

cotransfected with wild-type and mutant *PINK1* cDNA and a green fluorescent protein (GFP) reporter plasmid and then stressed with the peptide aldehyde Cbz-leu-leu-leucinal (MG-132), which inhibits the proteasome and induces apoptosis via distinct mechanisms, including mitochondrial injury (8). Analysis of TMRM fluorescence in GFP-positive cells revealed that the *PINK1* mutation had no significant effect on $\Delta\psi_m$ under basal conditions (Fig. 3A). However, after stress with MG-132, there was a significant decrease in $\Delta\psi_m$ from basal levels in cells transfected with G309D *PINK1* as compared with wild-type *PINK1* (G309D, $-44.1\% \pm 8.1$; versus wild-type *PINK1*, $13.0\% \pm 13.7$; $P < 0.01$; $n = 8$ data sets) (Fig. 3B and fig. S4).

We next studied apoptosis of MG-132-stressed SH-SY5Y cells transfected with either wild-type or G309D *PINK1* by FACS using annexin V conjugated to the fluorochrome phycoerythrin (annexin V-PE). Annexin V has a high binding affinity for the membrane phospholipid, phosphatidylserine, that is exposed on the surface of apoptotic cells. Consistent with the changes in $\Delta\psi_m$ after stress, overexpression of wild-type *PINK1* but not mutant *PINK1* significantly reduced the level of apoptotic cell death induced by MG-132 in GFP-positive cells [vector, $45.4\% \pm 5.0$; wild-type *PINK1*, $32.7\% \pm 4.0$; G309D, $45.8\% \pm 5.0$; $P > 0.05$; analysis of variance (ANOVA), $n = 12$ data sets] (Fig. 3C and fig. S5). These preliminary findings suggest that wild type *PINK1* may protect neurons from stress-induced mitochondrial dysfunction and stress-induced apoptosis and that this effect is abrogated by the G309D mutation.

Several lines of evidence suggest that impairment of mitochondrial activity could represent an early critical event in the pathogenesis of sporadic PD (2). Environmental toxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridin and the pesticide rotenone induce selective death of dopaminergic neurons through inhibition of complex I activity (9, 10). Complex I deficiency and a variety of markers of oxidative stress have been demonstrated in postmortem brains of PD patients (2, 11, 12). In addition, several reports have shown that mitochondrial dysfunction associated with oxidative stress can trigger α -synuclein aggregation and accumulation, although the exact mechanisms remain unclear (13).

The *PINK1* mutations described here occur in the putative serine/threonine kinase domain and thus conceivably could affect kinase activity or substrate recognition. Altered phosphorylation has been reported as a pathogenetic mechanism in other neurodegenerative diseases, including Alzheimer's

disease, tauopathy, and spinocerebellar ataxia (14, 15). The recent demonstration that phosphorylation of α -synuclein at serine 129 occurs in Lewy bodies in a variety of brains from humans with synucleinopathy (16) suggests that altered phosphorylation may also play a role in PD. We hypothesize that *PINK1* may phosphorylate mitochondrial proteins in response to cellular stress, protecting against mitochondrial dysfunction. *PINK1* was originally shown to be up-regulated by the tumor suppressor gene *PTEN* in cancer cells (6). In neurons, the PTEN signaling pathway is involved in cell cycle regulation and cell migration and promotes excitotoxin-induced apoptosis in the hippocampus (17). However, *PINK1* has not been shown to have any effects on *PTEN*-dependent cell phenotypes (6), and its role in the *PTEN* pathway therefore requires further investigation.

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Genetic Structure of the Purebred Domestic Dog

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We used molecular markers to study genetic relationships in a diverse collection of 85 domestic dog breeds. Differences among breeds accounted for ~30% of genetic variation. Microsatellite genotypes were used to correctly assign 99% of individual dogs to breeds. Phylogenetic analysis separated several breeds with ancient origins from the remaining breeds with modern European origins. We identified four genetic clusters, which predominantly contained breeds with similar geographic origin, morphology, or role in human activities. These results provide a genetic classification of dog breeds and will aid studies of the genetics of phenotypic breed differences.

The domestic dog is a genetic enterprise unique in human history. No other mammal has enjoyed such a close association with humans over so many centuries, nor been so substantially shaped as a result. A variety of dog morphologies have existed for millennia, and reproductive isolation between them was formalized with the advent of breed clubs and breed standards in the mid-19th century. Since that time, the promulgation of the "breed barrier" rule—no dog may become a registered member of a

breed unless both its dam and sire are registered members—has ensured a relatively closed genetic pool among dogs of each breed. At present, there are more than 400 described breeds, 152 of which are recognized by the American Kennel Club (AKC) in the United States (1). Over 350 inherited disorders have been described in the purebred dog population (2). Many of these mimic common human disorders and are restricted to particular breeds or groups of breeds as a result of aggressive in-

breeding programs used to generate specific morphologies. We have previously argued that mapping genes associated with common diseases, including cancer, heart disease, epilepsy, blindness, and deafness, as well as genes underlying the striking diversity among breeds in morphology and behavior, will best be accomplished through elucidating and taking advantage of the population structure of modern breeds (3). Understanding the genetic relationships among breeds will also provide insight into the directed evolution of our closest animal companions.

Mitochondrial DNA analyses have been used to elucidate the relationship between the domestic dog and the wolf (4–6), but the evolution of mitochondrial DNA is too slow to allow inferences about relationships among modern dog breeds, most of which have existed for fewer than 400 years (1, 7, 8). One previous study showed that nuclear microsatellite loci could be used to assign dogs from five breeds to their breed of origin, demonstrating large genetic distances among these breeds (9). Another study used microsatellites to detect the relatedness of two breed pairs in a collection of 28 breeds but could not establish broader phylogenetic relationships among the breeds (10). The failure to find such relationships could reflect the properties of microsatellite loci (10), the limited number of breeds examined, or the analytical methods used in the study. Alternatively, it may reflect the complex structure in purebred dog populations, resulting from the recent origin of most breeds and the mixing of ancestral types in their creation. Here, we show that microsatellite typing of a diverse collection of 85 breeds, combined with phylogenetic analysis and modern genetic clustering methods (11, 12), allows the definition of related groups of breeds and that genetic relatedness among breeds often correlates with morphological similarity and shared geographic origin.

To assess the amount of sequence variation in purebred dogs, we first resequenced 19,867 base pairs of noncontiguous genomic sequence in 120 dogs representing 60 breeds. We identified 75 single nucleotide polymorphisms (SNPs), with minor allele frequencies ranging from 0.4 to 48% (table S1). Fourteen

of the SNPs were breed specific. When all dogs were considered as a single population, the observed nucleotide heterozygosity (13) was 8×10^{-4} , essentially the same as that found for the human population (14, 15).

To further characterize genetic variation within and among breeds, we genotyped 96 microsatellite loci in 414 purebred dogs representing 85 breeds (five unrelated dogs that lacked any common grandparents were sampled from most breeds; table S2). We predicted that, because of the existence of breed barriers, dogs from the same breed would be more similar genetically than dogs from different breeds. To test this prediction, we estimated the proportion of genetic variation among individual dogs that could be attributed to breed membership. An analysis of molecular variance (16) in the microsatellite data showed that variation among breeds accounts for more than 27% of total genetic variation. Similarly, the average genetic distance between breeds calculated from the SNP data is $F_{ST} = 0.33$. These observations are consistent with previous reports that analyzed fewer dog breeds (9, 10), confirming the prediction that breed barriers have led to strong genetic isolation among breeds, and are in marked contrast to the much lower genetic differentiation (typically in the range of 5 to 10%) found among human populations (17, 18). Variation among breeds in dogs is on the high end of the range reported for domestic livestock populations (19, 20).

Strong genetic differentiation among dog breeds suggests that breed membership could be determined from individual dog genotypes (9). To test this hypothesis, we first applied a Bayesian model-based clustering algorithm, implemented in the program *structure* (11, 12, 21), to the microsatellite data. The algorithm attempts to identify genetically distinct subpopulations on the basis of patterns of allele frequencies. We applied *structure* to overlapping subsets of 20 to 22 breeds at a time (22) and observed that most breeds formed distinct clusters consisting solely of all the dogs from that breed (Fig. 1A). Dogs in only four breeds failed to consistently cluster with others of the same breed: Perro de Presa Canario, German Shorthaired Pointer, Australian Shepherd, and Chihuahua. In addition, six pairs of breeds clustered together in the majority of runs. These pairings—Alaskan Malamute and Siberian Husky, Belgian Sheepdog and Belgian Tervuren, Collie and Shetland Sheepdog, Greyhound and Whippet, Bernese Mountain Dog and Greater Swiss Mountain Dog, and Bullmastiff and Mastiff—are all expected on the basis of known breed history. To test whether these closely related breed pairs were nonetheless genetically distinct, we applied *structure* to each of these clusters. In all but one case, the clusters separated into two populations corresponding to the individual breeds (Fig. 1B). The

single exception was the cluster containing Belgian Sheepdogs and Belgian Tervurens. The European and Japanese Kennel Clubs classify these as coat color and length varieties of a single breed (23, 24), and although the AKC recognizes them as distinct breeds, the breed barrier is apparently too recent or insufficiently strict to have resulted in genetic differentiation.

We next examined whether a dog could be assigned to its breed on the basis of genotype data alone. Using the direct assignment method (25) with a leave-one-out analysis, we were able to assign 99% of individual dogs to the correct breed. Only 4 dogs out of 414 were assigned incorrectly: one Beagle as a Perro de Presa Canario, one Chihuahua as a Cairn Terrier, and two German Shorthaired Pointers as a Kuvasz and a Standard Poodle. All four errors involved breeds that did not form single-breed clusters in the *structure* analysis.

Having demonstrated that modern dog breeds are distinct genetic units, we next sought to define broader genetic relationships among the breeds. We first used standard neighbor-joining methods to build a majority-rule consensus tree of breeds (Fig. 2), with distances calculated using the chord distance measure (26), which does not assume a particular mutation model and is thought to perform well for closely related taxa (27). The tree was rooted using wolf samples. The deepest split in the tree separated four Asian spitz-type breeds, and within this branch the Shar-Pei split first, followed by the Shiba Inu, with the Akita and Chow Chow grouping together. The second split separated the Basenji, an ancient African breed. The third split separated two Arctic spitz-type breeds, the Alaskan Malamute and Siberian Husky, and the fourth split separated two Middle Eastern sight hounds, the Afghan and Saluki, from the remaining breeds.

The first four splits exceeded the majority-rule criterion, appearing in more than half of the bootstrap replicates. In contrast, the remaining breeds showed few consistent phylogenetic relationships, except for close groupings of five breed pairs that also clustered together in the *structure* analysis, one new pairing of the closely related West Highland White Terrier and Cairn Terrier, and the significant grouping of three Asian companion breeds of similar appearance, the Lhasa Apso, Shih Tzu, and Pekingese (fig. S1). A close relationship among these three breeds was also observed in the *structure* analysis, with at least two of the three clustering together in a majority of runs. The flat topology of the tree likely reflects a largely common founder stock and occurrence of extensive gene flow between phenotypically dissimilar dogs before the advent of breed clubs and breed barrier rules. In addition, it probably reflects the fact that some historically older breeds that died out during the famines, depressions, and wars of the 19th and 20th centuries have been recreated with

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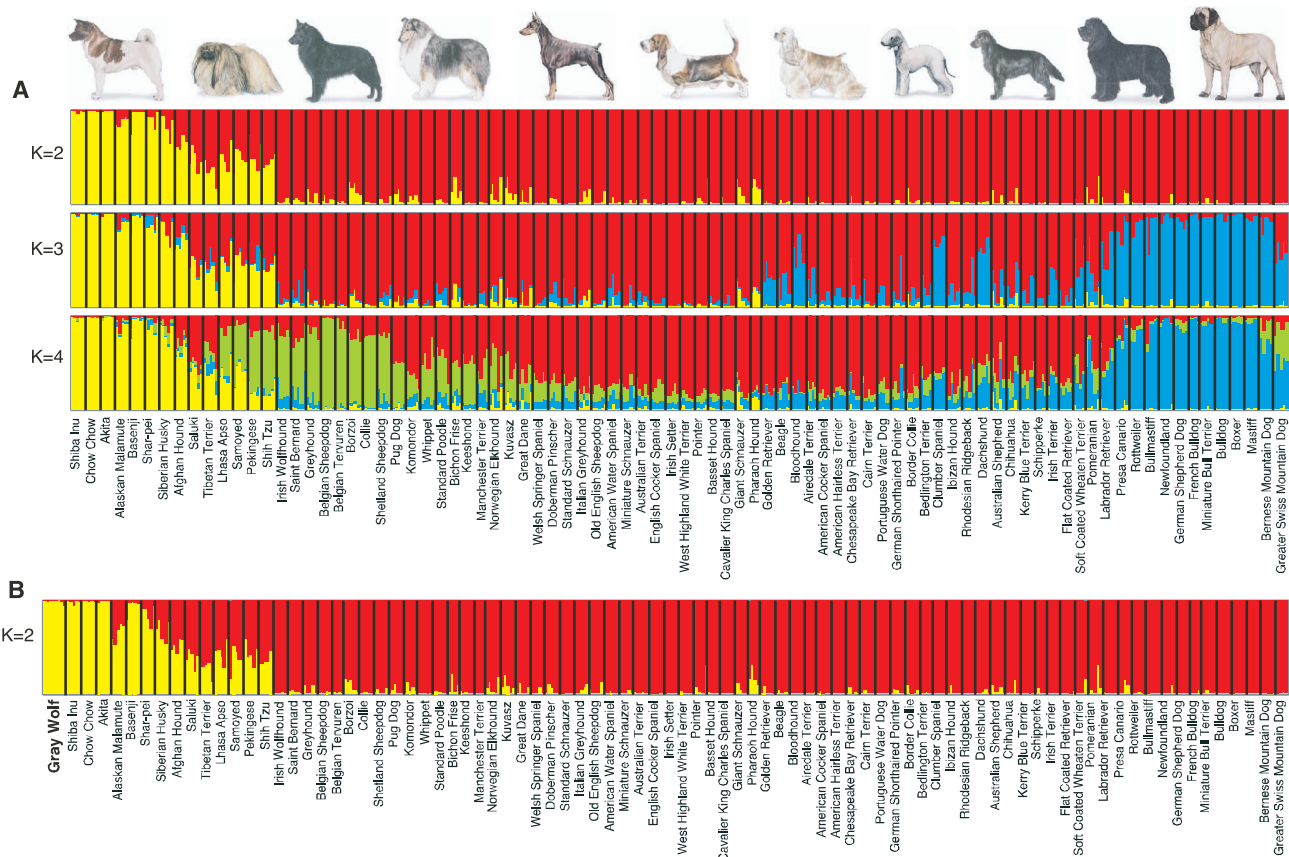


Fig. 3. (A) Population structure of 85 domestic dog breeds. Each individual dog is represented by a single vertical line divided into K colors, where K is the number of clusters assumed. Each color represents one cluster, and the length of the colored segment shows the individual's estimated proportion of membership in that cluster. Black lines separate the breeds that are labeled below the figure. Representative breeds pictured above

the graph from left to right: Akita, Pekingese, Belgian Sheepdog, Collie, Doberman Pinscher, Basset Hound, American Cocker Spaniel, Bedlington Terrier, Flat-Coated Retriever, Newfoundland, and Mastiff. Results shown are averages over 15 *structure* runs at each value of K . **(B)** Population structure, as in (A), but with gray wolves included. Graph shown is averaged over five *structure* runs at $K = 2$.

analysis, they fell entirely within this cluster (Fig. 3B). The branch leading to the wolf outgroup also fell within this group of breeds in the phylogenetic analysis (Fig. 2).

At $K = 3$, additional structure was detected that was not readily apparent from the phylogenetic tree. The new third cluster consisted primarily of breeds related to heritage and appearance to the Mastiff and is anchored by the Mastiff, Bulldog, and Boxer, along with their close relatives, the Bullmastiff, French Bulldog, Miniature Bull Terrier, and Perro de Presa Canario. Also included in the cluster are the Rotweiler, Newfoundland, and Bernese Mountain Dog, large breeds that are reported to have gained their size from ancient Mastiff-type ancestors. Less expected is the inclusion of the German Shepherd Dog. The exact origins of this breed are unknown, but our results suggest that the years spent as a military and police dog in the presence of working dog types, such as the Boxer, are responsible for shaping the genetic background of this popular breed. Three other breeds showed partial and inconsistent membership in this cluster across *structure* runs (fig. S2), which lowered the similarity coefficient to 0.84.

At $K = 4$, a fourth cluster was observed, which included several breeds used as herding dogs: Belgian Sheepdog, Belgian Tervuren, Collie, and Shetland Sheepdog. The Irish Wolfhound, Greyhound, Borzoi, and Saint Bernard were also frequently assigned to this cluster. Although historical records do not suggest that these dogs were ever used to herd livestock, our results suggest that these breeds are either progenitors to or descendants of herding types. The breeds in the remaining cluster are primarily of relatively recent European origins and are mainly different types of hunting dogs: scent hounds, terriers, spaniels, pointers, and retrievers. Clustering at $K = 4$ showed a similarity coefficient of 0.61, reflecting similar cluster membership assignments for most breeds but variable assignments for other breeds across runs (fig. S2). At $K = 5$, the similarity coefficient dropped to 0.26 and no additional consistent subpopulations were inferred, suggesting a lack of additional high-level substructure in the sampled purebred dog population.

Our results paint the following picture of the relationships among domestic dog breeds. Different breeds are genetically distinct, and individuals can be readily assigned to breeds on the

basis of their genotypes. This level of divergence is surprising given the short time since the origin of most breeds from mixed ancestral stocks and supports strong reproductive isolation within each breed as a result of the breed barrier rule. Our results support at least four distinct breed groupings representing separate “adaptive radiations.” A subset of breeds with ancient Asian and African origins splits off from the rest of the breeds and shows shared patterns of allele frequencies. At first glance, it is surprising that a single genetic cluster includes breeds from Central Africa (Basenji), the Middle East (Saluki and Afghan), Tibet (Tibetan Terrier and Lhasa Apso), China (Chow Chow, Pekingese, Shar-Pei, and Shi Tzu), Japan (Akita and Shiba Inu), and the Arctic (Alaskan Malamute, Siberian Husky, and Samoyed). However, several researchers have hypothesized that early pariah dogs originated in Asia and migrated with nomadic human groups both south to Africa and north to the Arctic, with subsequent migrations occurring throughout Asia (5, 6, 30). This cluster includes Nordic breeds that phenotypically resemble the wolf, such as the Alaskan Malamute and Siberian Husky, and shows the closest genetic relationship to the wolf, which is the direct

ancestor of domestic dogs. Thus, dogs from these breeds may be the best living representatives of the ancestral dog gene pool. It is notable that several breeds commonly believed to be of ancient origin, such as the Pharaoh Hound and Ibizan Hound, are not included in this group. These are often thought to be the oldest of all dog breeds, descending directly from the ancient Egyptian dogs drawn on tomb walls more than 5000 years ago. Our results indicate, however, that these two breeds have been recreated in more recent times from combinations of other breeds. Thus, although their appearance matches the ancient Egyptian sight hounds, their genomes do not. Similar conclusions apply to the Norwegian Elkhound, which clusters with modern European breeds rather than with the other Arctic dogs, despite reports of direct descent from Scandinavian origins more than 5000 years ago (1, 24).

The large majority of breeds appears to represent a more recent radiation from shared European stock. Although the individual breeds are genetically differentiated, they appear to have diverged at essentially the same time. This radiation probably reflects the proliferation of distinct breeds from less codified phenotypic varieties after the introduction of the breed concept and the creation of breed clubs in Europe in the 1800s. A more sensitive cluster analysis was able to discern additional genetic structure of three subpopulations within this group. One contains Mastiff-like breeds and appears to reflect shared morphology derived from a common ancestor. Another includes Shetland Sheepdog, the two Belgian Sheepdogs, and Collie, and may reflect shared ancestral herding behavior. The remaining population is dominated by a proliferation of breeds dedicated to various aspects of the hunt. For these breeds, historical and breed club records suggest highly intertwined bloodlines, consistent with our results.

Dog breeds have traditionally been grouped on the basis of their roles in human activities, physical phenotypes, and historical records. Here, we defined an independent classification based on patterns of genetic variation. This classification supports a subset of traditional groupings and also reveals previously unrecognized connections among breeds. An accurate understanding of the genetic relationships among breeds lays the foundation for studies aimed at uncovering the complex genetic basis of breed differences in morphology, behavior, and disease susceptibility.

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Materials and Methods

Figs. S1 and S2

Tables S1 to S5

References

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Mutational Analysis of the Tyrosine Phosphatome in Colorectal Cancers

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Tyrosine phosphorylation, regulated by protein tyrosine phosphatases (PTPs) and kinases (PTKs), is important in signaling pathways underlying tumorigenesis. A mutational analysis of the tyrosine phosphatase gene superfamily in human cancers identified 83 somatic mutations in six PTPs (*PTPRF*, *PTPRG*, *PTPRT*, *PTPN3*, *PTPN13*, *PTPN14*), affecting 26% of colorectal cancers and a smaller fraction of lung, breast, and gastric cancers. Fifteen mutations were nonsense, frameshift, or splice-site alterations predicted to result in truncated proteins lacking phosphatase activity. Five missense mutations in the most commonly altered PTP (*PTPRT*) were biochemically examined and found to reduce phosphatase activity. Expression of wild-type but not a mutant *PTPRT* in human cancer cells inhibited cell growth. These observations suggest that the mutated tyrosine phosphatases are tumor suppressor genes, regulating cellular pathways that may be amenable to therapeutic intervention.

Phosphorylation of tyrosine residues is a central feature of many cellular signaling pathways, including those affecting growth, differentiation, cell cycle regulation, apoptosis, and invasion (1, 2). This phosphorylation is coordinately controlled by protein tyrosine kinases (PTKs)

and phosphatases (PTPs). Although a variety of PTK genes have been directly linked to tumorigenesis through somatic activating mutations (3–6), only a few PTP genes have been implicated in cancer (7–10). Moreover, it is not known how many or how frequently members

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