effect of the E1021K mutation on the PI3Kδ activity may be cell-type or stimulus-specific, or it may be compensated for by effects of other PI3K isoforms or PTEN. Nevertheless, we cannot exclude that a subtle defect in neutrophil function may contribute to the disease pathogenesis in these patients.

In summary, we have described a PID caused by a recurrent autosomal-dominant germline mutation E1021K in the *PIK3CD* gene that encodes p110δ. We found it in 17 patients from seven unrelated families, suggesting that it is frequent among PID patients and may explain a substantial fraction of patients with recurrent respiratory infections and bronchiectasis. Our rapid genotyping assay should facilitate screening for the E1021K mutation in existing PID and bronchiectasis cohorts, as well as new patients. The E1021K mutation was previously noted in one Taiwanese patient with recurrent respiratory infections and PID; however, its causative and pathogenic role has not been demonstrated (*23*). Here, we have shown that E1021K increases PI3Kδ activity, augmenting the production of PIP_3 and activating the downstream AKT protein in lymphocytes. This leads to defects in T and B cell function and inefficient immune responses to bacterial pathogens, predisposing to recurrent respiratory infections and eventually to bronchiectasis. We named this disorder activated PI3K-δ syndrome (APDS).

Activation of the PI3K pathway is associated with malignant transformations, and it has been shown that overexpression of p110δ can transform cells (*24*). To date, only one of our APDS patients, P13, has been diagnosed with lymphoma (Table 1). Nonetheless, the oncogenic potential of PI3K up-regulation can be enhanced by additional mutations (*25*, *26*). Therefore, APDS patients may be at increased risk of leukemia or lymphoma if they acquire additional somatic mutations.

The APDS patients described here had been treated with immunoglobulin replacement and antibiotics. Despite this, there is evidence of considerable airway damage in most cases. Because of progressive severe disease after splenectomy, patient P8 underwent allogeneic hematopoietic stem cell transplantation (HSCT) at the age of 8 years. One year after HSCT, his clinical condition had improved dramatically, suggesting that HSCT may be a long-term treatment option for young patients. Nevertheless, our results raise the possibility that selective p110δ inhibitors, such as GS-1101, may be an alternative effective therapeutic approach in APDS patients. GS-1101 (CAL-101 or Idelalisib) has been tested in phase 1 and 2 clinical trials for treatment of chronic lymphocytic leukemia (www.clinicaltrials.gov). The possibility of treating APDS patients with p110δ inhibitors should therefore be considered.

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Supplementary Materials

www.sciencemag.org/342/6160/PAGE/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S9
Tables S1 and S2
References (*27*–*37*)

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Complete Mitochondrial Genomes of Ancient Canids Suggest a European Origin of Domestic Dogs

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The geographic and temporal origins of the domestic dog remain controversial, as genetic data suggest a domestication process in East Asia beginning 15,000 years ago, whereas the oldest doglike fossils are found in Europe and Siberia and date to >30,000 years ago. We analyzed the mitochondrial genomes of 18 prehistoric canids from Eurasia and the New World, along with a comprehensive panel of modern dogs and wolves. The mitochondrial genomes of all modern dogs are phylogenetically most closely related to either ancient or modern canids of Europe. Molecular dating suggests an onset of domestication there 18,800 to 32,100 years ago. These findings imply that domestic dogs are the culmination of a process that initiated with European hunter-gatherers and the canids with whom they interacted.

Dogs are one of the best known examples of domestication, the process of species modification over time by human-induced selection (*1*). Domestication often leads to increased phenotypic variation and a geographic distribution that can be heavily influenced by human dispersal. The extensive phenotypic variation among dog breeds hinders a simple inference of dog origins based on the presence of traits shared between dogs and any specific population of the

gray wolf (*Canis lupus*) from which dogs derive (*2*–*4*). Furthermore, inferences from genetic data are confounded by a long history of trade and admixture among dogs from disparate geographic areas, ancient and ongoing local admixture with wolves, intense inbreeding within some lineages, and the stochastic effects of incomplete lineage sorting. Nevertheless, centers of dog origins from genetic data have been proposed, including the Middle East and East Asia (*5*–*7*). However, the oldest putative dog remains are found in Western Europe and Siberia and date from 15,000 to 36,000 years ago (*2*, *8*), although the classification of these specimens remains contentious (*9*). The earliest putative dog remains from the Middle East and East Asia are no older than about 13,000 years ago [see table S3 (*10*)].

DNA extracted from the earliest canids showing phenotypic evidence of domestication (*2*, *8*, *11*–*14*) can potentially be used to test hypotheses about the origin of modern dogs. We generated complete and partial mitochondrial genomes from 18 prehistoric canids and 20 modern wolves of Eurasian and American origin (Table 1 and table S2) by performing DNA capture followed by high-throughput sequencing (*15*). The DNA fragments recovered from these samples show patterns expected of ancient DNA, including a correlation between sequence length and sample age (fig. S1) and deamination patterns typical of ancient DNA (*15*) (fig. S2). After filtering, iterative assembly, and exclusion of mitochondrial genomes with less than 50% of the length recovered, we obtained a median 12-fold (1.9 to 625.7) coverage of the 18 ancient genomes, with on average 15,014 (8667 to 16,415) nucleotides supported by at least twofold coverage. These mtDNA assemblies from ancient canids were compared with complete mitochondrial genome sequences from 49 wolves; 77 dogs, including divergent dog breeds such as Basenji and Dingo; three recently published Chinese indigenous dogs (*7*); and four coyotes totaling 148 mitochondrial genomes.

Phylogenetic analyses of the mitochondrial genome data using maximum likelihood, coalescence and Bayesian approaches all reveal a well-resolved phylogeny (Fig. 1). Although dogs and wolves are not reciprocally monophyletic, all modern dogs and many wolf populations fall within one of several well-supported clades (Fig. 1 and fig. S9). Within this tree topology, dogs fall within one of four clades (Dog A to D) (Fig. 1 and fig. S9), with clade A containing the majority of dog sequences (64%). Three haplotypes from ancient Belgian canids form the most deeply diverging group in the tree. Although the cranial morphology of one of these, the Goyet dog (Belgium *36,000*) (Table 1 and table S1) has been interpreted as dog-like (*2*), its mtDNA relation to other canids places it as an ancient sister-group to all modern dogs and wolves rather than a direct ancestor of dogs. One of the Belgian specimens (Belgium *26,000*) has been found to be uniquely large (*2*) and could be related to a genetically and morphologically distinct form of wolves from Late Pleistocene deposits of the High Arctic permafrost (*16*). However, none of the sequences from the three northerly permafrost wolves (Alaska *28,000*, Alaska *21,000*, and Alaska *20,800*) (Fig. 1) fall within or are sister to this clade. Given their mitochondrial distinctiveness, the Belgian canids, including the Goyet dog, may represent an aborted domestication episode or a phenotypically distinct, and not previously recognized, population of gray wolf.

Dog clades A, C, and D, which make up 78% of dog sequences in our study, are each sister to one or more ancient canids of Europe. The most diverse of these groups is clade A, which includes divergent breeds, such as Basenji and Dingo, and two of the Chinese indigenous dogs (*7*). Moreover, three pre-Colombian New World dogs, ranging in age from 1000 to 8500 years ago, fall within dog clade A (Table 1 and table S1). The calculated time to the most recent common ancestor (MRCA) of dog clade A and ancient New World dog sequences is ~18,800 years ago [95% highest posterior density (HPD): 15,100 to 22,600] (fig. S10), which supports the hypothesis that pre-Colombian dogs in the New World share ancestry with modern dogs. Thus, these dogs likely arrived with the first humans in the New World (*17*, *18*). The clade comprising these ancient New World dogs and modern dog clade A is most closely related to an ancient wolf sequence from the Kesslerloch cave in Switzerland (Switzerland 2 *14,500*) with a MRCA that existed ~32,100 years ago (95% HPD: 27,500 to 36,700).

The lowest diversity dog clade (D) contains only sequences from two Scandinavian breeds and is sister to an ancient wolflike canid from Switzerland with a common ancestor that existed ~18,300 years ago (95% HPD: 15,300 to 21,900). This grouping is most closely related to another sequence from ancient European wolves, as well as extant wolves from Poland and Italy, and is rooted with the sequence from a putative early dog from the Altai Mountains in Russia (*13*). The grouping of clade D with ancient wolf lineages and the association of the Altai specimen with this clade do not support recent common ancestry of the Altai specimen lineage with the great majority of modern dogs. However, clade D dog haplotypes could have been captured as a result of interactions between ancient wolves and early humans that migrated into Scandinavia (*19*).

The closest sister group for dogs in clade C, which makes up 12% (9 of 77) of modern dog sequences, are two morphologically distinct ancient dogs from Bonn-Oberkassel (*12*) and the Kartstein cave in Germany (*14*) (Germany *14,700* and Germany *12,500*, respectively) having a MRCA that existed ~16,000 to 24,000 years ago (95% HPD: 13,500 to 28,100). Last, dog clade B, which contains 22% (17 of 77) of dog sequences has the closest phylogenetic associations with sequences from modern wolves from Sweden and the Ukraine and shares a MRCA with them some ~9200 years ago (95% HPD: 6500 to 12,300).

The association of sequences from modern dogs in clades A, C, and D with ancient European canid specimens and of modern dogs from clade B with European wolves suggests an origin of dogs in Europe, rather than the Middle East or East Asia, as previously suggested (*5*–*7*). Critically, none of the modern wolf sequences from other putative centers of origins such as the Middle East (Saudi Arabia, Oman, Israel, Iran, and India) or East Asia (China, Japan, and Mongolia) show close affinity with modern dog clades. Bayesian analysis of divergence times implies a European origin of the domestic dog dating to as much as 18,800 to 32,100 years ago, given an upper limit of the MRCA of an ancient wolf sequence and dogs clustered in clade A and the MRCA of the most diverse dog clade as a lower limit (Fig. 1). Consequently, our results support the hypothesis that dog domestication preceded the emergence of agriculture (*20*) and occurred in the context of European hunter-gatherer cultures.

Previous research suggested that modern dogs experienced a two-phase bottleneck. The first was at the origin of the domestication process, and the second was more recent during breed formation over the past several hundred years (*21*). To investigate the demographic history of dogs, we used a Bayesian Skygrid analysis (*22*) applied to dog clade A and the closely related pre-Columbian dogs. We find a continuous population size increase from the

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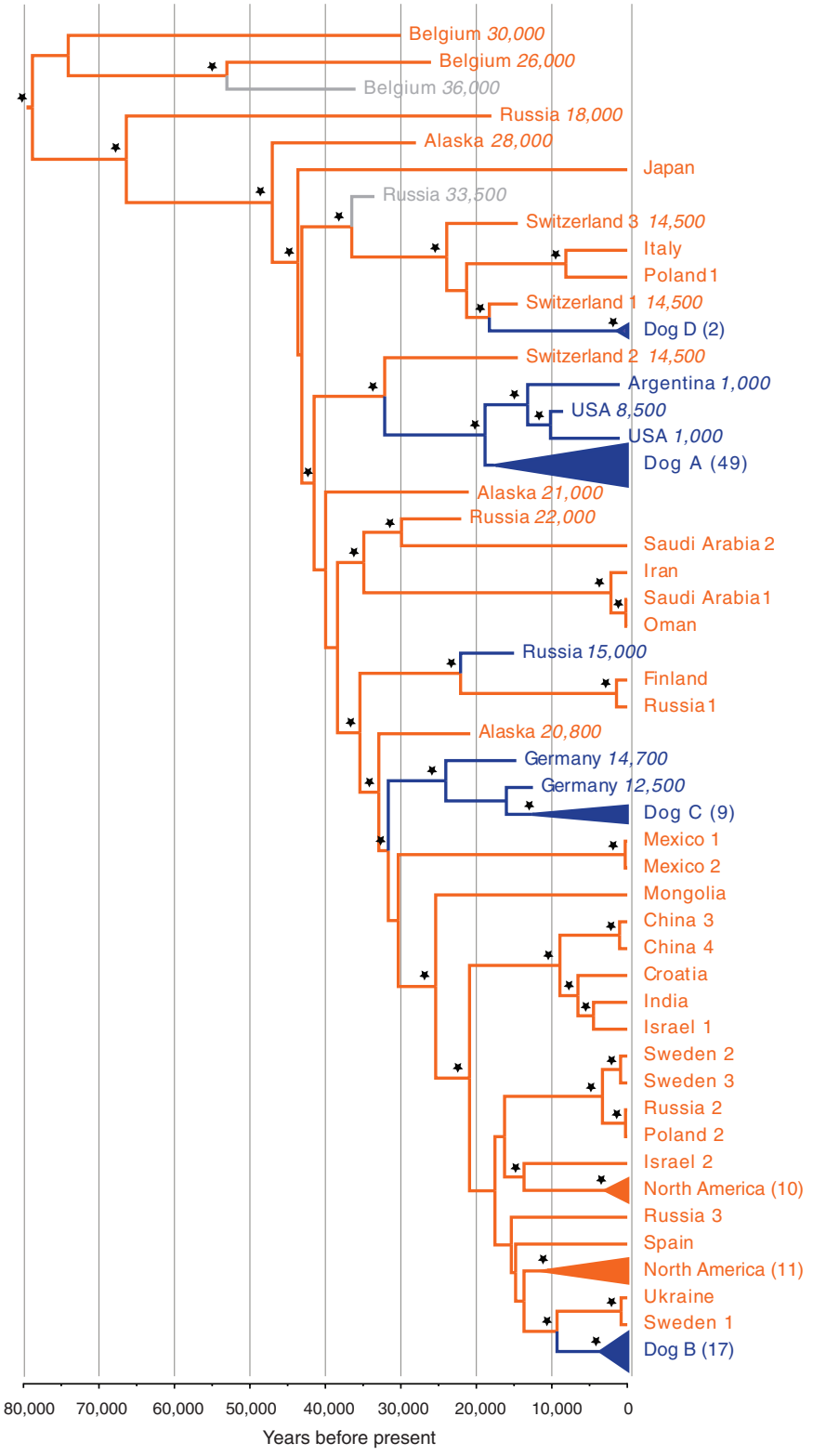

Fig. 1. Phylogenetic arrangement of modern and ancient dog (blue) and wolf sequences (orange) as obtained from coalescence-based, maximum likelihood, and Bayesian methods. The outgroup (four coyotes) and two Chinese wolf sequences were excluded [see (*15*) for more details]. Ancient specimens are labeled with the respective country of origin and their approximate reported age (italicized; in years before present). Fossil specimens with ambiguous taxonomic classification are indicated by a gray color. Whenever modern canid sequences form a monophyletic cluster, the number of sequences in the cluster is indicated in brackets. Asterisks highlight statistical support whenever both bootstrap values are >90% and posterior support is >0.9 for the maximum likelihood and Bayesian analyses, respectively.

time of the MRCA to about 5000 years ago, which may be attributable to the earliest domestication phase (Fig. 2). A more recent decline occurred between 5000 and 2500 years ago and was followed by a sharp increase in population size (Fig. 2). This increase parallels the trajectory of human population size (*23*), which suggests demographic dependence of dogs on human populations. In contrast, wolf numbers declined during this period, consistent with the emergence of agrarian cultures and the loss of vital wolf habitat and wild game.

Our findings support the conclusion that the mitochondrial legacy of dogs derives from wolves of European origin. Past mitochondrial and Y chromosome analyses that suggested a non-European location for the onset of domestication were more limited in sampling of modern or ancient wolves or prehistoric dogs and had weak statistical support for phylogenetic branching points (*4*, *6*, *24*). The modern dog clades A to D are well-supported in our tree of complete mtDNA sequence. We find that the sequence diversity that exists today in dogs can all be found in ancient (clades A, C, and D) or modern (clade B) European canids. The inferred recent divergence of clade B from wolves now found in Sweden and the Ukraine implies that it might represent a mitochondrial genome introgressed from wolves rather than one established by domestication, because dogs were clearly domesticated by this time (*8*, *12*, *14*).

Notably, our ancient panel does not contain specimens from the Middle East or China, two proposed centers of origin (*5*, *6*). In fact, no ancient dog remains older than ~13,000 years are known from these regions (*10*). However, ancient wolf and dog remains from these areas would need to be rooted more closely to the four dominant dog clades than any ancient or modern European canids to contradict our primary conclusions. We consider this scenario unlikely as it would require a common recent coalescence of these ancestral wolf and dog sequences from geographically disparate areas. Nevertheless, a more complete and nuanced picture of dog domestication will likely emerge with the addition of ancient canine mtDNA data from the Middle East and Asia. A further caveat to our conclusions is that although the mtDNA sequence tree is well supported, it represents a single genetic locus. The rapid coalescence of mtDNA genomes and the lack of recombination are important advantages; however, both mitochondrial and nuclear genomes suffer from incomplete lineage sorting, which, given the recent divergence of dogs and wolves, can potentially confound evolutionary inference. The availability of multiple independent loci in the nuclear genome potentially offers more power to resolve phylogenetic relations. We attempted to capture multiple nuclear loci using a densely tiled capture array, but were not able to obtain sufficient coverage to call genotypes confidently in any of the ancient specimens, which reflects their poor state of DNA preservation (*15*). Nonetheless, our mtDNA genome tree shows that three of four

Table 1. Ancient specimens used and summary of sequencing statistics. (**A**) Ancient specimens captured using custom designed capture arrays. (**B**) Specimens enriched for mtDNA using long range PCR-products and custom designed biotinylated adapters (*15*). Morphological classification and approximate age are from the respective references (see table S1). Ancient specimens with ambiguous morphological classification are shown in italic font. Nucleotides were retained with a minimum of two reads per base. Further information on filtering parameters is available (*15*).

Identification	Origin	Morphological classification	Approximate age (years B.P.)	Average mt-genome coverage	Retained nucleotides
A					
Belgium *26,000*	Belgium, Trou des Nutons	Wolflike	26,000	8.3	16,170
Belgium *36,000*	Belgium, Goyet niveau 4	*Doglike*	36,000	4.1	12,020
Belgium *30,000*	Belgium, Goyet niveau 4	Wolflike	30,000	20.4	16,348
Russia *18,000*	Russia, Medvezya cave	Wolflike	18,000	137.7	16,414
Russia *15,000*	Russia, Eliseevichi	Doglike	15,000	6.0	14,340
USA *8500*	USA; Koster site, Illinois	Doglike	8500	7.9	16,154
Argentina *1000*	Argentina, Cerro Lutz	Doglike	1000	27.8	16,369
Russia *22,000*	Russia, Kostenki	Wolflike	22,000	21.5	16,397
USA *1000*	USA, Florida	Doglike	1000	53.7	16,414
B					
Switzerland 1 *14,500*	Switzerland, Kesslerloch cave	Wolflike	14,500	14.7	16,357
Alaska *28,000*	Alaska, Eastern Beringia	Wolflike	28,000	90.1	16,415
Alaska *21,000*	Alaska, Eastern Beringia	Wolflike	21,000	2.1	9073
Alaska *20,800*	Alaska, Eastern Beringia	Wolflike	20,800	625.7	16,412
Switzerland 2 *14,500*	Switzerland, Kesslerloch cave	Wolflike	14,500	4.2	13,965
Russia *33,500*	Russia, Razboinichya cave	*Doglike*	33,500	100.8	16,411
Germany *14,700*	Germany, Bonn-Oberkassel	Doglike	14,700	1.9	8667
Germany *12,500*	Germany, Kartstein cave	Doglike	12,500	8.6	16,239
Switzerland 3 *14,500*	Switzerland, Kesslerloch cave	Wolflike	14,500	9.2	16,089

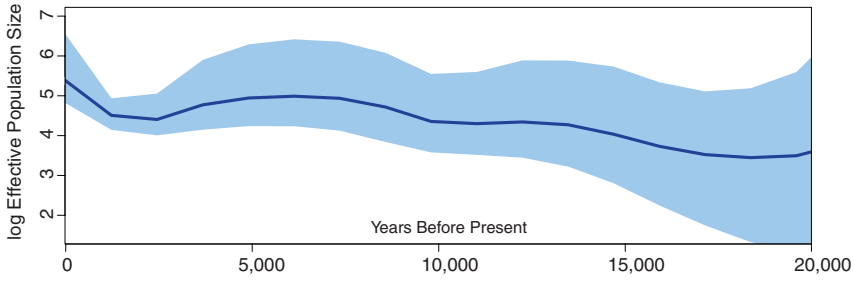

Fig. 2. Bayesian Skygrid plot depicting the demographic trajectory of dog clade A and closely related pre-Columbian dogs. Times are given in years before present and the effective population size is indicated in median $\log N_e$ (solid line) with the accompanying 95% HPD interval.

modern dog clades are more closely related to sequences from ancient European rather than extant wolves. Further, analysis of coalescence times support a dog-wolf divergence time of >15,000 years ago. An evolutionary scenario consistent with these results is that dog domestication was initiated close to the Last Glacial Maximum when hunter-gathers preyed on megafauna (*25*). Conceivably, proto-dogs might have taken advantage of carcasses left on site by early hunters, assisted in the capture of prey, or provided defense from large competing predators at kills. Finally, our results imply that some of the earliest putative dog remains, such as the Goyet dog from Belgium (*2*) or Altai Mountain specimen from Russia (*13*), may represent aborted domestication episodes. If true, this suggests that the conditions for dog domestication were not unique to one place or time and adds a role for serendipity to the process that led to the early and singular domestication of a large and dangerous carnivore.

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Supplementary Materials

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