Original Article

Early genetic sequencing in neonates with hyperkalemia: a retrospective cross-sectional study

Wenyi Liang[1], Fanshu Xu[1], Yulan Lu[1], Huiyao Chen[1], Xiang Chen[1], Hui Xiao[1], Liyuan Hu[1,2]

[1]Department of Neonatology, Children's Hospital of Fudan University, National Children's Medical Center, Shanghai, China; [2]National Health Commission (NHC) Key Laboratory of Neonatal Diseases, Fudan University, Children's Hospital of Fudan University, Shanghai, China

Contributions: (I) Conception and design: L Hu, W Liang, F Xu; (II) Administrative support: None; (III) Provision of study materials or patients: W Liang, Y Lu, H Chen, X Chen, H Xiao; (IV) Collection and assembly of data: W Liang; (V) Data analysis and interpretation: W Liang, L Hu, F Xu; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Liyuan Hu, PhD. Department of Neonatology, Children's Hospital of Fudan University, National Children's Medical Center, 399 Wanyuan Road, Minhang District, Shanghai 201102, China; National Health Commission (NHC) Key Laboratory of Neonatal Diseases, Fudan University, Children's Hospital of Fudan University, Shanghai, China. Email: nowadays921@126.com.

Background: The genetic etiology and clinical characteristics of neonates with hyperkalemia remain unknown. We aimed to implement early gene sequencing to identify genetic causes, optimize treatment and improve outcomes in this population.

Methods: We retrospectively studied the clinical characteristics and genetic etiology of neonates with hyperkalemia who underwent exome sequencing or targeted panel sequencing from January 1, 2016, to December 31, 2023, at the Department of Neonatology, Children's Hospital of Fudan University.

Results: Among 3,757 neonates with hyperkalemia, approximately 14.08% underwent sequencing. The average gestational age was 34.82±3.94 weeks, and the average birth weight was 2,375.22±864.09 grams. Males accounted for 56.0% of the cohort. The risk factors for hereditary hyperkalemia included dry skin, pigmentation and pseudohermaphroditism. Of these factors, only pigmentation independently predicted the genetic etiology of hyperkalemia; the presence of pigmentation increased the risk of hyperkalemia by 29.586 times [odds ratio (OR) 29.586, confidence interval (CI): 4.927–177.649, P<0.001]. According to the American College of Medical Genetics and Genomics (ACMG) guidelines, we found that 7.56% hyperkalemia neonates had a genetic diagnosis; 28 genes were identified, including 18 genes not previously reported. Among genetic diseases, congenital adrenal hyperplasia (CAH) had the highest incidence (1.7%). For neonates with mineralocorticoid deficiency, early treatment with hydrocortisone reduced adverse outcomes to some extent. Gene Ontology (GO) analysis indicated that these genes were enriched primarily in nephron development.

Conclusions: The genetic etiology of neonatal hyperkalemia is complex. When clinical manifestations involve risk factors, it is advisable to conduct hormone testing and provide symptomatic treatment. Early genetic testing can aid in the diagnosis of hyperkalemia and improve the treatment of neonates with atypical clinical manifestations.

Keywords: Genes; genetic etiology; hyperkalemia; neonates

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Introduction

The definition of neonatal hyperkalemia varies slightly according to gestational age, but this condition is generally defined as a plasma potassium (K^+) level >6 mmol/L. Hyperkalemia is a common clinical electrolyte disorder that can lead to severe arrhythmia and even death. Consequently, the early identification and treatment of hyperkalemia are essential to prevent mortality outcomes. Neonatal hyperkalemia commonly results from clinical causes such as hypoxia, acidosis, hypothermia, neonatal hemolysis, tissue necrosis, and immature renal function or sodium-potassium (Na-K) pumps in neonates (1).

In addition to these common causes of neonatal hyperkalemia, an increasing number of studies have shown that certain hereditary diseases, such as congenital adrenal hyperplasia (CAH) (2), Bartter syndrome type II (BS II) (3) and pseudohypoaldosteronism (PHA) (4,5), can lead to hyperkalemia. These patients often lack specific clinical manifestations, presenting with only feeding intolerance, vomiting, rash, and dry skin (4,6), creating challenges in early diagnosis and targeted treatment.

To date, no studies have explored the genetic diagnosis of hyperkalemia in neonates and the diagnostic value of early genetic sequencing. Moreover, the clinical features and risk factors for hyperkalemia in neonates remain unknown.

This study was a retrospective cross-sectional analysis with the goal of identifying the clinical features and risk factors for hyperkalemia in neonates with etiology and providing evidence supporting the importance of early genetic sequencing. We present this article in accordance with the STROBE reporting checklist (available at https://tp.amegroups.com/article/view/10.21037/tp-24-485/rc).

Highlight box

Key findings

- This study found that the risk factors for hereditary hyperkalemia were dry skin, pigmentation and pseudohermaphroditism.

What is known and what is new?

- Ten genes had ever known in neonatal hyperkalemia, such as CYP21A2, SCNN1A and so on.
- Eighteen genes is novel in neonatal hyperkalemia, and they enriched in the functions of the kidney and red blood cell membrane.

What is the implication, and what should change now?

- Early genetic sequencing can aid in the diagnosis of hyperkalemia and improve the treatment of neonates with atypical clinical manifestations.

Methods

Study population

From January 1, 2016, to December 31, 2023, neonates who were admitted to the Department of Neonatology, Children's Hospital of Fudan University, with hyperkalemia and met the following criteria were enrolled in our study: (I) age ≤28 days; (II) serum K^+ level >6 mmol/L; and (III) comprehensive next-generation sequencing (NGS) performed at the hospital. The exclusion criteria were as follows: (I) no signed informed consent; (II) incomplete clinical data; and (III) unqualified test samples, such as hemolyzed samples (*Figure 1*). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Research Ethics Committee of the Children's Hospital of Fudan University (No. 2015-130). Informed consent was taken from all the patients' legal guardians.

NGS, genetic diagnosis, and evaluation

Fragmented genomic DNA samples were enriched for clinical exome sequencing using the Agilent (Santa Clara, CA, USA) ClearSeq Inherited Disease Panel Kit, followed by NGS targeting 2,742 genes (7). The average on-target sequencing depth was 120× for embryo sequencing (ES) and 200× for clinical exome sequencing. The clinical significance of copy number variations (CNVs) or single-nucleotide variations (SNVs) was prioritized for interpretation (8). The criteria for variant classification followed the American College of Medical Genetics and Genomics (ACMG) guidelines (9). A genetic diagnosis of hyperkalemia was made when pathogenic or likely pathogenic variants were detected in a gene associated with hyperkalemia, the zygosity of the mutant allele matched the inheritance pattern, and the gene was associated with the patient's phenotype (10).

Statistical analysis

Statistical analyses were performed using IBM SPSS 26.0. Comparisons were conducted via Chi-squared tests, Fisher's exact tests, or independent sample *t*-tests as appropriate. Collinearity analysis was performed to test the collinearity of the conditioning factors. Variables with P<0.05 and no

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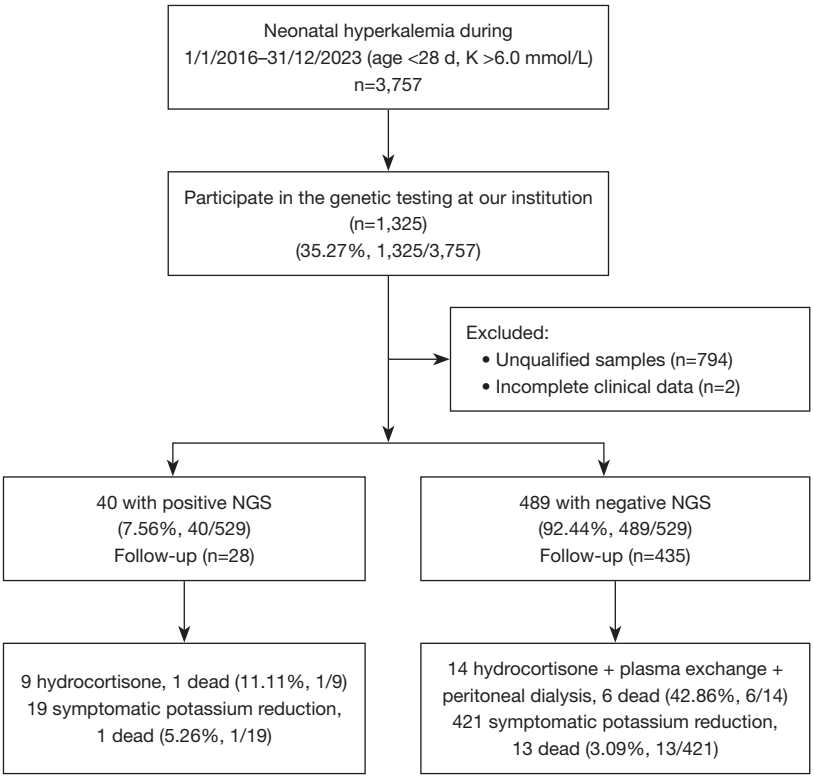

Figure 1 Inclusion and exclusion criteria for neonatal hyperkalemia patients with a genetic etiology. NGS, next-generation sequencing.

collinearity were entered into the binary logistic regression. Differences with a P value <0.05 were considered statistically significant.

Results

General characteristics

As shown in *Figure 1*, among 3,757 neonates with hyperkalemia admitted to the hospital from 2016 to 2023, 529 (529/3,757, 14.08%) were ultimately enrolled. The average gestational age was 34.82±3.94 weeks, and the average birth weight was 2,375.22±864.09 grams. Among the included neonates, 296 were males (296/529, 56.0%).

Genetic diagnosis was achieved in 40 neonates (40/529, 7.56%), and NGS returned negative results for 489 neonates (489/529, 92.44%); two deaths and 19 deaths, respectively, occurred in these groups (*Table 1*). Among the 40 neonates with a genetic etiology (28 with follow-up data), nine neonates received hydrocortisone treatment, with one death (1/9, 11.11%), and 19 neonates received only symptomatic potassium reduction treatment, with one death (1/19, 5.26%). Among the 489 neonates without a genetic etiology (435 with follow-up data), 14 neonates received hydrocortisone treatment (one was also treated with plasma exchange and peritoneal dialysis), with six deaths (6/14, 42.86%), and 421 neonates received only symptomatic potassium reduction treatment, with 13 deaths (13/421, 3.09%).

Risk factors for genetic etiologies

As shown in *Table 1*, dry skin (P=0.03), pigmentation (P<0.001), and pseudohermaphroditism (P=0.004) were significantly different between neonates with and without a genetic etiology of hyperkalemia. Additionally, neonates with a genetic etiology had a greater gestational age (P=0.04), whereas postnatal hypoxia was more common in neonates without a genetic etiology (P=0.007). Among the neonates tested for aldosterone levels, those without genetic disorders presented greater levels (P=0.04).

As shown in *Table 2*, the collinearity analysis of the six conditioning factors discussed above revealed that the tolerance ranged from 0.436 to 0.923, and the variance inflation factor (VIF) ranged from 1.083 to 2.292.

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Table 1 Comparison of neonates with hyperkalemia with and without a genetic etiology

Characteristic	Total (N=529)	With a genetic etiology (N=40)	Without a genetic etiology (N=489)	P value
Baseline information				
Male, n (%)	296 (56.0)	28 (70.0)	268 (54.8)	0.06
Gestational age (weeks), mean (SD)	34.82 (3.94)	36.04 (3.78)	34.72 (3.94)	0.04*
Birth weight (g), mean (SD)	2,375.22 (864.09)	2,565.00 (747.90)	2,359.64 (871.80)	0.15
Death, n (%)	21 (4.5)	2 (7.1)	19 (4.4)	0.37
Perinatal history				
Postnatal hypoxia, n (%)	239 (46.2)	10 (25.6)	229 (47.9)	0.007*
Premature rupture of membranes, n (%)	130 (26.9)	5 (14.3)	125 (27.8)	0.08
Apgar score 1 min, mean (SD)	7.91 (2.13)	7.87 (2.45)	7.92 (2.11)	0.90
Apgar score 10 min, mean (SD)	8.93 (1.29)	9.0 (1.12)	8.93 (1.30)	0.87
Low amniotic fluid, n (%)	24 (5.0)	2 (5.9)	22 (4.9)	0.68
Excessive amniotic fluid, n (%)	28 (5.8)	2 (5.9)	26 (5. 8)	>0.99
Adverse pregnancy and childbirth history, n (%)	205 (39.6)	14 (35.9)	191 (39.9)	0.63
Clinical manifestations, n (%)				
Vomiting	26 (5.0)	4 (10.3)	22 (4.6)	0.12
Pigmentation	9 (1.7)	7 (18.0)	2 (0.4)	<0.001*
Pseudohermaphroditism	5 (1.0)	3 (7.7)	2 (0.4)	0.004*
Dry skin	33 (6.4)	6 (15.4)	27 (5.7)	0.03*
Rash	23 (4.5)	3 (7.7)	20 (4.4)	0.41
Low muscle tone	251 (48.4)	15 (38.5)	236 (49.2)	0.20
High muscle tone	18 (3.5)	1 (2.6)	17 (3.5)	>0.99
Laboratory tests				
High ALT, n (%)	13 (2.5)	1 (2.5)	12 (2.5)	>0.99
High AST, n (%)	219 (42.0)	22 (55.0)	197 (41.0)	0.08
High CK, n (%)	329 (71.0)	24 (70.6)	305 (71.0)	0.97
High creatinine, n (%)	157 (30.1)	15 (38.5)	142 (29.4)	0.24
High cortisol, n (%)	56 (38.9)	6 (23.1)	50 (42.4)	0.07
Low cortisol, n (%)	20 (13.9)	6 (23.1)	14 (11.9)	0.21
High renin, n (%)	16 (84.2)	6 (85.7)	10 (83.3)	>0.99
High aldosterone, n (%)	34 (91.9)	10 (76.9)	24 (100.0)	0.04*
High 17-hydroxyprogesterone, n (%)	53 (63.1)	13 (65.0)	40 (62.5)	0.84
High dehydroepiandrosterone, n (%)	11 (68.8)	5 (62.5)	6 (75.0)	>0.99
High androstenedione, n (%)	11 (84.6)	5 (83.3)	6 (85.7)	>0.99
High ACTH, n (%)	21 (30.9)	8 (47.1)	13 (25.5)	0.10

Table 1 (*continued*)

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Table 1 (*continued*)

Characteristic	Total (N=529)	With a genetic etiology (N=40)	Without a genetic etiology (N=489)	P value
First hyperkalemia, mean (SD)	6.57 (0.67)	6.66 (0.70)	6.57 (0.67)	0.45
Peak K^+ level, mean (SD)	6.75 (0.84)	6.90 (0.82)	6.73 (0.84)	0.24
Hyperkalemia frequency, mean (SD)	1.98 (2.06)	2.76 (2.91)	1.92 (1.96)	0.08
Hyperkalemia recovery time (d), mean (SD)	4.83 (12.33)	4.74 (7.80)	4.83 (12.63)	0.97
First Na^+, mean (SD)	138.04 (43.95)	134.79 (5.60)	138.31 (45.67)	0.63
Minimum Na^+ level, mean (SD)	131.75 (4.65)	130.24 (6.27)	131.87 (4.49)	0.12
Hyponatremia frequency, mean (SD)	3.64 (7.78)	5.18 (5.46)	3.51 (7.93)	0.19
Hyponatremia recovery time (d), mean (SD)	6.67 (14.25)	8.01 (12.65)	6.56 (14.38)	0.55
Auxiliary examination, n (%)				
Tachyarrhythmia	13 (9.9)	3 (20.0)	10 (8.6)	0.17
Moderate abnormality on electroencephalogram	93 (26.1)	6 (25.0)	87 (26.1)	0.90
Severe abnormality on electroencephalogram	26 (7.3)	3 (12.5)	23 (6.9)	0.40

*, differences with a P value <0.05 were considered statistically significant. The above data were all results obtained after correcting the original data based on real follow-up numbers and excluding the missing data. SD, standard deviation; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; ACTH, adrenocorticotropic hormone.

Table 2 Collinearity test among the conditioning factors for a genetic etiology of neonatal hyperkalemia

Variable	Tolerance	VIF
Gestational age (weeks)	0.439	2.277
Postnatal hypoxia	0.689	1.451
Dry skin	0.641	1.560
Pigmentation	0.554	1.804
Pseudohermaphroditism	0.923	1.083
High aldosterone	0.436	2.292

VIF, variance inflation factor.

Therefore, these factors were included in the binary logistic regression analysis. *Table 3* shows that pigmentation was the only risk factor that independently predicted an underlying genetic etiology of hyperkalemia [odds ratio (OR) 29.586, confidence interval (CI): 4.927–177.649, P<0.001]. The OR indicated that pigmentation was 29.586 times greater in neonates with a genetic etiology than in those without a genetic etiology (*Table 3*).

Genetic etiology spectrum

Among the genetic etiologies identified in this study, CAH (*CYP21A2* gene) had the greatest incidence (9/529, 1.7%) and was accompanied by electrolyte disturbances and steroid hormone dysregulation. Among the patients in whom pseudohermaphroditism (n=7) and skin pigmentation (n=6) were observed, hydrocortisone treatment was empirically administered early for six patients. Additionally, one neonate with a gestational age of 27 weeks who was diagnosed with BS II (*KCNJ1* gene) accompanied by neonatal respiratory distress syndrome (NRDS) presented with severe hyperkalemia, hyponatremia, hypercortisolism and hyperaldosteronism immediately after birth. Despite timely treatment with mineralocorticoids and various potassium-lowering treatments, the infant ultimately died one day later. One neonate diagnosed with IMAGE syndrome (*CDKN1C* gene) presented with severe recurrent hyperkalemia and hyponatremia. The levels of multiple hormones, including renin, angiotensin, adrenocorticotropic hormone (ACTH), aldosterone, 17-hydroxyprogesterone, and sex hormones, were relatively high. The patient received treatments with hydrocortisone, various potassium-lowering agents and

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Table 3 ORs for risk factors for neonatal hyperkalemia with genetic etiology

Variable	B	P value	OR	95% CI
Pseudohermaphroditism	1.911	0.10	6.758	0.715–63.866
Postnatal hypoxia	–0.628	0.16	0.533	0.224–1.269
Dry skin	0.085	0.90	1.089	0.281–4.216
Pigmentation	3.387	<0.001	29.586	4.927–177.649
Gestational age (weeks)	0.008	0.89	1.008	0.903–1.125

Due to the low proportion of patients in whom aldosterone hormone levels were tested in the study, it is meaningless to conduct binary logistic analysis of high aldosterone levels (see the "Discussion" section). OR, odds ratio; CI, confidence interval.

sodium-replenishing agents and ultimately had a favorable prognosis. Our study revealed other genetic disorders, including generalized PHA1, renal PHA1, PHA2, X-linked adrenal hypoplasia congenita (X-AHC), congenital lipoid adrenal hyperplasia, and hereditary stomatocytoses (HSTs), with 18 genetic etiologies that were not reported previously.

Among the 28 genes associated with hyperkalemia identified in this study, 10 genes have been previously reported, whereas the remaining 18 genes have not been previously reported (*Table 4*). Enrichment analysis indicated that the 28 genes were predominantly enriched in nephron development ($P=10^{-8.537}$). Other enriched pathways/functions included glomerular basement membrane development, transmembrane transporter binding, monoatomic cation channel activity, multicellular organismal-level homeostasis, blood circulation, steroid binding, structural constituents of the cytoskeleton, hemopoiesis, and protein homodimerization activity (*Figure 2*). Enrichment analysis indicated that the 18 novel genes were predominantly enriched in the functions of the kidney and red blood cell membrane (*Figure 3*).

Discussion

To our knowledge, this study is the first to explore the initial risk factors and genetic diagnosis of hyperkalemia, increasing our understanding of the initial characteristics, broadening the genetic spectrum and indicating the importance of early determination of the genetic etiology of neonatal hyperkalemia.

Pigmentation: an independent risk factor

We found that dry skin, pigmentation and pseudohermaphroditism were risk factors for hyperkalemia with a genetic etiology, and among these, pigmentation was an independent risk factor (OR 29.586). Additionally, neonates without a genetic etiology had a lower gestational age and a greater probability of postnatal hypoxia. Neonates with a lower gestational age are more susceptible to clinical factors such as hypoxia, acidosis, hypothermia, neonatal hemolysis and immature renal function, which can lead to secondary hyperkalemia. We found that a greater proportion of these patients had increased aldosterone levels. However, owing to the low proportion of individuals in whom aldosterone hormone levels were tested in the present study (7%, 37/529), the results were likely biased. Therefore, these findings should be interpreted with caution, and further research is needed.

Among the six CAH patients who received hydrocortisone treatment, no deaths occurred. However, among the 17 non-CAH patients who received hydrocortisone treatment, seven deaths occurred (7/17, 41.18%), and 14 deaths occurred in 440 patients receiving only symptomatic potassium reduction treatment (14/440, 3.18%).

Therefore, early treatment with hydrocortisone for neonatal hyperkalemia appears to be important in neonates with CAH, and further research is needed.

New revelations of genetic etiology reported

CAH (OMIM #201910) is an autosomal recessive disease with an incidence of approximately 1:6,084 (2) in China. CAH is caused by mutations in the *CYP21A2* gene located on chromosome 6p21, resulting in a deficiency of 21-hydroxylase (aldosterone synthase), which reduces aldosterone synthesis and decreases renal potassium excretion. Patients with classic salt-wasting CAH exhibit pigmentation and pseudohermaphroditism and have severe complications, such as adrenal crisis, which requires timely glucocorticoid replacement, within 2–3 weeks after

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Table 4 Gene mutation information of 40 hyperkalemic neonates with genetic etiology

Gene number	Gene	Mutation	Inheritance pattern	Origin	Chromosome	Variant
23F00001	*ABCC8*	NM_000352:exon11:c.1671+1G>A	AD/AR(Het)	–	11	LP
22F04266	*CYP21A2*	NM_000500:exon3:c.293-13C>G	AR(Het)	–	6	P
22F04266	*CYP21A2*	NM_000500:exon10:c.1432C>T(p.Q478X)	AR(Het)	–	6	LP
22F03999	*COL4A4*	NM_000092:exon30:c.2590G>A(p.G864R)	AD/AR(Het)	–	2	P
22F03999	*COL4A4*	NM_000092:exon28:c.2380G>A(p.E794K)	AD/AR(Het)	–	2	VUS
22F04003	*ACTN4*	NM_004924:exon12:c.1416del(p.I473Sfs*36)	AD(Het)	–	19	VUS
22F04108	*SLC3A1*	NM_000341:exon7:c.1271dupT(p.Y435Vfs*2)	AD/AR(Het)	–	2	LP
22F03937	*KCNJ1*	NM_000220:exon2:c.592G>A(p.A198T)	AR(Het)	–	11	P
22F03937	*KCNJ1*	NM_000220:exon2:c.827G>T(p.S276I)	AR(Het)	–	11	LP
22F03530	*PKD1*	NM_001009944:exon45:c.12400del(p.L4134Cfs*64)	AD(Het)	–	16	P
21F17745	*CYP21A2*	NM_000500:exon3:c.293-13C>G	AR(Het)	–	6	P
21F16710	*COL4A3*	NM_000091:exon6:c.382_383insAAGTA(p.S128*)	AD/AR(Het)	–	2	LP
21F16653	*WNK4*	NM_032387:exon5:c.1218del(p.E407Rfs*36)	AD(Het)	–	16	LP
21F16653	*PIEZO1*	NM_001142864:exon27:c.3917dupA(p.Y1306*)	AD/AR(Het)	–	17	LP
21F12160	*CHD7*	NM_017780:exon3:c.1945G>A(p.A649T)	AD(Het)	Paternal	8	VUS
21F09042	*KLF1*	NM_006563.4:c.-154C>T	AD(Het)	–	19	P
21F09042	*SPTA1*	NM_003126:exon36:c.5093T>C(p.V1698A)	AD/AR(Hom)	–	1	LP
21F10242	*CYP21A2*	NM_000500.7:exon1:c.29_31delTGC	AR(Het)	–	5	P
21F10242	*CYP21A2*	NM_000500.7:exon1:c.138C>A(p.P46P)	AR(Hom)	–	5	P
21F00230	*NR3C2*	NM_000901:exon4:c.1951C>T(p.R651X)	AD(Het)	De novo	4	P
20F14704	*SALL1*	NM_002968:exon2:c.2060A>G(p.E687G)	AD(Het)	–	16	VUS
20F01242	*DSTYK*	NM_015375:exon4:c.1384C>T(p.R462X)	AD/AR(Het)	–	1	P
20F01034	*STAR*	NM_003126:exon6:c.713_714delA(p.K238Nfs*83)	AD/AR(Het)	–	8	P
20F01034	*STAR*	NM_000349:exon7:c.772C>T(p.Q258X)	AR(Het)	–	8	P
20F00557	*NR0B1*	NM_000475:exon1:c.139C>G(p.P47A)	XLR(Hemi)	–	X	VUS
20F00072	*CYP21A2*	NM_000500.7:exon1:c.118C>T(p.L40L)	AR(Hom)	–	6	P
20F00072	*CYP21A2*	NM_000500.7:exon1:c.138C>A(p.P46P)	AR(Hom)	–	6	P
19F15851	*ANK1*	NM_000037:exon31:c.3841C>T(p.R1281W)	AD/AR(Het)	–	8	VUS
19F14492	*CYP21A2*	NM_000500.7:exon1:c.188A>T(p.H63l)	AR(Het)	–	6	P
19F14492	*CYP21A2*	NM_000500.7:exon3:c.293-13C>G	AR(Hom)	–	6	P
19F14492	*CYP21A2*	NM_000500.7:exon3:c.332_339delGAGACTAC	AR(Het)	–	6	P
19F13643	*COL4A5*	NM_000495:exon30:c.2479C>T(p.P827S)	XLD(Hemi)	–	X	
19F11440	*SLC4A1*	NM_000342:exon6:C.350-7C>A	AD/AR(Het)	–	17	VUS

Table 4 (*continued*)

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Table 4 (*continued*)

Gene number	Gene	Mutation	Inheritance pattern	Origin	Chromosome	Variant
19F07587	*SPTB*	NM_000347:exon21:c.4513G>A(p.D1505N)	AD(Het)	Maternal	14	VUS
19F07060	*CYP21A2*	NM_000500.7:exon3:c.293-13C>AG	AR(Het)	–	6	P
19F07060	*CYP21A2*	NM_000500.7:exon8:c.1069C>T(p.R357W)	AR(Het)	–	6	VUS
19F07060	*CYP21A2*	NM_000500.7:exon1:c.118C>T(p.L40L)	AR(Hom)	–	6	VUS
19F05953	*SPTB*	NM_001355436:exon4:c.566+4_566+5dup	AD(Het)	–	14	VUS
19F05439	*SPTB*	NM_000347:exon15:c.3322G>A(p.D1108N)	AD(Het)	Maternal	14	VUS
19F02164	*COL4A4*	NM_000092: exon33:c.3029C>T (p.P1010L)	AD/AR(Het)	–	2	VUS
19F01226	*ETFDH*	NM_004453:exon12:c.1657T>C(p.Y553H)	AR(Het)	–	4	
19F01837	*MUT*	NM_000255:exon9:c.1663G>A(p.A555T)	AR(Het)	–	6	
18F14311	*SPTA1*	NM_003126: exon15:c.2012A>T (p.E671V)	AD/AR(Het)	–	1	VUS
16F11230	*CYP21A2*	NM_000500.7:exon1:c.138C>A(p.P46P)	AR(Het)	–	6	VUS
23F00907	*ABCC8*	NM_000352:exon4:c.502C>G(p.R168G)	AD/AR(Het)	–	11	
23F00275	*NPHS1*	NM_004646:exon19:2663G>A(p.R888K)	AR(Het)	Maternal	19	P
23F00275	*NPHS1*	NM_004646:exon24:3213del(p.L1072Ffs*71)	AR(Het)	Paternal	19	P
23F04071	*CYP21A2*	NM_000500.7:exon8:c.955C>T(p.Q319X)	AR(Het)	–	6	P
23F12250	*PKD1*	NM_001009944:exon4:c.412C>T(p.R138X)	AD(Het)	–	16	P
23F18738	*INF2*	NM_022489:exon22:c.3726_3729del(p.K1243Nfs*4)	AD(Het)	–	14	VUS
23F20173	*CDKN1C*	NM_000076:exon1:c.530_linsGCGGTC(p.V177_L178insRS)	AD(Het)	–	11	VUS
23F21348	*SCNN1A*	NM_001038:exon3:c.424del(p.142Kfs*50)	AD/AR(Het)	Paternal	12	LP
22F06007	*CYP21A2*	NM_000500:exon8:c.955C>T(p.Q319X)	AR(Het)	–	6	P
22F06007	*CYP21A2*	NM_000500:exon3:c.293-13C>G	AR(Het)	–	6	P

Het, heterozygous; Hom, homozygous; Hemi, hemizygous; AR, autosomal recessive inheritance; AD, autosomal dominant inheritance; XLR, X-linked recessive inheritance; XLD, X-linked dominant inheritance; P, pathogenic; LP, likely pathogenic; VUS, variant of uncertain significance.

birth (11). In our study, good clinical recognition and timely treatment were observed for patients with typical CAH. However, for neonates without typical clinical manifestations, early NGS testing is important.

PHA1 includes generalized and renal forms. Generalized PHA1 is caused by mutations in the *SCNN1A* gene on chromosome 12p13.31, which encodes the epithelial K^+ channel, resulting in severe systemic salt loss (12). Renal PHA1 (OMIM #177735) is caused by mutations in the *NR3C2* gene on chromosome 4q31.1, which encodes the mineralocorticoid receptor (MR) (12), resulting in mild salt wasting. The incidence of PHA1 is still unknown, but in our study, this rate was 0.38% in neonates with hyperkalemia.

PHA2 (OMIM #145260) is caused by mutations in the *WNK4* gene, which is located on chromosome 17q21 and encodes WNK kinase. Mutations in this gene increase the function of WNK kinase and the reabsorption of sodium chloride (NaCl) through the Na–Cl cotransporter in the distal convoluted tubule, consequently reducing potassium excretion in the collecting duct. Patients with PHA2 often present with hypertension and metabolic acidosis, but these manifestations may not be observable within a short hospital stay (13). Therefore, for patients with PHA2, close monitoring of blood pressure and mental status in the long

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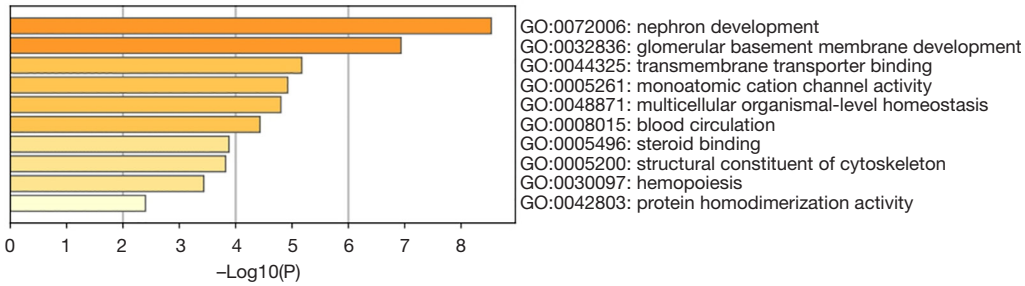

Figure 2 Enrichment analysis of the total genes associated with neonatal hyperkalemia. Nephron development ($P=10^{-8.537}$), glomerular basement membrane development ($P=10^{-6.935}$), transmembrane transporter binding ($P=10^{-5.174}$), monoatomic cation channel activity ($P=10^{-4.924}$), multicellular organismal-level homeostasis ($P=10^{-4.802}$), blood circulation ($P=10^{-4.432}$), steroid binding ($P=10^{-3.880}$), structural constituents of the cytoskeleton ($P=10^{-3.822}$), hemopoiesis ($P=10^{-3.435}$), and protein homodimerization activity ($P=10^{-2.398}$). The graph shows the functional enrichment pathways from high to low according to the significant difference ($P<0.01$), indicating that genetic disorders primarily affect these pathways, resulting in hyperkalemia in neonates.

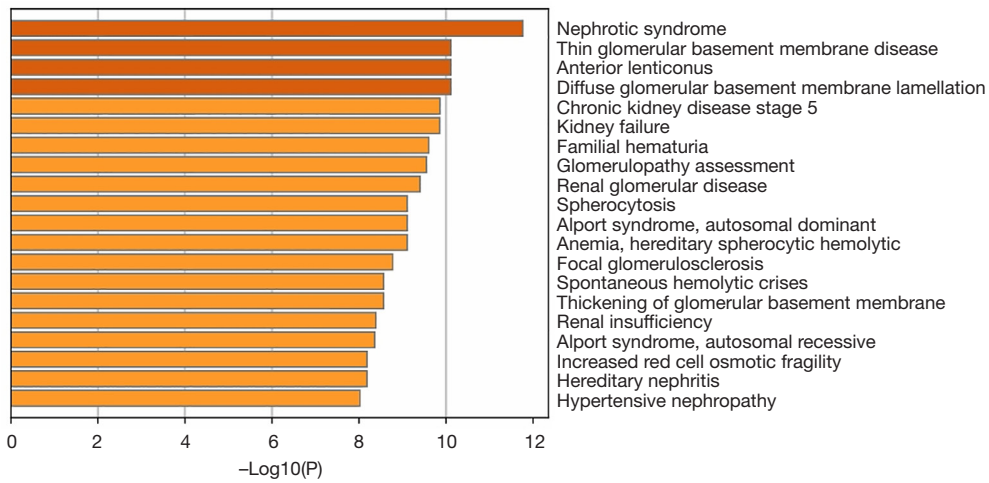

Figure 3 Enrichment analysis of novel genes. Nephrotic syndrome ($P=10^{-12.00}$); anterior lenticonus ($P=10^{-10.00}$); glomerular basement membrane disease ($P=10^{-10.00}$); diffuse glomerular basement membrane lamellation ($P=10^{-10.00}$); chronic kidney disease stage 5 ($P=10^{-9.90}$), kidney failure ($P=10^{-9.90}$); familial hematuria ($P=10^{-9.60}$); glomerulopathy assessment ($P=10^{-9.50}$); renal glomerular disease ($P=10^{-9.40}$); anemia, hereditary spherocytic hemolytic ($P=10^{-9.10}$); spherocytosis ($P=10^{-9.10}$); Alport syndrome, autosomal dominant ($P=10^{-9.10}$); focal glomerulosclerosis ($P=10^{-8.80}$); thickening of glomerular basement membrane ($P=10^{-8.60}$); spontaneous hemolytic crises ($P=10^{-8.60}$); renal insufficiency ($P=10^{-8.40}$); Alport syndrome, autosomal recessive ($P=10^{-8.40}$); hereditary nephritis ($P=10^{-8.20}$); increased red cell osmotic fragility ($P=10^{-8.20}$); hypertensive nephropathy ($P=10^{-8.00}$). The graph shows the functional enrichment pathways from high to low according to the significant difference ($P<0.01$), indicating that genetic disorders not previously reported primarily affect these pathways, resulting in hyperkalemia in neonates.

term is recommended.

BS II (OMIM #241200) is caused by mutations in the *KCNJ1* gene located on chromosome 11q24–25, which encodes the ROMK channel. Neonates with immature Na-K pump function and dysfunctional channels exhibit transient hyperkalemia. The incidence of BS II is approximately 1:1,000,000, and during the transient hyperkalemia phase, most patients do not require specific treatment (3). However, as observed in this study, neonates of low gestational age and with other underlying diseases presented severe electrolyte and hormonal disturbances. Therefore, not all BS II patients can be treated with the

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watchful waiting approach. Close monitoring for severe adverse outcomes and early intervention are essential in those with underlying disease.

X-AHC (OMIM #300200), with an incidence of 1:140,000–1,200,000, is caused by a mutation in *NR0B1* located on chromosome Xp21, which encodes the nuclear receptor transcription factor DAX-1 and regulates the adrenal cortex, hypothalamus, and pituitary gland. Patients with X-AHC exhibit adrenal insufficiency, characterized by low aldosterone levels, hyperkalemia and hyponatremia (14). For atypical X-AHC neonates, even if early electrolyte disturbance is less severe, steroid hormone testing is still recommended to avoid unpredictable hormonal imbalance or adrenal crisis in the future (15).

STAR (OMIM #600617) is located on chromosome 8p11.2 and is a key gene in steroidogenesis. Mutations in *STAR* decrease steroid production, resulting in congenital lipoid adrenal hyperplasia, which is characterized by salt wasting, such as hyponatremia and hyperkalemia, and even acidosis and adrenal crisis (16). In this study, neonates with *STAR* mutations presented significant salt-wasting symptoms and were promptly treated with hydrocortisone. It is difficult to distinguish patients with *STAR* mutations from those with adrenal hypoplasia, but hydrocortisone treatment can be prioritized for both conditions to prevent severe consequences in the early stages.

CDKN1C (OMIM #614732) is located on chromosome 11p15. Mutations of this gene can cause IMAGE syndrome, which includes congenital adrenal cortical hypoplasia (17). Similarly, early treatment with hydrocortisone in patients can prevent severe outcomes.

HSTs (OMIM #609153) are caused by mutations in the *PIEZO1* and *SLC4A1* genes, which encode ion channels that are crucial for red blood cell function. Patients with HSTs may exhibit mild potassium leakage or severe hemolysis. The incidence of HST is not well known (18). In this study, the incidence of HST in neonates with genetic hyperkalemia was 0.38%.

Novel genetic etiology findings

In our study, we identified several genes related to potassium metabolism pathways that have not been previously reported in neonates. In accordance with the ACMG guidelines, the mutations were classified as pathogenic or likely pathogenic: *COL4A3* (OMIM #120070), *COL4A4* (OMIM #120131), *COL4A5* (OMIM #303630), *ACTN4* (OMIM #604638), *PKD1* (OMIM #601313), *CHD7* (OMIM #608892), *SALL1* (OMIM #602218), *DSTYK* (OMIM #612666), *NPHS1* (OMIM #602716), and *INF2* (OMIM #610982). The functions of these genes are primarily related to kidney function. Mutations in these genes directly or indirectly affect the function of Na–K ion channels, ultimately leading to hyperkalemia.

The genes *ABCC8* (OMIM #600509), *SLC3A1* (OMIM #104614), *ETFDH* (OMIM #231675), and *MUT* (OMIM #609058) play roles in the adrenal glands, pancreas and kidneys. Mutations in these genes can lead to irregular levels of hormones, such as renin, aldosterone and cortisol, which are often accompanied by varying degrees of acidosis and hyperkalemia.

The genes *KLF1* (OMIM #600599), *SPTA1* (OMIM #182860), *ANK1* (OMIM #612641), and *SPTB* (OMIM #182870) encode proteins that are essential for the structure and function of the red blood cell membrane. Mutations in these genes result in the production of abnormally shaped red blood cells that are prone to rupture, releasing K^+ into the peripheral blood and ultimately leading to anemia and hyperkalemia. In our study, one patient was diagnosed with mutations in the *KLF1* and *SPTA1* genes and presented with hemolytic anemia and severe hyperkalemia. These findings suggest that neonates with two or more gene mutations may present more severe clinical manifestations.

Gene enrichment analysis was performed on the total set of 28 genes and the 18 novel genes identified in our study, which suggested both gene sets were enriched primarily in functions related to the function of nephrons.

Limitations

There are certain limitations in this study. First, NGS was used to identify potential pathogenic genes on the basis of the observed phenotypes of patients, but it cannot detect underlying variants that are not suspected. Second, this study was a retrospective single-center study with a limited number of participants, which could result in bias to a certain extent. The limited sample size and incomplete hormonal data may introduce selection bias and restrict the generalizability of the findings. Additionally, family gene testing was rare in the patients included in the present study, resulting in incomplete knowledge of the genetic mode of inheritance. Finally, a long-term follow-up study is necessary to evaluate patient prognosis and verify the value of early sequencing.

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Conclusions

Our study included a relatively large population and expanded the spectrum of pathogenic variants associated with neonatal hyperkalemia and possible genetic etiologies. Genetic hyperkalemia is common in neonates, and >20% of cases are caused by mutations in the *CYP21A2* gene, which is associated with CAH. Early-stage genetic sequencing has become important in diagnosis and therapy. A study with a larger and more representative sample size with standardized hormonal assessments obtained through multicenter collaboration is needed to strengthen the generalizability and robustness of the conclusions.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Research Ethics Committee of the Children's Hospital of Fudan University (No. 2015-130). Informed consent was taken from all the patients' legal guardians.

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