Full title: Wolcott Rallison Syndrome in Iran: a common cause of neonatal diabetes

Short title: Wolcott-Rallison Syndrome in Iran

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Abstract

Background: Wolcott-Rallison Syndrome is a rare autosomal recessive disorder characterized by neonatal/early-onset non-autoimmune insulin-dependent diabetes, multiple epiphyseal dysphasia and growth retardation. It is caused by mutations in the gene encoding eukaryotic translation initiation factor 2α kinase 3 (*EIF2AK3*). We aimed to study the clinical characteristics and frequency of the disease in the Iranian population.

Method: We recruited 42 patients who referred to the endocrine and metabolism clinic at Mashhad Imam Reza Hospital with neonatal diabetes. Molecular screening of *KCNJ11*, *INS*, *ABCC8* and *EIF2AK3* was performed at the Exeter Molecular Genetics Laboratory, UK. We calculated the frequency of the disease in 124 patients referred from Iran to the Exeter Molecular Genetics Laboratory for genetic screening and compared it to other countries worldwide.

Results: We identified 7 patients as having Wolcott-Rallison Syndrome. The genetic testing confirmed the clinical diagnosis and indicated five novel mutations. Only 2 patients developed clinical features of the syndrome by 6 months of age. Of all 124 cases of Iranian neonatal diabetes referred to the Exeter Molecular Genetics Laboratory for genetic screening, 28 patients (22.58%) had a recessive mutation in *EIF2AK3*.

Conclusion: The results of this study raises awareness of the condition and provides further accurate data on the genetic and clinical presentation of Wolcott-Rallison Syndrome in the Iranian population. Our study highlights the importance of genetic testing in patients from consanguineous families with diabetes diagnosed within the first 6 months of life.

Introduction:

Permanent neonatal diabetes mellitus (PNDM) diagnosed before 6 months of age is mainly monogenic rather than autoimmune (1, 2) and affects approximately one in 100,000 live births (3). The majority of patients have heterozygous mutations in the *KCNJ11* and *ABCC8* genes or the pre-proinsulin (*INS*) gene (4-9). However, in patients born to related parents, homozygous mutations in *EIF2AK3* are the most common cause of neonatal diabetes (10). *EIF2AK3* is located on chromosome 2p1 and encodes eukaryotic translation initiation factor 2α kinase 3 also known as PKR-like endoplasmic reticulum kinase (PERK). Functional studies have shown that Perk is essential for neonatal development of β cells and that loss of PERK expression in β cells reduces β cell mass and insulin secretion leading to neonatal diabetes independently of an autoimmune process(11). Homozygous mutations in *EIF2AK3* cause a syndrome called Wolcott-Rallison Syndrome characterized by neonatal non-autoimmune insulin-dependent diabetes, multiple epiphyseal dysphasia and growth retardation (12).

The clinical diagnosis of Wolcott-Rallison syndrome requires the presence of permanent neonatal diabetes associated with skeletal dysplasia and/or episodes of acute liver failure (12). Although diabetes in these patients is often diagnosed within the first 6 months of life, the key presenting features of the syndrome, including skeletal dysplasia and liver failure, can appear later during the course of the disease. Other clinical findings of a different nature and severity include renal dysfunction, pancreatic exocrine failure, neutropenia, recurrent infections, hypothyroidism, intellectual deficit, and bone fractures (12). The disease is thought to be underdiagnosed due to the deaths of patients prior to the appearance of the full spectrum of clinical manifestations. Early diagnosis of Wolcott-Rallison syndrome is of great value in predicting complications

and timely intervention in life-threatening episodes of liver failure. In contrast to other forms of diabetes, insulin treatment should not target a tight control of blood glucose as hypoglycemia could trigger episodes of acute aggravation of the disease(12).

Since familial marriage is common in Iran (37.4%) (13), Wolcott-Rallison syndrome is probably one of the more important causes of PNDM. We present here the clinical spectrum of this syndrome in 7 patients with a genetic diagnosis of Wolcott-Rallison syndrome and highlight the importance of genetic testing in patients from consanguineous families with diabetes diagnosed within the first 6 months of life.

Materials and methods

Patients

We recruited 42 patients with diabetes diagnosed before the age of 6 months who were referred to the Imam Reza Hospital, Mashhad University of Medical Science. We obtained written consent for clinical and genetic investigation from the patients' parents or their legal guardians. Clinical information was collected at the time of referral. Parental consanguinity was defined as parents being first or second cousins. The study was approved by the ethics committee of the Mashhad University of Medical Science.

Genetic analysis

Peripheral blood samples were collected from affected patients and their parents to extract DNA using standard procedures. Molecular screening of *KCNJ11*, *INS*, *ABCC8* and *EIF2AK3* was performed at the Exeter Molecular Genetics Laboratory, UK (http://www.diabetesgenes.org/content/genetic-testing-neonatal-diabetes). The coding and flanking intronic regions of the genes were analyzed by PCR followed by Sangers sequencing. Sequences were compared with the published template (NM_000525, NM_000207, NM_000352.2 and AF110146.1).

We checked the frequency of the identified variants in GnomAD (>120,000 individuals; http://gnomad.broadinstitute.org) and in human variant and mutation databases, such as ClinVar and Human Gene Mutation Database, as well as the relevant literature. We used the bioinformatics tools SIFT, PolyPhen-2 and Align GVGD which were accessed through the ALAMUT Visual software version 2.7.1 (Interactive Biosoftware, Rouen, France) to predict the effect of novel variants. Rare variants were tested for their presence in parents.

Statistical analysis

Clinical numeric data are given as median and interquartile range (IQR). We used the Chi-square test to assess the differences between the consanguineous and non-consanguineous groups.

Results

We identified seven new cases of Wolcott-Rallison Syndrome in North-East of Iran.

Of the 42 patients with neonatal diabetes, homozygous likely pathogenic and pathogenic *EIF2AK3* variants confirming a genetic diagnosis of Wolcott-Rallison syndrome were identified in 7 patients. Five novel mutations were detected: c.1745_1746del (p.Ser582fs), c.733dup (p.Arg245fs), c.1149_1150del (p.Asn383fs), c.869_870del (p.Glu290fs) and c.536C>A (p.Ser179*) (**table 1**). All parents were heterozygous carriers.

The median age at diagnosis of diabetes in patients with a mutation in *EIF2AK3* was 3 months (interquartile range (IQR) [2–6]) whilst the median age at diagnosis in the 35 patients without an *EIF2AK3* mutation was 2 months (IQR [1–5]). The median birth weight was 2,970 grams (IQR [2,470–3,375]) which was higher than in patients who did not have mutation in *EIF2AK3* (1650 grams [1650-3150]), although this difference was not statistically significant ($p_{difference} > 0.05$). The consanguinity rate in patients with mutations in *EIF2AK3* was 100% compared to 57% in the rest of the cohort ($p_{difference} = 0.03$). Full clinical description of patients is included in the **supplementary note**.

Case 1 was hospitalized at the age of 2 months with metabolic acidosis, hyperglycemia, elevated hepatic enzymes, hepatomegaly, renal dysfunction and spastic seizures. Currently she is 6 years old with severe neuromotor deficit, microcephaly and evident skeletal dysplasia, short trunk, kyphosis and genu valgum (**figure 1**). In her X-ray radiographies, we observed generalized osteoporosis, bilateral hip dislocation, delay in

bone age and epiphyseal dysplasia at radius end (**figure 2**). The CT scan revealed bilateral calcifications of basal ganglions (**figure 3**). She is homozygous for c.1745_1746del mutation.

Case 2 was hospitalized at age 54 days with edema and jaundice. Laboratory findings included elevated hepatic enzymes and direct hyperbilirubinemia. Hepatic biopsy revealed a hyperplasic biliary duct and sedimentation of large amounts of biliary pigments with no fibrosis. Currently he is 9 years old. He has mild intellectual disability. Clinical examination showed bilateral genu valgum. Bilateral hemi-epiphyseal of femur and proximal of tibia is evident in radiographic examination. He is homozygous for c.733dup mutation.

Case 3 was hospitalized for respiratory distress and fever at age 2 months after vaccination and diabetic ketoacidosis (DKA). The patient had cleft lip. Currently he is 9 years old with severe intellectual disability. His skeletal disorders include pigeon chest, genu valgum and short trunk. His condition resembles Mucopolysaccharidosis type IV though enzymatic test excluded Mucopolysaccharidosis type I, IV and VI and no progressive skeletal dysplasia was detected in his skeletal X-rays at 6 years old. He is homozygous for c.1149_1150del mutation.

Case 4 was hospitalized with fever and lethargy at the age of 2 months and diagnosed with DKA. She was admitted again at age of 8 months for edema and jaundice and showed hepatic cholestatic failure. Currently she is 10 years old with mild intellectual disability. She has severe motor limitations and skeletal deformities with short trunk, kyphosis and genu valgum. She is homozygous for c.869_870del mutation.

Case 5 was hospitalized due to fever and restlessness at age 20 days. He had a history of recurrent infections during the first year of life. He underwent bilateral surgery due to undescended testes at the age of 1 year. He is currently 24 months old. Generalized osteoporosis, epiphytical digenesis at femur ends and proximal of tibia, and bilateral dislocation of hip were evident in his radiography. The patient had motor developmental delay but mentally was within normal range. He is homozygous for c.536C>A mutation.

Case 6 presented with DKA at 6 months. At 7 months, she was admitted to the hospital with effusion in the right knee joint and septic arthritis. Culture of the synovial fluid grew pseudomonas aeruginosa. Currently she is 21 months with mild cognitive and motor delays. She has short trunk and bilateral genu valgus deformity. Skeletal survey revealed epiphyseal dysplasia involving proximal and distal femoral epiphysis and proximal tibial epiphysis, generalized osteoporosis, thoracic kyphosis and lumbar lordosis and mild platyspondyly. At the pelvic level, epiphyses are flattened with coxa vara (**figure 4**). She is homozygous for c.1149_1150del mutation.

Case 7 presented with DKA at 6 months. At 4 years old, after a common cold and fever, hepatic involvement presented with icterus and hepatomegaly and spontaneously resolved after 2-3 weeks. Currently, he is 6.5 years old. He has mild intellectual disability. He has kyphosis and genu valgus deformity. He is homozygous for c.536C>A mutation.

One out of four patients with neonatal diabetes from consanguineous parents have mutation in *EIF2AK3*.

We used the Exeter Neonatal Diabetes cohort to calculate the frequency of mutations in *EIF2AK3* in patients referred from Iran. A total of 124 patients with neonatal diabetes below age of 6 months have been referred to the Exeter Molecular Genetics Laboratory for genetic testing. The diagnostic genetic test identified a homozygous mutation in *EIF2AK3* for 28 patients (22.58%); among them 22 patients (78.57%) were reported to be born to consanguineous parents. We excluded the patients who were not known to be born to related parents or for whom data was missing. Of 77 children who were from consanguineous families, 22 patients (28.57%) had a recessive mutation in *EIF2AK3*. In contrast to neonatal diabetes in Europeans, mutations in *KCNJ11*, *ABCC8* and *INS* altogether accounted for only 31% of neonatal diabetes cases in 124 Iranian patients.

To compare the frequencies with other ethnicities, we investigated 999 cases of neonatal diabetes referred from 83 countries for genetic diagnostic test. Country of referral was missing in 7 patients. We divided patients into 14 geographical regions based on the country of referral including Asia, South Asia, East Asia, Middle East, Eurasia, Europe, Australia, East Africa, South Africa, North Africa, North America, South America, Central America and the Caribbean (**table 2**). The recessive mutations in *EIF2AK3* were most prevalent in North Africa (28.12% of 32 referrals), Middle East (18.5% of 173 referrals), and South Asia (15.22 % of 92 referrals). In contrast, the frequency of genetically diagnosed Wolcott-Rallison Syndrome was 6.12% (total number of referred samples = 49) in Asia, 3.38 % (385) in Europe and 1.02% (97) in North America. There were no cases of genetically diagnosed Wolcott-Rallison Syndrome in samples referred from Australia (39 patients), East Asia (19 patients), South America (58 patients), South Africa (26 patients), Central America (12 patients), Eurasia (2 patients), East Africa (3 patients) and the Caribbean (6 patients).

Discussion:

We reported 7 new cases of Wolcott-Rallison Syndrome from the north east of Iran identified by genetic testing. These patients had 5 novel homozygous mutations. Our study provides evidence that Wolcott-Rallison Syndrome is a common cause of neonatal diabetes in Iran. Among 124 neonatal diabetes patients referred to the Exeter Molecular Genetics Laboratory from Iran, 22.58% had a homozygous mutation in *EIF2AK3*. The prevalence of neonatal diabetes due to mutations in *EIF2AK3* was similar to countries where the rate of consanguinity is high, including countries in North Africa (28.12% of neonatal diabetes), Middle East (18.5% of neonatal diabetes) and South Asia (15.22% of neonatal diabetes).

Insulin dependent non-autoimmune diabetes is a cardinal feature of Wolcott-Rallison Syndrome; the median age of incident of diabetes has been reported as 10.5 weeks with the earliest age of presentation at 3 weeks (14). In all but two patients, diabetes was diagnosed within the first 6 months of life although there are 2 cases where diabetes was diagnosed at ages of 14 and 30 months (14, 15). There is no reported association between different types of mutation and age of presentation of diabetes (10). In our study, 4 patients presented with diabetes at approximately age 2 months, one patient was diagnosed at the age of 20 days and one at age of 6 months.

Although patients with Wolcott-Rallison Syndrome have a lower birth weight than the population (median -1.4 standard deviation score), low birth weight (<2500 grams) is not common (10). In our study, 5 cases had a birth weight lower than 3000 grams including 2 patients with low birth weight (<2500 grams). There was no significant correlation between birth weight and age at diagnosis of diabetes; consistent with previous studies (10).

Skeletal involvement characterized with multiple epiphiseo-metaphyseal dysplasia usually affects long bones, the pelvis and vertebrae without affecting the skull, and usually is detected between the ages of 1 and 2 years (12, 16). Skeletal deformities in all 4 patients in the present study, who are currently 6-10 years old, are evident in form of short trunk, kyphosis, pigeon chest, genu valgum and abnormal gait. Although evidence of skeletal deformities are currently not present in case 5 who is only 2 years old, delayed walking, short height and abnormal gait are evident.

Osteoid odontoideum in which the odontoid peg separates radiologically from the second cervical vertebra is common in patients with Wolcott-Rallison Syndrome. This condition can be clinically asymptomatic or associated with spinal cord compression symptoms even in transient form. It is recommended that all patients suffering Wolcott-Rallison Syndrome undergo regular appropriate evaluation for Os odontoideum screening including imaging (17). There was no evident of cord compression in neurological assessments of our patients although imaging was not performed.

Hepatic diseases are characteristic findings of Wolcott-Rallison Syndrome (10, 12). Hepatic involvement in the form of cholestasis, jaundice, higher levels of hepatic enzymes or hepatomegaly were detected in 3 patients in this study; 2 patients presented hepatic involvement at the time diabetes was diagnosed. This included elevated hepatic enzymes associated with hepatomegaly in case 1 and elevated hepatic enzymes and direct hyperbilirubinemia in case 2.

Intellectual disability and developmental delay, with a range of different severities, are very common in Wolcott-Rallison Syndrome patients (15). In our study, 4 cases developed neuromotor retardation and case 5 had developmental delay. Various central nervous system anomalies including cerebellar cortical dysplasia, microcephaly,

cerebral atrophy, pachygyria and cerebellar hypoplasia have been described in patients with Wolcott-Rallison Syndrome (18, 19).

Bilateral calcification of basal ganglion in one of the patients (case 1), who is under treatment for epilepsy, was reported for the first time. Case 1 in this study also underwent peritoneal dialysis due to a rise in blood urea nitrogen and creatinine; renal function was recovered almost immediately. Other clinical futures including hypothyroidism, neutropenia and exocrine pancreas insufficiency were not seen in our patients.

Many cases of Wolcott-Rallison Syndrome die before complete clinical presentation of the disease appears. Cases of Wolcott-Rallison Syndrome can develop variable clinical phenotypes starting at different time points after the initial diabetes diagnosis, causing many cases to be either misdiagnosed or undiagnosed. The most common implications include skeletal dysplasia and liver failure which is the most deadly feature of the disease. To prepare for right clinical intervention and management of the disease, a timely diagnosis is critical.

Wolcott-Rallison Syndrome is a common cause of neonatal diabetes in Iran due to the high rate of consanguineous marriage. Many cases of Wolcott-Rallison Syndrome might remain undiagnosed. We recommend genetic testing for all cases of neonatal diabetes born to consanguineous parents worldwide to be undertaken at the time of diagnosis, to predict the best available diabetes treatment and allow anticipation of the development of related features.

Disclosure

All authors declared no competing interests.

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Figure 1: **Skeletal dysplasia and growth retardation in proband 1 (age 6 years).** The patient has evident skeletal dysplasia, short trunk, kyphosis and genu valgum.

Figure 2: **Radiographs of proband 1.** (A) Pelvis: the acetabular roofs are hypoplastic with dysplastic capital femoral epiphyses. (B) Spine: dorsal region of spine shows flattening of the vertebral bodies with defects at the anterior edges and sever osteoporosis. (C) Hand: dysplasia in distal radius and ulna epiphyses; several phalanges are dysplastic with abnormal metaphyseal cupping at the proximal ends.

Figure 3: **CT scan of brain in proband 1.** The patient has bilateral calcifications of basal ganglions.

Figure 4: Radiographs of proband 7. (A) Short trunk, kyphosis, and bilateral genu valgus deformity. (B) Epiphyseal dysplasia involving proximal and distal femoral epiphysis and proximal tibial epiphysis. (C) At the pelvic level, epiphyses are flattened with coxa vara.

Table 1. Mutation description and phenotype of study participants.

Individual	Mutation description	Age of Diagnosis (months)	Gestational age (weeks)	Birth weight (grams)	Sex	Current age (years)	Current weight Kg (Z-score)	Current height cm (Z-score)	Reported features
Case 1	p.Ser582fs	2	39	2250	F	6	11 (-5.1)	100 (-3.2)	Microcephaly, hypotonia, absence of head control, leukodistrophy, liver disease, renal disease, seizure, skeletal anomalies
Case 2	p.Arg245fs	2	37	2460	M	9	13 (-6)	89 (-8.1)	liver disease, skeletal anomalies, developmental delay
Case 3	p.Asn383fs	2	38	2900	M	9	14.2 (-5.6)	98 (-6.3)	Intellectual disability, skeletal anomalies
Case 4	p.Glu290fs	2	36	2780	F	10	17.5 (-3)	110 (-4.5)	Intellectual disability, skeletal anomalies, liver disease
Case 5	p.Ser179*	1	39	3200	M	2	9.5 (-2)	76 (-3.7)	developmental delay, recurrent infections, skeletal anomalies
Case 6	p.Asn383fs	6	40	2740	F	2	9.9 (-0.2)	81 (-0.4)	Pseudomonas infections in knees, arthritis, skeletal anomalies, developmental delay
Case 7	p.Ser179*	6	41	3550	M	6.5	23 (0.6)	112 (-1.3)	Skeletal anomalies, liver disease

F: female; M: male; del: deletion; fs: frameshift. The * symbol indicates a Stop codon.

Table 2. The frequencies of recessive mutations in *EIF2AK3* in 999 cases of neonatal diabetes referred from 83 countries for genetic diagnostic test to the Exeter Molecular Genetics Laboratory. We divided patients into 14 geographical regions based on the country of referral including. We did not have information on country of referral for 7 patients.

Geographical regions	Patients with recessive mutations in *EIF2AK3* n (%)	Total number of referrals	Countries (number of referrals)
North Africa	9 (28.12%)	32	Egypt (9), Libya (6), Morocco (8), Sudan (8) and Tunisia (1)
Middle East	32 (18.5%)	173	Bahrain (2), Iraq (2), Israel (6), Jordan (16), Kuwait (8), Lebanon (1), Oman (8), Qatar (1), Saudi Arabia (34), Syria (1), Turkey (80), UAE (14)
South Asia	14 (15.22 %)	92	Bangladesh (10), India (75), Pakistan (7)
Asia	3 (6.12%)	49	Malaysia (9), Singapore (5), Thailand (5), Vietnam (29), Philippines (1)
Europe	13 (3.38 %)	385	Austria (10), Belgium (8), Bulgaria (4), Czech Republic (12), Denmark (3), France (5), Germany (82), Hungary (5), Bosnia (1), Croatia (3), Cyprus (2), Finland (1), Greece (3), Ireland (8), Netherlands (24), Kosovo (1), Latvia (3), Macedonia (2), Poland (20), Portugal (2), Romania (1), Serbia (3), Slovakia (8), Spain (5), Sweden (17), Switzerland (3), UK (142), Ukraine (7)
North America	1 (1%)	97	US (68), Canada (29)
Australia and New Zealand	0 (0%)	39	New Zealand (11), Australia (28)
East Asia	0 (0%)	19	China (14), Japan (4), Republic of Korea (1)
Eurasia	0 (0%)	2	Russia (1), Georgia (1)
East Africa	0 (0%)	3	Kenya (2), Mauritius (1)
South Africa	0 (0%)	26	South Africa (26)
Central America	0 (0%)	11	Costa Rica (5), Guatemala (1), Honduras (4), Mexico (1)
South America	0 (0%)	58	Argentina (20), Brazil (7), Chile (15), Colombia (1), Peru (1), Venezuela (14)
The Caribbean	0 (0%)	6	Puerto Rico (5), Trinidad and Tobago (1)

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