

# Krabbe Disease in the Arab World

Hatem Zayed<sup>1</sup>

<sup>1</sup> Department of Health Sciences, Biomedical Program, Qatar University, Doha, Qatar

Address for correspondence Hatem Zayed, PhD, Department of Health Sciences, Biomedical Program, Qatar University, Doha 2713, Qatar (e-mail: hatem.zayed@qu.edu.qa).

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## Abstract

The autosomal recessive inherited Krabbe disease (KD) is a devastating pediatric lysosomal storage disorder affecting white matter of the brain. It is caused by mutations in the gene coding for the lysosomal enzyme galactocerebrosidase. While most patients present with symptoms within the first 6 months of life, others present later in life throughout adulthood. The early infantile form of KD (EIKD) is frequent in the Muslim Arab population in Israel, with a very high prevalence of approximately 1/100 to 1/150 live births. The homozygous variant c.1582G > A (p.D528N) was found to be responsible for EIKD in Palestinian Arab patients. KD was reported in different Arab countries with much lower frequency. While most Arab patients presented with EIKD, late infantile and late onset KD forms were also reported. Most Arab patients presented with variable symptoms ranging from EIKD to late onset KD, with variable clinical findings. Based on literature studies, this review focuses on the clinical and molecular findings of KD patients with Arab ancestry, and highlights the need for developing universal genetic screening programs to overcome the under-reported status of KD prevalence in Arabia. This is expected to improve the prognosis of the disease and promote targeted molecular diagnostics to the Arab patients.

## Keywords

- ▶ Krabbe disease
- ▶ galactocerebrosidase
- ▶ genotype–phenotype correlation
- ▶ Arabs
- ▶ prenatal diagnosis

## Introduction

Krabbe disease (KD), also known as globoid cell leukodystrophy, is an autosomal recessive metabolic neurodegenerative disease that affects the nervous system. It is caused by the deficiency of galactocerebrosidase (or galactosylceramidase; GALC) enzyme.<sup>1</sup> The prevalence of KD is approximately 1 per 100,000 live births in Europe and USA, with noticeable variations among different countries: 1.00 in Turkey, 1.35 in the Netherlands, 1.21 in Portugal, 0.71 in Australia, and 0.40 in the Czech Republic.<sup>2</sup> It is found to be extremely high in two communities in Israel, where familial unions or the degree of consanguinity is relatively high among Druze and Muslim Arab populations. The incidence of early infantile form of KD (EIKD) is found to be approximately 1/100 to 1/150 live births.<sup>3</sup>

Three forms of KD have been identified. The first form is EIKD, which is the most common and severest form, and can

be diagnosed within the first 6 months of life.<sup>4–9</sup> It is clinically characterized by limb stiffness, feeding difficulties, seizures, excessive irritability, motor developmental delay, and peripheral neuropathy.<sup>7,10–12</sup> Most patients diagnosed with EIKD die before the age of 2 years. The second form is the late infantile KD (LIKD) with onset of symptoms presenting between ages 7 and 12 months. The third form is late onset KD (LOKD), which occurs between 13 months and 10 years of age, and into adulthood.<sup>13–19</sup> Both the LIKD and LOKD forms share symptoms with the EIKD form, but are characterized by a slow progression.<sup>18,20,21</sup>

## GALC Gene

GALC gene was mapped to chromosome 14 using multipoint linkage analysis.<sup>3,22</sup> The genomic locus consists of 17 exons, spanning 58 kb of DNA, and is transcribed into a 3.8 kb mRNA.<sup>16</sup> The predominant GALC transcript isoform encodes a

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669 amino acid protein.<sup>23,24</sup> The linear nucleotide sequence of the *GALC* open reading frame is 2,058 nucleotides and has a 45.92% GC content.

Molecular cloning of *GALC* gene<sup>25</sup> and cDNA<sup>23,24</sup> has simplified the genotyping of patients with KD. To date, the Human Gene Mutation Database has reported more than 130 variants in the *GALC* gene, which mostly associate with the KD disease spectrum representing 128 mutations.<sup>26</sup> The NHLBI Exome Sequence Project of the Exome Variant Server reported 209 variants in *GALC* gene (107 in European American and 102 in African American).<sup>27</sup> The *GALC* gene has two distinct translatable isoforms; both starting with two inframe ATG translational start codons of exon 1, and processed to a fully functional mature enzyme.<sup>16,23,24</sup>

Mutations that cause KD appear to be population specific. For example, people of European descent presenting with EIKD commonly harbor the large deletion: c.1161 + 6532\_polyA + 9kdel (IVS10del30kb) in combination with three other mutations (c.1586C > T, c.1700C > T, c.1472delA [1538C > T, 1652A > C, 1424delA]); all together accounting for approximately 60% of disease-causing alleles.<sup>7,28</sup> This genetic lesion was not noted in KD Japanese patients, instead, they show c.683\_694del12insCTC or c.2002A > C (635\_646del12insCTC and 1954A > C), representing approximately 30% of Japanese EIKD-associated alleles.<sup>29</sup> Recently, Liao et al<sup>30</sup> found that the symptoms of KD were heterogeneous across all ages of KD patients. EIKD was found to be the most prevalent form of KD among the Muslim Arabs and Druze communities, mostly due to the homozygous occurrence of two mutations: c.1582G > A (p.D528N) and c.1748 T > G (p.I583S), respectively.<sup>31,32</sup>

## GALC Enzyme

*GALC* is a lysosomal enzyme that catalyzes the hydrolysis of specific galactolipids including galactosylceramide (galactocerebroside) and galactosylsphingosine (psychosine).<sup>33</sup> It is primarily found in the brain and kidneys where galactolipids are hydrolyzed. The normal precursor enzyme protein is 80 kDa, which is processed into 50 kDa and 30 kDa subunits; both of which are required for full enzyme activity.<sup>24,34</sup> The crystal structures of murine *GALC* enzyme (PDB: 3ZR5) (83% identity with human *GALC* amino acid sequence) has been resolved,<sup>35</sup> which will allow for the determination of a structural basis of mutations and contribute to our understanding of the challenges associated with establishing clearer genotype–phenotype correlations.

The *GALC* activity is measured using the radiolabeled natural substrate galactocerebroside, or a synthetic substrate (6-hexadecanoylamino-4-methylumbelliferyl-β-D-galactopyranoside [HMGal]).<sup>36</sup> *GALC* activity can be measured using the stored newborn screening cards,<sup>12</sup> tandem mass spectrometry,<sup>37</sup> or fetal cells collected by chorionic villus sampling at 10 to 12 weeks gestation.<sup>8</sup> The level of *GALC* enzyme activity is used to confirm or establish the diagnosis of KD, especially for patients clearly deficient with 0 to 5% of normal activity as detected in leukocyte or fibroblast samples. The absolute level of *GALC* enzyme activity is not a reliable indicator for KD prognosis, espe-

cially for carrier testing, because of the wide range of enzymatic activity observed between carriers and non-carriers.<sup>38</sup> For example, when one or both parents of an affected child have a benign variant resulting in a low *GALC* enzyme activity, this does not necessarily predict an affected phenotype.

## Genotype–Phenotype Correlation of KD

Mutations in the *GALC* gene are responsible for the KD phenotype, mostly due to the deficiency of *GALC* enzyme activity from 0 to 5% of normal activity.<sup>7,39</sup> Although in some cases, there is a clear and meaningful correlation between the genotype and clinical presentation of patients with KD, however, in other cases, it is difficult to predict such correlation based on the underlying of *GALC* mutations alone, even for patients with identical mutations.

Nine families with history of KD were analyzed for variants in the *GALC* gene<sup>40</sup>; five LOKD patients harbored variants that completely abolished *GALC* enzyme were found. One would expect that LOKD patients would have residual enzyme activity. Therefore, the absence of the enzyme activity in LOKD patients may indicate a significant degree of genetic heterogeneity and/or the presence of modifier genes. The details of the pathogenic mechanism of the variants are detailed within this article. Another example of LOKD patients harboring *GALC* variants, which dramatically affected the *GALC* activity, was conducted by Lissens et al,<sup>41</sup> where they report five Italian patients with LOKD, out of which three were homozygous for the variant c.121A > G (p.G41S) and the other two patients were compound heterozygous for p.G41S with either the missense variant: c.1493T > C (p.F498S) or the frameshift variant c.907delT (p.Tyr303MetfsX6).<sup>41</sup> Although the p.G41S variant abolished the activity of the *GALC* enzyme and was predicted as possibly damaging by PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>), there was no correlation between the enzyme activity, in silico predictions, age of onset, and disease progression. Fiumara et al<sup>5</sup> performed a retrospective study on 26 patients with KD from Italian and Tunisian origins, including 17 patients with LOKD. Sequence analysis revealed that four patients were homozygous for the p.G41S mutation and four were compound heterozygous for this variant combined with other variants. Among 30 unrelated Italian patients with KD, the mutational heterogeneity was observed in 33 different mutations,<sup>39</sup> which made it difficult to conclude a meaningful relationship between the genotype and the clinical phenotype of the patients.

Xu et al<sup>29</sup> investigated the molecular underpinnings of KD in 17 unrelated Japanese patients and reviewed the mutations previously reported in 11 Japanese patients. The study identified variants specific to KD Japanese patients: c.12del3ins and p.I66M + p.I289V, which account for 37% of all known disease associated alleles. These mutant alleles, with two additional mutations: p.G270D and p.T652P, accounted for up to 57% of mutations among Japanese patients. Xu et al<sup>29</sup> observed these variants: p.I66M + p.I289V, p.G270D, and p.L618S associated with a mild phenotype.

Debs et al<sup>20</sup> investigated the genotype–phenotype correlation of 11 patients diagnosed with KD in French hospitals, originating from the Caribbean, Italy, Reunion Island, Morocco, Senegal, and Turkey; together with 30 patients previously reported in the literature. All patients showed zero GALC enzyme activity in association with the LOKD phenotype; yet only 59% of the adult patients presented with peripheral neuropathy, a symptom more distinctive to the EIKD form.<sup>6,42,43</sup>

Recently, Hossain et al<sup>44</sup> presented the largest study till date to understand the genotype–phenotype correlation of 51 Japanese patients with KD. They investigated 22 newly diagnosed patients and 29 previously reported patients.<sup>29,45–47</sup> By screening for c.635\_646delinsCTC and p.T652P (two common infantile variants) and p.G270D (common late-onset variant), they were able to predict the EIKD phenotype or the LOKD phenotype in 16 and 45% of patients, respectively, and the phenotype of 61% of the patients could be detected by screening only for seven different prevalent variants circulating among KD patients in Japan.<sup>44</sup>

The amount of psychosine in the brain<sup>48,49</sup> and blood<sup>50</sup> correlated with the severity of the clinical presentation of KD. Using psychosine degradation as an indicator of severity of GALC mutations, Harzer et al<sup>51</sup> studied 10 patients with KD, including seven LOKD patients, showing correlation between the rate of psychosine degradation and the phenotype of KD patients who harbored compound heterozygous mutations. Recently, Hossain et al<sup>44</sup> confirmed the same relationship using patients' individual mutations. They found that the severity of the phenotype correlated with the effect of mutations, manifested by the variations of the rate of psychosine degradation. There were higher rates of psychosine degradation observed among variants responsible for LOKD compared with the mutations responsible for EIKD.

Owing to the highly variable clinical profile of patients with KD, it is difficult to discern a direct genotype–phenotype correlation. This might be due to several reasons: most patients with KD are compound heterozygous;<sup>5,12,20,39–41,45</sup> variable enzyme activities intrinsic to the nature of the biochemical assay used to measure GALC activity, whether using radioactive<sup>52</sup> or fluorescent<sup>36</sup> galactosylceramide substrate. The molecular heterogeneity, combined with highly variable polymorphic background in KD patients<sup>12,39,40,45</sup> which might be responsible for variable clinical presentation<sup>3,10,12,13,30</sup> even within the same families.<sup>39</sup> Predictions of the phenotype of novel mutations is challenging, especially when patients are compound heterozygous for a known variant associated with a disease phenotype and a variant of unknown clinical significance.<sup>7</sup> Thus, it is sometimes not possible to predict the effect of private and population-specific mutations in other ethnic groups with different genetic makeups. Since the GALC enzyme consists of 30 kDa and 50 kDa subunits derived from the same precursor assembled in aggregates, and most of KD patients are compound heterozygous, the small variations of the enzyme activities described as residual activities make significant conclusions difficult.

This review sheds light on the prevalence of the KD in Arabia, draws the clinical and molecular picture of Arab

patients with KD, and discusses the importance of using intervening strategies to limit the spread of KD in the Arab world.

## Prevalence of KD in the Arab world

Determining the prevalence of KD in the Arab world is challenging due to the paucity of reports, the rarity of the disease, and the undeveloped infrastructure for genetic screening in the 22 Arab states.

The high frequency of consanguineous marriage,<sup>53</sup> which is responsible for the elevated frequencies of genetic diseases in Arabia,<sup>54</sup> is expected to increase the prevalence of KD. Two Muslim Arab villages in Israel, where consanguineous marriage is a common cultural practice, underwent targeted genetic testing for causative KD mutations and the incidence was significantly high.<sup>3</sup> The carrier rate was estimated to be one in six.<sup>31</sup> Macarov et al<sup>55</sup> presented the results of a four-decade investigation of prenatal diagnosis and carrier screening for KD in two Muslim Arab villages, which are part of the Jerusalem municipality. These authors found that half of the Arab women did not believe in the effectiveness of prenatal diagnosis, which is consistent with the findings of Jaber et al,<sup>56</sup> wherein half of the women expressed doubts regarding the accuracy of prenatal diagnosis. Interestingly, carrier screening based on the molecular identification of the KD founder mutations was very instructive for the Muslim Arab population when deciding whether to avoid marriage. This practice has led to a reduction in the incidence of KD from 1.6/1,000 to 0.82/1,000 live births.<sup>55</sup>

A recent study by Foss et al<sup>57</sup> reviewed the proper use of epidemiological measures to document the success of the genetic screening programs for KD in the two Arab Muslim villages. They provided an interpretation for the previous risk estimates reported by Zlotogora et al<sup>32</sup> and Macarov et al<sup>55</sup> to determine the life-risk estimator for these populations. The study compared different epidemiological methods and recommended the life-table method to produce the most accurate estimates<sup>58</sup> for studying KD prevalence. The life-table method was used because of its ability to correct errors resulting from censored cases (individuals who would have developed symptoms of KD but died of unrelated causes before symptom onset). Foss et al<sup>57</sup> estimated the risks for 3,600 live births (1999–2002) and 4,876 live births (2003–2007) in the Muslim Arab population in Israel (reported by Macarov et al<sup>55</sup>). They estimated these risk to be extremely high-reaching, 1,670/1,000,000 units (yielding six postnatal cases) and 820/1,000,000 units (yielding four postnatal cases) for the two periods, respectively. The epidemiological estimate analysis was extended<sup>57</sup> to predict six postnatal patients out of 3,731 births<sup>32</sup>; the risk estimate was again found to be significantly high: 1,610/1,000,000.

## Palestine and Syria

Zlotogora et al<sup>3</sup> reported on large Druze kindred in Northern Israel of Syrian origin (the Druze religion was founded in Egypt in the beginning of the 11th century, which then spread to Lebanon and Syria). The pedigree had 12 affected children

who mostly presented with the EIKD form between the ages of 4 and 6 months, except one child presented at 11 months. The GALC enzyme was measured only in five patients and was less than 10% of normal control, and referred to as deficient by the authors. Interestingly, the children from the same inbred family presented with a variable clinical profile at the age of onset (–Table 1). One would predict that all children, descended from a common ancestor in the same inbred family, will be homozygous for the same variant (no molecular studies were performed), that affected the GALC function. EIKD is a well-characterized form of the disease<sup>11</sup> and yet most EIKD patients presented with significant phenotypic variability (–Table 1), and similar variability levels were observed in different studies.<sup>12,30,45</sup>

The Druze community constitutes a religious isolate with an increased prevalence of first cousins marriage, approaching 35% of all marriages.<sup>59</sup> A study by Tirosh et al<sup>60</sup> reported on the consanguineous marriage among different ethnic groups in Northern Israel and found that the rates recorded were very high, reaching up to 85% in Druze, 77% in Christians, and 72% in Muslims. In 1991, Zlotogora et al<sup>32</sup> diagnosed 18 non-Jewish Israeli infants affected with KD over a period of 15 years. Six were Druze from the large kindred previously reported by Zlotogora et al.<sup>3</sup> The other 12 patients were Muslim Arabs. Seven were from two adjacent villages in Israel, most of whom were found to be related. The incidence of the disease for this segment of population was estimated to be 1/130 live births.

In 1997, Zlotogora<sup>61</sup> surveyed the distribution of autosomal recessive disorders among 2,000 different Palestinian Arab families. In 601 cases, an autosomal recessive disease was diagnosed or strongly suspected. KD was found to represent a high frequency of 21 families out of 601 families screened, of which 18 families of those originated from only two small neighboring villages.<sup>32</sup> Muslim Arabs seem to have two missense homozygous variants,<sup>31</sup> c.1582G>A (p.D528N), which seem to affect function, probably due to protein hyperglycosylation and misfolding.<sup>62</sup> The second variant is c.1637T > C (p.I546T), which exists in 35% of the alleles in a different sub-population. Interestingly, the p.I546T variant was found to affect the GALC expression as detected by *in vitro* assay, and has a cumulative effect when coexists in KD patients with other variants affecting GALC function.<sup>38,46</sup> Again, this is predicted as possibly damaging using PolyPhen-2 predictions (–Table 2). The Druze patients were found to harbor the homozygous variant c.1748 T > G (p.I583S). *In vitro* expression studies of both the Arab and Druze variants (p.D528N and p.I583S) in COS-1 cells showed that both variants abolished the GALC activity. These two variants are considered as founder mutations due to the frequent homozygous occurrences responsible for KD phenotype in the two communities.<sup>29</sup> Another KD patient was found among Israeli Arabs through prenatal diagnosis screening performed by Zlotogora and Reshef<sup>63</sup> from 1992 to 1996.

Korn-Lubetzki et al<sup>6</sup> studied eight children of Muslim Arab decent living in northern Israel (four boys and four girls), that presented symptoms of the disease at the age of 2.5 to 5 months. All were confirmed as EIKD patients at 2 weeks

to 10 months of age. Six patients demonstrated central nervous system signs and symptoms (–Table 1), who were blind and cognitively deteriorated by 6 to 7 months after first visit; and two patients had no central nervous system involvement for 9 to 10 months after initial symptom of peripheral neuropathy. All patients were homozygous for same c.1582 G > A (p.D528N) variant. Korn-Lubetzki et al<sup>6</sup> indicated that EIKD high frequency among the Muslim Arab Palestinians population in Israel should be considered in the differential diagnosis of early infantile peripheral neuropathy.

#### Saudi Arabia

Al-Essa et al<sup>64</sup> studied a 2.6-year-old Saudi Arabian male using fluorine-18-labeled-2-fluoro-2-deoxyglucose positron emission tomography scan. The patient is a product of a consanguineous marriage and the parents were distant cousins and had five older normal children. No family history of neurologic disease was reported. The scanning investigation revealed that the patient had brain atrophy and lack of cerebral growth (–Table 1). The fluorine-18-labeled-2-fluoro-2-deoxyglucose positron emission tomography study of the brain demonstrated a marked decrease in the metabolism of the left cerebral cortex, and no uptake in the caudate heads. There were no further molecular studies performed on this patient. Another Saudi Arabian infantile patient was reported by Wenger et al<sup>7</sup> as homozygous for the variant c.860 C > T (p.S287F), which abolished the GALC activity as detected by *in vitro* studies, but retained ~7% of the activity *in vivo*, and was predicted as probably damaging using PolyPhen-2 (–Table 2). This patient also harbored the c.1637T > C p.I546T variant. No clinical evaluation reported for this patient.

#### United Arab Emirates, Tunisia, and Morocco

Al Talabani et al<sup>65</sup> surveyed 24,233 newborn babies (1992–1995) in Abu Dhabi, UAE, to study the pattern of major congenital malformations. A total of 401 babies (289 Arabs) were seen with major malformation, including one patient with KD who was a product of a consanguineous marriage. No more data were reported for the patient. In a retrospective analysis of 26 patients diagnosed with EIKD in Italy, Fiumara et al<sup>5</sup> found three siblings (two boys and a girl), born to Tunisian first cousin parents, that presented with cerebral cry at 3, 4, and 5 months, respectively. The three patients showed symptoms and signs consistent with EIKD (–Table 1). *In vitro* studies of the patients' fibroblasts showed that the GALC activity was 1.9 to 2.5% of normal activity.

A 72-year-old Moroccan woman<sup>20</sup> presented with walking difficulties since the onset of the disease at 66 years. Neurological examination revealed motor weakness in lower limbs, and no motor deficit in upper limbs (–Table 1). Molecular analysis showed that this patient harbored a missense homozygous variant c.147G > C (p.G49G) (–Table 2), affecting the last nucleotide of exon 1 of GALC gene leading to missplicing. This mutation was found with a polymorphic variant c.1637T > C (p.I546T). Interestingly, the p.I546T variant led to what is known as GALC pseudo-deficiency<sup>38</sup> decreasing GALC enzyme activity, but did not result in deficient enzyme,<sup>31</sup> consistent with the *in silico* prediction (–Table 2).

**Table 1** Summary of clinical findings in Arab patients with Krabbe disease

Origin	Phenotype	Age of onset	PN	Clinical presentation (PN)	Method of diagnosis <sup>b</sup>	Reference
Muslims and Druze	EIKD	4–11 m	12	Irritability (4), lack of movement (2), regression (5), fever (1), tremor (2), head lag (10), increased deep tendon reflexes (4), and decreased deep tendon reflexes (3), elevated CSF (7)	Clinical, enzymatic	Zlotogora et al <sup>3</sup>
Muslims and Druze	EIKD	5–11 m	18	Motor regressions (12), hypotonia (2), fever (2) irritability (9), decreased tendon reflexes (14), elevated CSF (13)	Clinical	Zlotogora et al <sup>32</sup>
Muslim Arab	NA	22 wk	1 <sup>a</sup>	NA	Prenatal (CVS)	Zlotogora and Reshef <sup>63</sup>
Muslims and Druze	EIKD	3–5 m	8	Poor focusing (4), hypertonia (4), areflexia (1), head lag (1), seizures (2), hypertonia (1), scissoring (1), irritability (5), optic pallor (1), peripheral neuropathy (2)	Molecular, enzymatic	Korn-Lubetzki et al <sup>6</sup>
Saudi Arabia	LIKD	2.6 y	1	Developmental delay, microcephaly, difficulty to soothe, poor head control, truncal hypotonia associated with mild spasticity of the extremities, increased deep tendon reflexes, mild brain atrophy, and elevated CSF	Enzymatic, FDG PET scan	Al-Essa et al <sup>64</sup>
Tunisia	EIKD	3–5 m	3	Cerebral cry (2), fever (1), irritability (2), hypertonia (3), hyperreflexia (3), clonus (2), hypotonia (1), optic atrophy (3), nystagmus (3), motor deterioration (3), elevated CSF (2)	Clinical, enzymatic	Fiumara et al <sup>5</sup>
Morocco	LOKD	66 y	1	Motor weakness in lower limbs predominating on flexor muscles, urinary dysfunction, dementia, mental retardation, and spastic paraparesis	Clinical, molecular	Debs et al <sup>20</sup>

Abbreviations: CSF, cerebrospinal fluid; the increase in the amount of CSF is a clinical indicator for diagnosis of KD<sup>30</sup>; CVS, chorionic villus sampling; EIKD, early infantile form of Krabbe disease; FDG PET, Fluorine-18 labeled-2-fluoro-2-deoxyglucose positron emission tomography scan; LIKD, late infantile Krabbe disease; LOKD, late onset Krabbe disease; LOKD, late onset Krabbe disease; NA, not applicable; PN, number of patients studied or showing symptoms.

<sup>a</sup>This patient was captured during prenatal diagnosis at the 22<sup>nd</sup> week.

<sup>b</sup>Molecular diagnosis involved sequencing of the *GALC* gene and biochemical (enzymatic) diagnosis involved the in vitro measurement of *GALC* activity in either cultured leukocytes or fibroblasts from Krabbe disease patients.

**Table 2** Summary of the genotype–phenotype correlation of Arab patients with Krabbe disease

Origin	Phenotype	Nucleotide change	Amino acid change	PP (S,S,S)	GALC_EA	PN	References
Muslim Arab (Dx Israel)	EIKD	c.1582G > A	p.D528N <sup>a</sup>	PrD (1,0,1)	Loss	1	Rafi et al <sup>31</sup>
Muslim Arab (Dx Israel)	(EIKD)	c.1637 T > C	p.I546T	PsD (0.46,0.89,0.90)	<sup>b</sup>	1	Rafi et al <sup>31</sup>
Druze (Dx Israel)	EIKD	c.1748 T > G	p.I583S	PrD (0.99, 0.27, 0.99)	Loss	1	Rafi et al <sup>31</sup>
Morocco (Dx France)	LOKD	c.147G > C	p.G49G	NA	NR	1	Debs et al <sup>20</sup>
Morocco (Dx France)	LOKD	c.1637T > C	p.I546T	PsD (0.459,0.89,0.90)	<sup>c</sup>	1	Debs et al <sup>20</sup>
Saudi Arabia	EIKD	c.860 C > T <sup>d</sup>	p.S287F*	PrD (1,0,1)	Loss	1	Wenger et al <sup>7</sup> ; Jardim et al <sup>70</sup>
Arab	EIKD	c.154T > C	p.S52P*	PrD (1,0,1)	NR	1	Wenger et al <sup>8</sup>

Abbreviations: Dx, diagnosed in; EIKD, early infantile Krabbe disease; EIKD, the variant itself is not known to cause the EIKD phenotype and considered as polymorphic variant (see text), GALC\_EA, galactocerebrosidase enzyme activity of normal; LOKD, late onset Krabbe disease; Loss, indicates complete absence of GALC activity; NA, not applicable; ND, not determined due to lack of studies on the subject; NR, not reported; PN, number of patients studied; PP, PolyPhen-2 (<http://genetics.bwh.harvard.edu>) prediction; PrD, probably damaging; PsD, possibly damaging; S,S,S, score, sensitivity, specificity.

Note: The GALC sequence used as a template for the in silico prediction has accession # AAA16645, version: AAA16645.1, and GI: 431310. The GALC reference sequence has accession # L23116 (+1 as A of the ATG start codon), version: L23116.1.

<sup>a</sup>These mutations predicted with SIFT (<http://sift.jcvi.org>) as not tolerated.

<sup>b</sup>The in vitro expression of this mutant resulting in decreased but not deficient GALC activity, and has been found in the heterozygous and homozygous state in healthy individuals and is therefore considered to be a polymorphism.<sup>29</sup>

<sup>c</sup>The activity is reported to be reduced and there was no exact measurement of the GALC activity.<sup>70</sup>

<sup>d</sup>This variant mentioned as unpublished observation by Wenger et al,<sup>7</sup> described as infantile, no more information published about this patient thus far.

## Future Directions and Conclusion

Although KD is considered to be a rare neurodegenerative disease, it is reported in Arabs, Japanese,<sup>44</sup> Chinese,<sup>9</sup> South American,<sup>12</sup> and Europeans.<sup>12,39</sup> The clinical profile of KD Arab patients seems to draw heterogeneous pictures that include overlapping signs and symptoms ranging from the EIKD to LOKD forms (–Table 1). In most cases, there were meaningful correlations noted between the genotype and phenotype (–Tables 1 and 2), where the enzyme activity was abolished in all of the patients presented with symptoms consistent with an EIKD form. The three variants circulated in Arab patients, the infantile Israeli Arab variant c.1582G > A (p.D528N), the infantile Saudi Arabian variant c.860 C > T (p.S287F), and the infantile Arab variant c.154T > C (p.S52P),<sup>8</sup> seem to be specific to Arab patients and were not noted in other ethnic groups. The three variants were predicted with PolyPhen-2 as probably damaging (–Table 2) and with SIFT as not tolerated (data not shown). The carrier frequency for c.1582G > A (p.D528N) variant, among Arab patients in the two Muslim villages in Israel reported to be very high (0.07).<sup>32</sup> In addition, this mutation appears to be primarily responsible for most of the severe KD phenotype among Arabs in homozygous occurrences and also considered to be a founder mutation for KD among Muslim Arabs in Israel. Given the dominant intra-familial marriage culture among Arabs, the undeveloped infrastructure of molecular investigation in Arabia, and the few reports dealing with KD cases in Arabia, one would predict the appearance of other interesting founder mutations and variants which might be associated with

severe phenotypes. Therefore, the most appropriate way of molecular diagnosis of KD will remain the full GALC gene sequence,<sup>66</sup> not the targeted sequence, including the exons, introns and regulatory regions, at least in the near future. The outcome of these studies is expected to promote better understanding of the genotype–phenotype correlation of KD and will help in the development of customized molecular diagnostics approaches, specific to Arab patients with KD. Although, some studies have been dedicated to the understanding of the genotype–phenotype correlation of the KD in different ethnic groups,<sup>5,8,20,28,29,44,67</sup> the overall relationship lacks clarity, making it difficult for both researchers and clinicians to reach a clear-cut conclusion.<sup>40</sup> Therefore, a molecular analysis of the GALC gene aims at identifying more variants of both newly diagnosed, as well as well-characterized Arab patients with KD will be beneficial in predicting of the prognosis of KD, while broadening our understanding of the molecular pathology of the KD. One of the obstacles hindering the clinical application of preventive solutions for genetic diseases is restrictions inherent to Arab culture, and social stigma against patients presenting with the disease, especially to the Arab female population. Preventing or lowering the birth rate of children who are most likely to present with the disease is a significant challenge for many Arabs who see it as a crime—according to the way in which religious and/or Islamic laws are interpreted—to either participate in birth control methods or to abort the fetus. An exception is found primarily when there is an eminent threat to pregnant woman's health and well-being.<sup>68</sup> In addition, some Arabs view abortion as acceptable if performed before

or within the first 4 months, or 120 days of pregnancy.<sup>69</sup> Therefore, since participation in these programs are discouraged significantly by members of the Arab Muslim community, only a small number of Arab women have joined in the studies, and particularly when they already had at least one affected child.<sup>63</sup> Clearly, this makes early diagnosis problematic, and a true assessment or count of those affected by this disease inaccessible. Remedies, such as training medical personnel, increasing the level of religious and public health awareness, and emphasizing the necessity of participating in the prenatal diagnostic programs, are crucial in drawing out members of the Arab population where consanguineous marriage is a normal and prevalent part of cultural practice.

To this end, molecular studies of well-characterized Arab patients will advance our understanding of the molecular pathology of KD, contribute to the development of accurate and precise customized molecular diagnostics for the disease, and promote better understanding of the genotype-phenotype correlation of KD. Early prenatal diagnosis will be an efficient and preventative measure to decrease the prevalence of the disease in Arabia.

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