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Original Research

A Genotype-First Approach for Clinical and Genetic Evaluation of Wolcott-Rallison Syndrome in a Large Cohort of Iranian Patients with Neonatal Diabetes

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ABSTRACT

Objective: Wolcott-Rallison syndrome (WRS) is an extremely rare autosomal recessive condition, characterized by permanent neonatal diabetes *mellitus* (PNDM) associated with skeletal dysplasia, growth retardation and liver dysfunction. WRS is caused by biallelic mutations in the gene encoding eukaryotic translation initiation factor 2alpha kinase 3 (*EIF2AK3*).
Methods: As part of a comprehensive study on clinical and genetic investigation of neonatal diabetes in an Iranian population, 60 unrelated Iranian subjects referred with PNDM were analyzed. All the probands were screened for *KCNJ11*, *INS*, *ABCC8* and *EIF2AK3* using a polymerase chain reaction–based sequencing approach.
Results: We identified 9 different variants in *EIF2AK3* in 11 unrelated Iranian probands, of which 5 variants were shown to be novel and not reported previously. The diagnosis of WRS was made by molecular genetic testing and confirmed by clinical re-evaluation of the subjects. Clinical follow up of the affected individuals shows that in at least some of them, PNDM was associated with short stature, failure to thrive, neurodevelopmental delay, epilepsy and hepatic and renal dysfunction. There was a strong family history of neonatal diabetes in the families of the probands with a high mortality rate.
Conclusion: WRS is a common cause of PNDM in children of consanguineous parents. Furthermore, clinical diagnosis of WRS would have been delayed or possibly missed without genetic testing because this study shows that the associated features of WRS might be obscured by a diagnosis of PNDM. Therefore *EIF2AK3* should be considered for any infant and young child with PNDM, particularly if the parents are related.
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RÉSUMÉ

Objectif : Le syndrome de Wolcott-Rallison (SWR), une maladie autosomique récessive extrêmement rare, est caractérisé par un diabète sucré néonatal permanent (DSNP) associé à une dysplasie squelettique, un retard de croissance et un dysfonctionnement du foie. Le SWR est causé par des mutations bialléliques du gène codant pour le *EIF2AK*3 (de l'anglais, *eukaryotic translation initiation factor 2alpha kinase 3*).
Méthodes : Dans le cadre d'une étude exhaustive sur l'évaluation clinique et génétique du diabète néonatal chez une population iranienne, 60 sujets iraniens non apparentés ayant reçu un diagnostic de DSNP ont fait l'objet de l'analyse. Tous les probants ont subi un dépistage de *KCNJ11*, *INS*, *ABCC8* et *EIF2AK3* au moyen d'une approche de séquençage qui repose sur la réaction en chaîne par polymérase.
Résultats : Nous avons trouvé 9 variants différents de *EIF2AK3* et 11 probants iraniens non apparentés, parmi lesquels 5 variants se sont avérés nouveaux et n'ont jamais été signalés auparavant. La réévaluation

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clinique des sujets a permis de confirmer le diagnostic du SWR établi par les analyses de génétique moléculaire. Le suivi clinique des individus atteints montre que chez certains d'entre eux, le DSNP étaitassocié à une courte stature, un retard de croissance, un retard neurodéveloppemental, une épilepsie et un dysfonctionnement du foie et des reins. On observait des antécédents familiaux importants de diabète néonatal dans les familles des probants dont le taux de mortalité était élevé.

Conclusions : Le SWR est une cause fréquente de DSNP chez les enfants de parents consanguins. De plus, le diagnostic clinique du SWR aurait été retardé ou aurait possiblement été erroné sans les analyses génétiques puisque cette étude montre que les caractéristiques associées au SWR seraient dissimulées par un diagnostic de DSNP. Par conséquent, le *EIF2AK3* devrait être considéré chez les nourrissons ou les jeunes enfants atteints d'un DSNP, particulièrement si leurs parents sont apparentés.

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Introduction

Wolcott-Rallison syndrome (WRS [OMIM 226980]) is a rare monogenic disorder with a recessive mode of inheritance (1). It has been characterized by permanent neonatal diabetes mellitus (PNDM), multiple epiphyseal dysplasia, growth retardation and other variable multisystem clinical manifestations including recurrent episodes of acute liver failure (2,3). Additional clinical features associated with WRS, including renal impairment, neutropenia, exocrine pancreatic insufficiency, central hypothyroidism and neurodevelopmental delay, have been reported in patients with WRS (4,5). Even though WRS is the most frequent cause of neonatal or early-onset diabetes in patients with consanguineous parents, it is still recognized as an extremely rare disease, and only around 100 cases have been reported worldwide so far (6,7). Although most patients with WRS are diagnosed with diabetes in the first 6 months of life, skeletal dysplasia can manifest around 1 or 2 years of age (8), and liver dysfunction can present at any time after the neonatal diabetes has developed during intercurrent illness as recurrent episodes of acute liver failure (3). WRS is caused by mutations in the gene encoding eukaryotic translation initiation factor 2alpha kinase 3 (*EIF2AK3*), also known as protein kinase R (PKR)-like endoplasmic reticulum kinase (9). Genetic testing confirms the diagnosis, which is important for timely clinical management, genetic counselling and prenatal diagnosis. Prognosis is poor for this syndromic form of PNDM, and most patients die at a young age (2). Here, we describe 11 of 60 unrelated Iranian patients who presented with PNDM diagnosed in the first 6 months of life who were referred for genetic testing. These 11 patients were diagnosed with WRS on identification of predicted damaging homozygous variants in *EIF2AK3*. A molecular diagnosis of WRS was made for some of these patients before the full clinical picture of the syndrome was evident. This study highlights the importance of timely genetic diagnosis in patients with neonatal diabetes before development of related clinical features and helps to guide clinical management.

Methods

Clinical data

The study was conducted according to a research protocol approved by the institutional review board of Tehran University of Medical Sciences, and signed written consent forms for genetic testing were obtained from all families. Sixty probands with an initial diagnosis of neonatal diabetes (onset <9 months old) were recruited from different regions of Iran and referred to the Division of Endocrinology and Metabolism in the Department of Pediatrics at the Children's Medical Center in Tehran, Iran. Clinical data, including demographic information and family history, were collected for all patients. Apart from 2 individuals (WRS05 and WRS07), all patients were offspring of consanguineous marriages. A total of 11 patients with homozygous variants in *EIF2AK3*, including 6 males and 5 females, were evaluated here. WRS06 was born to parents who had type 2 diabetes. WRS08 had a maternal grandfather and a paternal grandmother with type 2 diabetes. Moreover, WRS09 also has a maternal grandmother with type 2 diabetes. All living patients were receiving insulin therapy. An overview of clinical features is provided in Table 1.

Genetic analysis

DNA was extracted from peripheral blood collected from the participants using standard procedures. Molecular screening of *KCNJ11*, *INS*, *ABCC8* and *EIF2AK3* was performed in the Exeter Molecular Genetics Laboratory (Exeter, United Kingdom) (http://www.diabetesgenes.org/content/genetic-testing-neonatal-diabetes).

The coding and flanking intronic regions of the genes were analyzed by polymerase chain reaction, followed by Sanger sequencing. Sequences were compared with the reference sequences (NM_000525, NM_000207, NM_000352.2 and AF110146.1). Any alteration in the sequence was checked against publicly available databases including the National Heart, Lung and Blood Institute Exome Sequencing Complete Genomics (February 2012), Project Exome Variant Server (September 2013), 1000 Genomes (May 2012), Database of Single Nucleotide Polymorphism (134–137) and Exome Aggregation Consortium and in-house human variant and mutation databases, such as ClinVar and Human Gene Mutation Database, as well as the literature.

Results

We identified 9 different homozygous variants in *EIF2AK3* in 11 patients with PNDM diagnosed before the age of 6 months. Five mutations have previously been reported (Table 2). Novel variants identified included 2 nonsense (c.679G>T, p. Glu227Ter and c.2476C>T, p. Arg826Ter), 2 frameshift (c.2589_2593delAAGTT, p. Phe1038fs and c.3112_3113insA, p. Phe1038fs) and 1 missense (c.2866G>C, p. Gly956Arg) variants (Table 2). The missense variant (p. Gly956Arg) has also been seen in another Middle Eastern patient (unpublished data from Exeter Molecular Genetics Laboratory) supporting its pathogenic role. All variants were absent in population databases including the National Center for Biotechnology Information database of genetic variation (Database of Single Nucleotide Polymorphism), Exome Aggregation Consortium, 1000 Genomes Project, Exome Sequencing Project (v2) and The Greater Middle East Variome *Project*. No variants were found in any of the remaining 3 PNDM-causing genes (*KCNJ11*, *INS* and *ABCC8*) sequenced in this study.

Discussion

The molecular causes underlying PNDM have been shown to vary across different populations and geographic regions. For example, in European and East Asian populations, adenosine triphosphate potassium channel mutations are the most common cause (10),

Table 1
Clinical characteristics of patients with Wolcott-Rallison syndrome

Patient ID	Sex	Age at diagnosis	Current age	Consanguineous family	Family history	Gestation (wk)	Birth weight (kg)	Current weight (kg)	Height (m)	Clinical features	Postprandial glucose levels (mmol/L)	Prognosis/Current management
Case 1	M	5 mo	6 yr	+	-	39	3.1	13	1.0	PNDM, liver disease	33.3	NPH:0.5–0.5 Reg-Lantus:4 nightsNovorapid:2-2-2
Case 2	M	3 mo	1 yr	+	+	40	3	12	0.85	PNDM	38.9	NPH:0.5–0.5 NPH:4–5
Case 3	F	2 mo	1 yr	+	+	37	2.2	8.5	0.77	PNDM, iron deficiency anemia	38.9	NPH:1-1NPH:2
Case 4	F	4 mo	1 yr	NA	NA	37	1.6	NA	NA	PNDM	16.7	died
Case 5	M	6 wk	1 yr	-	-	40	2.5	4.5	0.55	PNDM	33.3	NPH:2-2NPH:1.33
Case 6	F	3 mo	3 yr	+	-	39	1.5	NA	NA	PNDM, CKD, epilepsy (treated with phenobarbital) developmental delay, muscle weakness	<27.8	Died at the age of 3years due to CKD
Case 7	F	55 d	3 yr	-	+	37	1.5	9.3	0.81	PNDM	NA	NPH insulin:2.5–2.5
Case 8	M	4 mo	4 yr	+	+	40	2.4	12	0.90	PNDM and epilepsy (treated with phenobarbital)	NA	NPH insulin:6-2
Case 9	F	2 mo	5 yr	+	+	40	3.4	16	1.06	PNDM	37.2	NPH insulin:12–4 Regular insulin:>200
Case 10	M	40 d	4 yr	+	+	38	2.5	13	0.97	PNDM, liver disease	16.7	NPH insulin: 3.5–4.5
Case 11	M	5 mo	3.5 yr	+	+	37	3.6	10	0.80	PNDM, microcephaly, developmental delay, muscle weakness strabismus, abnormal brain MRI	38.9	NPH insulin:0/2-0/1
P1 Behnam et al 2013 (12)	F	40 d	3 yr	+	+	NA	NA	NA	NA	PNDM, ASD, hepatomegaly, ascites,walking difficulty	NA	Died at the age of 3 year old
P2 Behnam et al 2013 (12)	M	2 mo	4.5 yr	+	+	37	2.8	NA	NA	PNDM, hepatomegaly, anemia, neutropenia, Lymphocytosis, skeletal Dysplasia, walking difficulty	NA	NPH insulin: twice daily

ASD, atrial septal defect; CKD, chronic kidney disease; d, day; F, female; M, male; mo, month; MRI, magnetic resonance imaging; NA, not available; NPH, neutral pH suspension of crystalline zinc; PNDM, permanent neonatal diabetes mellitus; wk, week; yr, year; +, yes; -, no.

whereas in Arab and Middle Eastern populations in which the rate of consanguinity is high, *EIF2AK3* is the most commonly mutated gene (6,11). Data on incidence and genetic cause of neonatal diabetes and—in particular WRS—are very limited in Iran, but this study shows that the prevalence of WRS is relatively high in Iranian patients with neonatal diabetes and is similar to that of other consanguineous populations in the Middle East. Eleven of 60 Iranian patients with neonatal diabetes were found to have WRS by genetic testing. Overall, of 102 patients with PNDM from Iran who were referred to the Exeter Molecular Genetics Laboratory up to now, 21 were found to have homozygous mutations in *EIF2AK3* (Unpublished data from Exeter Molecular Genetics laboratory). With the addition of the Iranian sibling with WRS previously reported by Behnam et al and the exclusion of the nonconsanguineous families, approximately 25% of patients of Iranian consanguineous families had homozygous variants in *EIF2AK3* (12). This percentage is consistent with what was reported by De Franco et al in their international cohort study (10). Based on the family history records and pedigrees, the majority of patients in the present study had at least 1 first-, second- or third-degree relative with neonatal diabetes, but their samples were not available for genetic testing. Therefore, *EIF2AK3* should be kept in mind during testing of any Iranian patients with neonatal diabetes. This is the first large comprehensive study to investigate a genetic cause of neonatal diabetes in the Iranian population.

The occurrence of the known mutation Gly956Glu in 2 unrelated patients with similar ethnicity (Baluch) from the southeast of Iran in this study suggests the possibility of a founder effect. The previously reported patients with the same variant were from United Arab Emirates, which is near this part of Iran (13). In fact, the mutation was seen in patients from 6 countries, which will make it 1 of the most common mutations in *EIF2AK3* (Unpublished data from Exeter Molecular Genetics Laboratory). Whether this is a founder mutation or just a recurrent mutation can be investigated in the future by using haplotype analysis. In addition, 2 unrelated patients (WRS02 and WRS10) carry the same mutation—Leu1057Phe—which was previously reported as compound heterozygous with a loss-of-function mutation in a Caucasian patient (13). Although they do not have similar ethnicity, both patients were originally from the same region in the south of Iran, which increases the possibility of a founder effect for this allele as well. A Gly956Arg mutation identified in 1 patient with Baluch ethnicity was also seen in a patient from Oman, which is geographically close to the region, where our patient is originally from (unpublished data from Exeter Molecular Genetics Laboratory). Founder and common mutations causing rare mendelian disorders are of particular clinical genetic use because they provide a cost-effective and rapid tool for population-specific molecular diagnosis of genetic disorders and for developing community genetic programs.

The clinical phenotype associated with WRS can be extremely variable in nature and severity, as well as age at onset of related clinical features. To date, there appears to be no clear genotype-phenotype correlation, even within members of a family. All patients in this study presented with diabetes before the age of 6 months. Six patients displayed only diabetes, and only in some patients was diabetes accompanied by neurodevelopmental characteristics (n=2) and liver disease (n=3). Two patients with poor prognoses died at young ages—1 in infancy and 1 in early childhood. Although liver disease and skeletal dysplasia are common features of WRS, skeletal dysplasia was not observed in any of our patients, and only 3 patients were diagnosed with liver dysfunction. The most likely explanation for the lower frequency of liver disease and skeletal dysplasia in this cohort is unavailability of long-term follow-up information and poor patient compliance in this study. Additionally, the young age of the patients can be a second reason that the hepatic and skeletal defects were not observed. Mean age of subjects in this study is 3 years. Our patients without liver

Table 2
EIF2AK3 variations found in Iranian patients

Patient ID	Province of origin in Iran	*EIF2AK3* variation location	Nucleotide change (hg19) NM_004836	Amino acid change	Mutation reported previously
WRS01	Kerman	Exon 4	c.679G>T	p.Glu227Ter (p.E227)	No
WRS02	Bushehr	Exon17	c.3169C>T	p.Leu1057Phe (p.L1057F)	Senée et al (14)
WRS03	Sistan Baluchestan	Exon14	c.2866G>C	p.Gly956Arg (p.G956R)	No (Exeter database-Unpublished data)
WRS04	East Azerbaijan	Exon 13	c.2589_2593delAAGTT	p.leu863fs (p.L863fs)	No
WRS05	Fars	Exon 5	c.997C>T	p.Gln333*(p.Q333*)	Demirbilek et al (15)
WRS06	Hamedan	Exon 16	c.3112_3113insA	p.Phe1038fs(p.F1038fs)	No
WRS07	East Azerbaijan	Exon13	c.2476C>T	p.Arg826Ter (p.R826)	No
WRS08	Sistan Baluchestan	Exon14	c.2867G>A	p.Gly956Glu (p.G956E)	Rubio-Cabezas et al (13) and Habeb et al (16)
WRS09	Qazvin	Exon12	c.1894C>T	p.Arg632Trp (p.R632W)	Rubio-Cabezas et al (13) Dias et al (17) and Can et al (18)
WRS10	Kohgiluyeh and Boyer-Ahmad	Exon 17	c.3169C>T	p.Leu1057Phe(p.L1057F)	Senée et al (14)
WRS011	Sistan Baluchestan	Exon 14	c.2867G>A	p.Gly956Glu (p.G956E)	Rubio-Cabezas et al (13) and Habeb et al (16)
WRS012	-	Exon 3	c.604A>G	p.Gln166Arg (p.Q166R)	Behnam et al (12)

disease were younger than 3.5 years. Considering the disease course in WRS and the high mortality, it is possible that many of them will develop liver disease over time and fully develop the classical clinical picture of WRS.

Early diagnosis is critical for WRS to ensure clinical management and timely intervention for episodes of hepatic failure, which is the most life-threatening complication in WRS. WRS can be differentiated from other forms of neonatal and early-onset insulin-dependent diabetes based on clinical presentation and genetic testing. However, molecular analysis of *EIF2AK3* is often delayed until the full clinical picture of WRS is evident. WRS can present initially as isolated (nonsyndromic) neonatal diabetes mellitus, obscuring the other findings of this syndrome observed in these patients. The findings in these patients indicate that WRS should be considered in infants and children of consanguineous parents with PNDM, even when there is no evidence of other clinical features of the syndrome.

In the present study, we describe genetic causes, including 5 novel mutations in *EIF2AK3* and the clinical spectrum and prevalence of WRS in Iranian patients with neonatal diabetes. The study consolidates the significant role of early genetic testing for patients with neonatal diabetes and its implications for clinical management.

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Disclosure

All authors declared no competing interests.

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