Adaptive behavior and psychiatric comorbidities in *KCNB1* encephalopathy

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A B S T R A C T

Aim: KCNB1 encephalopathy encompasses a broad phenotypic spectrum associating intellectual disability, behavioral disturbances, and epilepsies of various severity. Using standardized parental

Abbreviations: ASD, autism spectrum disorder; CBCL, Childhood Behavioral Check-List; DE, developmental encephalopathy; DEE, developmental and epileptic encephalopathy; ID, intellectual disability; SCQ, Social Communication Questionnaire; VABS-II, Vineland Adaptive Behavioral Scale second edition.

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questionnaires, we aimed to capture the heterogeneity of the adaptive and behavioral features in a series of patients with *KCNB1* pathogenic variants.

Methods: We included 25 patients with a *KCNB1* encephalopathy, aged from 3.2 to 34.1 years (median = 10 years). Adaptive functioning was assessed in all patients using the French version of the Vineland Adaptive Behavior Scales, Second Edition (VABS-II) questionnaire. We screened global behavior with the Childhood Behavioral Check-List (CBCL, Achenbach) and autism spectrum disorder (ASD) with the Social Communication Questionnaire (SCQ). We used a cluster analysis to identify subgroups of adaptive profiles.

Results: VABS-II questionnaire showed pathological adaptive behavior in all participants with a severity of adaptive deficiency ranging from mild in 8/20 to severe in 7/20. Eight out of 16 were at risk of Attention Problems at the CBCL and 13/18 were at risk of autism spectrum disorder (ASD). The adaptive behavior composite score significantly decreased with age (Spearman's Rho=-0.72, $p<0.001$) but not the equivalent ages, suggesting stagnation and slowing but no regression over time. The clustering analysis identified two subgroups of patients, one showing more severe adaptive behavior. The severity of the epilepsy phenotype predicted the severity of the behavioral profile with a sensitivity of 70% and a specificity of 90.9%.

Conclusion: This study confirms the deleterious consequences of early-onset epilepsy in addition to the impact of the gene dysfunction in patients with *KCNB1* encephalopathy. ASD and attention disorders are frequent. Parental questionnaires should be considered as useful tools for early screening and care adaptation.

1. Introduction

The recent development of high throughput sequencing techniques led to the identification of a large number of genes involved in neurodevelopmental disorders, associated or not with epilepsy. The phenotype of these pathogenic variants needs further delineation as one gene can be associated with different phenotypes or a spectrum of phenotypes. This delineation can help to achieve better care path and outcome prediction.

KCNB1 codes for the α-subunit of the voltage-gated potassium channel subfamily 2 (Kv2.1). Kv2.1 channels are widely expressed across the central nervous system, mediating delayed rectifier potassium current which is an important modulator of membrane repolarization in neuronal cells [1,2]. Pathogenic variants in *KCNB1* have initially been described in patients with infantile-onset epileptic encephalopathy [3,4]. However, recent studies have shown that *KCNB1* pathogenic variants can be associated with a broader phenotypic spectrum [4–7]. All reported individuals show early psychomotor developmental delay and mild-to-profound intellectual disability (ID) has been reported without formal psychometric evaluation. In addition, autistic features, impulsiveness, hyperactivity, and aggressivity are clinically reported in more than half of individuals [5]. Most individuals (80%) develop epilepsy consisting either in developmental and epileptic encephalopathy (DEE) with infantile-onset drug-resistant seizures or in developmental encephalopathy (DE) with late-onset and often pharmacoresponsive epilepsy [5]. Individuals with *KCNB1* DEE have a more severe long-term cognitive outcome compared to those with *KCNB1* DE [5,8]. The former denomination emphasizes the impact of early-onset epilepsy on neuro-development in addition to the role of the gene dysfunction.[3] Pathogenic variants are either missense (80%) or truncating (20%) [6].

Despite small series reported, individuals with truncating variants seem to have less severe and less active epilepsy compared to those with missense variants [5].

We aimed to explore the developmental phenotype of individuals harboring pathogenic variants in *KCNB1* with standardized scales in order to better delineate their neurodevelopment profile and identify their special needs.

2. Patients and Methods

2.1. Participants

Individuals with *KCNB1* pathogenic variants were recruited through a European collaboration between the network of the French Reference Centre for Rare Epilepsies and other centers in Belgium, Switzerland and Luxembourg with the support of the families' association "KCNB1 France" [5]. French speaking families were included in this study.

For each individual, we collected demographic data, information on epilepsy history and developmental milestones, and details of the genetic *KCNB1* variant. We collected seizures' types, age at seizure onset and offset, epilepsy duration in months, number of current and tried anti-seizure medications (ASMs), current seizure frequency and drug response, age at sitting and walking achievement.

Seizures and epilepsy types were determined according to the International League Against Epilepsy (ILAE) classification proposal [8]. Individuals with "*KCNB1* developmental and epileptic encephalopathy" (DEE) had active epilepsy (seizures and EEG) thought to interfere with the ongoing neurodevelopment with often early-onset seizures. Individuals with "*KCNB1* developmental encephalopathy" (DE) had less active epilepsy, often with late onset and without regression or further slowing of development at seizure onset. This group also included individuals who did not develop seizures at the time of the study.

The study protocol was approved by the ethics committee of our institution and written informed consents were obtained for all participants.

2.2. Methods

Questionnaires were proposed during a face-to-face clinic or were directly sent to the family after their information and approval. In both situations, parents were asked to fill the scales autonomously. In case for any further information and when the questionnaire had missing answers, the neuropsychologist (DB) contacted the family to complete missing data.

2.2.1. VABS-II

Adaptive behavior was evaluated using the Vineland Adaptive Behavioral Scale second edition (VABS-II) [9]. This parental autoquestionnaire includes items clustered around 4 main domains: Communication (receptive, expressive, written communication skills), Daily Living Skills (personal, domestic and community interaction skills), Socialization (interpersonal relationships, play and leisure time, and coping skills) and Motor Skills (gross and fine). VABS-II provides an Adaptive Behavior Composite score that summarizes the individuals' skills in all four domains. Equivalent age corresponds to the developmental age, i.e., age at which a typically developing person usually acquires a certain skill or behavior. Adaptive Behavior Composite and domains standardized scores (mean =100, standard deviation=15), adaptive levels (Low adaptive level, standard score (SS)=[20;70]; Moderately low, SS=[71;85]; Adequate, SS=[86;114]; Moderately high, SS=[115;129]; High, SS=[130;160]) and mean domain equivalent age were reported. For subdomains, v-scores (mean =15, standard deviation =3) and equivalent ages were reported. The motor scale for VABS-II shows a ceiling effect over 6 years so that motor scores were not reported for these individuals. We categorized the scores in four severity degrees of adaptive deficiency: no deficiency (>70), mild deficiency (50–69), moderate (35–49), severe (20–34) and profound deficiency (<20; according to IDC-10) [10].

2.2.2. Childhood Behavioral Check-List

Childhood Behavioral Check-List (CBCL, Achenbach) was used in both preschool (18 months to 5 years old) and school (6 to 18 years old) versions [11].

CBCL questionnaires were not completed for individuals older than 18 years according to the age limitations of the scale. We explored in this study the scores common to the two age-related CBCL versions: Withdrawn/Depressed, Somatic complaints, Anxious/Depressed, Attention Problems, Aggressive Behavior, Internalized Behavior, Externalized Behavior and Total Score. Standardized T-scores were obtained (mean=50, standard deviation=10). T-scores over 70 are pathological and T-scores between 60 and 69 are considered as borderline scores.

2.2.3. Social Communication Questionnaire

Social Communication Questionnaire (SCQ) "Life span version" is a tool used for ASD screening in individuals aged over 4 years [12]. A total score over 15 indicates a possible ASD and suggest further ASD diagnosis evaluation. We reported the number of individuals at risk of ASD (scores above threshold).

2.3. Data analysis

We used descriptive analysis to characterize our series. Variables assessed included gender, age, age of major developmental milestones, results of the screening of behavior and autism spectrum disorders (pathological or not), and the presence of epilepsy including current seizure frequency (more or less than 1 per month), epilepsy duration (quoted at 0 for individuals without epilepsy), total number of anti-seizure medications previously and currently used, and the presence of drug resistance. Normal and non-normal data were represented as median [25th−75th percentiles]. We performed a hierarchical cluster analysis in order to determine the number of clusters present in the subdomain of the VABS-2 (quoted as equivalent age). We chose the equivalent age based on our previous study on Dravet syndrome showing a stagnation of the neurodevelopment development after the age of three with no loss of abilities [13]. This stagnation leads to a stability of the equivalent age scores, whereas the standardized age score decreases over time due to the continuation of neurodevelopmental acquisitions in the control group. Then, we used nonhierarchical k-means clustering to classify the individuals. To limit the impact of the selected start partitioning, the algorithm was repeated 500 times with a random start partitioning each time. Differences between the equivalent age scores in VABS-II subdomains of children belonging to different cluster subgroups were retrieved using Wilcoxon non-parametric test for numerical variables and chi-squared test for categorical variables. A p value less than 0.05 was considered a significant difference and a p value less than 0.1 a trend.

3. Results

3.1. Population

Twenty-one individuals (10 males, 11 females) aged from 3.2 to 34.1 years (median = 10.1 [6.3-13.8] years) were included in this study. Nineteen of them have been identified from our previously reported series [5]. The two individuals not reported previously were female monozygotic twins aged 6 years and who carried the pathogenic variant p.Arg312His. They had quite similar developmental phenotype with autonomous walk acquired at 36 and 38 months and a severe language disorder. The first had only two febrile seizures at 18 months of age (individual #20) while the second had yearly febrile and non-febrile seizures since the age of 2 years (individual #21). They did not receive ASM.

All individuals achieved independent sitting at a median age of 13 [11–22] months and autonomous walk at a median age of 25 [21-37] months. Twelve individuals (12/21) were nonverbal after the age of 3. Eighteen individuals (18/21) had epilepsy. Median age at seizure onset was 29.5 [18-48] months (range = 10 months-24 years). The median epilepsy duration was 37 [16-95] months. Eight individuals had *KCNB1* DEE and13 had *KCNB1* DE including 3 without epilepsy at the time of this study (Table 1). Eight individuals had drug-resistant epilepsy. Seizure frequency reported with the questionnaire was daily in 6 individuals, monthly in 3 and yearly in 4. Seizures had remitted in 4 individuals who showed no seizures since at least 1 year.

3.2. Questionnaires results

3.2.1. Vabs-II

The adaptive behavior composite score was in the low range for 20/21 participants (median = 46 [25-58], Table 1). Individual #5, aged 3 years, had an adaptive behavior composite score in the moderately low range (71). Scores were in the low range in 21/21 for Communication, 20/21 for Daily Living Skills, 19/21 for Socialization, and 6/6 for Motor Skills. For participants aged 7 years and older, none reached the maximum score for the motor scale. The severity of the global adaptive deficiency was mild for 8/20, moderate for 5/20, and severe for 7/20.

Considering subdomains, mean v-scores ranged from 3.95 (Expressive in Communication; median = 4 [1–5]), to 7.48 (Domestic in Daily Life Skills; median = 8 [3–10]).

3.2.1.1. Cbcl. CBCL questionnaire was filled by 16/21 caregivers whose children were aged < 18 years to fulfill the questionnaire age limits. Median standardized scores ranged from 51.5 [50-61.5] for Anxious/Depressed to 71 [66-80.3] for Attention Problems. For Attention Problems, 8/16 participants obtained scores in the pathological range and 6/16 in the borderline range. Second higher proportion of behavioral disturbances in CBCL was for the Aggressive behavior: 3/16 participants obtained scores in the pathological range and 3/16 in the borderline range.

Table 1
Clinical data and scores of adaptative functioning and behavioral scales.

No.[a]	Age (y)	Gender	*KCNB1* variant	Epilepsy/ phenotype	Age at sz onset (m)	Current number of AED	VABS-II						CBCL	SCQ
							Communication	Daily Life skill	Socialization	Motor skills[b]	Global Composite score	Level of adaptive deficiency	Attention score[c]	Total score[c]
1 *(1)*	29.2	M	p.Glu43Gly	Yes/DEE	16	5	21	21	20	-	20	Severe	-	20**
2 *(3)*	11.0	M	p.Thr210Arg	Yes/DE	48	2	57	62	59	-	59	Mild	-	7
3 *(5)*	3.8	F	p.Arg306Cys	Yes/DE	20	1	69	81	85	64	71	-	73*	-
4 *(6)*	7.1	F	p.Arg306Cys	Yes/DE	48	1	30	65	76	-	58	Mild	77*	9
5 *(7)*	24.1	M	p.Arg312Cys	Yes/DE	288	0	21	29	23	-	20	Severe	-	37**
6 *(8)*	34.1	M	p.Arg312His	Yes/DEE	18	2	21	21	20	-	20	Severe	-	20**
7 *(9)*	13.5	M	p.Arg312His	Yes/DE	60	1	31	47	47	-	40	Moderate	78*	24**
8 *(11)*	3.6	F	p.Arg312His	Yes/DEE	26	2	49	51	66	43	50	Mild	67	-
9 *(12)*	19.3	M	p.Arg312His	Yes/DE	60	1	26	25	32	-	23	Severe	-	17**
10 *(13)*	31.8	M	p.Ser319Phe	Yes/DEE	13	1	21	21	20	-	20	Severe	-	25**
11 *(14)*	3.2	M	p.Thr323Ile	Yes/DEE	21	1	42	51	59	43	47	Moderate	80*	-
12 *(16)*	4.8	F	p.leu334Pro	Yes/DE	33	0	42	43	57	40	43	Moderate	57	16**
13 *(17)*	10.8	M	p.Ser347Arg	Yes/DE	18	1	57	59	59	-	58	Mild	81*	26**
14 *(19)*	13.8	M	p.Trp369Arg	Yes/DEE	10	3	31	43	34	-	33	Severe	69	23**
15 *(23)*	7.7	F	pVal378Leu	Yes/DE	92	0	54	62	55	-	58	Mild	91*	9
16 *(28)*	8.7	F	p.Gly394Arg	Yes/DEE	42	2	42	48	50	-	46	Moderate	82*	21**
17 *(29)*	10.1	F	p.Gly395Arg	Yes/DEE	12	1	42	40	45	-	41	Moderate	68	20**
18 *(32)*	8.7	M	p.Ile409Thr	No/DE	-	0	70	62	62	-	64	Mild	92*	23**
19 *(36)*	12.4	F	p.Arg583Term	No/DE	-	0	31	23	34	-	25	Severe	57	26**
20	6.3	F	p.Arg312His	No/DE	-	0	52	64	68	56	57	Mild	66	13
21	6.3	F	p.Arg312His	Yes/DE	36	0	52	62	68	61	58	Mild	66	14
Median [IQ]	10,1 [6.3-13.8]				29.5 [18-48]	1 [0-2]	42 [30-52]	48 [29-62]	55 [34-62]	49.5 [43-59.8]	46 [25-58]		71 [66-80.3]	20 [14.5-23.8]

IQ, interquartile range; y, years; sz : seizure; AED, antiepileptic drugs; VABS-II, Vineland Adaptive Behavioral Scale second edition; CBCL, Childhood Behavioral Check-List; SCQ, Social Communication Questionnaire.
*Scores above the pathological threshold, ** "at risk" scores.
[a] The number reported in parentheses corresponds to the patient's number in the paper of Bar et al., 2020.
[b] VABS-II motor score was not reported for patients older than 6 years due to ceiling effect
[c] Missing data are related to the age limits of the questionnaire

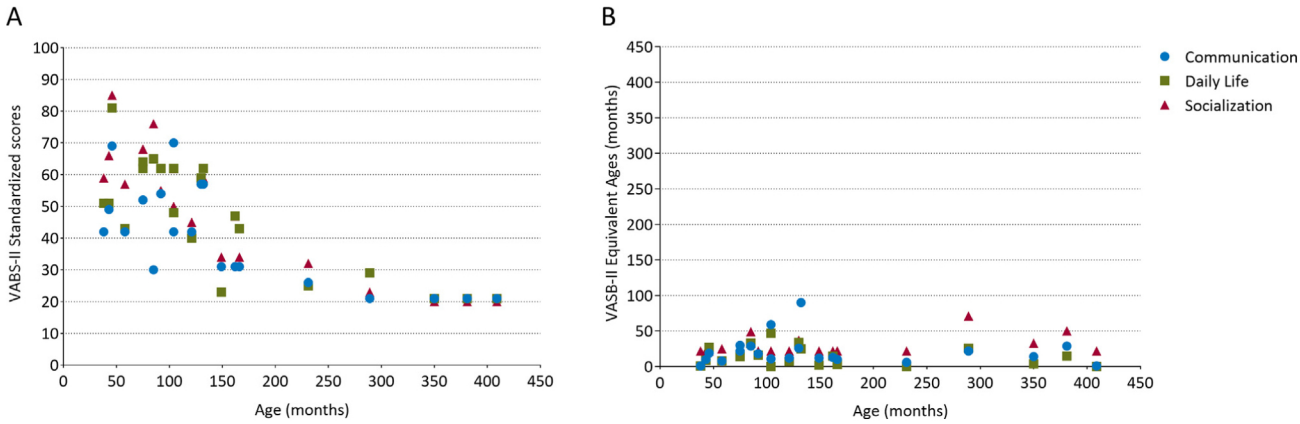

Fig. 1. Standardized scores (A) and equivalent ages (B) at each subdomain of the VABS-II questionnaire in our series.

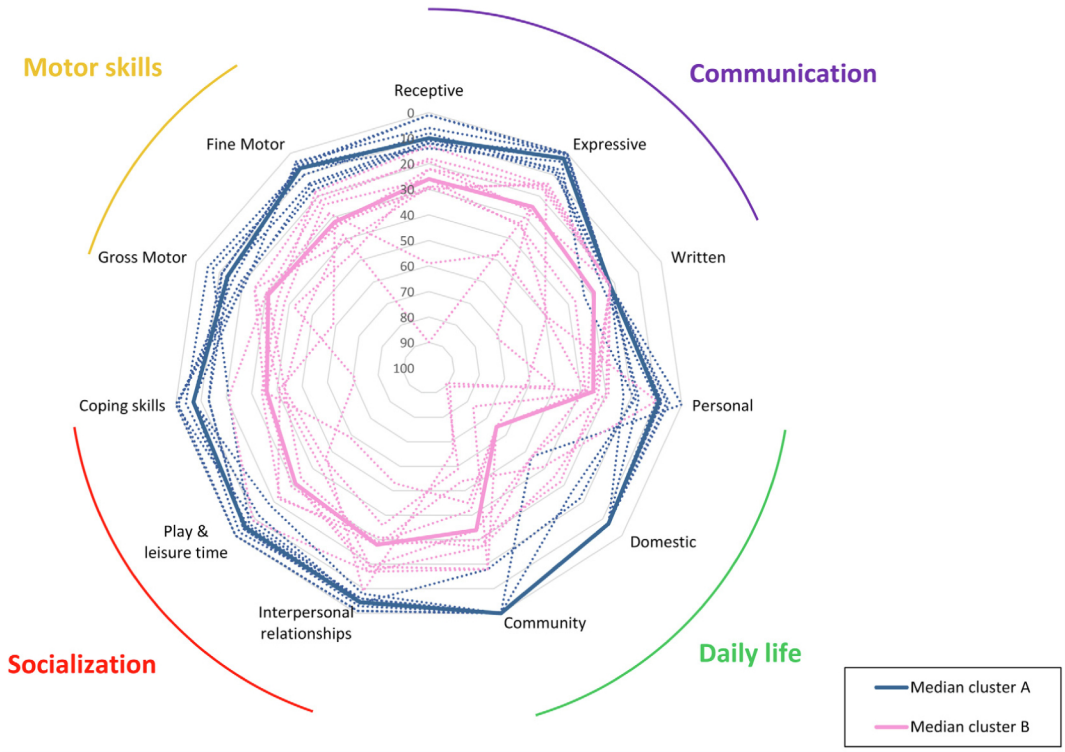

Fig. 2. Comparison of the equivalent age (in months) for each subdomain of the VABS-II questionnaire between cluster A (blue) and cluster B (pink).

3.2.1.2. Scq. Social Communication Questionnaire was filled by 18/21 caregivers according to the age criteria for this questionnaire (> 4 years). Raw scores ranged from 7 to 37 (median = 20 [14.5-23.8]). Thirteen out of 18 participants had a score above the threshold of risk of ASD.

3.3. VABS-II and correlation to age

Given the large age interval present in our population, we studied correlation between age at study and adaptive behavior scores. The adaptive behavior composite score decreased significantly with increasing age (Spearman's Rho=-0.72, p<0.001). Regarding domains' standardized scores, a significant decrease in the Communication score (Spearman's Rho=-0.67, p<0.001), the Daily Living Skills score (Spearman's Rho=-0.74, p<0.001), and the Socialization scores (Spearman's Rho=-0.86, p<0.0001) were observed. However, the equivalent age in Communication, Daily Living Skills, and Socialization domains were not correlated with the chronological age (Fig. 1). Finally, we did not find any significant difference (Mann–Whitney, p >0.05) between the chronological age of individuals "at risk" of attention problems (CBCL questionnaire) and ASD (SCQ questionnaire) compared to those "not at-risk".

3.4. Clustering analysis

The clustering analysis based on equivalent ages for each subdomain of the VABS-II identified two clusters of individuals. The median adaptive profile of these 2 clusters is shown in Fig. 2 and the comparison of the median score for each subdomain is reported in Table 2.

Table 2
Comparison of median equivalent ages (in months) across dimensions of functioning of the VABS-2 questionnaire between clusters.

Domains	Subdomains	Cluster A	Cluster B	p-value
Communication	Receptive	10[6,5–11,75]	26[20,5–29,5]	$<10^{-4}$
	Expressive	2,5[0,25-6,25]	25[15,5–30]	1.10^{-4}
	Written	22[22–22]	29[22–49,5]	0,049
Daily life	Personal	9[6,5–17,75]	35[31,5–38]	$<10^{-4}$
	Domestic	7[7–18,25]	65[44-71]	$<10^{-4}$
	Community	0[0-0]	34[27–49,5]	$<10^{-4}$
Socialization	Interpersonal relationships	4,5[3,25-5,75]	28[18-30]	1.10^{-4}
	Play & leisure time	5[2,5–8,5]	31[26–44,5]	$<10^{-4}$
	Coping skills	7[1,75–11,5]	36[35-42]	$<10^{-4}$
Motor skills	Gross Motor	13,5[11–18,25]	31[30-40]	1.10^{-4}
	Fine Motor	7[6–13]	32[23-36]	1.10^{-4}

Table 3
Clinical characteristics of the population and analysis of between-cluster differences.

	Cluster A (n=10)	Cluster B (n=11)	p-value
Sex (M/F)	5/5	6/5	1.0
Age at study (y)	11.3 [5.8-17.9]	8.7 [6.7-12.3]	0.78
Age at sitting position (m)	20 [11.5-24]	12 [9.5-17]	0.09
Age at independent walk (m)	35.5 [24.8-48.5]	24 [19.5-33]	0.17
Epilepsy	9	9	1.0
Epilepsy duration (m)	85.5 [17-153]	16 [0-54.5]	**0.08**
Number of AEDs tested	7 [3.3-13]	2 [0-4]	**0.04**
Drug-resistant epilepsy	7	1	**0.008**
Seizure frequency (>1 per m)	7	0	**0.001**
DEE phenotype	7	1	**0.008**

M, male; F, female; y, years; m, months; AED, antiepileptic drug; DEE, developmental and epileptic encephalopathy.

Cluster A included 10 individuals, 5 males and 5 females, and cluster B included 11 individuals, 6 males and 5 females. Cluster A individuals exhibited a lower level of adaptive behavior than cluster B individuals with significantly lower developmental ages in all subdomains (Table 2). The median adaptive behavior composite score was 37 [23.5-45.3] in cluster A and 58 [48.5-58.5] in cluster B ($p = 0.02$).

In addition, more individuals in cluster A had a score indicating a risk of ASD at SCQ questionnaire (8/8) compared to cluster B (5/10, $p = 0.04$). However, the number of individuals presenting pathological score for Attention Problems at CBCL questionnaire was not significantly different between the 2 clusters ($p = 0.131$).

We then compared the demographic, clinical, and genetic data of the individuals of the 2 clusters (Table 3). There was no significant difference in terms of age at the time of the study. Epilepsy was present similarly in both clusters (9/10 individuals in cluster A and 9/11 in cluster B). However, individuals with epilepsy in cluster A showed a longer duration of epilepsy with an earlier onset, a higher frequency of seizures, and more frequent drug resistance compared to individuals of cluster B. Epilepsy have been classified as DEE in 7/10 individuals in cluster A against 1/11 individual in cluster B. Therefore, the classification of the epilepsy phenotype into DEE vs DE predicted the severity of the behavioral profile with a sensitivity of 70% and a specificity of 90.9%.

3.5. Genotype–phenotype analysis

Twenty individuals had a missense variant while one had a truncating variant. Six individuals aged from 3.6 to 34.1 years had the recurrent variant p.Arg312His, localized in the voltage-sensor S4 domain of the protein. They were distributed similarly in cluster A and B (3 versus 3). The 8 individuals with a mild adaptative deficiency (VABS-II adaptative composite score > 50) had variable localization of the pathogenic variant: Four in the voltage-sensor S4 domain, three had variants in the S5 or S6 pore domains, and one in the S1-S2 extramembrane linker domain. The small numbers did not allow further statistical analyses.

4. Discussion

We used standardized scores and tests based on parental questionnaires to delineate the neurodevelopmental phenotype of individuals with *KCNB1* encephalopathy. All individuals exhibit global dysfunction of their adaptive behavior in all domains (communication, daily living skills, socialization and motor skills), present attention disorders and are at high risk of ASD. However, the adaptive behavior level was heterogeneous and ranged from mild-to-severe adaptative deficiency.

4.1. Use of parental questionnaires

The evaluation of adaptive skills is recommended for the diagnosis of intellectual disability together with the evaluation of reasoning skills (calculating an intellectual quotient) [9,14]. We did not perform intellectual evaluation in this study. This formal evaluation of cognitive functions using standardized performance tests is difficult to perform in this population because of a high rate of intellectual disability and behavioral disorders often leading to floor effects in scores. In another developmental encephalopathy, Dravet syndrome, the use of parental questionnaires as VABS-II show a high consistency with cognitive evaluation [15]. The use of such questionnaires relies on the care giver's report with a risk of subjectivity, but has the clear benefit of avoiding unreliable results due to the condition of the individual on the day of the test, the difficulty to perform such tests due to the fatigue of difficulties of engagement in test sessions (child refuses, important psychomotor agitation, do not answer to questions, do not maintain enough attention on the tasks), and finally the floor effect in classical tests. Moreover, the families are usually the best party to evaluate the impact of chronic diseases as epilepsy on the everyday life [16]. These questionnaires might be useful also as remote evaluation avoiding long travel time for patients and families to reference centers that have the expertise and the human resources to perform such evaluations.

4.2. ASD and attention problems

We show here that 72% (13/18) of individuals are at risk of ASD at the SCQ questionnaire, supporting the need to screen for ASD in this group and perform diagnostic evaluations using gold standard tools such as ADOS-2 and ADI-R [17,18]. Early detection is recommended for a necessary specific care path and rehabilitation. Our study shows a consistent number of individuals with attention disorders on CBCL and potential attention deficit hyperactivity disorder (ADHD). Almost half of individuals (8/18) show a high score for

attention disorders at parental hetero-questionnaires. This finding emphasizes the high occurrence of inattentive behavior and the possible benefit of targeted therapies. The use of methylphenidate should be evaluated and could be helpful in some individuals. The worsening effect of seizures is very unlikely and an individual balance benefit risk should be weighted [19]. Other behavioral domains are less frequently impaired in our population. Studies in populations with ID show behavioral complaints by caregivers such as aggressive behavior, social problems, or anxiety [20,21]. On the CBCL questionnaire, assessment of psychopathology is less reliable for persons with moderate, severe, or profound ID [22]. Therefore, we cannot exclude the presence of other psychiatric disorders and a child psychiatrist or psychiatrist evaluation should be proposed.

4.3. Adaptive behavior and relation with age at study

We explored in this study the correlation between the adaptive behavior and psychiatric profile and the age of the individuals at study (Fig. 1). Standardized scores for VABS-II, which are scores corrected for age, were lower for older participants in our population for Communication, Daily Life Skills, Socialization as well as for global Adaptive behavior score. Yet, the use of standardized scores or developmental quotients cannot confirm the assumption of a worsening and regression over time [13]. When we considered the equivalent ages, not corrected for the chronological age of the participant, this correlation was no longer significant. These results suggest rather a progressive widening gap over time for adaptive profile between *KCNB1* patients and the typically developing reference population and not a progressive cognitive deterioration. More longitudinal data are needed to confirm this hypothesis. Concerning ASD and attentional screening, age of the participant did not differ between at-risk and not at-risk groups, in favor of a relative stability of these comorbidities through age.

4.4. Adaptive behavior and relation to epilepsy phenotype

To go further in understanding the developmental spectrum of individuals with *KCNB1* variants, we performed an original clustering approach. This allowed the delineation of two subgroups with homogeneous profiles based on the interrelatedness of children's abilities across domains of functioning (Fig. 2). Cluster A is composed of individuals with the most severe adaptive deficiency with median equivalent age below 15 months in all domains at an age range of 38 months to 34 years. Cluster B exhibited a higher level of adaptive behavior with median equivalent age ranging from 25 months in expressive communication domain to 65 months in domestic daily life domain (Table 2). The risk of ASD was higher in cluster A compared to cluster B.

Individuals in both clusters, including those with a few seizures or even showing no epilepsy, showed abnormal development and an adaptive behavior deficiency. However, individuals with the most severe profile of adaptive behavior (cluster A) had the most severe epilepsy phenotype. This emphasizes that the early control of seizures might be crucial to limit the additional deleterious impact of seizures beyond the impact of the gene dysfunction. This shows mainly the relevance of the recent terminology of DEE versus DE with or without epilepsy [8]. This adds to this concept of a cumulative effect of the early-onset epilepsy and of the pathogenic variant as shown in other conditions such as Dravet syndrome [13]. These findings support on one hand, the need for the development of effective anti-seizure medications dedicated to early onset and refractory seizures and on the other hand, for the development of mechanistic and gene therapies hoping that this will offer a rescue of the gene defect and might improve globally the different aspects of the disorder including the seizures. The role of these mechanistic approaches in avoiding epilepsy development is to be further demonstrated.

5. Limitations

This study included a limited number of individual and most of them have been referred by tertiary epilepsy centers, which may have selected mostly patients with epilepsy and probably the severe spectrum of the developmental profile. The support and the participation of a young families' association (KCNB1 France) might have moderated this tendency and opened for a more holistic individual characteristics. The algorithm of the cluster analysis has been chosen due to its robustness in small sample size studies [23], but the two adaptive behavioral profiles should be validated on larger groups. Two main limits can be drawn with the VABS-II scale. The written communication subscale shows a floor effect as young participants obtain scores in the normal range or low limit while written language is not developed. The motor standardized scores showed a ceiling effect after the age of 7 years. Future studies might use more comprehensive evaluations exploring different dimensions of functioning to complete the picture of the behavioral profiles of individuals with *KCNB1* and other DEs.

6. Conclusion

This study describes the adaptive and behavioral profile of individuals with *KCNB1* encephalopathy using standardized hetero-questionnaires filled by caregivers. Individuals show an adaptive behavior deficiency and a high risk of attention disorders and ASD. Cluster analysis based on adaptive behavior scores delineated a more severe profile in DEE group compared to DE. This finding confirms the deleterious consequences of early and severe epilepsy in addition to the impact of the gene dysfunction. Our cross-sectional study emphasizes a trajectory of stagnation of the adaptive behavior without regression over age. The role of parental questionnaire and of the impact on adaptive behavior should be further explored in order to better understand the natural history of these diseases, the outcome, and to establish the most adapted care path.

Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] King AN, Manning CF, Trimmer JS. A unique ion channel clustering domain on the axon initial segment of mammalian neurons. J Comp Neurol 2014;522 (11):2594–608. https://doi.org/10.1002/cne.23551.

[2] Trimmer JS. Immunological identification and characterization of a delayed rectifier K+ channel polypeptide in rat brain. Proc Natl Acad Sci U S A 1991;88 (23):10764–8.

[3] Torkamani A, Bersell K, Jorge BS, Bjork RL, Friedman JR, Bloss CS, et al. De novo KCNB1 mutations in epileptic encephalopathy. Ann Neurol 2014;76 (4):529–40. https://doi.org/10.1002/ana.24263.

[4] de Kovel CGF, Syrbe S, Brilstra EH, Verbeek N, Kerr B, Dubbs H, et al. Neurodevelopmental disorders caused by de novo variants in *KCNB1* genotypes and phenotypes. JAMA Neurol 2017;74(10):1228. https://doi.org/10.1001/jamaneurol.2017.1714.

[5] Bar C, Kuchenbuch M, Barcia G, Schneider A, Jennesson M, Le Guyader G, et al. Developmental and epilepsy spectrum of KCNB1 encephalopathy with long-term outcome. Epilepsia 2020;61(11):2461–73. https://doi.org/10.1111/epi.16679.

[6] Bar C, Barcia G, Jennesson M, Le Guyader G, Schneider A, Mignot C, et al. Expanding the genetic and phenotypic relevance of KCNB1 variants in developmental and epileptic encephalopathies: 27 new patients and overview of the literature. Hum Mutat 2020;41(1):69–80. https://doi.org/10.1002/humu.23915.

[7] Marini C, Romoli M, Parrini E, Costa C, Mei D, Mari F, et al. Clinical features and outcome of 6 new patients carrying de novo KCNB1 gene mutations. Neurol Genet 2017;3(6):e206. https://doi.org/10.1212/NXG.0000000000000206.

[8] Scheffer IE, Berkovic S, Capovilla G, Connolly MB, French J, Guilhoto L, et al. ILAE classification of the epilepsies: position paper of the ILAE Commission for Classification and Terminology. Epilepsia 2017;58:512–21. doi: 10.1111/epi.13709.

[9] Sparrow SS, Balla DA, Cicchetti DV. Vineland-II adaptive behavior scales. AGS Publishing; 2005.

[10] World Health Organization. The ICD-10 classification of mental and behavioural disorders: clinical descriptions and diagnostic guidelines. Geneva: World Health Organization; 1992.

[11] Achenbach TM, Rescorla LA. ABESA school age forms and profiles. University of Vermont; 2001.

[12] Rutter M, Bailey A, Lord C. The social communication questionnaire: Manual. Western Psychological Services; 2003.

[13] Nabbout R, Chemaly N, Chipaux M, Barcia G, Bouis C, Dubouch C, et al. Encephalopathy in children with Dravet syndrome is not a pure consequence of epilepsy. Orphanet J Rare Dis 2013;8(1):176. https://doi.org/10.1186/1750-1172-8-176.

[14] Schalock RL, Borthwick-Duffy SA, Bradley VJ, Buntinx WHE, Coulter DL, Craig EM, et al. Intellectual Disability: Definition, Classification, and Systems of Supports. American Association on Intellectual and Developmental Disabilities: Eleventh Edition; 2010.

[15] Ouss L, Leunen D, Laschet J, Chemaly N, Barcia G, Losito EM, et al. Autism spectrum disorder and cognitive profile in children with Dravet syndrome: Delineation of a specific phenotype. Epilepsia Open 2019;4(1):40–53. https://doi.org/10.1002/epi4.12281.

[16] Nabbout R, Auvin S, Chiron C, Irwin J, Mistry A, Bonner N, et al. Development and content validation of a preliminary core set of patient- and caregiver-relevant outcomes for inclusion in a potential composite endpoint for Dravet Syndrome. Epilepsy Behav EB 2018;78:232–42. https://doi.org/10.1016/j.yebeh.2017.08.029.

[17] Lord C, Rutter M, DiLavore PC, Risi S, Gotham KY, Bishop SL. ADOS 2. Autism Diagnostic Observation Schedule-2. Manual. Western Psychological Services. USA: 2015.

[18] Le Couteur A, Lord C, Rutter M. The autism diagnostic interview-revised (ADI-R). Los Angeles, CA: Western Psychological Services; 2003.

[19] Besag F, Gobbi G, Caplan R, Sillanpää M, Aldenkamp A, Dunn DW. Psychiatric and behavioural disorders in children with epilepsy (ILAE Task Force Report): epilepsy and ADHD. Epileptic Disord Int Epilepsy J Videotape 2016. https://doi.org/10.1684/epd.2016.0811.

[20] Dekker MC, Koot HM, Ende JVD, Verhulst FC. Emotional and behavioral problems in children and adolescents with and without intellectual disability. J Child Psychol Psychiatry 2002;43(8):1087–98. https://doi.org/10.1111/1469-7610.00235.

[21] Green SA, Berkovits LD, Baker BL. Symptoms and development of anxiety in children with or without intellectual disability. J Clin Child Adolesc Psychol 2015;44(1):137–44. https://doi.org/10.1080/15374416.2013.873979.

[22] Koskentausta T, Iivanainen M, Almqvist F. CBCL in the assessment of psychopathology in Finnish children with intellectual disability. Res Dev Disabil 2004;25(4):341–54. https://doi.org/10.1016/j.ridd.2003.12.001.

[23] Steinley D, Brusco MJ. Evaluating mixture modeling for clustering: recommendations and cautions. Psychol Methods 2011;16:63–79. https://doi.org/10.1037/a0022673.