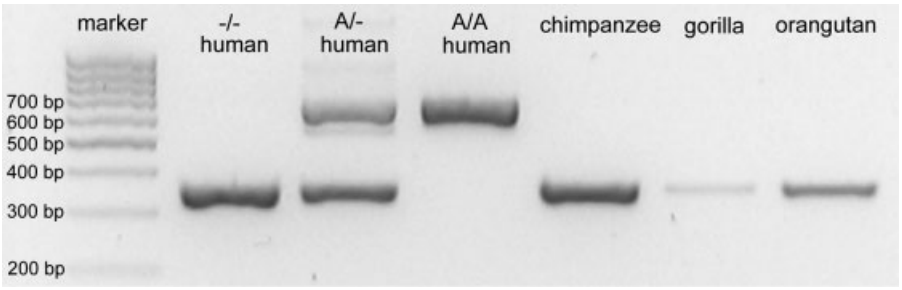

Figure 1. Detection of the presence of *WNK1* intron 10 *AluYb8* insertion in primates. Agarose gel (3%) electrophoresis of *WNK1* intron 10 PCR products amplified from human, chimpanzee, gorilla, and orangutan genomic DNAs. In humans, alternative genotype carriers are shown: wild-type homozygote without *AluYb8* insertion (−/−, PCR product 353 bp); heterozygous (A/−) and homozygous (A/A, PCR product 660 bp) carriers of the insertion

from ECACC), for an orangutan (*Pongo pygmaeus*; primary cell line AG12256, purchased from ECACC) and for 11 western chimpanzees (*Pan troglodytes verus*) using identical PCR setup as in human genotyping. DNA sample of one chimpanzee originates from a wild-born male specimen (Pino) from Tallinn Zoo, Estonia. Ten samples of wild-caught and unrelated animals (Annaclara, Frits, Hilko, Louise, Marco, Oscar, Regina, Socrates, Sonja, and Yoran) are from the collection stored at the Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany, and were kindly shared by Dr. Svante Pääbo. This sample collection is described in detail elsewhere [Becquet et al., 2007; Ptak et al., 2004].

Ancestral sequence of the targeted genomic region (*WNK1* exon10–intron10–exon11) was assessed by the comparative sequencing of the genomic DNA from *WNK1 AluYb8* insertion noncarrier (−/−) and carrier (Alu/Alu) human homozygotes as well as from a chimpanzee (Pino). Sequencing primers are listed in Supp. Table S4. PCR cycling conditions, product purification, and sequencing have been described elsewhere [Hallast et al., 2005]. Sequences were aligned using Web-based global alignment program ClustalW2 (http://www.ebi.ac.uk/Tools/clustalw2/). Human WNK1 alternative sequences of the region including exon 10/intron 10/exon 11 (without and with *AluYb8* insertion) were compared with available genome sequences from multiple species using the BLAST tool blastn (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The searches were performed against the following sequence databases: NCBI Genomes, Whole-Genome-Shotgun Sequences. Nucleotide substitution rates between human and chimpanzee were calculated as the percentage of the number of substitutions divided with the total number of aligned nucleotides in the specific genomic region. The number of substitutions and the total number of aligned nucleotides were calculated using EMBOSS stretcher (http://emboss.sourceforge.net/) [Rice et al., 2000].

Stage 1 Association Analysis in HYPEST

In Stage 1, the association of the *WNK1 AluYb8* insertion with BP was addressed using HYPEST (HYPertension in ESTonia) case–cohort sample collection (Table 1; recruitment details in Supp. Text S1). The HYPEST study has been approved by the Ethics Committee on Human Research of University of Tartu (no. 122/13, 22.12.2003; 137/20, 25.04.2005) and it was carried out in compliance with the Helsinki Declaration. All the participants have given their written informed consent. HYPEST subjects were recruited across Estonia during 2004–2007 (1,823 individuals; age range: 18–85 years) with the aim to analyze genetic–epidemiological risk factors for essential hypertension and related cardiovascular disease in Estonian population. In the current study, the total number of genotyped HYPEST subjects was $n = 1{,}747$. At the recruitment, the resting BP of each participant has been measured by trained clinicians using a standard mercury column sphygmomanometer and size-adjusted cuffs. HYPEST individuals possessed a documented history of multiple systolic and diastolic BP readings (on average, 4.31 readings per individual during mean 3.17 years). For the analysis, the median across the longitudinal BP readings as well as the median of the subject's age during the readings were used.

Association analysis with SBP and DBP was performed using 1,211 individuals (803 women, 408 men) derived from the population-based cohort across Estonia consisting of long-term blood donors not receiving any antihypertensive medication (Table 1). For binary analysis with essential hypertension, cases ($n = 673$) were defined as untreated subjects with BP readings $\geq 160/100$ mmHg based on the median of several measurements or patients receiving antihypertensive therapy. Normotensive controls ($n = 601$; SBP ≤ 140 mmHg/DBP ≤ 90 mmHg) were selected from the population-based HYPEST cohort among the subjects that have never been prescribed antihypertensive treatment.

Stage 2 Replication

In Stage 2, association testing of the *WNK1 AluYb8* insertion and BP was performed in two European samples—the BRIGHT (BRItish Genetics of HyperTension) and the CADCZ (Coronary Artery Disease in Czech)—and the results were combined in meta-analysis with Stage 1 study samples. The final sample size in meta-analysis with SBP and DBP was 3,494 subjects (2,088 women, 1,406 men; none treated with antihypertensive medication), and with hypertension 3,181 cases/2,720 controls (women, 1,851/1,692; men 1,330/1,028).

CADCZ study has been approved by the Ethics Committee of Charles University—First Faculty of Medicine (December 1996) and the BRIGHT study was approved by the Ethics Committee from local research committees of all partner institutes. All BRIGHT and CADCZ participants have given their written informed consent. The MRC British Genetics of Hypertension case–control samples have been recruited across the United Kingdom (http://www.brightstudy.ac.uk). Case ascertainment and phenotyping has been described elsewhere [Caulfield et al., 2003]. Briefly, cases originated from severely hypertensive families (1,700 sibpairs and 800 families collected for transmission disequilibrium test) were defined as patients under antihypertensive treatment and with BP readings $\geq 150/100$ mmHg based on one reading or $\geq 145/95$ mmHg based on the mean of three readings. Healthy normotensive controls ($n = 2{,}000$; BP $< 140/90$ mmHg,