ORIGINAL ARTICLE

Severe plasma prekallikrein deficiency: Clinical characteristics, novel *KLKB1* mutations, and estimated prevalence

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Abstract

Background: Severe plasma prekallikrein (PK) deficiency is an autosomal-recessive defect characterized by isolated activated partial thromboplastin time prolongation. To date, no comprehensive methodologically firm analysis has investigated the diagnostic, clinical, and genetic characteristics of PK deficiency, and its prevalence remains unknown.

Patients/Methods: We described new families with PK deficiency, retrieved clinical and laboratory information of cases systematically searched in the (gray) literature, and collected blood of these cases for complementary analyses. The Genome Aggregation Database (gnomAD) and the population-based Gutenberg Health Study served to study the prevalence of mutations and relevant genetic variants.

Results: We assembled a cohort of 111 cases from 89 families and performed new genetic analyses in eight families (three unpublished). We identified new *KLKB1* mutations, excluded the pathogenicity of some of the previously described ones, and estimated a prevalence of severe PK deficiency of 1/155 668 overall and 1/4725 among Africans. One individual reported with PK deficiency had, in fact, congenital kininogen deficiency associated with decreased PK activity. One quarter of individuals had factor XII clotting activity below the reference range. Four major bleeding events were described in 96 individuals, of which 3 were provoked, for a prevalence of 4% and an annualized rate of 0.1%. The prevalence of cardiovascular events was 15% (6% <40 years; 21% 40-65 years; 33% >65 years) for an annualized rate of 0.4%.

Conclusions: We characterized the genetic background of severe PK deficiency, critically appraised mutations, and provided prevalence estimates. Our data on

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laboratory characteristics and clinical course of severe PK deficiency may have clinical implications.

KEYWORDS

bleeding, intrinsic pathway, *KLKB1* mutations, prekallikrein deficiency, thrombosis

1 | INTRODUCTION

Prekallikrein (PK; also called "Fletcher factor" after the first family with a severe deficiency described in 1965)[1] is the zymogen of the serine protease plasma kallikrein, which participates in the initiation of the intrinsic pathway of coagulation together with factors XII (FXII), XI (FXI), and the non-enzymatic cofactor high-molecular-weight kininogen (HK), and mediates the inflammatory response by liberating the vasodilatory and proinflammatory nonapeptide bradykinin from HK.[2,3] This mechanism is involved in several diseases,[2] notably hereditary angioedema,[4] for which plasma kallikrein inhibitors may represent an effective therapy.[5,6]

Severe PK deficiency is an autosomal-recessive defect caused by *KLKB1* mutations. An isolated prolongation of the activated partial thromboplastin time (aPTT) is often the initial hint.[3] A swift confirmation of PK deficiency is essential to prevent time-consuming investigation of other potential causes of aPTT prolongation, possibly delaying invasive procedures and prolonging the in-hospital stay.[7] The preoperative administration of prohemostatic agents may represent a useless or even harmful intervention, as excess of bleeding is considered to be absent.

The absence of overt severe clinical disturbances has led to a gradual decrement of clinical research on PK deficiency (Figure S1 in supporting information), although plasma kallikrein inhibition is emerging as an appealing therapy to modulate coagulation[8] and control bradykinin release.[5,6] Novel data concerning the genetic background and diagnostic criteria of PK deficiency are lacking. Because publication bias cannot be excluded, the most common scientific repositories used in prior reviews[9] are unlikely to contain all available studies. In this context, the prevalence of severe PK deficiency in the general population is thought to be very low, but remains unknown.

Our goal is to conduct a comprehensive analysis of severe PK deficiency by (a) identifying *KLKB1* mutations in novel cases or after having obtained blood material of published families, and estimating

> **Essentials**
>
> - Severe plasma prekallikrein (PK) deficiency is a rare autosomal-recessive defect.
> - We analyzed genetic, laboratory, and clinical data of 111 individuals with severe PK deficiency.
> - The estimated prevalence of severe PK deficiency is 1/155 668 (overall) and 1/4725 (Africans).
> - Major bleeding occurred in 4% (0.1% yearly), cardiovascular events in 15% (0.4% yearly).

the prevalence of causal mutations in large-scale sequencing projects, (b) critically evaluating all reported *KLKB1* mutations based on the predicted effect on the protein and estimating their prevalence, and (c) studying the laboratory features and prevalence of clinical events in cases identified by a systematic review of the literature.

2 | METHODS

2.1 | Study population

The study population of individuals with severe PK deficiency was assembled based on the following sources: (1) novel cases of severe PK deficiency diagnosed at two German centers, from whom complete clinical, laboratory, and genetic information was established in this study; (2a) cases identified from a systematic review of the literature from whom blood samples were obtained to perform complementary genetic analyses and functional tests after contact with the authors; (2b) cases identified from a systematic review of the literature contributing only clinical or laboratory data, as reported in the original publication. The study flow is summarized in Figure 1.
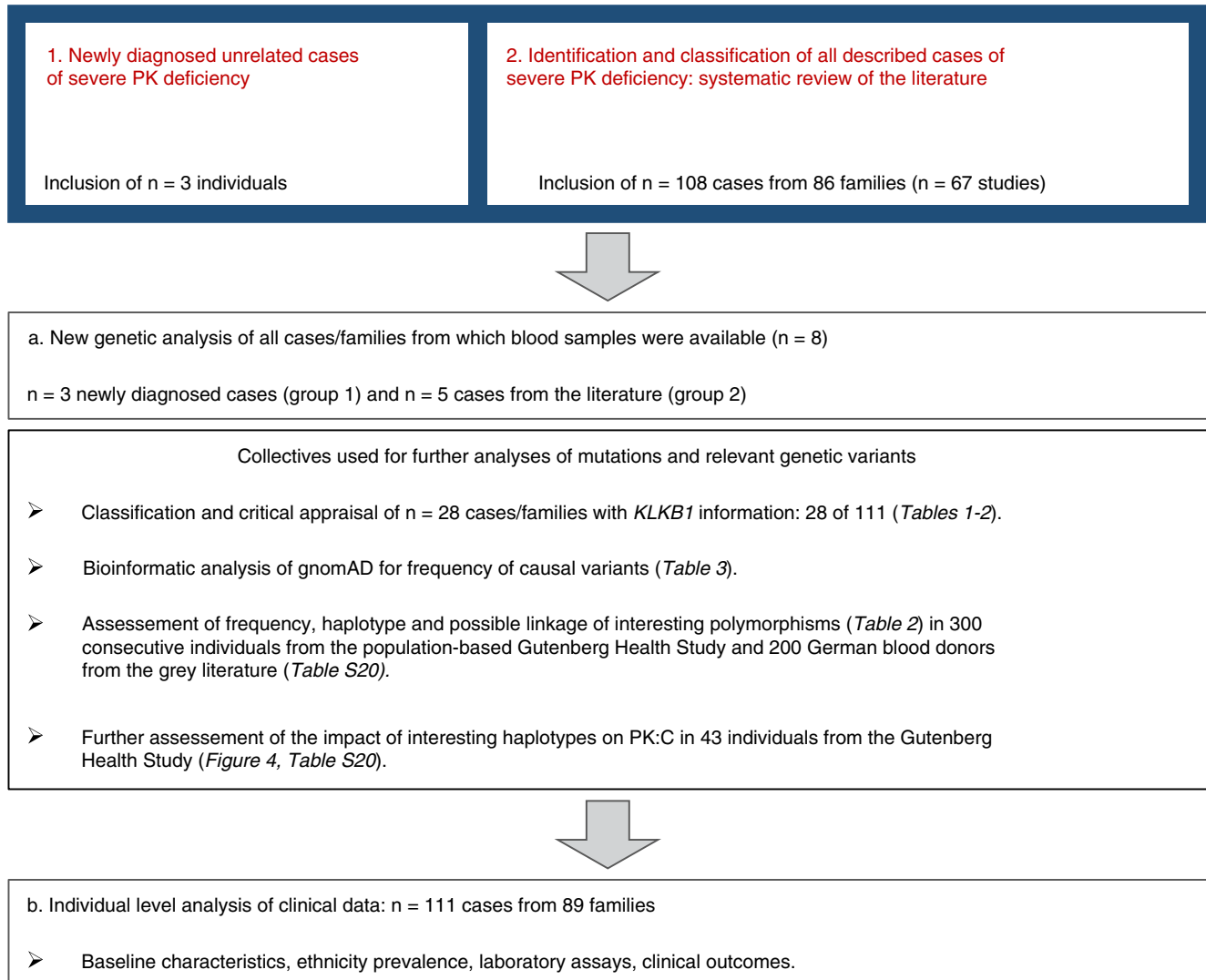
1. Newly diagnosed unrelated cases of severe PK deficiency	2. Identification and classification of all described cases of severe PK deficiency: systematic review of the literature
Inclusion of n = 3 individuals	Inclusion of n = 108 cases from 86 families (n = 67 studies)

a. New genetic analysis of all cases/families from which blood samples were available (n = 8)

n = 3 newly diagnosed cases (group 1) and n = 5 cases from the literature (group 2)

Collectives used for further analyses of mutations and relevant genetic variants

➢ Classification and critical appraisal of n = 28 cases/families with *KLKB1* information: 28 of 111 (*Tables 1-2*).

➢ Bioinformatic analysis of gnomAD for frequency of causal variants (*Table 3*).

➢ Assessment of frequency, haplotype and possible linkage of interesting polymorphisms (*Table 2*) in 300 consecutive individuals from the population-based Gutenberg Health Study and 200 German blood donors from the grey literature (*Table S20).*

➢ Further assessment of the impact of interesting haplotypes on PK:C in 43 individuals from the Gutenberg Health Study (*Figure 4, Table S20*).

b. Individual level analysis of clinical data: n = 111 cases from 89 families

➢ Baseline characteristics, ethnicity prevalence, laboratory assays, clinical outcomes.

FIGURE 1 Overview of the process of study selection to assess clinical events and perform new genetic studies and gene frequency analysis in individuals with severe prekallikrein (PK) deficiency

In our systematic review of the literature, we searched MEDLINE (PubMed), EMBASE (Ovid), Web of Science, the gray literature, and user-based or institutional repositories without time/language restrictions. Detailed methods are reported in Appendix S1 in supporting information. The study selection consisted of three steps: (a) evaluation of titles/abstracts, (b) selection of full texts, and (c) contact with the authors to obtain additional clinical information or blood material for laboratory analyses.

All potentially eligible cases of severe PK deficiency were discussed by five investigators and included if the diagnostic tools were adequately described and the diagnosis was supported by a combination of specific diagnostic criteria. Because several tests, notably PK clotting activity (PK:C) and genetic analyses of *KLKB1*, became available only recently, we could not apply a single set of criteria to cases described over a 50-year span. The presence of at least one of these criteria, possibly in individuals screened for the presence of other coagulation factor deficiencies and lupus anticoagulant, sufficed for inclusion in the present analysis: (a) PK:C or PK amidolytic activity

test < 15% of normal (irrespective of the PK:Antigen [PK:Ag] value), or mixing studies with deficient plasmas; (b) normalization or quasi-normalization of aPTT after prolonged pre-incubation before recalcification; (c) presence of causal, homozygous or compound heterozygous, *KLKB1* mutations. We excluded cases with a diagnosis not substantiated by laboratory tests (ie, PK:C performed but values not reported), or key inclusion criteria were not met (ie, PK:C ≥ 15%). These cases are listed and discussed in Tables S1-S2 in supporting information.

2.2 | Quantitative analysis of clinical events

We focused on three main aspects of the clinical presentation of severe PK deficiency: major bleeding events, thromboembolic events, and other comorbidities (including arterial hypo- and hypertension). Bleeding events were retrospectively classified into major and non-major[10] by two independent investigators. Thromboembolic events were classified according to the site of presentation.

2.3 | Laboratory methods

Prothrombin time (HemosIL RecombiPlasTin 2G reagent); derived fibrinogen; aPTT (SynthASil reagent); thrombin time; clotting activity of coagulation factors FVIII:C, FIX:C, FXI:C, FXII:C; lupus anticoagulant testing; and further mixing studies (aPTT in mixtures of index cases and normal plasma before and after incubation at 37°C for 1 hour to exclude inhibitors) were performed on an ACL TOP 700 instrument (Instrumentation Laboratory Company) using IL reagents and following the manufacturer's instructions. PK:C and HK:C were measured with reagents supplied by IL, George King Bio-Medical Inc (PK Deficient Plasma), and CoaChrom Diagnostica GmbH (HK Deficient Plasma).[11] Clotting times were obtained by a KC4A Macro coagulation analyzer (SYCOmed e.K.). PK:Ag and HK:Ag were determined by enzyme-linked immunosorbent assay (ELISA) using the matched-pair antibody set for ELISA of human PK and HK (Affinity Biologicals Inc).

Genetic testing was performed based on genomic DNA from ethylenediaminetetraacetic acid (EDTA) blood samples. When no whole blood was available, cell-free DNA was extracted and isolated from 1-2 mL citrated plasma using QIAamp® MinElute® ccfDNA Mini Kit (Qiagen): comprehensive details on the genotyping procedure on cell-free DNA are provided in Appendix S1. Depending on the accessible material, variant detection in *KLKB1* or *KNG1* was done by Sanger Sequencing (Beckman CEQ8000, Sciex), Pyrosequencing (PyroMark Q96 ID, Qiagen), or whole exome sequencing (WES, MedExome, Roche). Digital droplet polymerase chain reaction (ddPCR; droplet generator, QX200 droplet-reader, Bio-Rad) was used for the confirmation of gross deletions detected by WES. Detailed information on laboratory methods and all the assays used is given in Tables S3-S14 in supporting information and Figure S2 in supporting information.

2.4 | Statistical and bioinformatic analyses

We analyzed individual level data; the prevalence of clinical events was accompanied by 95% confidence interval (95% CI, Wilson method) for the entire population and across age groups. Based on the years of observation which each individual contributed, we calculated annualized incidence rates (95% CI, Poisson formula) of clinical events.

To provide initial estimates of the prevalence of mutations causing PK deficiency according to the Hardy-Weinberg principle, the allele frequencies of the most common *KLKB1* mutations were retrieved from the Genome Aggregation Database (gnomAD; https://gnomad.broadinstitute.org/). The gnomAD aggregates exome and genome sequencing data from several large-scale sequencing projects spanning 125 748 exome and 15 708 whole-genome sequences from unrelated individuals from population and disease-specific genetic studies not including individuals (or their relatives) with severe pediatric diseases;[12] it represents the most comprehensive population dataset with genetic information of individuals of different ethnicity and was used for population estimates.[12]

The frequency and, if applicable, the possible haplotype linkage (GenePOP 4.2) of three common non-pathogenic *KLKB1* variants (c.428G>A, p.Ser143Asn; c.1679G>A, p.Arg560Gln; c.759-12dupT) were deduced from a prior analysis of the 200 youngest blood donors from a cohort of 750 consecutive German donors and extrapolated from.[13] In addition, we analyzed PK:C activity and the genotype for three relevant variants in 300 individuals included in the population-based Gutenberg Health Study (GHS). The GHS is a population-based, prospective, single-center cohort study that started in 2007 at the University Medical Center Mainz and enrolled participants from the German Rhine-Main area according to the local residents' registration office. It primarily aimed to investigate the prevalence and course of cardiovascular diseases, cancer, eye diseases, metabolic disorders, diseases of the immune system, and mental illnesses in the general population. A total of 15 000 individuals was enrolled after 2007. Inclusion criteria were an age between 35 and 74 years and a written consent; exclusion criteria were insufficient knowledge of the German language to understand explanations and instructions, and physical or psychic inability to participate in the examinations in the study center. The samples analyzed for the present study were collected from 300 individuals who had been recruited for GHS consecutively from April to July 2019 and consented to genetic analyses. Additional details on the database, variant calling tools, gene variant sorting, prediction of variant dignity, and GHS are given in Table S14.[14]

The nomenclature of all previously described mutations was adapted to HGVS using NM_000892.4 as reference, starting with the first nucleotide in the translation initiation codon. Older numbering of the mutations, however, had often been reported omitting the first 57 nucleotides/19 amino acids.

Genotyping as well as functional and protein analyses were performed for diagnostic purposes at the Institute of Clinical Chemistry and Laboratory Medicine, University Medical Center Mainz, in individuals who gave explicit consent also for the use of their anonymized data for research purposes. The GHS was approved by an independent ethics committee and required separate consent: all individuals provided written informed consent to participate in parent studies. The use of anonymized retrospective data or of data already published in the literature followed local regulation at each participating institution. The studies were designed and executed in accordance with all local legal and regulatory requirements, notably the General Data Protection Regulation (EU 2016/679) and the Declaration of Helsinki, 7th revision.

3 | RESULTS

3.1 | Identification of cases of severe PK deficiency

We identified 67 studies reporting a total of 108 cases diagnosed with severe PK deficiency from 86 families.[1,11,13,15-79] The process of study selection is summarized in Figure 1; the reasons for study exclusion are detailed in Tables S1-S2.

After the incorporation of three novel unrelated cases (Cases #1-3 described below and in Tables S15-S19 in supporting

information), we assembled a cohort of 111 cases (89 families) with severe PK deficiency, who contributed for clinical events and laboratory characteristics. Women represented 47.5% of the overall study population (n = 10 missing values for sex); median age at diagnosis was 39 (Q1-Q3: 16-58) years. Of 51 cases with available individual case level information on ethnicity, the majority were reported to have either an African-American (n = 20) or Caucasian (n = 25) background. Of 17 cases with pedigree information, 9 (53%) had consanguineous parents. Severe PK deficiency was diagnosed after routine pre-surgical screening in 46% of cases.

In 15 individuals from eight families, including the three novel cases, we performed new genetic analyses (Table 1).[11,13,15-17]

3.2 | Molecular genetic analyses in novel and not previously investigated cases of severe PK deficiency

3.2.1 | Case #1

A 68-year-old woman from Ghana underwent routine coagulation tests before nephrectomy (renal cell carcinoma). Personal and familial history of bleeding and thrombotic events was negative. Severe PK deficiency was diagnosed based on aPTT prolongation, normal activity of coagulation factors, absence of heparinemia and lupus anticoagulant, quasi-normalization of aPTT with increasing pre-incubation time (Figure 2), and PK:C and PK:Ag<1% each. Nephrectomy was performed without complications. WES revealed the *KLKB1* homozygous mutation c.451dupT, p.Ser151Phefs*34.[13,63]

3.2.2 | Case #2

A 17-year-old German boy was referred for an isolated aPTT prolongation. Coagulation factor activities were normal with no heparinemia and lupus anticoagulant. PK:C was <1%, PK:Ag was 10%, HK:C and HK:Ag were normal. We detected the homozygous *KLKB1* mutation c.1643G>A, p.Cys548Tyr (Sanger), previously described with the legacy name Cys529Tyr (Cys548 corresponds to Cys529 in mature PK).[13,18,21,62,63] His parents were heterozygous for p.Cys548Tyr with normal PK:C.

3.2.3 | Case #3

A 26-year-old man from Somalia without personal or familial history of thrombotic/bleeding disorders underwent routine coagulation tests before sinus surgery. Severe PK deficiency was diagnosed based on aPTT prolongation (Figure 2), normal activity of coagulation factors except for FXII:C (39%-50%), neither heparinemia nor lupus anticoagulant, decreasing aPTT with increasing pre-incubation time, and <1% for PK:C and PK:Ag. Surgery was performed without complications. WES revealed the homozygous *KLKB1* mutation

c.451dupT, p.Ser151Phefs*34 (as in Case #1), as well as a functional polymorphism c.-4C>T and a variant of unknown significance c.413C>A in *F12*.

3.2.4 | Case #4

Asmis and colleagues[11] reported on a 71-year-old Swiss man with primary myelofibrosis, who was hospitalized for splenectomy. No personal or familial history of bleeding or thrombotic events was reported. PK deficiency was diagnosed based on prolonged aPTT, aPTT shortening after prolonged pre-incubation, PK:C 5%, and PK:Ag 2%. Blood samples were obtained from two living offsprings, in whom the heterozygous *KLKB1* mutation c.1203_1204delGT, p.Trp402Alafs*35 was found (Sanger). Therefore, the index case should have been either homozygous or compound heterozygous for p.Trp402Alafs*35 and a further, unknown mutation.

3.2.5 | Case #5

Tomao and colleagues[15] described the case of a 72-year-old Italian woman with endometriosis and no prior bleeding/thrombosis. The diagnosis of PK deficiency was made based on a prolonged aPTT, normal activity of coagulation factors (HK:C not tested), aPTT shortening after 15 minutes of pre-incubation, and PK:C 7%. We analyzed previously stored EDTA blood. No *KLKB1* mutations were found (Sanger), but we identified two *KNG1* mutations (WES): c.1038+1G>A and c.1165C>T, p.Arg389*. The mutations have never been reported in the literature for HK deficiency; their pathogenicity is predicted because they may lead to a nonsense-mediated mRNA decay (NMD) or a truncated protein without binding region for PK/FXI. These results suggest that this individual had HK deficiency, but was erroneously classified as severe PK deficiency based on a low PK:C value.

3.2.6 | Case #6

In 1993, Wuillemin and colleagues reported on a 68-year-old Swiss man with myocardial infarction.[16] The diagnosis of PK deficiency was based on aPTT prolongation, normal activity of coagulation factors, aPTT shortening after prolonged pre-incubation, PK:C<1%, and PK:Ag 10%. Only citrated plasma from the deceased case was stored. Pyrosequencing, which allows sequencing of short DNA fragments, detected heterozygous c.1643G>A, p.Cys548Tyr. A library preparation protocol for next-generation sequencing from ancient DNA was adapted to our sequencing procedure.[80] WES confirmed c.1643G>A, p.Cys548Tyr and detected c.1196G>A, p.Trp399*, later confirmed by pyrosequencing. c.1196G>A, p.Trp399* has not been reported yet. The case's daughter had PK:C and PK:Ag in the low reference range, and was heterozygous for c.1643G>A, p.Cys548Tyr (pyrosequencing).

TABLE 1 Cases and families with severe PK deficiency with molecular genotyping analyses reported or performed in this study

Family ID (reference)	Allele 1	Allele 2	Method	Mat.	PK:C, %	PK:Ag, %	HK:C, %	HK:Ag, %	Conclusions
Unpublished cases									
1 — Index case (68, F)	c.451dupT, p.Ser151Phefs*34	c.451dupT, p.Ser151Phefs*34	WES, Sanger	EB	<1	<1	94	95	homozygous PK deficiency
2 — Index case (17, M)	c.1643G>A, p.Cys548Tyr	c.1643G>A, p.Cys548Tyr	Sanger	EB	<1	10	70	68	homozygous PK deficiency (CRM+)
2-2: Index case's mother (45, F)	c.1643G>A, p.Cys548Tyr	KLKB1: —	Sanger	EB	72	87	98	87	heterozygous carrier
2-3: Index case's father (52, M)	c.1643G>A, p.Cys548Tyr	KLKB1: —	Sanger	EB	70	93	175	93	heterozygous carrier
3 — Index case (26, M)	c.451dupT, p.Ser151Phefs*34	c.451dupT, p.Ser151Phefs*34	Sanger	EB	<1	<1	142	76	homozygous PK deficiency
Published cases not previously tested for mutations									
4[11] — Index case (71, M)	c.1203_1204delGT, p.Trp402Alafs*35	Unknown	Deduced from the children's genotype	—	5	2	—	—	prekallikrein deficiency (homozygous or compound heterozygous)
4-2: Index case's daughter (58, F)	c.1203_1204delGT, p.Trp402Alafs*35	KLKB1: —	Sanger	EB	66	43	91	73	heterozygous carrier
4-3: Index case's son (56, M)	c.1203_1204delGT, p.Trp402Alafs*35	KLKB1: —	Sanger	EB	59	34	93	58	heterozygous carrier
5[15] — Index case (72, F)	KLKB1: — **KNG1: c.1165C>T, p.Arg389***	KLKB1: — **KNG1: c.1038+1G>A**	Sanger WES, Sanger	EB EB	7	—	—	—	*compound heterozygous: HK deficiency*
6[16] — Index case (68, M)	**c.1196G>A, p.Trp399***	c.1643G>A, p.Cys548Tyr	WES + PSQ	CP	1	6-10	91	70	compound heterozygous PK deficiency (CRM+)
6-2: Index case's daughter (—, F)	KLKB1: —	c.1643G>A, p.Cys548Tyr	PSQ	CP	55	61	100	76	heterozygous carrier
7[17] — Index case (3, F)	**c.143_221+128del, p.Gln48Argfs*11**	**c.143_221+128del, p.Gln48Argfs*11**	WES, ddPCR, Sanger	EB	1	<1	110	—	homozygous PK deficiency
7-2: Index case's mother (35, F)	**c.143_221+128del, p.Gln48Argfs*11**	KLKB1: —	WES, ddPCR, Sanger	EB	—	79	—	—	heterozygous carrier
7-3: Index case's father (50, M)	**c.143_221+128del, p.Gln48Argfs*11**	KLKB1: —	WES, ddPCR, Sanger	EB	—	72	—	—	heterozygous carrier
7-4: Index case's brother (5, M)	KLKB1: —	KLKB1: —	WES, ddPCR	EB	—	95	—	—	not affected

TABLE 1 (Continued)

Family ID (reference)	Allele 1	Allele 2	Method	Mat.	PK:C, %	PK:Ag, %	HK:C, %	HK:Ag, %	Conclusions
Published case with genetic data (gray literature), resequenced for this publication and complemented by functional data									
8[13] Index case (13, M)	**c.689T>A, p.Ile230Asn**	c.1643G>A, p.Cys548Tyr	Sanger, PSQ	EB	<1	9	84	73	compound heterozygous PK deficiency (CRM+)
Published cases with genetic data (gray literature)									
9[13] 9-1 Index case (34, F)	c.451dupT, p.Ser151Phefs*34	c.451dupT, p.Ser151Phefs*34	dHPLC, Sanger	EB	–	–	–	–	homozygous PK deficiency
9-2 Index case's daughter (9, F)	c.451dupT, p.Ser151Phefs*34	KLKB1: –	dHPLC, Sanger	EB	–	–	–	–	heterozygous carrier
9-3 Index case's daughter (13, F)	c.451dupT, p.Ser151Phefs*34	KLKB1: –	dHPLC, Sanger	EB	–	–	–	–	heterozygous carrier
9-4 Index case's daughter (11, F)	c.451dupT, p.Ser151Phefs*34	KLKB1: –	dHPLC, Sanger	EB	–	–	–	–	heterozygous carrier
10[13] 10-1 Index case (64, M)	c.717_719delCTT, p.Phe240del	c.1643G>A, p.Cys548Tyr	dHPLC, Sanger	EB	<1	–	–	–	compound heterozygous PK deficiency (CRM + likely)
10-2 Index case's son (42, M)	KLKB1: –	c.1643G>A, p.Cys548Tyr	dHPLC, Sanger	EB	–	–	–	–	heterozygous carrier
11[13] Index case (30, F)	c.1165delA, p.Thr389Hisfs*18	c.1643G>A, p.Cys548Tyr	dHPLC, Sanger	EB	<1	–	–	–	compound heterozygous PK deficiency
12[13] Index case (62, F)	c.337C>T, p.Arg113*	c.689T>A, p.Ile230Asn	dHPLC, Sanger	EB	–	–	–	–	compound heterozygous PK deficiency
13[13] Index case (73, M)	c.1643G>A, p.Cys548Tyr	c.1643G>A, p.Cys548Tyr	dHPLC, Sanger	EB	<1	–	–	–	homozygous PK deficiency (CRM + likely, no data on PK:Ag available)
14[13] Index case (23, F)	c.337C>T, p.Arg113*	c.337C>T, p.Arg113*	dHPLC, Sanger	EB	–	–	–	–	homozygous PK deficiency
Published cases with genetic data (PubMed, Embase)									
15[18] 15-1 Index case (14, M)	c.1205G>A, p.Trp402*	c.1643G>A, p.Cys548Tyr	Sanger	BL	<1	traces	Norm	–	compound heterozygous PK deficiency
15-2 Index case's mother (–, F)	KLKB1: –	c.1643G>A, p.Cys548Tyr	Sanger	BL	68	72	Norm	–	heterozygous carrier
15-3 Index case's father (–, M)	c.1205G>A, p.Trp402*	KLKB1: –	Sanger	BL	50	60	Norm	–	heterozygous carrier
15-4 Index case's brother (–, M)	KLKB1: –	c.337C>T, p.Arg113*	Sanger	BL	90	95	Norm	–	not affected
16[61] 16-1; Index case (47, M)	c.1259G>A, p.Gly420Glu	c.1259G>A, p.Gly420Glu	Sanger	WB	<1	25	–	–	homozygous PK deficiency (CRM+)
16-2; Index case's sister (–, F)	c.1259G>A, p.Gly420Glu	c.1259G>A, p.Gly420Glu	ASO	WB	1	21	–	–	homozygous PK deficiency (CRM+)

(Continues)

TABLE 1 (Continued)

Family ID (reference)	Allele 1	Allele 2	Method	Mat.	PK:C, %	PK:Ag, %	HK:C, %	HK:Ag, %	Conclusions
16-3 to 16-14; 12 relatives from three generations	c.1259G>A, p.Gly420Glu	KLKB1: —	ASO	WB	23-50	50-76	—	—	heterozygous carriers
16-15 to 16-18; 4 relatives from three generations	KLKB1: —	KLKB1: —	ASO	WB	88-150	82-140	—	—	not affected
17-1; Index case (79, M)	c.337C>T, p.Arg113*	c.337C>T, p.Arg113*	Sanger	—	<1	4	79	—	homozygous PK deficiency
17-2; Index case's wife (—, F)	KLKB1: —	KLKB1: —	RFLP	—	—	—	—	—	not affected
17-3 to 17-7; Index cases' five children	c.337C>T, p.Arg113*	KLKB1: —	RFLP	—	58-78	48-72	—	—	heterozygous carriers
18-1; Index case (53, M)	c.[367G>A;428=], p.[Gly123Arg;Ser143=]	c.[367G>A;428=], p.[Gly123Arg;Ser143=]	Sanger	BL	1	—	75	—	homozygous PK deficiency
18-2, 18-3; Index case's sisters	c.[367G>A;428=], p.[Gly123Arg;Ser143=]	c.[367G>A;428=], p.[Gly123Arg;Ser143=]	Sanger	BL	1; 3	—	—	—	homozygous PK deficiency
18-4, 18-5; Index case's mother and brother	c.[367G>A;428=], p.[Gly123Arg;Ser143=]	c.[367G>A;428G>A], p.[Gly123Arg;Ser143Asn]	Sanger	BL	74; 90	—	—	—	heterozygous carriers
18-6, 18-7; Case's daughters	c.[367G>A;428=], p.[Gly123Arg;Ser143=]	c.[367=;428G>A], p.[Gly123=;Ser143Asn]	Sanger	BL	90; 100	—	—	—	heterozygous carriers
19[62]	c.1643G>A, p.Cys548Tyr	c.1643G>A, p.Cys548Tyr	Sanger	EB	<1	7	—	—	homozygous PK deficiency (CRM+)
20[62]	c.1643G>A, p.Cys548Tyr	c.1643G>A, p.Cys548Tyr	Sanger	EB	<1	7.5	—	—	homozygous PK deficiency (CRM+)
21[20]	c.1259G>A, p.Gly420Glu	c.1259G>A, p.Gly420Glu	—	—	<1	—	—	—	homozygous PK deficiency
22[21]	c.1643G>A, p.Cys548Tyr	c.1643G>A, p.Cys548Tyr	—	—	13	—	Norm.	—	homozygous PK deficiency (CRM + likely, no data on PK:Ag available)
23-1; Index case (14, M)	c.451dupT, p.Ser151Phefs*34	c.1643G>A, p.Cys548Tyr	Sanger	BL	<1	—	68	—	compound heterozygous PK deficiency
23-2; Index case's mother	KLKB1: —	c.1643G>A, p.Cys548Tyr	Sanger	BL	77	—	—	—	heterozygous carrier
23-3; Index case's father	c.451dupT, p.Ser151Phefs*34	KLKB1: —	Sanger	BL	85	—	—	—	heterozygous carrier
23-4; Index case's brother	KLKB1: —	KLKB1: —	Sanger	BL	81	—	—	—	not affected

23[63]

(Continues)

TABLE 1 (Continued)

Family ID (reference)	Allele 1	Allele 2	Method	Mat.	PK:C, %	PK:Ag, %	HK:C, %	HK:Ag, %	Conclusions
23-5; Index case's brother	c.451dupT, p.Ser151Phefs*34	c.1643G>A, p.Cys548Tyr	Sanger	BL	<1	–	–	–	compound heteozygous PK deficiency
24[68]; Index case (40, M)	c.1731T>G, p.Asp577Glu	Unknown; c.759-12dupT variant is a common polymorphism[a]	Sanger	BL	5	95	60	–	suspected PK deficiency, *second mutation unknown: the reported variant is a common polymorphism*
24-2, 24-3 Index case's sisters	c.1731T>G, p.Asp577Glu	Unknown; c.759-12dupT variant is a common polymorphism[a]	Sanger	BL	~4	Norm.	Norm.	–	suspected PK deficiency; *second mutation unknown: the reported variant is a common polymorphism*
24-4 Index case's brother	c.1731T>G, p.Asp577Glu	Unknown; c.759-12dupT variant is a common polymorphism[a]	Sanger	BL	~50	Norm.	–	–	heterozygous carrier: *the other allele has a common polymorphism*
25[31] Index case (40, M)	c.1553G>A, Trp518*	c.1553G>A, Trp518*	Sanger	–	<10	–	–	–	homozygous PK deficiency
26[64] Index case (32, M)	c.[428=;1679G>A], p.[Ser143=;Arg560Gln][a]	c.[428=;1679G>A], p.[Ser143=;Arg560Gln][a]	Sanger	BL	1	<1	93	–	*PK deficiency; homozygosity for two common polymorphisms most likely not the cause of severe PK deficiency*
26-2; Index case's aunt (55, F)	c.[428=;1679G>A], p.[Ser143=;Arg560Gln][a]	c.[428=;1679G>A], p.[Ser143=;Arg560Gln][a]	Sanger	BL	1	<1	–	–	*PK deficiency; homozygosity for two common polymorphisms most likely not the cause of severe PK deficiency*
26-3 to 26-9; 7 relatives from three generations	c.[428=;1679G>A], p.[Ser143=;Arg560Gln][a]	KLKB1: –	Sanger	BL	68-86	–	–	–	heterozygous carriers?
26-10 to 26-12; 3 relatives from three generations	KLKB1: –	KLKB1: –	Sanger	BL	88-126	–	–	–	not affected
27[59] Index case (4, M)	c.1259G>A, p.Gly420Glu	c.1198G>T, p.Gly400*	Sanger	WB	<1	3-4	–	–	compound heterozygous PK deficiency
28[79] Index case (58, F)	c.417_418insCATTCTTA, p.Arg140Hisfs*3	c.821delA, p.Asn274Thrfs*9	Sanger	–	<1	<1	–	–	compound heterozygous PK deficiency

Note: The following mutations have been identified in this study for the first time (shown in the table in bold): c.1203_1204delGT, p.Trp402Alafs*35 (*KLKB1*); c.1165C>T, p.Arg389* (*KNG1*); c.1038+1G>A (*KNG1*); c.1196G>A, p.Trp399* (*KLKB1*); c.143_221=128del, p.Gln48Argfs*11 (*KLKB1*); c.689T>A, p.Ile230Asn (*KLKB1*).

Abbreviations: ASO, allele-specific oligonucleotide hybridization; BL, blood leukocytes; CP, citrated plasma; CRM, cross-reacting material; ddPCR, digital droplet polymerase chain reaction; dHPLC, denaturing high pressure liquid chromatography; EB, EDTA-blood; HK, high-molecular-weight kininogen; PK, prekallikrein; PSQ, pyrosequencing; RFLP, restriction fragment length polymorphism; Sanger, Sanger sequencing; WB, whole blood; WES, whole-exome-sequencing.

[a]The following polymorphisms previously described in the literature in association with severe PK deficiency are unlikely to be causal mutations: c.[428=;1679G>A], p.[Ser143=:Arg560Gln>A], p.[Ser143=:Arg560Gln] (*KLKB1*); c.759-12dupT (*KLKB1*); *KLKB1*: –, no mutation detected; c.: coding DNA reference sequence used (NM_000892.4); p.: protein reference sequence used (NP_000883.2).

TABLE 2 Critical reevaluation of all the KLKB1 variants reported to cause PK deficiency and likely consequences on protein level

KLKB1 mutation	Exon	MAF (dbSNP), %	rsID (dbSNP)	Bioinformatic analyses					Consequences on protein level	Ref.	Classification (ACMG/AMP)[82]
				ClinVar	Poly Phen2	SIFT	PROVEAN	Mutation Taster			
Missense											
c.1643G>A, p.Cys548Tyr	14	0.03-0.08	rs121964951	P/VUS	D	D	D	D	amino acid exchange	13,18,21,62,63	pathogenic
c.689T>A, p.Ile230Asn	7	0.02-0.05	rs142420360	—	D	D	D	D	amino acid exchange	13	likely pathogenic
c.1259G>A, p.Gly420Glu	11	0.02-0.0008	rs186254196	P	D	D	D	D	amino acid exchange	20,59,61	pathogenic
c.[367G>A;428=], p.[Gly123Arg;Ser143=]	5	—	rs121964952; rs3733402	P	D	D	D	D	two amino acid exchanges on one allele	67	likely pathogenic
c.367G>A, p.Gly123Arg	5	0.0008	rs121964952	P	D	D	D	D	amino acid exchange	67	variant of uncertain significance
c.428G>A, p.Ser143Asn	5	39-46	rs3733402	P	B	B	B	B	amino acid exchange, 143Ser: possibly less active protein	64,67	benign
c.1731T>G, p.Asp577Glu	15	—	—	—	VUS	D	D	D	amino acid exchange	68	likely pathogenic
c.[428=;1679G>A], p.[Ser143=;Arg560Gln]	5/14	—	rs3733402; rs4253325	—	B	B	B	B	two amino acid exchanges on one allele	64	benign
c.1679G>A, p.Arg560Gln	14	16-22	rs4253325	—	B	B	B	B	amino acid exchange	64	benign
Frameshift											
c.451dupT, p.Ser151Phefs*34	5	0.13-0.56	rs560588447	—	—	—	—	—	truncating mutation, presumably NMD	13,63	pathogenic
c.1203_1204delGT, p.Trp402Alafs*35	11	0.01	rs768319200	—	—	—	—	—	truncating mutation, presumably NMD	—	pathogenic
c.1165delA, p.Thr389Hisfs*18	11	—	—	—	—	—	—	—	truncating mutation, presumably NMD	13	pathogenic
c.143_221+128del, p.Gln48Argfs*11	3	—	—	—	—	—	—	—	truncating mutation, presumably NMD	—	pathogenic
c.417_418insCATTCTTA, p.Arg140Hisfs*3	5	0.002-0.004	rs777280545	—	—	—	—	—	truncating mutation, presumably NMD	79	pathogenic
c.821delA, p.Asn274Thrfs*9	8	—	—	—	—	—	—	—	truncating mutation, presumably NMD	79	pathogenic

(Continues)

TABLE 2 (Continued)

KLKB1 mutation	Exon	MAF (dbSNP), %	rsID (dbSNP)	Bioinformatic analyses					Consequences on protein level	Ref.	Classification (ACMG/AMP)[82]
				ClinVar	Poly Phen2	SIFT	PROVEAN	Mutation Taster			
Truncation/Nonsense											
c.1196G>A, p.Trp399*	11	–	rs1303557405	–	–	–	–	–	truncating mutation, presumably NMD	–	pathogenic
c.337C>T, p.Arg113*	5	0.002-0.007	rs121964949	P	–	–	–	–	truncating mutation, presumably NMD	13,19	pathogenic
c.1205G>A, p.Trp402*	11	0.0008	rs121964950	P	–	–	–	–	truncating mutation, presumably NMD	18	pathogenic
c.1553G>A, p.Trp518*	13	–	–	–	–	–	–	–	truncating mutation, presumably NMD	31	pathogenic
c.1198G>T, p.Gly400*	11	–	–	–	–	–	–	–	truncating mutation, presumably NMD	59	pathogenic
In frame deletion											
c.717_719delCTT, p.Phe240del	7	–	–	–	–	–	D	D	deletion of Phe at position 240	13	likely pathogenic
Splice site											
c.759-12dupT	IVS7	34.7- 38.7	rs3214676	–	–	–	–	–	splice defect?	68	benign

Note: KLKB1 mutations: numbered according to RefSeq NM_000892.4; Genome Build GRCh37/hg19: total exon count for *KLKB1*: 15; MAF: minor allele frequency according to dbSNP; ClinVar: P = likely pathogenic, VUS = variant of uncertain significance; D = deleterious/damaging/disease causing; B = benign/polymorphism/neutral/tolerated; NMD = nonsense mediated mRNA decay; ACMG/ AMP = American College of Medical Genetics and Genomics/Association of Molecular Pathology. The following KLKB1 mutations have been identified in this study for the first time (shown in the table in bold): c.1203_1204delGT, p.Trp402Alafs*35; c.1196G>A, p.Trp399*; c.143_221+128del, p.Gln48Argfs*11; c.689T>A, p.Ile230Asn. The following polymorphisms previously described in the literature in association with severe PK deficiency are unlikely to be causal mutations: c.[428=;1679G>A], p.[Ser143=;Arg560Gln] (KLKB1); c.759-12dupT (KLKB1). – = no data available; c.: coding DNA reference sequence used (NM_000892.4); p.: protein reference sequence used (NP_000883.2).

3.2.7 | Case #7

Shahverdi and colleagues[17] reported on a 3-year-old Iranian girl presenting with recurrent epistaxis, bruising, thrombocytopenia, and giant platelets due to Bernard-Soulier syndrome. Severe PK deficiency was diagnosed based on a prolonged aPTT which normalized upon prolonged pre-incubation, normal activity of coagulation factors, and PK:C 1.3%. Sanger sequencing resulted in low quality electropherograms due to sample degradation during shipment. WES revealed the homozygous missense mutation c.284G>A, p.Tyr95Cys in exon 3 of *GP9*, which causes Bernard-Soulier syndrome,[81] and the homozygous partial deletion of exon 3 of *KLKB1* (c.143_221+128del,

p.Gln48Argfs*11), confirmed by ddPCR. The parents were heterozygous for both mutations.

3.2.8 | Case #8

Complete analysis of citrated plasma and EDTA blood of a 13-year-old German boy confirmed the diagnosis of PK deficiency[13] based on aPTT prolongation, normal activity of coagulation factors (HK:C not tested; PK:C<1%, PK:Ag 9%), and genetic analysis. Sanger sequencing confirmed compound heterozygosity for c.1643G>A, p.Cys548Tyr and c.689T>A, p.Ile230Asn.[13]

3.3 | Genetic analysis: critical appraisal, nomenclature, and allele frequency

All index cases of PK deficiency with novel and previously published genetic data of the *KLKB1* gene are summarized in Table 1, including pedigree information, DNA source, genotyping method, and the results of PK/HK:C and antigen. Table 2 and Figure 3 include detailed information on all *KLKB1* variants, the minor allele frequency (dbSNP), the results of bioinformatics testing, and a critical appraisal concerning their dignity according to the American College of Medical Genetics and Genomics/Association of Molecular Pathology guidelines.[82]

We categorized four alleles, which had been suggested to be disease-causing, as not pathogenic (benign) or variants of uncertain significance, namely c.428G>A, p.Ser143Asn; c.[428=;1679G>A], p.[Ser143=;Arg560Gln]; c.1679G>A, p.Arg560Gln; and c.759-12dupT. The reevaluation of the genotypes of 200 German blood donors, as well as the analysis of the genetic and functional data of 300 participants of the GHS revealed that the four abovementioned alleles were too frequent to cause severe PK deficiency

FIGURE 2 Measurement of activated partial thromboplastin time (aPTT) using different preincubation times of plasma with aPTT reagent before addition of calcium. Plasma of the index individuals and several control plasmas were mixed with aPTT reagent for various time intervals before addition of Ca^{2+}. Clotting time almost normalized upon preincubation for ≥ 480 seconds (SyntAsil, Instrumentation Laboratory, Bedford, US)

FIGURE 3 Summary and localization of *KLKB1* mutations. Variants reported in [brackets] refer to common polymorphisms, which have been described in association with severe prekallikrein (PK) deficiency, but have been shown in the present work not to be causal/pathogenic mutations. The following *KLKB1* mutations have been identified in this study for the first time: c.1203_1204delGT, p.Trp402Alafs*35; c.1196G>A, p.Trp399*; c.143_221+128del, p.Gln48Argfs*11; c.689T>A, p.Ile230Asn. The following polymorphisms previously described in the literature in association with severe PK deficiency are unlikely to be causal mutations: c.[428=;1679G>A], p.[Ser143=;Arg560Gln] (*KLKB1*); c.759-12dupT (*KLKB1*). c.: coding DNA reference sequence used (NM_000892.4); p.: protein reference sequence used (NP_000883.2)

(c.[428=;1679G>A], p.[Ser143=;Arg560Gln]: no homozygotes, but ≥11 heterozygotes/500; c.[428=;759-12dupT]: ≥15 heterozygotes/200, 5 homozygotes/200; Table S20 in supporting information). None of the haplotypes defined by the variants p.Ser143Asn and p.Arg560Gln caused reduced PK activity comparable to that observed in heterozygotes for PK deficiency (Figure 4). While some functional effect cannot be ruled out, we exclude that any of these variants causes severe PK deficiency.

3.4 | Estimated prevalence of severe PK deficiency

The data from gnomAD (Table 3) depicted a higher-than-expected prevalence of mutations causing PK deficiency in individuals of African ancestry (1 per 4725 population), as compared to non-Finnish Europeans (1 per 242 786 population) or Latinos (1 per 536 587 population). We estimated an overall prevalence of severe PK deficiency of 1 per 155 668 (6 per 1 000 000) population. The high estimated prevalence among Africans is primarily due to the frequency of c.451dupT, p.Ser151Phefs*34 (rs560588447),[83] which we detected in homozygous form in two individuals of African origin (Cases #1, #3).

3.5 | Laboratory parameters

Median aPTT using non-ellagic acid-based assays was 119 (Q1-Q3: 96-150; n = 64; reference ranges 31-60) seconds. Median aPTT was
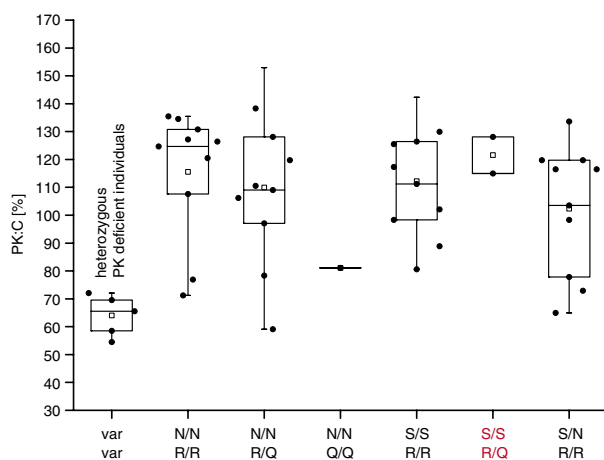
FIGURE 4 Impact of different *KLKB1* haplotypes (defined by the common polymorphisms p.Ser143Asn and p.Arg560Gln) on prekallikrein clotting activity (PK:C). Comparison of PK:C between *KLKB1* heterozygotes (var: various genotypes for p.Ser143Asn and p.Arg560Gln, also shown in Figure 5, left panel) and control individuals from the Gutenberg Health Study (GHS), who carry combinations of the common *KLKB1* polymorphisms p.Ser143Asn (N/N, S/N, and S/S) and p.Arg560Gln (R/R, R/Q, Q/Q). In homozygosity, the haplotype 143Ser-560Gln (S-Q) was suggested to cause PK deficiency.[64] This graph shows that heterozygosity (S/S-Q/R, shown in red) is not associated with a reduction of PK:C as expected for heterozygotes of *KLKB1* mutations (left column, var). p.: protein reference sequence used (NP_000883.2)

normal or only mildly prolonged if ellagic acid-based reagents were used: median 40 (Q1-Q3: 32-45; n = 17; reference ranges 21-42) seconds. PK:C was low (≤5%) in more than 95% of cases tested (Table S16). One case with PK:C of 7% (Case #5) had compound heterozygous *KNG1* mutations suggesting severe hereditary HK deficiency with secondary PK:C reduction (Figure 5).

The median (Q1-Q3; min-max) clotting activity of the other clotting factors was: FVIII:C 100% (85%-160%; 47%-330%), FIX:C 92% (80%-110%; 38%-173%), FXI:C 89% (72%-100%; 49%-200%), FXII:C 80% (66%-104%; 25%-180%), and HK:C 90% (79%-110%; 31%-153%).

3.6 | Clinical events

After excluding cases with no clinical information reported, a total of 14 thromboembolic events were described in 95 individuals with severe PK deficiency (Table S17) for a prevalence of 14.7% (95% CI 9.0%-23.2%) and an annualized incidence rate of 0.4 cases/100 person-years (95% CI 0.2-0.6). Of these, four had familial history of cardiovascular disorders. Among individuals aged <40 years, three had thromboembolic events for a prevalence of 6.3% (95% CI 2.1%-16.8%): one was described as "low-extremity thrombophlebitis" and two were ischemic strokes. A total of 6 (20.7%; 95% CI 9.8%-38.4%) events occurred in individuals aged 40-65 years, including three ischemic strokes, one myocardial infarction, and two venous thromboembolic events. Among individuals older than 65 years, the prevalence was 33.3% (95% CI 15.2%-58.3%; n = 5 arterial cardio- or cerebrovascular events).

Four (4.2%; 95% CI 1.6%-10.2%) major bleeding events were described in 96 individuals during their lifetime for an annualized incidence rate of 0.1 cases/100 person-years (95% CI 0.03-0.3). They consisted of a spontaneous recurrent hematemesis with hypovolemic shock in a 13-year-old child and of three provoked events: (a) an anticoagulant-associated intracranial hemorrhage after cerebral vein thrombosis in a 43-year-old individual, (b) a prolonged bleeding after tonsillectomy requiring transfusion of fresh frozen plasma (6-month-old child), (c) an intracranial hemorrhage in a premature newborn with no further bleeding events until the age of 9 (Table S18). The prevalence of major bleeding was 6.3% (95% CI 2.1%-16.8%; n = 3) in individuals aged <40 years, 3.3% (95% CI 0.6%-16.7%; n = 1) in individuals aged 40-65 years, and 0% (95% CI 0%-20.4%; n = 0) in the elderly. The prevalence of other (non-major) bleedings was 14.5%, 16.6%, and 13.3%, respectively.

The prevalence of other comorbidities seemed sporadic; the quality of reporting was deemed to be too poor for quantitative synthesis (Table S19).

4 | DISCUSSION

Our individual level analysis of 111 individuals with severe PK deficiency represents the largest study ever performed on this condition.

TABLE 3 Allele frequencies (%) of the most common pathogenic *KLKB1* mutations and estimated prevalence of homozygotes or compound heterozygotes for mutations causing severe prekallikrein (PK) deficiency

KLKB1 mutations	African	European (non-Finnish)	Latino	East Asian	South Asian	European (Finnish)	Ashkenazi Jewish	Other	TOTAL
Variant allele count out of total allele number (variant allele frequency, %)									
rs121964951[a]	2/24 756 (0.01)	**128/128 518 (0.1)**	**35/35 060 (0.1)**	0/19 766 (0)	2/30 382 (0.01)	0/25 028 (0)	0/10 348 (0)	**13/7134 (0.18)**	180/280 992 (0.06)
rs142420360	2/24 970 (0.01)	44/129 198 (0.03)	3/35 440 (0.01)	0/19 954 (0)	0/30 616 (0)	0/25 122 (0)	0/10 370 (0)	2/7226 (0.03)	51/282 896 (0.02)
rs186254196	0/16 106 (0)	0/112 678 (0)	0/34 544 (0)	2/18 364 (0.01)	0/30 580 (0)	0/21 632 (0)	0/10 008 (0)	0/6108 (0)	2/250 020 (0)
rs121964952	0/16 254 (0)	1/113 676 (0)	0/34 588 (0)	0/18 394 (0)	0/30 612 (0)	0/21 648 (0)	0/10 078 (0)	0/6134 (0)	1/251 384 (0)
rs560588447[b]	**358/24 954 (1.43)**	37/129 130 (0.03)	9/35 436 (0.03)	0/19 954 (0)	8/30 614 (0.03)	2/25 120 (0.01)	0/10 370 (0)	5/7226 (0.07)	419/282 804 (0.15)
rs768319200	0/24 878 (0)	34/128 406 (0.03)	1/35 422 (0)	0/19 934 (0)	0/30 596 (0)	0/25 118 (0)	0/10 330 (0)	0/7204 (0)	35/281 888 (0.01)
rs777280545	0/24 966 (0)	0/129 156 (0)	0/35 438 (0)	8/19 954 (0.04)	0/30 612 (0)	0/25 122 (0)	0/10 370 (0)	0/7226 (0)	8/282 844 (0)
rs121964949	1/24 956 (0)	16/129 046 (0.01)	0/35 434 (0)	0/19 950 (0)	0/30 612 (0)	0/25 120 (0)	1/10 366 (0)	1/7218 (0.01)	18/282 702 (0.01)
rs121964950	0/16 160 (0)	1/112 832 (0)	0/34 564 (0)	0/18 376 (0)	0/30 592 (0)	0/21 642 (0)	0/10 038 (0)	0/6120 (0)	1/250 324 (0)
Estimated prevalence of compound heterozygotes or homozygotes for mutations causing severe PK deficiency									
One case per N population	N = 4725	N = 242 786	N = 536 587	N = 3 847 226	N > 5 000 000	N > 5 000 000	N > 5 000 000	N = 116 521	N = 155 668
Cases per 1 000 000 population	210	4	2	<1	<1	<1	<1	9	6

Note: The bold values represent allele counts resulting in variant allele frequencies ≥ 0.1%. c.: coding DNA reference sequence used (NM_000892.4); p.: protein reference sequence used (NP_000883.2).

[a]c.1643G>A, p.Cys548Tyr emerged as the most common mutation among non-Finnish Europeans.

[b]c.451dupT, p.Ser151Phefs*34 emerged as the most common mutation among Africans.
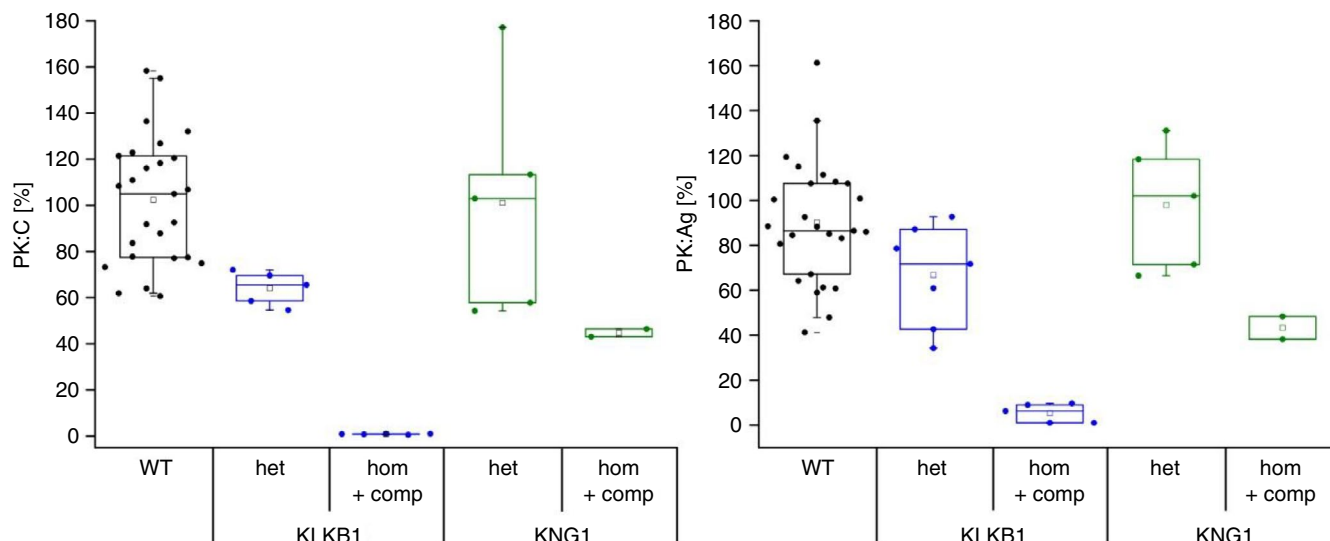
FIGURE 5 Clotting activity (PK:C) and antigen level (PK:Ag) of prekallikrein (PK) in individuals with a defect in *KLKB1*, *KNG1*, and control individuals. Comparison of PK:C (left panel) and PK:Ag (right panel) between control individuals (WT) and individuals, who carry mutations either in the *KLKB1* or in the *KNG1* gene; het: heterozygous, hom: homozygous, comp: compound heterozygous; blank square: mean value. The graphs illustrate that there is a significant difference in PK:C and PK:Ag between individuals with no, heterozygous and homozygous, or compound heterozygous *KLKB1* mutations. Heterozygous carriers of *KNG1* mutations do not show reduced PK:C or PK:Ag, but homozygotes and compound heterozygotes for *KNG1* mutations show a PK:C and PK:Ag similar or even lower than heterozygotes for *KLKB1* mutations

We identified novel pathogenic mutations and confirmed that some of those previously described as "causal" or "pathogenic" represent common variants. We provided prevalence estimates, suggesting that severe PK deficiency is a frequent cause of prolonged aPTT in individuals of African ancestry. As preliminary as our evidence is, the low prevalence of bleeding events observed in individuals with PK deficiency does not support routine administration of prohemostatic products before surgical procedures.

Our estimate of the prevalence of homozygotes or compound heterozygotes with severe PK deficiency suggests a much higher prevalence of causal *KLKB1* mutations among individuals of African ancestry (1 per 4725 versus 1 per 155 668 overall). In a population survey published in 1977, the authors identified 1 case of PK deficiency among 40 522 individuals from a Nigerian community.[40] If confirmed, these results may have consequences for individuals of African origins undergoing routine coagulation screening, especially considering that the prevalence at birth for non-severe hemophilia A and B is approximately 1 case per 6600 males and 1 case per 28 600 males, respectively.[84] All cases of PK deficiency with homozygosity and compound heterozygosity for c.1643G>A, p.Cys548Tyr[13,18,21,62,63] presented with low non-functional PK antigen (Cross Reacting Material+) and were more often European [69]; c.451dupT, p.Ser151Phefs*34 explained most cases in individuals of African ancestry.[13,63]

We excluded that any of the three common variants 143Ser, 560Gln,[64] and 759-12dupT[68] (or a haplotype combining these variants) cause PK deficiency, in contrast to prior reports.[64,67,68] With a frequency of >1% for 143Ser-560Gln,[64] which is by far the rarest of the aforementioned common alleles, their involvement in the genesis of PK deficiency is not plausible (Table S20). In the setting of a population-based study, we showed that individuals heterozygous for 143Ser-560Gln had PK:C and PK:Ag levels twice as high as in the presence of a confirmed heterozygous PK defect and identical to non-PK-deficient individuals (Figure 4). Prior reporting on 143Ser-560Gln as a pathogenic variant was based on its co-segregation in homozygosity with severe PK deficiency (Case #26).[64] However, it is commonly observed that benign variants are in linkage with the disease-causing variant and co-segregate with the phenotype. Some mutation types missed by conventional sequencing were not tested, including gross deletions.

A further report described four siblings (Case #24)[68] with the same, allegedly PK deficiency-causing genotype, but only three of four affected individuals showed PK deficiency; therefore, at least one of the described variants was not disease-causing. While c.1731T>G, p.Asp577Glu (in the original paper described as p.Asp-558Glu) is rare, and bioinformatics predicts pathogenicity, the other variant, c.759-12dupT, is common (33% in a collective of German blood donors, Table S20), intronic, and not predicted to alter splicing. As 143Ser co-segregates with 759-12dupT in the family, the authors speculated that the haplotype 143Ser/759-12dupT was disease causing, but it does not co-segregate with the phenotype and is frequent in German blood donors.

There is one report[67] suggesting a role of 143Ser, whenever located in cis with the rare variant c.367G>A, p.Gly123Arg (in the original paper described as Gly104Arg). Three siblings (Case #18) with PK:C ≤ 3% were homozygous for 123Arg and 143Ser, while homozygosity for 123Arg alone did not co-segregate with PK deficiency. Analysis of the recombinantly expressed A2 domain of PK showed the lowest affinity to HK when the amino acids at position 143 and 123 were both substituted, but the presented data was not significant. Gross deletions were not investigated.[67]

A correct diagnosis of PK deficiency may prevent unnecessary delay in surgical procedures. However, functional PK assays may not be widely available and a prolonged aPTT, a normalization of aPTT following increased preincubation time, and the exclusion of other factor deficiencies may represent the only diagnostic information available at most centers. Although plasma kallikrein inhibition may not have an impact on hemostasis in humans, a selective reduction of PK induced an antithrombotic effect in animal models.[85-87] Cases of PK-deficient dogs and horses with excessive bleeding were described.[88,89] We showed that the prevalence of major bleeding events was very low, approximately 4%, corresponding to an annualized estimated incidence rate of 0.1%, which was calculated based on the years contributed by each individual. This rate is similar to that observed in the general population for nonfatal major bleeding among individuals aged 30-39 years who were not exposed to antithrombotic therapy.[90] It must be noted that only one of four major bleeding events was spontaneous, whereas the other three individuals had a provoked event, namely an anticoagulant-associated intracranial hemorrhage, a prolonged bleeding after tonsillectomy, and an intracranial hemorrhage related to prematurity. In contrast, the prevalence of arterial cardiovascular and cerebrovascular events appeared higher with a prevalence of 15% and an estimated annualized incidence rate of 0.4% in a population characterized by a median age of 39 years. Although these findings can only serve to generate hypotheses, they may not support the routine administration of prohemostatic products to individuals with severe PK deficiency who undergo invasive procedures.

Of five individuals/families from whom we performed additional genetic analysis, we identified one carrier of compound heterozygous mutations in *KNG1*, likely associated with a truncated HK protein, and no mutation in *KLKB1*. PK-deficient samples are characterized by almost normalization of the severely prolonged aPTT following increased preincubation time, which is due to autoactivation of FXII.[11] The case had low PK:C (7%) and only moderate aPTT shortening after prolonged pre-incubation.[15] We confirmed the reduction of PK:C in hereditary HK deficiency (Figure 5) and attributed it to the lack of chaperone function of HK, resulting in PK:C values between 10%-45%.[91-99] Of note, we confirmed prior reports showing that ellagic acid-based aPTT reagents may be relatively insensitive to PK deficiency as ellagic acid may accelerate FXII autoactivation.[100,101] Other cases of HK deficiency may have been mistaken for PK deficiency and PK deficiency cases missed if ellagic acid-based aPTT reagents were used for screening.

Severe PK deficiency is a rare benign condition, as reflected by several reports in the gray literature identified by our literature search. Underreporting may be substantial and we acknowledge publication bias as a key limitation of our analysis with only exceptional cases, ie, those with overt clinical manifestations, beeing reported. This is also indirectly suggested by the number of publications having appeared over the past 50 years (Figure S1). This may have substantially influenced the prevalence of clinical events. Furthermore, the type and severity of clinical events were extrapolated, if and when available, from information in the original reports. In this context, we cannot exclude misclassification bias, over- or underreporting of comorbidities, and the presence of confounding by indication, as many individuals indeed received prohemostatic products before invasive procedures. We also acknowledge limitations concerning our prevalence estimates. GnomAD contains data from population studies and disease-specific genetic studies not including pediatric diseases: our estimates may not fully reflect the prevalence in the general population, especially considering that Africans are less genetically homogeneous than other groups. The fact that the prevalent pathogenic *KLKB1* mutation c.451dupT, p.Ser151Phefs*34 was found in individuals from African regions that are far apart, Ghana and Somalia, is only partially reassuring.

We identified novel mutations causing severe PK deficiency and unveiled that some of those previously described in the literature represent common variants. Prevalence of severe PK deficiency may be as high as 1 per 4725 among individuals of African ancestry. From a diagnostic perspective, it must be considered that ellagic-acid based aPTT reagents are relatively insensitive to PK deficiency, that FXII:C levels may be spuriously low, and that variably low PK:C levels are encountered in individuals with severe hereditary HK deficiency. These findings will play a role for the standardization of the diagnostic criteria of severe PK and HK deficiency. Individuals with PK deficiency are not characterized by a bleeding diathesis: delaying surgical procedures and the routine administration of prohemostatic agents before invasive procedures may not be justified. Future trials on plasma kallikrein inhibitors should include cardiovascular events as a safety outcome, as their prevalence was not negligible in individuals with severe PK deficiency.

Our data on mutations, prevalence estimates, laboratory characteristics, and clinical course of severe PK deficiency may have clinical implications. Based on these results we plan to start an international registry, ideally supported by the International Society on Thrombosis and Haemostasis, including individuals with suspected or confirmed PK and HK deficiencies. In the setting of a registry study, it will be possible to standardize diagnostic criteria, profit from the accumulated experience to serve as a platform for institutions where specific PK/HK tests are not available, and allow the prospective evaluation of clinical aspects and interpretation of functional and genotype data.

CONFLICTS OF INTEREST

None of the authors report any conflicts of interest with the present work. SB received travel and congress payment from Daiichi Sankyo and Bayer HealthCare, and personal honorarium from BTG Pharmaceuticals, Leo Pharma, and Bayer HealthCare, outside the present work. BL received travel and congress payment or lecture fees from Baxter, Ablynx, Alexion, Siemens, Bayer HealthCare, Roche, outside the present work.

AUTHOR CONTRIBUTIONS

Concept and design of the study, data analysis, and writing of the manuscript: SB, SS, AT, AA, BL, HR. Acquisition and interpretation of data: SB, SS, AT, AA, HA, LC, FH, JAKH, KJL, FL, EM, NVS, LT, WAW, BZ, BL, HR. Discussion concerning potentially eligible cases of severe PK deficiency: SB, SS, AT, AA, BL. Laboratory and bioinformatics analysis: SS, AA, FH, HR. Other statistical analyses: SB. Critical revision for important intellectual content and final approval: SB, SS, AT, AA, HA, LC, FH, JAKH, KJL, FL, EM, NVS, LT, WAW, BZ, BL, HR.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.