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Severe high-molecular-weight kininogen deficiency: clinical characteristics, deficiency–causing *KNG1* variants, and estimated prevalence

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Abstract

Background: Severe high-molecular-weight kininogen (HK) deficiency is a poorly studied autosomal recessive contact system defect caused by pathogenic, biallelic *KNG1* variants.

Aim: We performed the first comprehensive analysis of diagnostic, clinical, genetic, and epidemiological aspects of HK deficiency.

Methods: We collected clinical information and blood samples from a newly detected HK-deficient individual and from published cases identified by a systematic literature review. Activity and antigen levels of coagulation factors were determined. Genetic analyses of *KNG1* and *KLKB1* were performed by Sanger sequencing. The frequency of HK deficiency was estimated considering truncating *KNG1* variants from GnomAD.

Results: We identified 48 cases of severe HK deficiency (41 families), of these 47 have been previously published (*n* = 19 from gray literature). We genotyped 3 cases and critically appraised 10 studies with genetic data. Ten HK deficiency–causing variants (one new) were identified. All of them were truncating mutations, whereas the only known HK amino acid substitution with a relevant phenotype instead causes hereditary angioedema. Conservative estimates suggest an overall prevalence of severe HK deficiency of approximately one case per 8 million population, slightly higher in Africans. Individuals with HK deficiency appeared asymptomatic and had decreased levels of prekallikrein and factor XI, which could lead to misdiagnosis.

Conclusion: HK deficiency is a rare condition with only few known pathogenic variants. It has an apparently good prognosis but is prone to misdiagnosis. Our understanding of

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its clinical implications is still limited, and an international prekallikrein and HK deficiency registry is being established to fill this knowledge gap.

1 | INTRODUCTION

Severe high-molecular-weight kininogen (HK) deficiency is an autosomal recessive defect of the contact system caused by pathogenic variants in *KNG1* [1–4]. This condition is considered rare because only a few cases are documented in well-known scientific databases such as PubMed/MEDLINE. Recently, interest in contact phase defects has increased as plasma prekallikrein (PK), the main HK cleaving protease zymogen, represents a therapeutic target in hereditary angioedema [5,6]. Moreover, elevated bradykinin (BK) levels are suspected to exacerbate COVID-19 symptoms [7–9], and β-amyloid-induced HK-mediated contact system activation has been suggested to enhance fibrin formation and BK release in Alzheimer`s disease [10]. HK/PK levels have been studied repeatedly in thromboembolic events with nonconclusive results [11–14].

HK and low-molecular-weight kininogen (LK) arise from alternatively spliced transcripts of the same gene, *KNG1*. LK accounts for the majority of kininogens in plasma (∼1.3 μmol/L), whereas HK is present at a lower concentration (∼0.67 μmol/L). Both isoforms are synthesized in hepatocytes and share domains 1-4. They only differ in their respective light chains (D5H, D6H in HK; D5L in LK), which are created by excision of a kinin. Despite their structural similarities, they are involved in distinct processes. HK is a scaffold protein in the intrinsic coagulation cascade and forms a complex with PK or factor XI (FXI) in plasma [3,15–17]. It also inhibits platelet aggregation [18–21], is involved in angiogenesis [22], has antimicrobial properties [23], and influences endothelial cell or leukocyte adhesion [24–26]. Moreover, HK is implicated in endotoxemia [27], brown adipose tissue activity [28], and acetaminophen-induced acute liver failure [29]. By contrast, LK does not participate in the contact activation system but, similar to HK, inhibits platelet aggregation [19,30]. Furthermore, various proteases release kinins from both kininogens, primarily plasma kallikrein and tissue kallikrein, releasing BK and lysyl-bradykinin [17]. These kinins are mainly known for their involvement in inflammatory processes, vasodilation, and increased vascular permeability. They also

Essentials

- High-molecular-weight kininogen (HK) deficiency is a recessive benign trait leading to isolated activated partial thromboplastin time prolongation.
- We performed the first comprehensive analysis of HK deficiency (*n* = 48).
- Only truncating variants are known, and HK deficiency significantly reduces prekallikrein and factor XI.
- Our estimates suggest an overall prevalence of 1 case per 8 million population.

affect smooth muscle contraction, hypotension, natriuresis and diuresis, blood glucose levels, and stimulation of nociceptors [31-35]. Despite these numerous processes in which HK is involved, its deficiency only seems to cause an isolated prolongation of the activated partial thromboplastin time (aPTT) in humans, neither accompanied by bleeding nor other overt clinical symptoms.

Since a systematic evaluation of known cases of HK deficiency is lacking so far, we performed a comprehensive analysis by (i) identifying HK deficiency–causing *KNG1* variants in a new family and, after obtaining blood samples, also in families from the literature, (ii) critically appraising all reported HK deficiency–causing variants, (iii) estimating its prevalence using the genome aggregation database (GnomAD), and (iv) assessing laboratory and clinical characteristics.

2 | METHODS

2.1 | Literature search and study population

We searched MEDLINE (Pubmed), EMBASE (Ovid), Web of Science, the "gray literature", and user-based or institutional online

repositories to perform a comprehensive literature review on HK deficiency. For more details regarding the implementation, refer to our previous systematic review on PK deficiency [36] and the Supplementary Material. Titles and abstracts of retrieved publications were screened and sorted out. Potentially interesting studies were reviewed in full text, and corresponding authors were contacted to obtain additional information and blood samples. We used the following inclusion criteria to identify cases of severe HK deficiency: HK clotting activity (HK:C) and/or HK antigen (HK:Ag) ≤5% of normal, or presence of biallelic causal KNG1 variants.

The study population includes a novel case of severe HK deficiency identified in Mainz, selected cases from the literature for whom we performed additional functional and/or genetic analyses, and all reported cases with only clinical and laboratory data available (Supplementary Tables S3-S8). All newly analyzed patients provided appropriate informed consent for genetic testing, biochemical examinations, and the anonymized publication of their data. The study meets all local legal and regulatory requirements (General Data Protection Regulation [EU 2016/679], Declaration of Helsinki [7th revision]). The use of anonymized retrospective data or of already published data followed local regulation at each participating institution.

2.2 | Laboratory methods

The HK and PK clotting activity (HK:C, PK:C) was determined using deficient plasmas (CoaChrom Diagnostica GmbH; George King Bio-Medical Inc.) and antigen levels (HK:Ag, PK:Ag) using enzyme-linked immunosorbent assays (Affinity Biologicals Inc.). Causative variants in KNG1 and KLKB1 were examined applying Sanger sequencing (Beckman CEQ8000, Sciex) or medical exome sequencing (MedExome, Roche; Nextseq500, Illumina). When a copy number variation (CNV) could not be ruled out by family studies in apparent homozygous patients, an additional digital droplet polymerase chain reaction (ddPCR) (QX200 Droplet Digital PCR System, Bio-Rad) was performed. All analyses were carried out as described previously, except for different primers used (Supplementary Table S1) and additional details on the databases, variant calling tools, gene variant sorting, and prediction of variant dignity can be found in Reference [36].

New genetic and functional analyses were performed for diagnostic purposes at the Institute of Clinical Chemistry and Laboratory Medicine of the University Medical Center Mainz. The nomenclature of reported KNG1 variants was updated according to the recommendations of the Human Genome Variation Society (HGVS) (version 20.05, http://www.HGVS.org/ [accessed April 2022]) and the reference sequence NM_001102416.3 (HK).

2.3 | Clinical events and statistical and bioinformatic analyses

The systematic analysis of individual clinical data is described in more detail in Barco et al. [36]. The life-time prevalence of clinical events

with 95% confidence intervals, as well as a separation by age groups or an annualized incidence rate, was computed where applicable. For statistical analysis, an independent t-test with a significance level of 0.05 was used (SPSS, version 27). Owing to the small and methodologically heterogeneous collective, data were not adjusted for confounders or multiple testing. Thus, all statistics have to be considered descriptive. In parallel to the HK-deficient collective, a PK-deficient collective was extracted from literature data (Supplementary Table S10) and used for the comparative analysis of factors VIII, X, XI, and XII. PK levels were compared with healthy controls [36].

A calculation based on the Hardy-Weinberg principle and the frequency of HK deficiency–causing KNG1 variants in different ethnicities was applied to estimate the prevalence of HK deficiency. GnomAD (https://gnomad.broadinstitute.org/, accessed July 2022) [37], the most comprehensive data set on genetic information and ethnicities, was used to extract frequency data. Since version 3.1.2 offered less data than version 2.1.1 for all ethnicities except "African/African-American," version 3.1.2 was used only for the latter and version 2.1.1 for all other ethnicities. Small ethnic groups were excluded because they would be overrepresented in a global generalized estimate.

All clearly pathogenic KNG1 variants were selected from GnomAD and used for a more comprehensive prevalence estimate. Only stop gained, frameshift or canonical splice site variants from the start codon to the last known deficiency–causing codon were included (amino acid (aa) 1-498), and their pathogenicity was reconfirmed with prediction tools.

3 | RESULTS

3.1 | Literature search and study population

The comprehensive literature search revealed 711 studies mentioning HK and deficiency (Figure 1A). After the described two-phase selection process and exclusion of duplicates, we identified 28 eligible studies. Detailed criteria for the selection process, inclusion (based on the highest reasonable levels found for HK:Ag and HK:C in deficient patients or KNG1 analyses), and exclusion are provided in the *Methods section* and Supplementary Table S2.

The 28 selected studies describe 47 cases of severe HK deficiency from 40 families. Approximately 40% of these index cases were published in the "gray literature" (Figure 1A, Supplementary Table S3). Most reports were single-family studies, and only 3 covered multiple families (n = 4-7) [38–40]. Including our newly identified case (case 1), a collective of 48 HK-deficient cases from 41 families constitutes the study population (Figure 1B).

Women accounted for ∼62% (n = 29) of the study population, and the average age at diagnosis of cases discovered independently of family examinations was 37.4 years (Q1-Q3 = 21.25-55.75, Supplementary Table S3). Ethnicity was reported for 33 index patients without apparent clustering of HK deficiency in any ethnic group (7 Mestizo, 7 East Asians, 6 whites, 6 Arabs, 4 African-Americans, etc.).
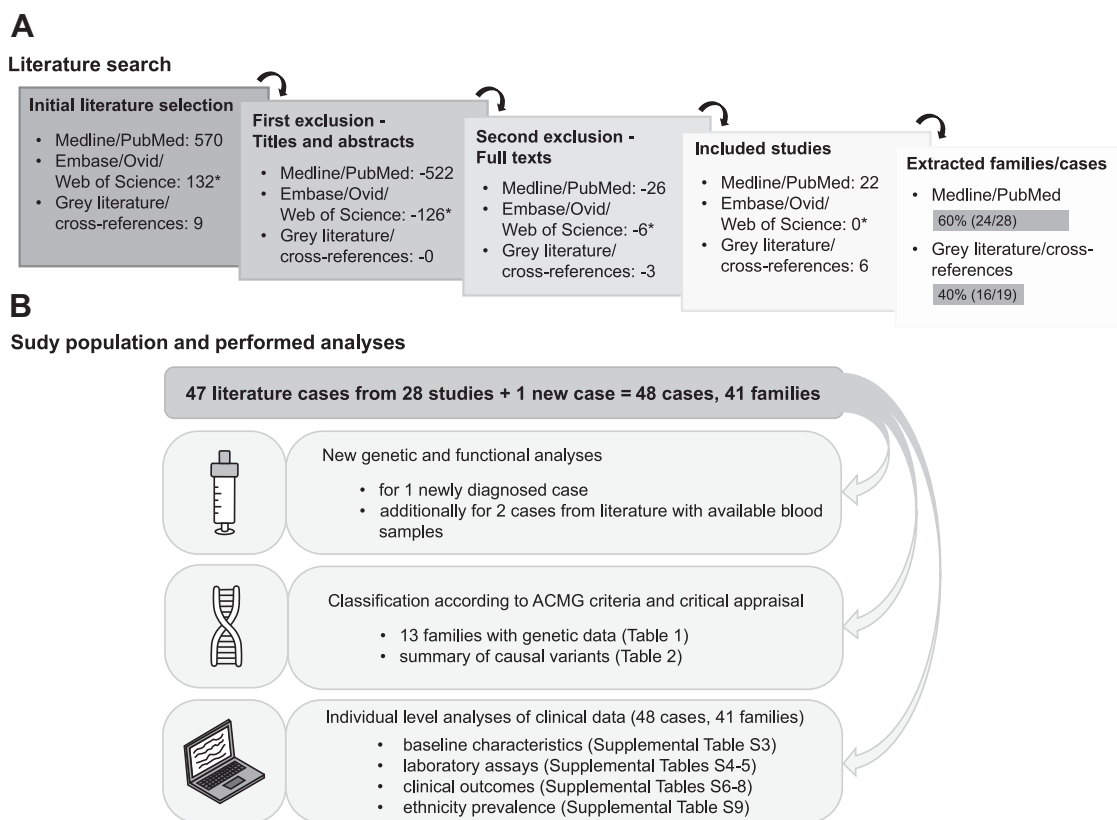
A

Literature search

B

Study population and performed analyses

FIGURE 1 The procedure and specifics of the literature search (A) and the composition of the study population and the analyses performed with specific sub collectives (B). Studies found in the gray literature were already prefiltered before the first step described here. *, without overlap with PubMed; ACMG, American College of Medical Genetics

10 of 23 index cases (43%) with pedigree information had consanguineous parents, and the initial diagnosis of HK deficiency was usually made incidentally during preoperative work-up, as seen in 70% of cases with respective information (Supplementary Table S7; 23/33 cases found independently of family examinations).

We performed new genetic and/or functional analyses for 8 individuals from 3 families.

3.2 | Genetic and biochemical analyses in a newly diagnosed and 2 reported cases

Case 1

In a routine coagulation screening before scrotal hernioplasty, the index patient (87-year-old man) was found to have an isolated aPTT prolongation (63.9 seconds; reference value: 20-32 seconds). Therefore, hemostatic therapy with desmopressin (20 μg intravenous) was applied before an uncomplicated surgery. Previous surgeries without preventive treatment also triggered no excessive bleeding (percutaneous transluminal coronary angioplasty and stent for angina pectoris, dental extractions, appendectomy, and transurethral resection of the prostate). He has been treated with platelet aggregation inhibitors for years, but he has not experienced severe bleeding or venous thromboembolic complications. In addition, he suffered from exertional dyspnea. Occasionally, he shows epistaxis lasting a few minutes and

ecchymoses but no hematuria or hematochezia. His daughter had a normal aPTT, no hemorrhagic diathesis, and an uncomplicated cesarean section. His son's aPTT was unremarkable, but since the age of 43, he had suffered from ischemic heart disease. Laboratory analyses of the index patient excluded a lupus anticoagulant and showed normal or increased levels of the intrinsic pathway factors, except for PK and HK (HK:C = 2.3%, HK:Ag < 1.0%, PK:C = 46.4%, PK:Ag = 48.4%). Sequencing revealed homozygosity for *KNG1* c.306+2T>A, a novel variant in the canonic acceptor splice site of exon 2, which presumably causes incorrect splicing and a rapidly degraded, truncated protein or nonsense-mediated messenger RNA decay (NMD), and hence, both HK and LK deficiency (Tables 1 and 2, Figure 2). ddPCR of exon 2 revealed no CNV and confirmed homozygosity. The index patient's daughter and son are heterozygous carriers of *KNG1* c.306+2T>A.

Case 2

The case of a 21-year-old Arab woman from Oman was published by Nazir et al. [40] (case 7). Her aPTT was prolonged (>180 seconds), and mixing studies with normal plasma revealed no inhibitor. Intrinsic coagulation factors were within the reference range except for a borderline decreased FXI:C and PK:C (57%, 51.6%), and a HK:C of <0.7%, indicating total HK deficiency. She had no history of bleeding or thrombosis but 4 miscarriages and one healthy offspring. Sanger sequencing was performed at our laboratory in Mainz and revealed the homozygous variant *KNG1*: c.586C>T p.(Arg196*). This is the first and

TABLE 1 Cases and families with severe HK deficiency with molecular genotyping and biochemical analyses performed in this study or reported in the literature.

Family ID		KNG1 allele 1	KNG1 allele 2	Method	Material	HK:C (%)	HK:Ag (%)	PK:C (%)	PK:Ag (%)	Conclusions
Unpublished case (this study)										
1 Mainz/Italy	Index patient (87, M)	**c.306+2T>A**	**c.306+2T>A**	Sanger, ddPCR	EDTA blood	<2[a]	<1[a]	46[a]	48[a]	homozygous HK and LK deficiency
	Son (50, M)	**c.306+2T>A**	—	Sanger	EDTA blood	85[a]	37[a]	177[a]	67[a]	heterozygous carrier
	Daughter (47, F)	**c.306+2T>A**	—	Sanger	EDTA blood	76[a]	44[a]	103[a]	72[a]	heterozygous carrier
Published cases with complementing analyses performed (this study)										
2 Nazir [40]	Index patient (21, F)	c.586C>T, p.Arg196*	c.586C>T, p.Arg196*	Sanger, ddPCR	EDTA blood	<1	—	52	—	homozygous HK and LK deficiency
3 Pancione [41]	Index patient (22, M)	c.718C>T, p.Arg240*	c.718C>T, p.Arg240*	Sanger	EDTA blood	1[a]	<1[a]	43[a]	38[a]	homozygous HK and LK deficiency
	Father (58, M)	c.718C>T, p.Arg240*	—	Sanger	EDTA blood	74[a]	50[a]	113[a]	131[a]	heterozygous carrier
	Mother (51, F)	c.718C>T, p.Arg240*	—	Sanger	EDTA blood	49[a]	29[a]	54[a]	118[a]	heterozygous carrier
	Brother (29, M)	c.718C>T, p.Arg240*	—	Sanger	EDTA blood	57[a]	39[a]	58[a]	102[a]	heterozygous carrier
Published cases with reported genetic data (PubMed, Embase)										
4 Tomao [36,42]	Index patient (72, F)	c.1165C>T, p.Arg389*	c.1038+1G>A	MES, Sanger	EDTA blood	—	—	7	—	compound heterozygous HK and LK deficiency
5 Colman [43]/Cheung [4] (Williams trait)	Index patient (64, F)	c.586C>T, p.Arg196*	c.586C>T, p.Arg196*	Sanger, PCR based RFLP	liver cDNA and whole blood	<1	<1	43	45	homozygous HK and LK deficiency
	Daughter 1 (F)	c.586C>T, p.Arg196*	—	PCR based RFLP	whole blood	54	54	—	—	heterozygous carrier
	Daughter 2 (F)	c.586C>T, p.Arg196*	—	PCR based RFLP	whole blood	60	60	—	—	heterozygous carrier
	Daughter 3 (F)	c.586C>T, p.Arg196*	—	PCR based RFLP	whole blood	60	35	—	—	heterozygous carrier
	Granddaughter (F)	—	—	PCR based RFLP	whole blood	87	63	—	—	not affected
6 Fukushima [44]	Index patient (67, M)	c.523-524dupTC, p.Leu176Profs*8	c.523-524dupTC, p.Leu176Profs*8	Sanger	whole blood	<1	—	28	—	homozygous HK and LK deficiency
	Son (M)	c.523-524dupTC, p.Leu176Profs*8	—	Sanger	whole blood	62	—	130	—	heterozygous carrier

(Continues)

TABLE 1 (Continued)

Family ID		KNG1 allele 1	KNG1 allele 2	Method	Material	HK:C (%)	HK:Ag (%)	PK:C (%)	PK:Ag (%)	Conclusions
	Daughter 1 (F)	c.523-524dupTC, p.Leu176Profs*8	—	Sanger	whole blood	44	—	100	—	heterozygous carrier
	Daughter 2 (F)	c.523-524dupTC, p.Leu176Profs*8	—	Sanger	whole blood	62	—	135	—	heterozygous carrier
7 Hayashi second [45,46] (Kishino)	Index patient (39, F)	c.586C>T, p.Arg196*	c.586C>T, p.Arg196*	PCR based RFLP	whole blood	<1	not detectable	34	—	homozygous HK and LK deficiency
8 Jeong [47]	Index patient (37, M)	c.488delG, p.Gly163Alafs*20	c.1165C>T, p.Arg389*	Sanger	whole blood	<1	~10[b]	48	~90[b]	compound heterozygous HK and LK deficiency
9 Krijanovski [48]	Index patient (6, M)	c.1493delA, p.Lys498Serfs*54	c.1493delA, p.Lys498Serfs*54	Sanger	whole blood	<1	not detectable	27 (chromogenic)	~25[b]	homozygous HK deficiency
	Father (M)	c.1493delA, p.Lys498Serfs*54	—	Sanger	whole blood	38	—	24 (chromogenic)	~36[b]	heterozygous carrier
	Mother (F)	c.1493delA, p.Lys498Serfs*54	—	Sanger	whole blood	59	—	24 (chromogenic)	~47[b]	heterozygous carrier
	Sister (F)	c.1493delA, p.Lys498Serfs*54	—	Sanger	whole blood	65	—	21 (chromogenic)	~62[b]	heterozygous carrier
10 Nakamura [46,49] (Tachibana)	Index patient (31, F)	c.586C>T, p.Arg196*	c.586C>T, p.Arg196*	PCR based RFLP	whole blood	<1	markedly low	25 (chromogenic)	57	homozygous HK and LK deficiency
11 Shigekiyo [50] (Tsukai)	Index patient (35, F)	c.1216dupC, p.His406Profs*10	c.1216dupC, p.His406Profs*10	Sanger	whole blood	<1	—	39	—	homozygous HK deficiency
	Father (M)	c.1216dupC, p.His406Profs*10	—	Sanger	whole blood	62	—	—	—	heterozygous carrier
	Mother (F)	c.1216dupC, p.His406Profs*10	—	Sanger	whole blood	44	—	—	—	heterozygous carrier
	Brother (M)	c.1216dupC, p.His406Profs*10	—	Sanger	whole blood	50	—	—	—	heterozygous carrier
	Son (M)	c.1216dupC, p.His406Profs*10	—	Sanger	whole blood	52	—	—	—	heterozygous carrier
	Daughter (F)	c.1216dupC, p.His406Profs*10	—	Sanger	whole blood	47	—	—	—	heterozygous carrier
12 Yang [51]	Index patient (67, M)	c.1456C>T, p.Gln486*	c.1456C>T, p.Gln486*	NGS	whole blood	<1	—	26	—	homozygous HK deficiency
	Sister 1 (63, F)	c.1456C>T, p.Gln486*	c.1456C>T, p.Gln486*	NGS	whole blood	1	—	42	—	homozygous HK deficiency

TABLE 1 (Continued)

Family ID	KNG1 allele 1	KNG1 allele 2	Method	Material	HK:C (%)	HK:Ag (%)	PK:C (%)	PK:Ag (%)	Conclusions
Sister 2 (58, F)	c.1456C>T, p.Gln486*	c.1456C>T, p.Gln486*	NGS	whole blood	<1	–	29	–	homozygous HK deficiency
Other relatives (II5, II7, III1-7, III9-10)	c.1456C>T, p.Gln486*	–	NGS	whole blood	33-88	–	78-98	–	heterozygous carriers
Other relatives (II4, II6, II8, III11-14)	–	–	NGS	whole blood	75-107	–	84-99	–	unaffected
Published cases with genetic data (gray literature)									
13 Geisen [52] Index patient (19, F)	c.718C>T, p.Arg240*	c.1038+1G>A	Sanger	whole blood	<1	–	52	–	homozygous HK and LK deficiency
Mother (F)	–	c.1038+1G>A	Sanger	whole blood	68	–	85	–	heterozygous carrier
Father (M)	c.718C>T, p.Arg240*	–	Sanger	whole blood	61	–	88	–	heterozygous carrier

ddPCR, digital droplet polymerase chain reaction; MES, medical exome sequencing; NGS, next generation sequencing; RFLP, restriction fragment length polymorphism; in bold, KNG1 variant which has been newly identified in this study.

a Parameters measured in Mainz; b Calculated using the mean of the given reference range in the individual papers as 100% of normal.

most frequently published HK variant (Cases 5, 7, and 10) [4,43,45,46,49] and well known to cause total kininogen deficiency (Tables 1 and 2). A ddPCR of exon 5 revealed no CNV and confirmed homozygosity.

Case 3

Pancione et al. [41] reported the case of a 22-year-old Italian man with an isolated prolongation of the aPTT (>120 seconds, normal PT) during a routine screening before rhinoplasty. Factors XII, XI, IX, and VIII were within the reference range. Thromboelastography indicated a defect in the intrinsic coagulation cascade. A 1:1 mixture with normal plasma resulted in a correction of the aPTT (29 seconds), excluding inhibitors. Mixing 1% of normal plasma with 99% of patient plasma was insufficient to normalize the aPTT, even after a prolonged incubation period, whereas 12.5% of normal plasma corrected the aPTT. Therefore, the authors suspected HK deficiency rather than PK deficiency. Further analyses performed in our laboratory in Mainz now complement and confirm the diagnosis. Coagulation activity and antigen levels of HK and PK are absent or reduced (HK:C = 2.0%, HK:Ag <1.0%, PK:C = 43.0%, PK:Ag = 38.3%). Sanger sequencing revealed no PK deficiency–causing variants in KLKB1, but the homozygous defect KNG1: c.718C>T p.(Arg240*) (rs761496908). This variant has only been described in compound heterozygosity (Case 13) in an abstract of the "ISTH2005" congress [52]. It causes combined HK and LK deficiency because it affects parts of the heavy chain and both light chains, leading to rapid degradation or NMD (Table 2, Figure 2). The index subject's father, his mother, and his brother are heterozygous carriers.

3.3 | Critical appraisal of variants, allele frequency, and estimated prevalence

Aside from the aforementioned 3 index cases genotyped by us (Cases 1-3), genetic data from 10 further index cases were extracted from the literature (Cases 4-13, Table 1). Ten of these 13 index cases were homozygous (consanguineous parents: n = 6, nonconsanguineous parents: n = 2, unknown: n = 2), and only 3 were compound heterozygous, revealing 10 different deficiency–causing KNG1 variants (Figure 2, Table 2). Nine of these have already been published, including one in our group's recent publication [36]. The variant c.306+2T>A, located in the canonical donor splice site of exon 2, was newly identified (Case 1). In addition, Krijanovski et al. [48] discovered the deep intronic variant NM_001102416.3(KNG1) c.1126-538_1126-525delinsGGTGGTGGTGGTGGTGG (rs869320718, nomenclature updated to current reference sequence and HGVS) in one of the earliest reported HK-deficient patients (Fitzgerald [1,48,53]). Although Fitzgerald is undoubtedly a case of HK deficiency (Supplementary Table S4), the discovered variant lacks functional validation. Therefore, we agree with the recommendation of the authors and classify it as a variant of unknown significance, not listing it among the HK deficiency–causing variants.

Fitzgerald is also the only plausible case with a residual antigen (HK:Ag ~13%, Supplementary Table S4). In 15 other cases with

TABLE 2 Critical reevaluation of all reported HK deficiency–causing variants according to ACMG/AMP criteria and likely consequences on protein level.

KNG1 variant	Exon	MAF (dbSNP) (%)	rsID	ClinVar	Prediction tool (Mutation taster)	Consequences on protein level	References	Classification (ACMG/AMP)	Unrelated cases	Deficiency
Frameshift										
c.488delG, p.Gly163Alafs*20	4	0.0008-0.05	rs756599757	—	disease causing	truncating, presumably NMD	[47]	pathogenic	1	HK + LK
c.523-524dupTC, p.Leu176Profs*8	4	—	—	—	disease causing	truncating, presumably NMD	[44]	pathogenic	1	HK + LK
c.1216dupC, p.His406Profs*10	10b	—	rs797044430	affects	disease causing	truncating	[50]	pathogenic	1	only HK
c.1493delA, p.Lys498Serfs*54	10b	—	rs797044429	affects	disease causing	truncating	[48]	pathogenic	1	only HK
Nonsense										
c.586C>T, p.Arg196*	5	0.0016-0.007	rs121918131	pathogenic	disease causing	truncating, presumably NMD	[4,45,49], this study	pathogenic	4	HK + LK
c.718C>T, p.Arg240*	6	0.0008-0.0014	rs761496908	—	disease causing	truncating, presumably NMD	this study, [52]	pathogenic	2	HK + LK
c.1165C>T, p.Arg389*	10a	0.0008	rs752411996	—	disease causing	truncating	[36,47]	pathogenic	2	HK + LK
c.1456C>T, p.Gln486*	10b	—	—	—	disease causing	truncating	[51]	pathogenic	1	only HK
Splice site										
c.306+2T>A	IVS2	—	—	—	disease causing	splice defect	this study	pathogenic	1	HK + LK
c.1038+1G>A	IVS8	0.0016-0.008	rs377594184	—	disease causing	splice defect	[36,52]	pathogenic	2	HK + LK
Possible variant of unknown significance (needs confirmation)										
c.1126-538_ 1126-525 indelGGTGGT GGTGGTGGTGG	IVS9	—	rs869320718	affects	*Fruitfly.org:* no change *HSF:* potential alteration, intronic cryptic donor site	no functional analysis available, a splice defect can neither be ruled out nor confirmed	[48]	variant of unknown significance	1	potential HK and partial LK defect

ACMG/AMP, American College of Medical Genetics and Genomics/Association of Molecular Pathology; exon count, 10 (HK), 11 (LK); genome build, GRCh37/hg19; HSF, human splicing finder; IVS, intervening sequence; MAF, minor allele frequency; NMD, nonsense-mediated messenger RNA decay; RefSeq., NM_001102416.3 (HK), NM_000893.4 (LK); rsID, identifier from dbSNP.
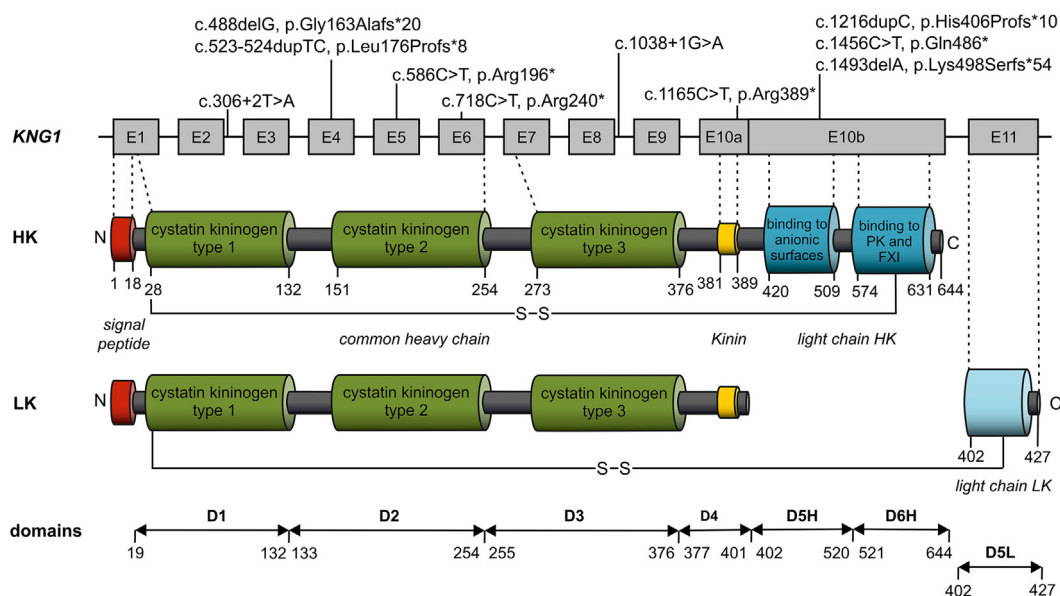
FIGURE 2 Summary and localization of *KNG1* variants. The following *KNG1* variant has been identified in this study for the first time: c.306+2T>A. The last 3 variants in exon 10b lead only to HK deficiency and do not cause LK deficiency. S-S: disulfide bridge connecting the heavy chain (aa19-380) to the light chain of HK (aa390-644 individual part: aa402-644) or LK (aa390-427, individual part: aa402-427); numbers beneath protein structure: amino acid (aa) positions; E: exon; c.: coding DNA; reference sequence used (NM_001102416.3 [HK], NM_000893.4 [LK]); p.: protein, reference sequence used (P01042-1 [HK], P01042-2 [LK]); D: domain

reported levels, no HK:Ag could be detected. The HK:Ag described by Jeong et al. [47] (~10%) was deemed unspecific as the patient's causal variants are unlikely to lead to measurable HK. Ten index cases are deficient in both kininogens, whereas 3 are only HK deficient (Tables 1 and 2). The presence of LK has been proven in 2 of 3 HK-only deficient cases. Shigekiyo et al. [45,50] report 100% LK/BK and Krijanovski et al. [48] found a strong LK band in Western blot analysis. Solely HK-deficient cases are caused by 3 variants, located in the HK-specific part of *KNG1* (aa402-644, exon 10b). All other collected variants cause total kininogen deficiency (Figure 2). In addition, 5 cases without sequencing data have high (3 cases [1,54,55]) or residual (~10%, 2 cases [2,56]) levels of LK or releasable BK. Whether a residual LK is truly present in the latter 2 cases remains questionable, which leaves 6 cases that reliably have a mere HK deficiency and express LK at least partially (Supplementary Table S4).

To date, no missense variants are known to cause HK deficiency. All currently reported HK deficiency–causing variants are either truncating, NMD-inducing, or severely affect the protein structure (splice site, nonsense, and frameshift mutations). Consequently, no HK:Ag is measurable in plasma (Figure 2, Table 2).

Using all identified HK deficiency–causing variants, we calculated the frequency of HK deficiency as no prevalence estimates existed. This revealed a prevalence of one case per 156 million worldwide (Table 3, upper part). However, the mere fact that 10 *KNG1* variants were observed in only 13 cases of severe HK deficiency, and the high rate of homozygous and consanguineous individuals suggests that the molecular basis of HK deficiency is not homogeneous. This leads to a substantial underestimation when considering solely the few reported HK deficiency–causing variants to estimate the prevalence. Moreover, it becomes evident that most collectives are poorly studied. For example,

based on these calculations, there should be no cases among Latinos, but one Venezuelan publication reports 7 unrelated cases (Supplementary Table S3) [39]. Therefore, as a more reasonable estimation, we calculated the prevalence of HK deficiency using all potentially HK deficiency–causing variants listed in GnomAD (truncating variants in *KNG1*, including indels, nonsense, and canonical splice site variants located in that part of the gene, where relevant mutations have been described; Methods section and Supplementary Table S9). Despite the fact that this is a conservative approach, it revealed a significantly greater frequency of HK deficiency worldwide (1 case per ~8 million) and among different ethnic groups (Table 3, lower part). It confirmed that HK deficiency might be slightly more common among Africans (1 case per ~2 million) and that Latinos have the lowest prevalence (1 case per 22.4 million). The most common HK deficiency–causing variant based on this analysis is *KNG1* c.1038+1G>A (rs377594184, 9/254 024 alleles [GnomAD]), rather than c.586C>T p.(Arg196*), the most frequently reported one (minor allele frequency: 0.0015%; 4/256 548 alleles) (Table 3) [4,43,45,46,49]. Among Africans, c.1038+1G>A even reaches a frequency of 0.0169% (7/41 444).

3.4 | Laboratory parameters

We measured HK:C and HK:Ag in 14 healthy donors, 5 heterozygous and 2 homozygous carriers of HK deficiency–causing *KNG1* variants, as well as in 6 heterozygous and 6 homozygous/compound heterozygous carriers of PK deficiency–causing *KLKB1* variants (Figure 3A). Heterozygous carriers of a HK deficiency–causing variant showed approximately half the HK:Ag levels (39.6%; Q1-Q3 = 37.0-43.6) compared with the control group (77.9%; Q1-Q3 = 70.3-86.7; *p* < .0001), which is consistent with heterozygosity and the fact that all

TABLE 3 Prevalence estimates of severe HK deficiency based on all known HK deficiency–causing *KNG1* variants and extended prevalence estimates based on all putative HK deficiency–causing *KNG1* variants reported in GnomAD.

Prevalence estimates based on all known HK deficiency–causing *KNG1* variants

	African/African-American			East Asian			European, non-Finnish			Latino			South Asian			Total		
	VAC	TAC	MAF (%)	VAC	TAC	MAF (%)	VAC	TAC	MAF (%)	VAC	TAC	MAF (%)	VAC	TAC	MAF (%)	VAC	TAC	MAF (%)
c.488delG	0	16 256	0.000	2	18 390	0.011	0	113 724	0.000	0	34 592	0.000	0	30 616	0.000	2	213 578	0.001
c.586C>T	1	41 418	0.002	1	19 954	0.005	1	129 128	0.001	0	35 436	0.000	1	30 612	0.003	4	256 548	0.002
c.718C>T	0	41 446	0.000	1	19 936	0.005	2	129 060	0.002	0	35 428	0.000	0	30 598	0.000	3	256 468	0.001
c.1038+1G>A	7	41 444	0.017	0	18 378	0.000	2	113 620	0.002	0	34 558	0.000	0	30 584	0.000	9	238 584	0.004
c.1165C>T	0	41 378	0.000	0	18 168	0.000	1	112 596	0.001	1	33 314	0.003	0	29 986	0.000	2	235 442	0.001
Frequency mutant alleles	0.00019305			0.00020903			4.97248E-05			3.0017E-05			3.2667E-05			8.28705E-05		
Frequency wild type alleles	0.99980695			0.99979097			0.99995028			0.99996998			0.99996733			0.99991713		
Frequency heterozygotes	0.00038602			0.00041797			9.94446E-05			6.0033E-05			6.5332E-05			0.00016573		
Frequency deficient individuals	3.7267E-08			4.3694E-08			2.47255E-09			9.0104E-10			1.0671E-09			6.86752E-09		
Prevalence, 1 Case:x	26 833 369			22 886 560			404 440 360			1 109 822 596			937 094 544			145 613 067		
Extended prevalence estimates																		
Frequency variant alleles	0.00063026			0.00037216			0.00027337			0.00021126			0.00043562			0.00035102		
Frequency wild type alleles	0.99936974			0.99962784			0.99972663			0.99978874			0.99956438			0.99964898		
Frequency heterozygotes	0.00125972			0.00074404			0.00054659			0.00042244			0.00087086			0.00070180		
Frequency deficient individuals	3.97223E-07			1.385E-07			7.4732E-08			4.4632E-08			1.8977E-07			1.23216E-07		
Prevalence, 1 Case:x	2 517 478			7 220 182			13 381 082			22 405 587			5 269 630			8 115 828		

MAF, minor allele frequency; TAC, total allele count; VAC, variant allele count.
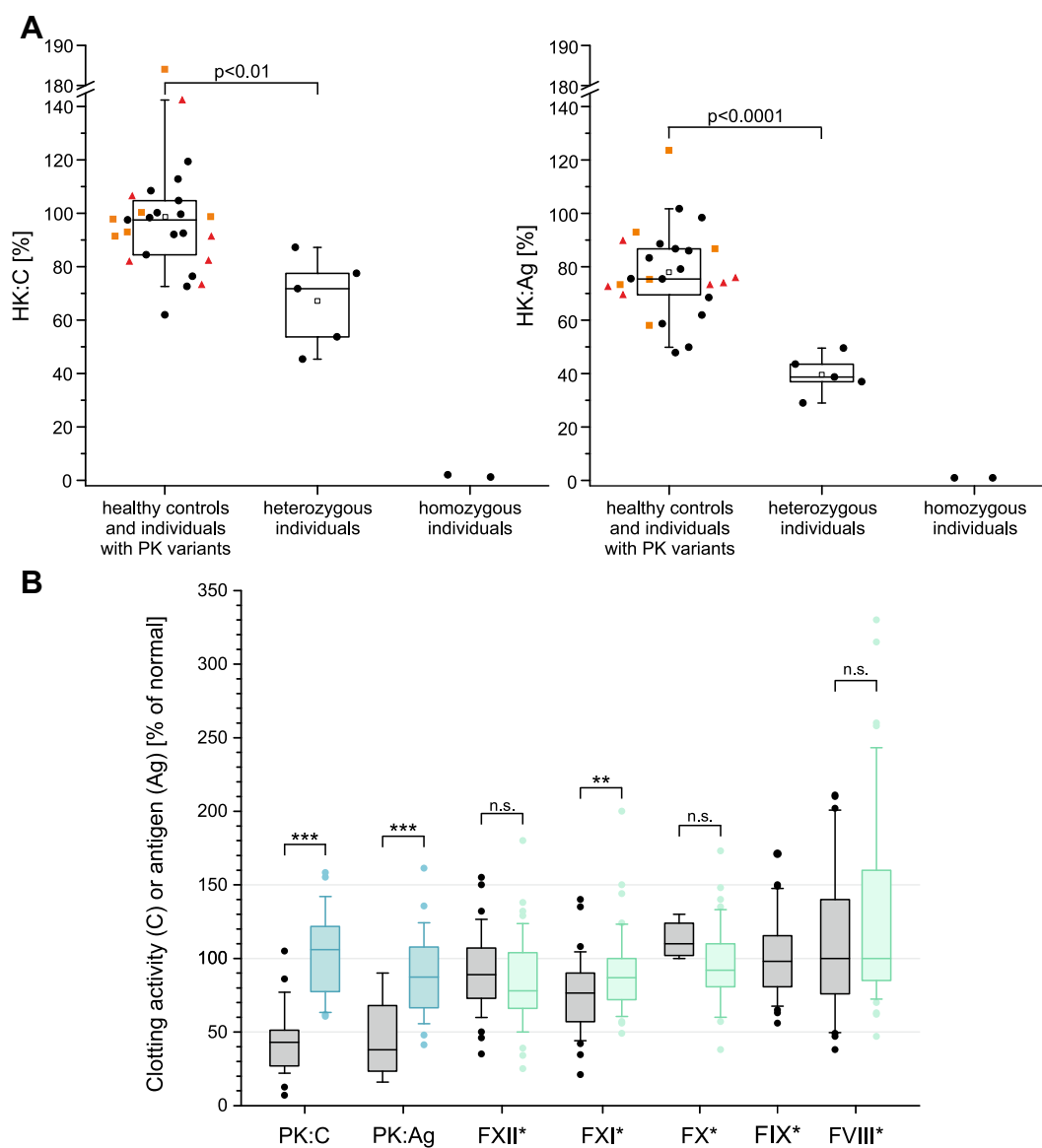
FIGURE 3 (A) HK clotting activity (HK:C; left) and HK antigen (HK:Ag; right) measured in our laboratory (Mainz) in healthy controls (*n* = 14) and individuals carrying PK variants (*n* = 6, heterozygous, orange squares; *n* = 6, homozygous, red triangles), and in subjects carrying heterozygous (*n* = 5) or homozygous (*n* = 2) HK deficiency–causing variants. Blanc square: mean. (B) Distribution of coagulation activity (C) and antigen (Ag) of different coagulation factors in HK-deficient families reported in the literature and in our new case (gray) compared with PK-deficient cases (light green, *n* = 37-40) or a normal collective (light blue, *n* = 26) in percent of normal. * = no uniform methodology of measurement or assay not mentioned, ** = *p* < .01, *** = *p* < .001, n.s. = *p* > .05

observed variants were truncating and therefore HK:Ag negative. HK:C was reduced in heterozygous carriers (67.1%; Q1-Q3 = 53.7-77.5) (HK:C controls: 98.6%; Q1-Q3=86.2-103.6; *p* < .01) but showed a wider variance than HK:Ag.

Considering our entire study population, we also evaluated the effect of HK deficiency on the level of other coagulation factors (Figure 3B, Supplementary Table S5). Most factors were within the standard reference ranges (factors I, II, V, VII, VIII, IX, X, XII, and XIII) and did not differ significantly from a similar collective of PK-deficient literature cases (Figure 3B, Supplementary Table S10; factor VIII, X, and XII). By contrast, factor XI and PK levels of HK-deficient patients were below the reference range and significantly

reduced compared with the PK-deficient collective or healthy donors (FXI: *p* = .0071; PK:C/Ag: *p* ≤ .0001; Figure 3B). The median PK:Ag was 38%, and the median PK:C was 43%, with substantial heterogeneity ranging from 7% [42] to 10% [1] to completely normal values. As a result, one of the cases with low PK:C [42] had been misdiagnosed as PK deficient as described earlier [36]. Conversely, our measurements confirm that PK deficiency does not affect HK because HK:C and HK:Ag in PK-deficient cases do not differ from healthy controls (Figure 3A). Three cases of HK deficiency (Cases 24-26 [39], Supplementary Table S5) showed low factor VIII:C (28%-38%) levels. These were 3 female siblings known to carry heterozygous factor VIII deficiency.

TABLE 4 Summary of clinical data of all collected HK-deficient cases.

	Number of cases with data	Event	No event	Life-time prevalence (%)	Mean age with event (min-max) (YRS)	Mean age all cases (Q1-Q3) (YRS)
Thrombotic complications	33	3	30	9.1	45.7 (6-67)	41.9 (25-66)
NONMAJOR AND UNCLASSIFIED BLEEDING EVENTS	40	15	32	37.5	34.8 (4-67)	40.4 (22.8-59.8)
MAJOR BLEEDING EVENTS	43	3	40	7.0	39.3 (25-59)	40 (24-59)
SURGERY COMPLICATION WITHOUT HEMOSTATIC PROPHYLAXIS	15	2	13	13.3	–	–
SURGERY COMPLICATION WITH HEMOSTATIC PROPHYLAXIS	4	0	4	0	–	–
HYPOTENSION	12	0	12	0	–	–
HYPERTENSION	13	4	9	30.8	54 (28-81)	51.3 (31-67)
OTHER COMORBIDITIES	23	19	4	82.6	46.8 (4-87)	44 (29.5-64)

Mean age all cases, mean age of all cases with reported data, respectively; Mean age with event, mean age of cases that experienced an event; yrs, years of age.

3.5 | Clinical events

After excluding cases with no clinical information, three of 33 individuals with severe HK deficiency had experienced one thromboembolic event each (Table 4, Supplementary Table S6, Cases 5, 6, and 9), resulting in a life-time prevalence of 9.1% (95% CI: 2.4%-25.5%) and an annualized incidence rate of 2.17 events/1000 person-years (95% CI: 0.6-6.9). One of these 3 died due to pulmonary embolism (age 64 years), one had a spontaneous splenic infarction (age 67 years), and one had a vertebral/basilar artery thrombosis with a left vertebral artery dissection provoked by cervical trauma (age 6).

Major bleeding events were described in three of 43 individuals (life-time prevalence: 7.0%; 95% CI: 1.8%-20.1%) for an annualized incidence rate of 1.7 events/1000 person-years (95% CI: 0.4-5.5) (Table 4). They consisted of a "transfusion dependent chronic iron deficiency anemia due to unexplained chronic melena" [38] (age 25 years), a vaginal bleeding with hematoma and postsurgical bleeding (age 34 years) and a massive postoperative hemorrhage after hysterectomy due to uterine myoma (age 56 years) (Supplementary Table S7; Cases 18, 20, and 34). The prevalence of cases with nonmajor and unclassified bleeding events was 37.5% (age <40 years, cases/events = 8, n = 26), 14.3% (age 40-65 years, case/events = 1, n = 7), and 22.2% (age >65 years, cases/events = 2, n = 9), respectively. In total, 21 patients underwent one or more surgical procedures (exclusion of 2 hemophilia A carriers), 4 of whom received bleeding prophylaxis (desmopressin or fresh frozen plasma). Of the remaining 15 patients with no preventive therapy, 2 experienced a postoperative bleeding, which was due to an uterine myoma in one patient and without specification in the other.
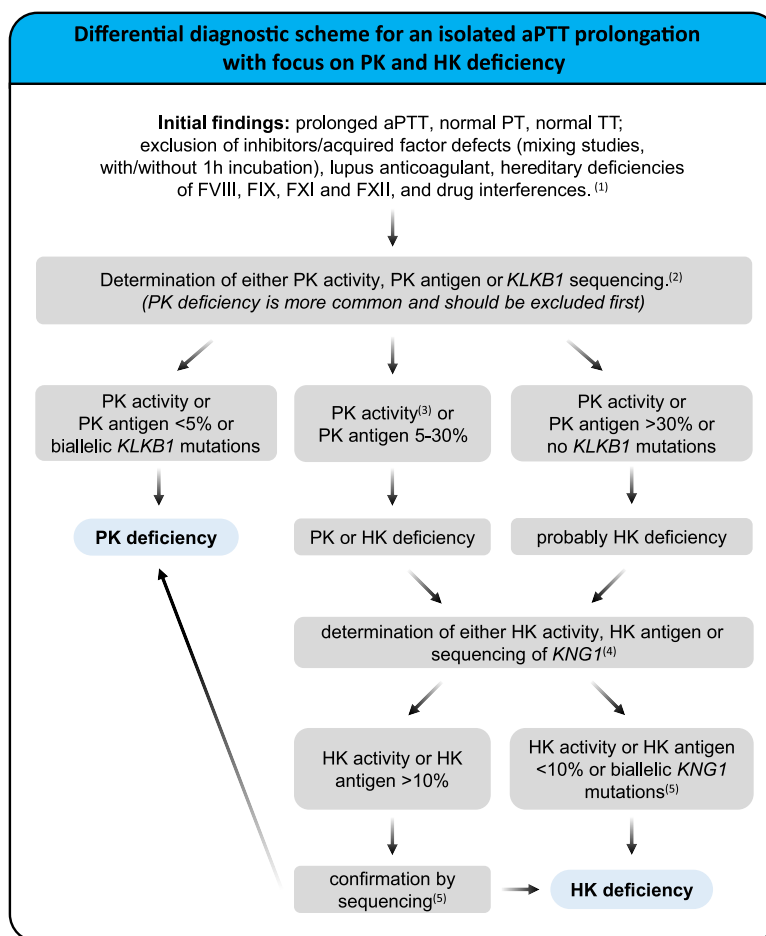
No HK-deficient patient showed hypotension (n = 12), whereas four of 13 patients suffered from hypertension (prevalence: 30.8%; 95% CI: 10.4-61.1) (Table 4). The quality of the reports on malignancies, blood counts, or other comorbidities was deemed insufficient for quantitative analysis (Supplementary Table S8).

4 | DISCUSSION

This is the only extensive and comprehensive study of HK deficiency. We compiled and analyzed all published HK-deficient cases, added a new case, identified a novel pathogenic KNG1 variant, defined diagnostic criteria, and provided prevalence estimates.

HK deficiency was typically discovered during a preoperative examination because of an isolated aPTT prolongation that prompted a hematological work-up. Because data on the consequences of HK deficiency are often deemed insufficient, a preventive prohemostatic treatment is initiated before surgery. Our meta-analysis, however, substantiates that HK-deficient individuals do not have an increased tendency to bleed, as evidenced by the low prevalence of major bleeding events as well as the high number of surgical procedures reported without postoperative bleeding. Therefore, administration of prohemostatic products before surgical procedures is not indicated and carries a disproportionate thrombotic risk, given the negligible bleeding

FIGURE 4 Differential diagnostic scheme for patients with an isolated prolongation of the aPTT and suspected HK or PK deficiency. (1) Variable aPTT results do not necessarily exclude hereditary PK/HK deficiency. No reports of isolated acquired PK or HK deficiency exist. FXI is significantly decreased in HK deficiency; FXII slightly in PK deficiency. Combined factor deficiencies or factor deficiency + lupus anticoagulant are possible. Heterozygous/moderate factor deficiencies often do not cause prolonged aPTT. (2) Measurement of aPTT with prolonged incubation time without HK/PK-deficient controls is no reliable diagnostic test. SNP or single exon analysis for "European" or "African" PK deficiency variant might be economical. Activity and antigen tests should have sufficient standards for low range discrimination (0%-10%). (3) PK level can be very low in HK-deficient cases. (4) ELISA-antibody should be directed exclusively against the HK light chain. Some manufacturers label their assays poorly and sell assays that detect both LK and HK. (5) All known HK-deficient cases have almost undetectable HK and truncating KNG1 variants; therefore, missense variants need functional confirmation.

Differential diagnostic scheme for an isolated aPTT prolongation with focus on PK and HK deficiency

Initial findings: prolonged aPTT, normal PT, normal TT; exclusion of inhibitors/acquired factor defects (mixing studies, with/without 1h incubation), lupus anticoagulant, hereditary deficiencies of FVIII, FIX, FXI and FXII, and drug interferences. (1)

Determination of either PK activity, PK antigen or KLKB1 sequencing.(2) (PK deficiency is more common and should be excluded first)

PK activity or PK antigen <5% or biallelic KLKB1 mutations → PK deficiency

PK activity(3) or PK antigen 5-30% → PK or HK deficiency

PK activity or PK antigen >30% or no KLKB1 mutations → probably HK deficiency

determination of either HK activity, HK antigen or sequencing of KNG1(4)

HK activity or HK antigen >10% → confirmation by sequencing(5)

HK activity or HK antigen <10% or biallelic KNG1 mutations(5) → HK deficiency

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risk associated with severe HK deficiency. Case 1, who received desmopressin before surgery, although it was contraindicated based on the patient's comorbidities, exemplifies this problem.

Thrombotic events did not appear to occur more frequently or earlier in life in HK-deficient patients. We could not find evidence that HK deficiency might protect against venous and/or arterial thrombosis, as previously suspected [11], because the annual incidence overlaps with that of the general population (1-3/1000 patient years) [57]. However, our cohort is small and limits the precision of these estimates. In a somewhat larger cohort with PK deficiency, for example, an elevated venous thrombosis risk also seemed unlikely, but an increased risk for cardiovascular events could not be ruled out [36].

The average age at diagnosis of severe HK deficiency is similar to that of patients with severe PK deficiency [36], reflecting that both deficiencies are usually diagnosed incidentally. Other clinically mild inherited disorders, such as heterozygous glucokinase deficiency [58] or Gilbert syndrome [59], are also diagnosed in the fourth decade of life, inferring that HK deficiency may also have a mild or late-onset phenotype that might be masked by age-related morbidity.

This type of meta-analysis has significant limitations, most notably publication bias. Patient data were not collected systematically, and cases with abnormal medical histories may have been detected more frequently than asymptomatic ones. In addition, patients with suspected hemostasis disorders (family history; many cases with mild bleeding events) are more likely to undergo a hematologic work-up. Because the study cohort is too small for confounder or multiple testing adjustment, the aforementioned clinical implications should be considered descriptive.

HK is part of the contact and kallikrein-kinin system, involved in coagulation, fibrinolysis, complement activation, inflammation, and blood pressure regulation. It binds to FXI and PK and acts as a cofactor for the FXIIa and PK-dependent initiation of contact activated plasmatic coagulation and as a substrate for the release of BK [60]. HK consists of 6 domains (D) with individual properties, such as a low-affinity calcium-binding site (D1), inhibition of cysteine proteases (D2+3), binding to platelets and endothelial cells (D3), or interaction with bacterial cell walls and anionic surfaces (D5, aa402-441, aa471-498) [17]. Mediated by uPAR, cytokeratin1, gC1qR, and heparan sulfate, HK also binds to endothelial cells, platelets, and neutrophils (D3-5), leading, eg, to the inhibition of angiogenesis. In addition, HK can interfere with vitronectin. Domain 6 harbors a PK (S583-K613) and FXI binding site (P574-M631), containing a polymorphism (rs710446, c.1742T>C, p.Ile581Thr) associated with shorter aPTT and thrombosis (C allele) [61–64]. Thus, HK affects multiple physiological pathways, most of which are poorly studied in HK deficiency.

All coagulation factor levels were unaffected by HK deficiency, except for FXI and PK. They were significantly decreased, which could be because of a faster clearance caused by the loss of binding to HK [15,16]. Variability of PK levels in HK deficiency is high and ranges from 7% to normal, potentially triggering misdiagnosis. The reason for this is unclear but may reflect a wide biological range and the use of varying, nonstandardized assay systems. Distinct HK variants do not seem causative as all known HK deficiency–causing variants are truncating, and, with one exception, no HK:Ag was detectable in HK-deficient patients. The molecular characterization of the sole HK-deficient case with residual HK antigen (~13%; Fitzgerald) was not entirely conclusive because of scarce material [1,48,53]. The authors suspected a splice site defect caused by NM_001102416.3(KNG1) c.1126-538_1126-525delinsGGTGGTGGTGGTGGTGG in intron 9 (updated nomenclature). This variant is located in a repeat region with numerous frequent indels far from the canonical splice sites, and Western blot analysis did prove a truncated protein but no splice defect. Owing to missing whole-gene sequencing and mRNA analysis, it remains unclear whether this variant is responsible for HK deficiency and a residual HK antigen.

The prevalence estimate of HK deficiency using GnomAD resulted in one case per 2 million to 22 million people, depending on genetic ancestry. According to this intentionally conservative prevalence estimate, HK deficiency may qualify as an ultra-rare condition. However, it is far more common than the few PubMed listed cases suggest and more relevant than this classification implies because numerous aPTTs are screened daily in many nations, making it pertinent for the requesting physicians to be aware of the implications of this deficiency. In addition, because 40% of the cases originate from the "gray literature", a publication gap is evident. Since this prevalence estimate relies on the generalizability of the underlying database data, it also may be prone to miss or overinterpret local effects (eg, founder effects).

For example, a slight clustering of HK-deficient cases is present in 3 publications that report above-average numbers of cases (3-7 index cases) [38–40], especially compared with the usually more frequent PK deficiency in the same publications (1-3 index cases) [36]. Possible reasons might be consanguinity or founder effects. A genetic advantage is also conceivable because HK deficiency–causing variants may be beneficial, eg, in infectious diseases or inflammation. Although HK deficiency may not exhibit a clear clinical impact in humans, animal models imply a protective effect, eg, against reactive arthritis [65], enterocolitis [66], allergic asthma [67], and thrombosis [11,68]. Kininogen-deficient rats also show resistance against encephalitis by a coronavirus (JHM strain) and a shorter delay in lymphocyte proliferation [69]. Because SARS-CoV-2 infections reduce angiotensin-converting enzyme 2, increasing des-Arg(9)-bradykinin levels, which is associated with lung injury and inflammation [9], HK deficiency may be beneficial. However, by using constraint metrics calculated by a GnomAD-based algorithm, no significant heterozygote survival advantage or disadvantage could be observed for KNG1 because the mathematical model indicates as many existing as expected KNG1 missense variants. Solely a slight suppression of truncating loss-of-

function variants is observed, which might indicate a minor selection pressure [70] and, considering the evolutionary persistence of HK, suggests a physiological significance in humans despite the absence of overt symptoms in HK deficiency.

Only truncating variants causing HK deficiency have been reported so far. The only amino acid substitution with presumed functional consequence, c.1136T>A p.Met379Lys, does not cause HK deficiency. It most likely mediates a gain of function resulting in hereditary angioedema (HAE) [71]. Therefore, HAE is the only known disease with a distinct clinical phenotype associated with KNG1 variants. The pathogenicity of a second, recently reported HAE-related KNG1 missense variant (Pro574Ala) remains uncertain because the American College of Medical Genetics and Genomics criteria were not met and a potential mechanism for a gain of function is missing [72]. Consulting the Gene Damage Index Score [73] reveals that approximately 90% of all genes are more intolerant against deleterious variants than KNG1. A possible reason might be that HK, as a scaffold protein, is less dependent on its secondary structure and thus largely resistant against function loss through amino acid exchanges. Only if those affect specific sites of HK (eg, signal peptide, BK peptide, or FXI/PK binding domain), they may alter functionality, causing HAE (gain of function) or perhaps other defects affecting the inflammatory role of HK. This is consistent with the observation that as many amino acid exchanges as expected were detected in KNG1 in databases, but only one amino acid exchange mediating a gain of function causes an apparent condition [71].

Because HK deficiency is considered rare, awareness is missing, and only a few hospitals/laboratories have established a diagnostic strategy. Such a strategy is important to avoid surgery delays and unnecessary prohemostatic treatment, especially in emergencies, because HK deficiency is usually an incidental finding. Reliable assays for diagnosis are the determination of HK:C with HK-deficient substrate plasma, HK ELISAs, or sequencing of KNG1. Since commercial assays are often difficult to obtain or unavailable, especially in low-income countries, alternative methods have been proposed, eg, mixing studies [41] or the more common measurement of the aPTT with a prolonged preincubation time. This modified aPTT measurement typically leads to normalization in PK deficiency but not in HK deficiency [74]. However, this assay is troublesome because it largely depends on the individual assay set up and the quality of the local evaluation process, requiring precharacterized plasma samples and positive controls analyzed in parallel, which prevents most laboratories from setting up a reliable assay [75]. In addition, often no complete normalization is seen in PK-deficient cases and a substantial shortening of the aPTT might also occur in HK deficiency [42,74], making the interpretation of the results difficult and ambiguous [75]. Thus, the determination of an aPTT with prolonged preincubation time (10 minutes incubation) is only a reliable diagnostic assay if validated properly. Furthermore, the case of Tomao et al. [42] illustrates that even the PK clotting activity is sometimes not sufficient for a final diagnosis. Their case, with a PK:C of 7%, turned out to be secondary to HK deficiency when KNG1 was sequenced in a

subsequent study [36]. Therefore, in cases with residual PK activity (PK:C >5%), HK should always be determined since the PK level in HK-deficient cases can be substantially decreased (lowest values 7%-10%). Heterozygous carriers of HK deficiency are not detectable on a functional level because they show a normal aPTT and inconclusive HK:C. HK antigen levels are decreased to ∼50% in heterozygotes, but a definitive diagnosis of carrier status can only be determined by genotyping. To unify and improve the diagnostic work-up of an isolated aPTT prolongation with suspected HK or PK deficiency, we propose the diagnostic scheme given in Figure 4.

In conclusion, we performed the first comprehensive analysis of HK deficiency, compiling, and complementing clinical and molecular genetic experience from more than 50 years. HK deficiency is a rare condition (1 in every 8 million people) with only a few pathogenic variants known. It has an apparently good prognosis but is prone to misdiagnosis. Based on clinical data, a prohemostatic treatment before surgery does appear disproportionate in HK-deficient patients in light of the low risk of bleeding.

Our understanding of clinical implications is still limited, and an international register on PK and HK deficiency is being established in collaboration with the International Society on Thrombosis and Haemostasis to fill this gap of knowledge. It aims to provide comprehensive diagnostics to healthcare facilities, to contribute to standardize diagnostic criteria, and to obtain a large, bias-free study population suitable to evaluate potential clinical and physiological consequences.

AUTHOR CONTRIBUTIONS

A.A. and S.B. contributed equally to this work. B.L. and H.R. also contributed equally as co-senior authors. Study design and writing of the manuscript: A.A., S.B., B.L., and H.R. Acquisition and interpretation of patient data: A.A., S.B., A.T., S.K., H.F.N., C.A., V.R., Y.P., L.T., A.R.-S., M.E., S.A., S.S., E.D.E., B.L., and H.R.. Laboratory and bioinformatics analysis: A.A., S.S., F.H., and H.R. Literature search and statistical analyses: A.A., S.B., and S.K. Critical revision and final approval: A.A., S.B., A.T., S.K., H.F.N., C.A., V.R., Y.P., L.T., A.R.-S., M.E., S.A., S.S., E.D.E., F.H., K.J.L., B.L., and H.R. Project Administration: K.J.L., B.L., and H.R.

DECLARATION OF COMPETING INTERESTS

None of the authors reports any conflicts of interest with the present work.

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SUPPLEMENTARY MATERIAL

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