1.02–1.55 (mean = 1.30; SD = 0.18), compatible with heterozygosity. No individuals homozygous for the Cys282Tyr mutation were identified. To validate the method for detection of the homozygous mutant genotype, the assay was performed on a blood sample from a known homozygote. The NSS ratios for this individual, in two evaluations, were 9.1 and 7.4, within the predicted range.

The results of PCR-RFLP testing were in complete agreement with those obtained with the cross-linking assay for all 101 samples.

The cross-linking assay has several advantages. It allows detection of the Cys282Tyr mutation without the laborious steps of DNA purification, PCR, and RFLP analysis, and it eliminates problems of sample inhibition of polymerases and sample contamination by amplicons. An additional advantage is the large-scale simultaneous processing of DNA samples, using the microtiter plate format. With automated detection, the cross-linking assay can be finished within 4 h.

Further work is needed to fully define the set of NSS ratio ranges that determine the three genotypes. Data from the blood sample assays showed wider variation among samples of the same genotype than was seen with the PCR samples. Presumably, this indicates that signal intensity is influenced by factors such as the efficiency of the overall sample preparation procedure and variation in blood volume and leukocyte concentration. Further sample data will allow us to set finer intervals for genotype assignment and to set "gray zone" values for repeat testing.

Large-scale, presymptomatic screening of blood donors for the Cys282Tyr mutation could identify individuals at risk for HH, who are then candidates for prophylactic phlebotomy, which increases the life expectancy to that of the general population. If such a screening regimen was to be implemented, the tests needed to perform genotype analysis will have to be accurate, inexpensive, and automatable. The cross-linking assay used here is an efficient, simple, and rapid method of genotyping *HFE* mutations that, with automation, would be suitable for routine genetic analysis in a large-scale manner.

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New Nomenclature for the Human Tissue Kallikrein Gene Family, *Eleftherios P. Diamandis,[1,2*] George M. Yousef,[1,2] Judith Clements,[3] Linda K. Ashworth,[4] Shigetaka Yoshida,[5] Torbjorn Egelrud,[6] Peter S. Nelson,[7] Sadao Shiosaka,[5] Sheila Little,[8] Hans Lilja,[9] Ulf-Hakan Stenman,[10] Harry G. Rittenhouse,[11] and Hester Wain[12]* ([1] Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, Ontario M5G 1X5, Canada; [2] Department of Laboratory Medicine and Pathobiology, University of Toronto, Ontario M5G 1L5, Canada; [3] Centre for Molecular Biotechnology, School of Life Sciences, Queensland University of Technology, Brisbane, Australia 4001; [4] Human Genome Center, Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore, CA 94551; [5] Division of Structural Cell Biology, Nara Institute of Science and Technology, 8916-5 Takayama Ikoma, Nara 630-0101, Japan; [6] Department of Dermatology, University Hospital, S-901 85 Umeå, Sweden; [7] Department of Molecular Biotechnology, University of Washington, Seattle, WA 98105; [8] Central Nervous System Research, Lilly Research Laboratories, Indianapolis, IN 46285; [9] Department of Laboratory Medicine, Division of Clinical Chemistry, Lund University, S-20502 Malmö, Sweden; [10] Department of Clinical Chemistry, Helsinki University Central Hospital, FIN-00290 Helsinki, Finland; [11] Hybritech Inc., PO Box 269006, San Diego, CA 92196; [12] Human Gene Nomenclature Committee, The Galton Laboratory, University College, London NW1 2HE, United Kingdom, * address correspondence to this author at: Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, 600 University Ave., Toronto, Ontario M5G 1X5, Canada; e-mail ediamandis@mtsinai.on.ca)

The human kallikrein gene family is important to the discipline of clinical chemistry because it contains genes that encode for valuable cancer biomarkers, including the best tumor marker available today, prostate-specific antigen (PSA). Despite reports of numerous kallikrein-like genes in the mouse *(1)*, until 2–3 years ago, only three human kallikrein genes were recognized: pancreatic/renal kallikrein (*KLK1*), human glandular kallikrein 2 (*KLK2*), and prostate-specific antigen (*KLK3*) *(1, 2)*. The proteins encoded by the three kallikrein genes are now known as hK1, hK2, and hK3 (PSA). These three genes encode for serine proteases with either trypsin-like (hK1, hK2) or chymotrypsin-like (hK3) activity. Traditionally, kallikreins have been defined as enzymes that can act on high-molecular weight substrates and release bioactive peptides, known as kinins *(3)*. Among the known kal-

likrein enzymes, only plasma kallikrein (encoded by a single gene localized on human chromosome 4q35; official symbol *KLKB1*) and pancreatic/renal kallikrein (hK1) have significant kininogenase activity. The proteins encoded by the *KLK2* and the *KLK3* genes have minimal or no kininogenase activity *(4, 5)*.

Why then are the *KLK1*, *KLK2*, and *KLK3* genes classified together into one gene family (tissue kallikrein gene family), when two of the three enzymes have no significant kallikrein enzymatic activity? This grouping is justified, based on the extensive homologies between the three genes at both the DNA and protein levels [reviewed in Ref. *(2)*] as well as their clustering in a 60-kb region on human chromosome 19q13.3-q13.4 *(6)*. It should then be emphasized that the term "kallikrein" does not necessarily imply that the gene product has kininogenase activity.

Rittenhouse et al. *(2)* have recently published a revised nomenclature for the three classical kallikrein genes. More recently, it has become apparent that several other kallikrein-like genes, encoding for serine proteases, are clustered in the same chromosomal locus along with the three classical kallikreins. We now know that there are at least 14 different genes exhibiting significant homologies and other similarities between them that are clustered within a 300-kb region on human chromosome 19q13.3-q13.4 *(7)*. The similarities between these 14 genes are summarized below [for more details, see Diamandis et al. *(7)*]:

- All genes localize to the same chromosomal region (19q13.3-q13.4), starting at ~55.6 Mb on the metric physical map of the chromosome *(8)*.
- All genes encode for putative serine proteases with a conserved catalytic triad (histidine, aspartic acid, and serine in the appropriate positions).
- All genes have five coding exons (some members contain one or more 5′ untranslated exons).
- Coding exon sizes are similar or identical.
- Intron phases are fully conserved among all 14 human members and among members of the rodent kallikrein gene families. Intron phase refers to the location of the intron within the codon. Intron phase I means that the intron occurs after the first nucleotide of the codon; phase II means that the intron occurs after the second nucleotide; phase 0 means that the intron occurs between codons.
- All genes have significant sequence homologies at the DNA and amino acid levels (40–80%).
- Many of these genes are regulated by steroid hormones.

Because many of these new genes were cloned independently by different investigators, various empirical names were initially used for their description.

The three classical human kallikrein genes as well as the rodent kallikrein genes have a loop region designated the "kallikrein loop", which is thought to be important for substrate specificity of these enzymes *(1)*. Given the diversity of substrates identified for the rodent families, the presence of the loop does not imply that all kallikreins have kininogenase activity but merely that this region, which is one of the most divergent regions in these enzymes, is important for a particular substrate specificity of each enzyme. The 11 new members of this family do not have the kallikrein loop, suggesting that this family has diverged further in humans than in rodents.

The Human Genome Organization (HUGO) recently proposed guidelines for human gene nomenclature. Initially, some members of the new kallikrein gene family were classified by HUGO, along with other serine proteases, under the prefix "PRSS", which stands for protease serine. It is now clear that this designation does not serve well the needs of the future because members of this multigene family are classified together with other serine proteases that map in different locations of the genome.

The construction of the first detailed map of the human kallikrein gene locus *(7, 9)* allows for a more rational assignment of official gene symbols. Because the tissue kallikrein multigene families of rodents and other animal species were known before 1992, an international working party had reached agreement in 1992 on uniform nomenclature of the animal kallikreins *(10)*. On the basis of this paradigm and the guidelines of HUGO (details available at the HUGO Gene Nomenclature Committee Website at: http://www.gene.ucl.ac.uk/nomenclature/), an international group of scientists working in the field agreed to adopt the new human kallikrein gene nomenclature, as shown in Table 1. Also included in Table 1 are previous symbols, based on the PRSS system as well as names originally proposed by the discoverers of these genes. Gene numbering starts from centromere to telomere on chromosome 19q13.3-q13.4 with the exception of the three classical kallikreins, for which the existing nomenclature was retained. It is possible that in the future, new members of this gene family may be identified, either centromeric to *KLK1* or telomeric to *KLK14*. If new kallikrein genes are identified in this locus, they will be numbered sequentially, starting with *KLK15*. No other kallikrein-like or other genes map between the 14 kallikrein genes described by Diamandis et al. *(7)*.

Recent developments related to the new human kallikrein gene family, including structure, function, and association of the various kallikreins with human diseases, have recently been reviewed *(7)*. It is possible that some new kallikrein genes and their products may be used in the future as valuable biomarkers for various cancers and other chronic diseases. We hope that expansion of the new human kallikrein gene family, identification of new members, and the new nomenclature presented here will facilitate progress in this field and improve our understanding of the physiological functions and the connections of the new kallikrein genes to various human diseases.

As with everything else in science, this newly proposed nomenclature should not be written in stone. As more developments become available, we will consider modifying this proposal to reflect the "state-of-the-art" knowledge and to fulfill the needs of easy communication in this field of investigation.

Table 1. New nomenclature for human kallikreins.

New gene symbol[a,b]	Previous gene symbol(s)	New protein symbol	Other protein names/symbols	GenBank Accession No.	OMIM entry[c]	Reference(s)
KLK1	*KLK1*	hK1	Pancreatic/renal kallikrein, hPRK	M25629, M33105	147910	*11, 12*
KLK3	*KLK3*	hK3	Prostate-specific antigen, PSA	X14810, M24543, M27274	176820	*13–15*
KLK2	*KLK2*	hK2	Human glandular kallikrein 1, hGK-1	M18157	147960	*16*
KLK4	*PRSS17, KLK-L1, KLK4*	hK4	Prostase, KLK-L1 protein, EMSP1	AF113141, AF135023, AF148532	603767	*17–20*
KLK5	*KLK-L2*	hK5	KLK-L2 protein, HSCTE	AF135028, AF168768		*21, 22*
KLK6	*PRSS9*	hK6	Zyme, protease M, neurosin	AF013988, AF149289, U62801, D78203	602652	*23–26*
KLK7	*PRSS6*	hK7	HSCCE	L33404, AF166330	604438	*27, 28*
KLK8	*PRSS19*	hK8	Neuropsin, ovasin, TADG-14	AB009849, AF095743, AB010780, AF055982		*29, 30*
KLK9	*KLK-L3*	hK9	KLK-L3 protein	AF135026		*9*
KLK10	*PRSSL1, NES1*	hK10	NES1 protein	AF055481, NM_002776	602673	*31–33*
KLK11	*PRSS20*	hK11	TLSP/hippostasin	AB012917, AF164623	604434	*34, 35*
KLK12	*KLK-L5*	hK12	KLK-L5 protein	AF135025		Submitted[d]
KLK13	*KLK-L4*	hK13	KLK-L4 protein	AF135024		*36*
KLK14	*KLK-L6*	hK14	KLK-L6 protein	AF161221		Submitted[e]

[a] Genes are presented in their order on chromosome 19q13.3-q13.4 from centromere to telomere.

[b] Full gene names are as follows: *hPRK*, human pancreatic/renal kallikrein; *hGK-1*, human glandular kallikrein-1; *KLK-L*, kallikrein-like; *EMSP1*, enamel matrix serine proteinase 1; *HSCTE*, human stratum corneum tryptic enzyme; *HSCCE*, human stratum corneum chymotryptic enzyme; *TADG-14*, tumor-associated, differentially expressed gene-14; *NES1*, normal epithelial cell-specific 1 gene; *TLSP*, trypsin-like serine protease; *PRSS*, protease serine; *PRSSL*, protease serine-like.

[c] The Online Mendelian Inheritance in Man (OMIM) Website is http://www.ncbi.nlm.nih.gov/omim/.

[d] Yousef GM, Magklara A, Diamandis EP. KLK-L5 is a novel serine protease and a new member of the human kallikrein gene family—differential expression in breast cancer. Submitted for publication.

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Note Added in Proof: A detailed review on the tissue human kallikrein gene family will appear in *Endocrine Reviews* [Yousef AM, Diamandis EP. The new human tissue kallikrein gene family: function and association to disease (in press)].

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Association of a Missense Glu298Asp Mutation of the Endothelial Nitric Oxide Synthase Gene with End Stage Renal Disease, *Hiromichi Suzuki, Sohji Nagase,* Shuichi Kikuchi, Ying Wang, and Akio Koyama* (Division of Nephrology, Institute of Clinical Medicine, University of Tsukuba, 1-1-1 Tennoudai, Tsukuba, Ibaraki 305-8575, Japan; * author for correspondence: fax 81-298-53-3202, e-mail sohjin@md.tsukuba.ac.jp)

Glomerular microcirculation is involved in the deterioration of renal function. Among several factors that regulate renal hemodynamics, NO has been reported to be critical *(1, 2)*. In the vascular endothelium, NO is produced by endothelial NO synthase (eNOS). NO production can be influenced by polymorphisms of the eNOS gene. Polymorphisms in exons may alter the three-dimensional structure of the enzyme, and those in introns may change the transcriptional activity. These can lead to a decrease in NO production and, subsequently, an increase in arterial pressure or intraglomerular hypertension.

Several studies have shown that the polymorphisms of the eNOS gene are associated with hemodynamics. Homozygosity of the eNOS4 *a* allele has been shown to be a risk factor for coronary artery disease among smokers *(3)*. We *(4)* and Yokoyama et al. *(5)* demonstrated that a polymorphism of the variable numbers of tandem repeats in intron 4 of the eNOS gene is associated with the progression of nondiabetic end stage renal disease (ESRD), but we failed to demonstrate the association between diabetic renal failure and the polymorphism. The genetic background for the progression of diabetic nephropathy to ESRD is still unclear. Furthermore, in some patients diabetic nephropathy does not show any association with conventional risk factors such as hyperglycemia *(6)*. We speculate that there must be a genetic factor that causes the deterioration of diabetic nephropathy to ESRD. Recently, a Glu298Asp missense polymorphism within exon 7 of the eNOS gene was reported to be associated with essential hypertension, vasospastic angina, and myocardial infarction in a Japanese population *(7–9)*. In this study, we explore the association between the Glu298Asp mutation and ESRD.

To evaluate whether the Glu298Asp mutation was associated with ESRD, we examined 159 patients with ESRD undergoing maintenance hemodialysis (96 men and 63 women; mean age, 57.1 years; age range, 17–85 years) and 270 genetically unrelated, apparently healthy control subjects (195 men and 75 women; mean age, 49.3 years; urine or blood examinations of the subjects were normal). All patients and controls were Japanese. Informed consent was obtained from each person enrolled in this study. The underlying causes of end stage renal failure were chronic glomerulonephritis (CGN; n = 68), diabetes mellitus (DM; n = 48), hypertension (n = 17), polycystic kidney disease (n = 13), lupus nephritis and vasculitis (n = 7), reflux and obstructive nephropathy (n = 5), and interstitial nephritis (n = 1; Table 1A).

Genomic DNA was extracted from peripheral mononuclear cells with a DNA extraction reagent set (Wako Pure