which (including *IER3IP1*) are very rare and have variable extra-pancreatic features (1). As the cost of next generation sequencing continues to fall, using such methodology as the initial approach is likely to be more cost-effective and efficient and could also facilitate efforts to identify novel causes. As was the case here, a genetic diagnosis does not always change medical treatment or the course of disease, but it can have an impact on the interaction of the patient and parents with the medical community as they desperately seek new information about their disease; furthermore it will inform reproductive planning and genetic counseling for the parents and other family members.

Acknowledgments

These studies were supported in part by a grant from the American Diabetes Association (1-11-CT-41), grants from National Institute of Diabetes and Digestive and Kidney Diseases (DK020595 and K23 DK094866), the National Heart Lung and Blood Institute (HL102419) and National Human Genome Research Institute (HG003273 and

HG006542) of the National Institutes of Health, and gifts from the Kovler Family Foundation and Lewis-Sebring Foundation.

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Table 1

Patients with microcephaly, epilepsy and neonatal diabetes due to mutations in *IER3IP1*.

	1	2	3	4	5	6	7
Published report (ref)	Poulton (2 and also 6)		Abdel-Salam (3)				Current report
			Family MEDS-1251		Family MEDS-1578		
Patient designation	Patient 1	Patient 2	Patient 1	Patient 2	Patient 1	Patient 2	UC0207
Parental Origin	Morocco	Argentina	Egypt	Egypt	Egypt	Egypt	Libyan-Tangier, Colombian
Parents related to eachother	Yes	Presumed	Yes	Yes	Yes	Yes	No
***IER3IP1* Mutations**	V21G	L78P	L78P	L78P	L78P	L78P	V21G/p.Phe27fsSer*25
Diabetes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Age of onset	Infantile	Infantile	Days of life	Infantile	6 wks	2 wks	8 wks
Treatment	Insulin	Insulin	NR	Insulin	Insulin	NR	Insulin (CSII)
Severe Developmental delay	Yes	NR	Yes	Yes	Yes	Yes	Yes
Microcephaly	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Simplified gyri on brain MRI	Yes	Yes	Yes	Yes	Yes	Yes	Yes
EEG abnormal/pattern of hypsarrythmia*	Yes/Yes	Yes/NR	Yes/Yes	Yes/Yes	Yes/Yes	Yes/Yes	Yes/Yes
Seizures (myoclonic?)	Yes (NR)	NR (NR)	Yes (Yes)	Yes (Yes)	Yes (Yes)	Yes (Yes)	Yes (Yes)
Skeletal abnormalities	NR	NR	Yes	Yes	Yes	Yes	Unknown
Facial Features(such as narrow/short forehead with bitemporal grooving, anteverted nares, deep philtrum)	NR	NR	Yes	Yes	Yes	Yes	Yes
Age of death (yrs)	1.5	2.3	5.5	2.3	3.5	NR	8

NR = not reported. CSII = continuous subcutaneous infusion of insulin. MRI = magnetic resonance imaging. EEG = electroencephalogram.

* = burst suppression pattern considered consistent with hypsarrythmia on EEG.