LOCALIZATION OF THE GENETIC DEFECT IN A CANINE CEREBELLAR ATAXIA

by

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(Under the direction of Royal A. McGraw)

ABSTRACT

Primary granule cell degeneration (PGD) is an autosomal recessive cerebellar ataxia that has been described in Jack Russell Terriers. This ataxia differs from existing mouse models and most other ataxias in that it has a very early onset and it exhibits primary granule cell loss rather than Purkinje involvement. Homozygosity mapping was used to analyze the canine genome of a small panel of affected animals. This screen included 496 markers, of which 377 were ruled unlikely to be linked to the defective locus. An unexpected level of homozygous and otherwise uninformative markers was seen in this screen, attributable to the close relationship between the animals studied. We were able to rule out 24 of the 38 autosomal chromosomes, leaving 16 possible regions containing the defective locus. Within these regions are 12 relevant genes suggested for further study.

INDEX WORDS: cerebellum, ataxia, Jack Russell Terrier, granule cell, apoptosis, homozygosity mapping, PGD, primary granule cell degeneration

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SECTION 1

SIGNIFICANCE

The significance of genetic diseases in the domestic dog is manifold, and for the purposes of this paper, they have been divided into two broad categories; the study of canine hereditary disease for the sake of the animal, and the use of such diseases as models for the study of similar human disorders. We will first examine the impact of this study on the future health of the Jack Russell Terrier breed.

For the Breeder

Today, the American Kennel club recognizes some 150 distinct breeds, only a portion of those recognized worldwide, each of which consists of a relatively large group of animals that have been selectively bred by man for certain key characteristics. Often this selective breeding has been in practice for over a century, with a loyalty to the integrity of type that has seen some breeds through human catastrophes of war and famine where perhaps little else of a culture remained intact. A case in point is the Leonburger, a European breed that was decimated to but a handful of dogs following World War II. All Leonburgers alive today are thought to be descendents of those few, following careful breeding by their dedicated human companions (Ostrander 2000). Likewise, the modern Rottweiler, Akita, Irish wolfhound, and many, many others, have been seen through severe bottlenecks intact, through the care of human enthusiasts. When

looking at the vast histories of modern dog breeds, it is clear that much human energy and affection belongs to the domestic dog, and it has been so for much of human history. Today, most significant regulators of the gene pool of purebred dogs are show and working dog enthusiasts, each of whom often has a different notion of what the ideal representation of a breed should be. However, in the words of veteran canine geneticist Donald F. Patterson, "the only objective in dog breeding with which essentially all breeders can be predicted to agree is that genetic diseases should be eliminated" (Patterson 2000). In fact, it is heavily stressed by the AKC and breed clubs that all potential dog owners should purchase dogs only from breeders who are actively working to eliminate heritable problems in their breed. This is most often done through testing of their animals by certified veterinarian boards like those in the Orthopedic Foundation for Animals (OFA) which monitors the hip structure of individual dogs for hip dysplasia, a very common, debilitating problem in many breeds. Another such certification is through Canine Eye Registration Foundation (CERF), which employs board certified veterinary ophthalmologists to determine the presence or lack of heritable eye disease. Both the OFA and CERF are national registries, and it is believed by many that certification of all breeding animals by these foundations is essential to the future elimination of the diseases and health defects they monitor. In fact, in Europe, there exist national kennel clubs (particularly in Switzerland) that will not allow the registration, and thus the registration of any progeny of a dog that has not passed a hip certification (similar to that of the OFA) successfully (Brooks 2001). Today, genetic counseling services are available at many institutions, including the nonprofit Josephine Deubler Genetic Disease Testing Laboratory at the University of Pennsylvania's School of Veterinary Medicine

(PennGen), which provides testing and counseling services for both cat and dog breeders. This facility currently offers testing for 13 genetic diseases in the dog, as well as a number of others for cats alone. Another company, VetGen, based in Ann Arbor, Michigan, offers twenty breed-specific disease tests (www.vetgen.com). These tests consist of two different types; direct and linkage. The former are used when the specific gene or mutation behind a disease or phenotype is known. An example of such is the test for von Willebrand's Disease (vWD) (a hereditary bleeding disorder also seen in humans) in Scottish Terriers (Venta 2000). However, the actual causative mutation behind inherited disease is often not known, and in these cases, a marker that maps close to the disease locus can be used. This method, termed a linkage test, can be used with a high probability of detecting carriers, but cannot be said to be absolutely certain. An example of such a test is Vetgen's linkage test for copper toxicosis (CT) in Bedlington Terriers (Yusbasiyan-Gurkan, 1997), which is said to detect carriers with a 95% certainty.

There are now over 370 canine genetic diseases known, the majority of which (70%) have been recognized and characterized as clinically distinct (Patterson 2000). In the spirit of this technological age, these diseases have been chronicled for easy reference and diagnosis in the Canine Genetic Disease Information System (CGDIS), which represents 189 breeds, and is available as a commercial software program for veterinarians (Patterson 2001). Clearly, the resources available to modern breeders and veterinarians are growing by leaps and bounds, and with it, the frequency of many genetic diseases common not 3-4 decades ago, are becoming rarer by the day (PennGen's web site, www.vet.upenn.edu). Why then, with all of this information and awareness, are canine genetic diseases still a problem? The answer is twofold, and is as follows. Be it

intentional or unintentional, not all breeders would be considered "responsible" when it comes to careful breeding practices. As one might imagine, many breedings occur without any extensive research, and may be based on affection for the animals involved, a part time hobby, etc. The only requirement the AKC has for registering a litter is that both parents be registered as the same breed the pups are to be registered. Over one million purebred dogs are registered per year in the United States (Ostrander 2000). The majority of these animals are not coming from breeders who have definitively cleared their breeding stock of genetic disease. Therefore, though genetic diseases are certainly reduced, there are still a number of carriers still successfully passing on their genes. This becomes especially important with a phenomenon termed the popular sire effect. This occurs when a particular stud becomes popular due to show or working trial successes and therefore may be commissioned to father a hundred litters or more (Ostrander 2000). When said stud also happens to be a carrier for a genetic disease, it is easy to see how what was once a rare allele might become prevalent within a certain breed. In fact, 50% of all known inherited diseases in the dog are seen to be breed specific. Likewise, the majority of tests for genetic disease detect only the disease mutation present in a specific breed, even though other breeds may show a similar pathology (The exception to this rule is in occasional incidences of closely related breeds, which may share a disease mutation present before they became distinct and separate breeds). With all of the study of current canine hereditary disease, it must also be noted that, as always in the medical fields, the disease process is far from complete. The veterinary profession sees five to ten new canine genetic diseases recognized each year, in addition to a near logarithmic growth of information learned concerning existing diseases (Patterson 2000).

It is clear, then, that canine genetic disease represents an important subject of study and scrutiny in veterinary medicine. When speaking of Primary Granule cell Degeneration (PGD) specifically, the importance of this study to the Jack Russell Terrier Club of America (JRTCA) is quite evident. Their website (www.jrtca.org), which also houses the Jack Russell Terrier Research Foundation, spotlights but 3 diseases in the JRT being studied currently, of which PGD is one. The other two are Glaucoma/Lens Luxation and hereditary hearing loss. The Georgia Jack Russell Terrier Club graciously donated a portion of their funding for this project, clearly demonstrating the support of the JRT community here in the southeast. It is our hope to localize the genetic defect behind PGD in the Jack Russell, which will provide the vital first step towards the development of a linkage test for carriers. Additionally, a close relative of the JRT, the Smooth Fox Terrier, also have a recognized cerebellar ataxia similar to PGD (Bjork 1957). A linkage test for the JRT may work within this breed as well. Though the linkage test may not work in less related breeds, several other breeds, including the Rough Coated Collie (Hartley 1978), Brittany (Tatalick 1993), Beagle (Tago 1993), and Border Collie (Sandy 2002) have each all had diseases similar to PGD described. To those who care for these animals, a genetic test means at the very least a reduced frequency of this disheartening disease from a beloved breed of companions, and is therefore an effort of some significance.

Canine Models of Human Disease

Throughout the lengthy and complex relationship between man and dog, there has ever existed a certain mutually beneficial reciprocity. Certainly the dog has aided man in every pursuit from agriculture to crime fighting, from helping the disabled to entertainment. Likewise, we have undeniably aided the dog, kept him safe, warm, and healthy, and for the majority of dog owners, welcomed them with open arms into our families. This relationship has now carried over into the fields of medicinal therapy and research, where both species have now become able to both help and benefit from the other in our similar battles against disease, particularly those of a genetic basis. In the past, it has been the human disease studies which have helped elucidate canine diseases, due to a far greater information base previously established for human disease genes. (Patterson 2000). However, the tides are turning. With the recent flood of information concerning the canine genome and a number of inherited diseases, humans may now turn to their canine companions for aid. Dogs offer a singular opportunity as models for human disease, and are one of few species that can likewise gain from their study.

Animal models have been in evidence in medicinal research throughout the majority of its history. In the more recent developments of genetic research, animal models have become indispensable, with mouse being the undeniable flagship. Human genetic diseases often depend on not a single, simple genetic defect, but a number of interacting intrinsic (genetic) factors, and are often further complicated by uncontrollable environmental factors. In contrast, animal studies offer the ability to control not only the environment of the study subjects, but their genetics as well. Unsurprisingly, animal studies are often notably more successful than similar human studies, with animal studies

frequently revealing significant single-locus effects which are often reproducible across species and/or strains (Williams 2004). Genetic studies have also been transformed by the ability to manipulate the genomes of several species, including *C. elegans* and the mouse using a number of techniques including knock out genes. However, the phenotypes generated by these methods more often show the effects of induced disease, and may not be as useful as naturally occurring, spontaneous disease mutations (Williams 2004). Of the animals commonly seen in research today, the dog is undeniably the most studied in terms of genetic medical conditions, thanks to its long history of surveillance by the veterinary profession. With over 370 known canine inherited diseases, the dog has shown the highest number of naturally occurring genetic disease of any nonhuman animal to date. At least 50% of these diseases described show distinct similarities to specific human diseases (Ostrander 2000). Unlike human genetic disorders, which are often complex, involving multiple gene effects, the breed specificity of canine inherited disease suggests the presence of a small number, or perhaps only a single disease gene in most canine disorders. These rare alleles become prevalent due to the fore mentioned bottle neck, founder, and popular sire effects within breeds, which themselves have a pedigree barrier. An integration of these effects can easily, then, lead to increased frequencies of rare alleles within given breeds, and therefore the incidence of breed specific diseases seen today (Ostrander 2000). Such cultivated consanguinity is ideal for genetic studies. In human genetic disease, geographically isolated, inbred families are often the most successfully studied (Marazita 2004). Of course, most human diseases cannot be conveniently elucidated in this manner. Humans often have poor records of matings, with unlimited outside genetic and environmental factors complicating matters. In

contrast, canine models often come complete with complex and well documented pedigrees. Also, dog litters, depending on the breed, may have up to ten or more pups, which is quite helpful when studying inheritance schemes.

It must be noted here that although the canine model presents a unique opportunity for the study of a number of diseases, human and canine, the dog is not the ideal laboratory animal, for a number of reasons. For one, the dog is a relatively large animal, whose consistent care, especially in long term studies, is quite expensive. Also, one cannot forget that dogs are highly sentient and emotive creatures, and often require a great deal of mental stimulation and interaction to keep them satisfied and healthy (Collins 2003). Additional differences between the dog and other popular laboratory choices like the mouse include the inability to genetically manipulate or transform the dog, and with a lengthier lifespan, dogs can typically be ruled out for short term, rapid turn around studies. However, it is for these very reasons that the dog often makes the ideal subject in select investigations.

The scientific community has, indeed, begun to embrace the dog as a significant contributor to the study of hereditary defects. Several years ago, The Whitehead Institute/MIT Center for Genome Research took up the challenge of sequencing the canine genome following the National Human Genome Research Institute (NHGRI)'s designation of the dog as a high priority genome. This project is scheduled to be completed Spring 2004. In response to this, the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/) included the dog on its website among its genome resources, which include the human, mouse, rat, fly and several other popular laboratory animals in genetic research. With all of this growth, it is not

surprising that a number of new articles appear each month in the literature concerning canine models for human genetic disease. An example is the study of canine Hemophilia A by A.M. Gallo-Penn of Queen's University in Canada (Gallo-Penn, 2001). Canine Hemophilia A is a close mimic of the human disease, which is a severe, life threatening, bleeding disorder found to be X-linked and afflicting 1 in 4,000 males of all populations. This disease has been known in dogs for over 50 years, and those demonstrating the disease phenotype, a deficiency in clotting factor VIII, have been used in the past to develop FVIII pharmaceutical products. The currently accepted method to treat bleeding crises in these patients is an infusion of plasma-derived or recombinant FVIII protein. However, this expensive treatment is in such a short supply that it is available to less than 10% of the human hemophiliac population worldwide. However, the work of Gallo-Penn and others with adenoviral vector-mediated gene therapy in canine patients afflicted with hemophilia A may help to determine the feasibility of such treatments in human populations. Another human disease with a close relative in the dog is Mucopolysaccharidosis IIIA (MPS IIIA, Sanfilippo A), studied by A. Fischer, K.P. Carmichael, and J.F. Munnell of the University of Georgia's College of Veterinary Medicine, among others (Fischer 1998). MPS IIIA, which is the most severe of four subtypes of MPS III, causes severe progressive neurological disease, with death usually occurring before age twenty. The canine model discovered and characterized by Fischer et al represents the first animal model of MPS IIIA, and was discovered in a family of Wire-haired Dachshunds. Animal models for other MPSs have proven invaluable in the study of the pathogenesis and therapeutic approaches associated with these diseases. Such metabolic disorders in animal models are especially valuable in the determining the

safety and efficacy of therapeutic approaches such as enzyme replacement and bone marrow transplant (Fischer 1998). Yet another canine model is that for progressive retinal atrophies (PRAs), which are found in over 80 breeds (Jack Russell Terriers included). In a 2003 article in the Journal of Heredity, J.W. Kijas and colleagues proposed the English and Bull Mastiff breeds in particular as a model for a particular form of PRA involving a mutation in the rhodopsin gene. This disease displays the exact phenotypic characteristics of retinitis pigmentosa (RP), a disease afflicting humans. The significance of this paper is their aggressive approach to studying the canine population, by developing a deliberate breeding program that would help elucidate the seemingly complex mode of inheritance of this disease, as well as developing a colony of animals for further investigation. Another excellent example is Duchenne's Muscular Dystrophy (DMD) in humans, which has a number of animal models. However, though the most studied example is the *mdx* mouse, which shows a molecular pathology similar to human DMD patients, the mouse does not present with the debilitating phenotypic characteristics of this disease. Conversely, a colony of similarly afflicted Golden Retriever dogs demonstrates the same muscular weaknesses and diminished life span as human patients, and is expected to be the best experimental model for human DMD. It is likely that the mouse model will remain in use due to the expenses incurred with canine subjects, though pre-clinical trials may now all be screened through the dog for efficacy and safety (Collins 2003). The list goes on; canine models are now being extensively used to facilitate understanding of human diseases including several forms of cancer (Rosol 2003), cyclic and severe congenital neutropenia (Horwitz, 2004), GM1 gangliosidosis

(Yamoto, 2003), and carbohydrate-deficient glycoprotein syndrome (Yang 1998), just to name a mere few.

Though it should be noted that cautions must be taken when comparing diseases and especially therapeutic effects between species, it is undeniable that animal models, canine included, are indispensable in the study of human and veterinary diseases. This study may potentially contribute to the growing pool of information gathering concerning human inherited ataxias. Cerebellar ataxias affecting humans include a variety of pathologies, both similar and dissimilar to PGD in the Jack Russell Terrier. Such diseases will be discussed further in the following sections.

SECTION II

PRIMARY GRANULE CELL DEGENERATION

Background

Primary Granule Cell Degeneration (PGD) can be detected in pups as early as two weeks of age, when they become distinct from their siblings who are likely actively learning to walk and run. The ataxic pup will stagger and fall as he attempts to join in their play, and will present with a number of other signs indicative of cerebellar dysfunction. This is a particularly saddening disease, as all other systems appear unaffected, and the pup seems alert and fully cognizant of his surroundings and interactions (Carmichael 2003). In this section, we will discuss both the outward signs as well as the pathology of this neurological disease. However, in order to fully comprehend the impact of Primary Granule cell Degeneration on the nervous system, and its potential causes, one must first have an understanding of the mammalian cerebellum itself, as well as its developmental processes.

Functionally, the cerebellum acts as the master conductor of body coordination. Though the voluntary aspects of the cerebral cortex may make the decision to move, it is the cerebellum that makes that move possible with at least a modicum of grace. The cerebellum must integrate literally millions of inputs to determine the proper response of limb and core muscles to shifts in the effects of gravity and other environmental factors.

The cerebellum, often called the "little brain", comprises a mere 15% of CNS mass, and yet contains more than 50% of its neurons (Schmahmann 2000). With such an extensive population to organize, the cerebellum is highly ordered, and is of uniform structure. In the most basic sense, the cerebellum is comprised of a central medulla surrounded by the cerebellar cortex, which is folded into thin sheets called folia. As it is within the cortex that the pathology of PGD is most evident, it is this region that will be the focus here. The cortex is comprised of three clearly distinct layers. The outermost is the molecular layer, which is a thick layer that has a relatively low cell count. The cells within this layer are primarily the basket and stellate cells (interneurons). The remaining bulk of this layer is humming with nerve tracts from other layers, especially the parallel fibers of the granule cell axons. The innermost layer, the inner granule cell layer (IGL) is densely packed with granule cell bodies. Sandwiched between the molecular and granule cell layers is a sheet of giant Purkinje cells, forming the 1-cell thick Purkinje or ganglionic layer (Manto 2002). Information enters the cerebellar cortex from one of two systems; the mossy fiber or climbing fiber system. The mossy fibers have numerous origins including somesthetic, acoustic, visual, vestibular, and cortical. These afferents have their termini within the granule cell layer, where they form club-like "rosettes". Each rosette synapses with roughly 100 granule cell dendrites (each cell contributing 4-5 dendrites), and is encompassed by a network of glial membranes, forming a glomerulus. The excitatory neurotransmitter here is glutamate. The granule cell's axon (unmyelinated) extends outward to the molecular layer where it bifurcates into parallel fibers, which run along the axis of the folium. These fibers run through the vast arborizations (so named for the fantastically enormous tree-like dendrites) of the Purkinje cells, where they may form

upwards to 80,000 (from 80,000 granule cells) synapses on a single Purkinje cell. On the other hand, the climbing fiber system originates in the inferior olive and can directly excite the Purkinje cells. The neurotransmitter of choice here is gamma-aminobutyric acid (GABA). Many regulatory circuits exist among the cells of the cerebellar cortex, including both inhibitory and excitatory, and involving not only the major players mentioned above, but a number of inhibitory interneurons as well (Manto 2002).

As PGD is a disease of the very young, it is important to also understand the normal processes of cerebellar development, especially those of the granule cell layer. The embryonic formation of the cerebellum begins first with the formation of the rhombic lip from tissues bordering the early fourth ventricle. As the cerebellum grows caudally from these tissues, a transient, embryonic structure is formed, called the external granule cell layer (EGL). This layer is superficial to the molecular layer and is composed of densely packed progenitor granule cells. Following these cells' final mitosis, they begin to migrate towards what will be their permanent home, the internal granule cell layer (IGL) of the adult. This migration is guided by specialized radial glial fibers (Bergman glia) and the cell-to-cell interactions between these cells and those migrating. The close apposition of the two cell membranes during this process suggests membrane molecule interactions governing granule cell migration. In fact, Santiago et al were able to stall granule cell migration through the immunoblockage of one such implicated membrane molecule, the ganglioside 9-O-acetyl GD3 (Santiago 2004). Another membrane molecule commonly associated with granule-glial cell adhesion is astrotactin (Adams 2002). Extracellular proteins too can play a role in migration events. Reelin, for example, is an extracellular matrix protein which acts directly on the radial glial cell

scaffolding and has been shown to be essential to proper neuronal positioning (Frotscher 2003). Also involved in this process are neuregulins, like NRG1, which is specifically involved with radial glial cell-mediated granule cell migration (Huang 2001). A number of neurotrophins, including BDNF and NT-3 also play key roles in migration (Borhesani 2002). Clearly, there exist an overwhelming number of interactions involved in this process. Therefore, an examination of the histology of Jack Russell Terriers affected by PGD is required before candidate genes could be selected. The following section discusses this further.

However, there is yet another important process in cerebellar development that must be discussed; apoptosis. Apoptosis, or programmed cell death, is a normal and vital process in both the development and maintenance of the nervous system. Apoptosis provides an orderly, clean, non-inflammatory means of clearing cells that are not optimal in some way and have therefore triggered an apoptotic response. The control of apoptosis must obviously be quite finely tuned to avoid either the unchecked growth of cells (ex. Neoplasias) or an overkill of cells, which could obviously result in nasty business. Both pro-apoptotic (ex. BAX) and anti-apoptotic (ex. BCL-2) signals constantly compete for dominance, acting in accordance with both extra- and intra-cellular signaling. Apoptosis is most often begun at the mitochondrial membrane, where apoptotic factors have the effect of controlling cytochrome c release, which begins a caspase cascade resulting in several cellular changes preparatory to a tidy death. These include but are not limited to chromatin condensation, nuclear shrinkage, DNA fragmentation, and cleavage of cytoskeletal proteins, all of which lead to cell fragmentation (while maintaining membrane integrity) and the formation of apoptotic

bodies. These express "eat me" signals (exposure of phohphatidylserine and changes in surface sugars, for instance) which signal "buffet" to roaming phagocytes, which promptly engulf them. This is in stark contrast to necrotic cells, which are characterized by swelling, lysis, and an inflammatory response. Apoptosis, on the other hand, can occur without local disruption of cells. In fact, the neighbors are none the wiser. In the developing cerebellum, apoptosis is an expected phenomenon, especially in the EGL. Though the vast majority of progenitor cells in the EGL migrate to their respective permanent domains, some remain where they are. These underachievers are cleared away by means of apoptosis, leaving the molecular layer as the outermost layer of the adult cerebellum. The action doesn't quite stop there, though. Some apoptosis can be seen in the IGL, as well. However, this form demonstrates few detectable dying cells and has been described as autophagic degeneration (Wood 1993; Muller, 1995). This is probably a final step in cerebellar organization.

The question remains, what determines which cells are lucky enough to avoid the apoptotic machine? Though the obvious answer is those cells which are serving the overall organism well and are operating within acceptable ranges, the answer most likely specifically lies in the availability of certain survival factors, usually shared through key cell-to-cell interactions (a sort of neighborhood watch program for malfunctioning or unwelcome cells). Growth factors, neurotrophins, as well as their respective receptors are just a few of the potential survival factors important to neuronal longevity. As to which factor is deficient in the ataxic JRT, a closer look at the distinct pathology of this disease is in order.

Pathology of PGD

Ataxia, from the Greek, meaning "lack of order" succinctly summarizes the critical importance of neuronal organization in the powerhouse of efficiency that is the healthy cerebellum. Given the sheer volume of information that must be integrated and processed within such infinitesimal timeframes and in such a small space, it is one of nature's great wonders that the cerebellum accomplishes what it does when optimal, and it is not surprising that even tiny errors can cause the entire system to go awry. In this section we will examine the specific pathology of Primary Granule Cell Degeneration in Jack Russell Terriers, as well as several of the many potentially defective genes that may be its cause.

As mentioned previously in this paper, PGD is an early onset ataxia, with signs typical of cerebellar dysfunction occurring as early as two weeks of age. The dysfunctional cerebellum has several trademark signs evident in the affected animal's behavior. For one, the pup may demonstrate intention tremors, where the limb will shake previous to intended motion. One must recall that the cerebellum is not responsible for the decision to move, but the coordination of that movement. Therefore, the animal by no means lacks the presence of mind to move forward, or to eat, but the ability to do so successfully and with coordinated effort. Another sign along the same lines is the hypermetric gait, often a clear indication of a cerebellar disorder. This has the pup over-reaching his intended goal, resulting in limbs flung high and past where the pup intended to step. Oscillatory spontaneous nystagmus, generally noticed as a twitching of the eye, is also common in affected animals. Interestingly, nystagmus is also seen in individuals who have impaired proprioceptors in their inner ears (Rhoades 2003). The cerebellum

also receives somatosensory information (via the mossy fibers) (Manto 2002), which it normally integrates into its coordination of motion. However, the eye has a reflex to rotation, or perceived rotation, which causes it to move from center, to side, to center. This, in a normal animal accurately perceiving a rotational movement, allows the eye to constantly focus on new landmarks to maintain a discernable visual field and balance. The eye motion, in the absence of true rotational movement, is seen as nystagmus in the affected pup. Other general signs of the ataxic animal include head bobbing and overall spasticity.

The original characterization (Carmichael 2003) of this disorder was conducted in 10 sire related animals (3 male, 7 female), showing probable recessive inheritance. The pups ranged from 5 weeks to 16 months of age at euthanasia, and were immediately necropsied. Gross examination of the cerebellum showed symmetrical and slightly smaller sizes in the affected animals. Normally the cerebellum comprises 10-12% of a pup's weight, whereas the cerebella of affected animals were 4-9%. Upon closer inspection, the cerebellar folia of affected pups were noticeably thinner than age matched controls. In older pups (5wk, 8wk, 5mo and 11mo old), the internal granule cell layer was depleted, with younger dogs showing a moderate scattering of small, dark cells within the depleted IGL. In pups less than 8 weeks old, the EGL was still present, and no abnormalities in migration were noted. There appeared to be no loss of Purkinje cells (except the oldest dog at 16 mos), though Purkinje cells showed vacuolization and swelling. This is in contrast the majority of ataxias, which demonstrate primary Purkinje cell loss, with possible secondary granule cell loss (Carmichael 2003). Glial fibrillary acidic protein (GFAP) immunoreactivity was intense in the depleted IGL of the younger

animals. Though it is not entirely understood how GFAP expression is triggered, it is generally expressed by astrocytes in the presence of damaged neurons or their processes. During his doctoral work, Hasan Ozen (Ozen 2002) examined a number of histological aspects of the PGD affected cerebellum. For one, he used TUNEL assay to determine the loss of granule cells in the IGL was, in fact, due to apoptosis. He also found an over-expression of BAX (pro-apoptotic BCL family member), with the majority of BAX-immunoreactive cells being the granule cells remaining in the depleted IGL. Most of the reactive cells had heterochromatic nuclei, were oddly shaped, and were more pyknotic than other cells (These are all three possible visual signs of apoptosis). The number of BAX immunoreactive cells within the IGL layer of ataxic pups was significantly larger than that seen in normal pups ($p<0.05$) (Ozen 2002). The BAX-immunoreactivity of Purkinje cells and pre-migratory granule cells in the EGL were similar to those in normal cerebella. The levels of BCL-2 in ataxic pups were similar to those in normal animals. Lacking an understanding of why BAX is overexpressed in these cells, the step was to compare levels of known survival factors, including neurotrophins. Neurotrophins, including BDNF (brain derived), NGF (nerve growth factor), and NT-3, are all derived from a single gene and demonstrate marked sequence and structural similarities. Such similarity means they can all bind common receptors, like Trk (tropomyosin related kinase) receptor tyrosine kinase-A, B and C (Trk A, TrkB, TrkC) as well as $p75^{NTR}$, another common neurotrophins receptor. As the young granule cell migrates, it changes the expression of receptors. For example, early in migration, the majority of cells express TrkB. As they mature, though, their preference switches significantly to TrkC.

In his study, Ozen focused on NGF, which is involved in granule cell survival and NT-3 expression (Gao 1995); NGF which has been shown to be involved in the mediation of apoptosis (Bredesen 1997; Carter 1997; Bamji 1998; Agerman 2000); and NT-3, which primarily promotes Purkinje cell survival. The results of immunoreactivity assays for these three molecules found no significant difference in the expression of NT-3 between ataxic and normal dogs. However, there were significantly less cells expressing NGF in the molecular layer in ataxic dogs versus normal dogs. Also, there is more $p75^{NTR}$ expressed in the depleted IGL of the ataxic dog than in the unaffected animals.

An important resource in studying cerebellar ataxias also exists in mouse mutants. Several mutants exist that demonstrate cerebellar ataxia, each with apparently different pathologies. The *waggler* mouse, for instance, demonstrates severe ataxia at an early age, but its granule cells migrate normally and live a normal adult lifespan. The error in these animals appears to be with the synapses between granule cells and both Purkinje cells and mossy fibers. Synaptic development is arrested in an early stage, and never fully matures, impairing the signaling between these neurons (Chen 1999). Another mouse mutant, *lurcher*, presents with severe ataxia, and primary Purkinje cell loss. The lurcher mouse has a defective GlueRdelta2 gene, which encodes a glutamate receptor subunit (Yue 2002). In the *weaver* ataxic mouse model, GIRK2 is defective. GIRK2 is a G-coupled inwardly rectifying K+ channel, present in granule cells and other neurons. The *weaver* cerebellum has no granule cell migration, resulting in a near total loss of granule cells, which never reach the IGL (Liesi 2000). Staggerer ataxic mice, on the other hand, have severely delayed Purkinje cell development, with some granule cell degeneration. The affected gene in this case is ROR A, a member of the nuclear hormone

receptor family (Hamilton 1996). Another mutant, the *reeler* mouse, has some granule cell degeneration (progressive) as well as markedly malpositioned neurons, including Purkinje cells. Reelin, encoded by *RELN,* is an extracellular matrix protein which signals migrating neurons to stop, therefore ensuring their correct positioning. Reelin is mutated in the *reeler* mouse (Magdaleno 2002). Another mouse, the astrotactin null mouse, lacks astrotactin, which is crucial to timely migration in granule cells. These mice have poor coordination accompanied by increased apoptosis of granule cells. This gene is referred to later, in the conclusions section (Adams 2002). The final mouse mutant we will discuss here is the *stargazer* mouse. The stargazer's defect lies in the CACNG2 gene, which encodes the gamma subunit of a voltage-gated calcium channel. These animals have greatly reduced BDNF in granule cells (Black 2003). While the stargazer expresses normal levels of the BDNF-receptor, TrkB, these receptors are significantly less phosphorylated. Highly immature, undifferentiated granule cells have also been reported (Hashimoto 1999). Clearly, there is not an exact homolog mutant in the mouse for PGD, expressing primary granule cell degeneration, though it would be well to further examine the astrotactin, reelin, and TrkB genes.

In this study, we will be using the homozygosity mapping technique (described in the following section) to find regions of homozygosity in PGD affected dogs. These regions will be compared to possible candidate genes including NGFr, NGFβ, NTrk3, NTrk1, TFG (Trk fused gene), PACAP, Cystatin B, NAIP, Reelin, BDNF and several others. These genes will be discussed in the Conclusions section of this paper.

SECTION III

HOMOZYGOSITY MAPPING

Homozygosity mapping was first described by Lander and Botstein (Lander 1987) as a means of localizing recessive defects. In this approach, the genome is scanned by amplifying known polymorphic microsatellite markers in a small set of consanguineous affected individuals. If the marker in question demonstrates homozygosity in affected animals, with carriers having only one copy of the same allele, it is considered likely that the marker is closely linked to the mutated locus. This method has been used successfully in both humans and other species, including the dog, to localize and even specifically identify mutated alleles in recessive diseases. An example is the identification of the defective gene in acheiropodia, a recessive disorder in humans where the hands and feet fail to develop. The DNA of seven individuals (including 3 affected) from a consanguineous family was examined using homozygosity mapping. A homozygous region spanning 11.7 cM was identified (Escamilla 2000). Further testing led to the identification and cloning of the gene (Ianakiev 2001). Another recent example is the localization of the defect in Joubert syndrome, a human neurological disorder. Homozygosity mapping of affected individuals and carriers identified a region of homozygosity spanning 13.1 cM (Lagier-Tourenne 2004). Another human disease, Chediak-Higashi Syndrome, was localized to a homozygous region spanning 18.8 cM

(Fukai 1996). Meanwhile, Steiner and colleagues were able to use this technique to localize the defective locus behind spondylocarpotarsal synostosis syndrome to a region of merely 5.7 cM (Steiner 2004). In familial horizontal gaze palsy with progressive scoliosis, a human disorder, originally published homozygosity results defined a homozygous region spanning 30 cM (Jen 2002). However, further work again narrowed this region, with the final results reported as defining a 9 cM region (Lo 2004). Clearly, homozygosity mapping is best and most often approached as a multi-step process, with a broad scan followed by refining steps to increase the resolution. This project will follow a similar pattern. In 2001, Richman and coworkers of the Fred Hutchinson Cancer Research Center (FHCRC) published a minimal screening set (MSS-1) of 172 microsatellite markers for genome-wide screens of the canine genome (Richman 2001). This set includes members of both the meiotic linkage and radiation hybrid maps. In 2003, a new minimal screening set (MSS-2) composed of 325 markers (311 autosomal) was published by the same group. This set was comprised of markers from the radiation hybrid panels only (Guyon 2003). These markers are said to be highly polymorphic, with reliable PCR results. The primer sequences are available on the FHCRC website (www.fhcrc.org). It is important to note that the maps created by this group have been published with calculated (estimated) mega base (Mb), as opposed to centimorgan (cM) units. This differs from much of the published literature where the centimorgan is commonly used. Though the cM can be said to be roughly equivalent to the Mb, genetic distance does not equal physical distance, and therefore potential differences must be taken into account.

METHODS

DNA Samples

This study used DNA from blood taken from 9 affected pups, though due to quality concerns, the majority of work included only 7 of these. A further 5 obligate carriers and 5 possible carriers (siblings) were used as well (Table 1). Some of the DNA samples were extracted several years ago (1996), with further blood samples unavailable (dogs euthanized). For this reason, in the case of Dudley and Pirate, whose DNA was of poor quality and gave unreliable PCR results, little could be done with these samples.

Blood was collected in EDTA, and DNA extracted with Bio-Fast™ DNA Extraction Kit, available from Biosynthesis Incorporated (Lewisville, Tx). Every 500µL of whole blood (in EDTA or Heparin) yields 5-30µg of DNA in 100µL of water.

Microsatellite Markers

Primers were designed based on the sequences provided by the FHCRC for 496 microsatellite markers present on both the meiotic linkage and RH canine genome maps. Fifty four of these markers have been taken from the meiotic linkage maps. Markers from the meiotic linkage map are not included in the newest radiation hybrid maps (Guyon 2003), and exact distances cannot be determined from these. For this reason, they are not included in distance calculations. The average distances between selected markers are presented in Table 2, with the overall average being 5.7 Mb.

Polymerase Chain Reaction

All markers were amplified using polymerase chain reaction. Promega PCR MasterMix was used for the majority of reactions with success. In several incidences where no product was amplified, PCR was run again with Applied Biosystems' AmpliTaq Gold® PCR Master Mix instead. In these incidences, no difference was seen between the two products. Most markers amplified at an annealing temperature of 50 C. In cases where multiple bands were seen, Ta was raised up to 58 C, while in cases where no product was amplified, Ta was lowered to 50 C with occasional successes. The thermocycler used in this study was a Programmable Thermal Controller by MJC Research. Reactions were performed in 0.5 mL flat cap PCR tubes (Fisher) in a volume of 10 μL, topped with oil.

Electrophoresis

Both agarose and poly-acrylamide gel electrophoresis (PAGE) were performed on samples. As an initial screen, all samples were analyzed on an agarose gel (3% BioRad Analytical Grade Agarose), with at least 2 affected animals. A 0.5x TBE running buffer with ethydium bromide was used. Results from these were recorded on black and white Polaroid 667 film. As the maximum resolution of these agarose gels is about 4 bases, animals seeming to be homozygotes in all affected animals in the agarose screen were then examined using denaturing PAGE, which can resolve a single base difference between alleles. For this, forward primers were labeled with ^{32}P gamma dATP using polynucleotide kinase (PNK). PAGE gels were done in two formats, 8"x11" (macro, at 6%) and 3"x3" (mini, at 8%). The acrylamide mixes used BioRad Electrophoresis grade

acrylamide and Fisher Electrophoresis grade urea. Results were visualized with autoradiography.

Interpretation of Results

Markers were typically run with at least 2 affected animals and at least one carrier. Appendix 1 of this paper contains 38 tables, one for each canine autosome. Each table provides the marker locus, its position on the chromosome (in Mb), the distance to the nearest Mb above each marker, references the location of all results from the marker (Notebook volumes I-III and 75 films), as well as a status designation. The PGD status possibilities are "candidate", "rule out", "uninformative", and "no data". "Candidate" refers to those markers that demonstrate homozygosity in all affected animals, heterozygosity in all carriers, and a common allele among them all. Possible carriers may either appear as carriers or share no common alleles. Markers may only be "rule out's" if either an affected animal appears definitively heterozygous, or if there is no common allele among all carriers and affected animals. If, perchance, all animals appear the same, even if they all seem to be heterozygous, the marker will be ruled uninformative. This is due to the possibility of artifact bands. For a marker to be ruled out there must be an affected that appears heterozygous, and different from other animals, such that it is clear that the additional band is actually an additional allele. Uninformative markers include markers for which there may be homozygous carriers, as well as all homozygous affected. Markers, for which no viable results have been seen, either due to too many bands or a lack of products in enough animals, are given the designation "no data".

RESULTS

Of 496 markers examined in this study, 377 were determined to be ruled out. No definite candidate markers were found that showed homozygosity in all affected animals (9) and heterozygosity in all carriers (5) with a common shared allele. A total of 91 markers were labeled "uninformative", with a further 27 having shown no viable results. While the average distance between all markers is 5.7 Mb, the average distance between ruled out markers is 7.8 Mb (Table 2), with 16 regions spanning more than 15 Mb that were unable to be ruled out. These regions are presented in Table 3 and further discussed in the conclusions chapter of this paper.

Several genes that were suspected candidates due to previous work by Dr. KP Carmichael and colleagues were examined indirectly in this study. After finding the gene loci in the human gene maps (using Locus Link at NCBI), we found the corresponding homologous location in the canine map (Guymon 2003). The results of these comparisons are also contained within the conclusions chapter.

CONCLUSIONS

In this study, we found a high degree of uninformative markers, such that instead of the single region of homozygosity that was our goal, we have found no less than 16 such regions. These regions, with their corresponding homologous regions and possible genes, are all listed in Table 3. Though no single locus examined in this study immediately presented with apparent direct linkage with the disease mutation behind PGD, there is quite a bit of valuable information to be gleaned from our results. To begin with, the results of previous work done by Dr. KP Carmichael and colleagues suggest several possible families of genes that may play a role in this disease. Many of these genes could be found in the human maps, and their homologous region identified in the dog map and examined.

One such possibility is any member of the tyrosine kinase receptor family (Trk). Several of these act as receptors for neurotrophins. TrkA in humans maps to 1q21, for which the canine homolog is unknown. TrkB maps to 9q22.1, also unknown in the dog. However, a large (28.5 Mb) region on chromosome 1 of the dog was unable to be ruled out in this study. The homologous region in the human includes 9q21.11-q13. TFG, a Trk receptor associated protein, maps to 3q11-q12 in the human. The canine homolog is on chromosome 31, in a region which has been ruled out. Animals deficient in another gene, Cystatin B, demonstrate extensive granule cell death, in addition to myoclonic seizures. This gene, in the human, maps to 21q22.3. Human regions 21q21.3 through 21q22.3 lie in a region of 13.4 Mb that contains no microsatellites. However, there is a

clearly ruled out marker immediately beside 21q22.3, making it likely that the PGD defect is not in the human region 21q22.3 (Lieuallen 2001). A similar situation exists with Reelin, which maps to the human region 7q22. The canine homolog is in the upper region of CFA 6, in a region spanning 11.6 Mb containing no microsatellites. The flanking markers are both clear rule outs, however. Given the disparities in pathologies between Reelin deficient mice and the PGD dog, plus this very narrow window, it is unlikely that Reelin is the gene in question, but it cannot be definitively ruled out, either. There are countless genes that could potentially cause the phenotype seen in the PGD affected pups that we could discuss here. However, given the fact that there are 16 regions of homozygosity that had to be ruled "uninformative" in this study, we will focus on the human homologues for those regions, and the genes they contain.

Referring to Table 3, there is a region in chromosome 1 that is intriguing. This region is quite large (28.5 Mb) and contains 6 uninformative markers. However, the canine map provides no homologous human region for much of this stretch. Those regions that are indicated contain no apparently relevant genes. However, as it is mentioned above, there is a human region here that maps close to the TrkB gene in the human. It might be possible to contact the FHCRC and determine if the TrkB gene and its immediate neighbors have been assigned a canine homolog as of yet unpublished. Further testing of markers within this region may also further narrow this region.

The next region is on chromosome 2, spanning 16.6 Mb. Within this region is the human homolog of a G-protein coupled receptor (#158) with unknown function (Hirosawa 1999). Another region can be found on CFA 4, spanning 15.3 Mb and containing the homolog to the region where human NRG3 is found (HS 10q22-q23).

Neuregulins are involved in granule cell migration (Yacubova 2003) and in coordinating inhibitory and excitatory interactions between neurons (Ozaki 2001). The next region, on chromosome 9 in the dog, is homologous to the human region containing BCN1, a neuronal cation channel, of the degenerin family. This gene in the nematode *C. elegans* is mutated to cause neurodegeneration. The channel is permeable to K+ and Na+ (Waldman 1996). The next region, also on CFA 9 is homologous to Gas (growth arrest specific 7), which is primarily present in mature Purkinje cells. This protein is involved in stimulating neuronal outgrowth (Chao 2003). Another portion of this same region on CFA 9 (22 Mb total) is homologous to the region containing astrotactin 2, which was discussed previously in this paper. Recall that this protein is critical to timely migration of granule cells. Reading Table 3, note that there are seven remaining genes of interest. This includes PTP receptor delta, a receptor type tyrosine phosphatase. These are involved in cell cycle regulation, and exist in many tissues. Also there is also a glutamate receptor possibly within one of our regions (CFA 18), GRM8. This is a metabotropic receptor whose gene was first discovered in a retinal cDNA library of the mouse. It is being studied in association with retinal disorders (Scherer 1997). Another gene, neuron navigator 2 (NAV2) has homology on CFA 21, in a large uninformative region (20.8 Mb). NAV 2 is involved in guiding neuron growth and migration of axons. This gene has been best studied in the nematode (Maes 2002). Yet another is a CNS vacuolar sorting protein receptor domain VSP10 containing protein, SORCS3. Though this protein is highly expressed in the developing brain, it is widely spread throughout the CNS (Rezgaoui 2001). Another gene with a homolog in our remaining uninformative region is neuroligin 1, which is involved in the formation of synapses in the CNS (Dean 2003).

Finally, also within one of these regions is the homolog for the human version of the gene mutated in the *lurcher* ataxic mouse model. GRID2 (Table 3) is an ionotropic glutamate receptor, expressed primarily in cerebellar Purkinje cells. The *lurcher* mouse presents with primary Purkinje cell loss, however, so had been ruled out as a model of PGD in dogs. However, further examination of this gene and its region in the dog is worthwhile, as a different mutation in the same gene may cause different effects, especially across species.

This study has certainly narrowed the search for the defect behind PGD, but there is still much work to be done. The unexpected level of homozygosity in these animals, which caused there to be such extensive uninformative regions, may be due to the fact that the canine genome maps were constructed using mongrel DNA, while the Jack Russells, though apparently from different areas, may all have common ancestors in their very recent pedigrees. The regions found here can be narrowed further with the use of more animals in the scan, more markers, or selective outcross breeding to introduce a greater amount of heterozygosity. As to the candidate genes reported in these regions, a direct approach may be used to either clone or sequence these genes in order to locate any potential mutations conserved in the affected animals.

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Table 1: PGD Jack Russell Terriers

Animal Name	**PGD Status**
Brownie	Affected
Penelope	Affected
Fergie	Affected
Di	Affected
Flipper	Affected
Ginger	Affected
A1	Affected
Pirate	Affected
Dudley	Affected
Pearl	Carrier
Hombre	Carrier
Rio	Carrier
Britches	Carrier
C1	Carrier
Left-side Spot	Possible Carrier
Saddle	Possible Carrier
Tricolor	Possible Carrier
PC1	Possible Carrier
PC2	Possible Carrier

TABLE 2: Screened Markers by Chromosome

CFA	Markers (RH)	Markers (ML)	Avg Distance	Avg RO
1	22	0	6.0	9.1
2	19	3	5.0	6.6
3	16	2	6.2	6.6
4	16	2	5.9	7.7
5	26	3	3.7	5.5
6	16	2	5.4	5.4
7	15	3	6.3	7.9
8	11	2	7.2	7.2
9	13	1	5.5	12.8
10	10	2	7.3	10.0
11	14	2	5.7	10.8
12	15	0	5.3	6.1
13	13	1	5.4	6.8
14	10	2	6.5	8.0
15	15	1	4.7	7.5
16	14	1	4.9	7.3
17	13	0	6.2	6.7
18	12	1	5.1	6.6
19	12	1	5.1	6.6
20	11	3	5.5	5.5
21	7	1	7.6	10.2
22	10	0	5.6	7.6
23	8	3	6.8	8.7
24	11	0	6.1	8.1
25	10	3	5.5	6.0
26	13	2	3.4	3.7
27	9	1	5.7	5.7
28	9	0	5.5	9.2
29	8	0	5.7	5.7
30	8	2	5.2	6.7
31	7	3	6.3	7.1
32	11	1	4.6	9.3
33	7	1	5.1	6.9
34	10	0	4.9	10.7
35	6	0	5.4	9.5
36	5	3	6.8	13.7
37	5	1	6.7	6.7
38	5	1	7.4	11.1
Overall	442	54	5.7	7.8

TABLE 3: CANDIDATE GENES

CFA	Region size (Mb)	Number of markers	Human regions	Known Genes
1	28.5	6	9q21.11-13 4p16.2 19q13.33-32 12.4 Mb unknown homology	9q22.1 TrkB None related None related
2	16.6	2	10p12.2-10p13	10p12.31 (Unknown function GPCR 158)
4	15.3	1	10q21.2-23.1	10q22-10q23 (NRG-3)
9	22.3	3	17q12 17q21	17q11.2-17q12 (BCN1 cation channel, neuronal) None related
9	22.4	3	17p13 9q32-34	17p13.1 (Gas-7) 9q33.2 (Astrotactin 2)
10	18.6	1	12q13.2-12q14.1	None related
11	19.6	1	9p21-23	9p23-9p24.3 (PTP delta)
18	15.8	2	7p13-14.1 7q31.1	None related 7q31.3-7q32.1 (GRM8)
21	20.8	1	11p15.1-4	11p15.1 (Neuron Navigator 2)
23	20.7	1	3q22.1	None listed* Linkage rule out
28	16.4	2	10q23-25	10q23-25 (VPS10 domain receptor protein SORCS 3)
32	15.1	2	4q22.1	4q22 (Glutamate receptor delta 2)
32	17.7	3	4q24	None listed
34	15.4	3	3q25-27	3q26.32 (Neuroligin 1)
36	19.9	2	2q31-32	None related
38	29.6	2	1q32.1-1q41	1q32-q41 (K+ Channel H1)

APPENDIX

CHROMOSOME TABLES

Key:

Locus:	Locus ID published by the Fred Hutchinson Cancer Research Center (Guyon 2003)
ID #:	Internal ID number by which primers are stored (-20 C)
Position #:	Ordered position number on a given chromosome, designated by FHCRC
Position (Mb):	Distance (Mb) from the top of the chromosome
DNM Up:	Distance to the nearest marker above (or to the top of the chromosome, for the first marker) in Mb.
Status:	Homozygosity mapping results. (Described in Methods Section)
Reference:	Locations of all experimental results for the primer set. P refers to the page number. These are presented as a roman numeral, which describes one of three notebooks (I, II, III), followed by the page number. F refers to autoradiography film, which are labeled 1-75.

CFA 1

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
FH3325	1	353	2	2.1	2.1	Rule Out	P:III22
REN138G03	1	265	6	8	5.9	Uninformative	P:III13; F:22
FH2016	1	78	13	16.4	8.4	Rule Out	P:II05
FH2313	1	nn	22	26	9.6	Rule Out	P:I20
C01.673	1	131	30	34.3	8.3	Rule Out	P:II16
FH3922	1	266	38	43.3	9	Uninformative	P:III13
REN162B09	1	193	42	47.5	4.2	Rule Out	P:II42
C01.246	1	132	46	52.1	4.6	Rule Out	P:II16; F:1,2,3,4
REN112I02	1	225	50	58.2	6.1	Rule Out	P:III09
C01.424	1	125	64	68.7	10.5	Rule Out	P:II15
FH3314	1	267	76	78.6	9.9	Rule Out	P:III14
REN159F24	1	226	87	89.5	10.9	Rule Out	P:III09
FH2309	1	mm	99	98.7	9.2	Rule Out	P:I20
FH3883	1	268	112	108.7	10	Uninformative	P:III14; F: 22
AHT138	1	141	113	109.8	1.1	Uninformative	P:II33,II41; F:5,6,7,8,9
C01.643	1	441	118	114.5	4.7	Uninformative	P:III28
C01.164	1	269	121	117	2.5	Uninformative	P:III14; F: 22
REN04I24	1	305	123	118.9	1.9	No Data	P:III18,III27
FH2326	1	442	126	121.8	2.9	Uninformative	P:III28; F:75
FH2598	1	70	133	127.2	5.4	Rule Out	P:II04
FH2294	1	110	138	129.4	2.2	Rule Out	P:II13
REN143K19	1	270	145	135.9	6.5	Rule Out	P:III14; F: 22
Terminus	1		z	137	1.1		

CFA 2

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
FH2274	2	U	2	3	3	Rule Out	P:I16
REN244F02	2	271	8	11.4	8.4	Rule Out	P:III14; F:48
CPH7	2	306	17	19.9	8.5	Rule Out	F:62
FH2890	2	272	18	21.3	1.4	Uninformative	P:III14; F:48
C02.609	2	142	23	29.9	8.6	No Data	P:II33,II41; F:2,4,5,6,7
REN44A17	2	228	31	36.5	6.6	Rule Out	P:III09, F:10
REN107M12	2	223	41	45.4	8.9	Uninformative	P:II44; F:10
C02.466	2	222	45	48.5	3.1	Rule Out	P:II44
FH2613	2	221	47	49.9	1.4	Rule Out	P:II44
FH2237	2	I	57	57.6	7.7	Rule Out	P:I41; F:11
REN70M14	2	227	59	58.6	1	No Data	P:III09; F:48
FH2608	2	273	69	65.8	7.2	Rule Out	P:III14
C02.894	2	144	77	72.4	6.6	Rule Out	P:II33
FH2132	2	X	79	74.4	2	Rule Out	P:I17
C06605	2	143	82	77.2	2.8	Uninformative	P:II33, F:12
AHT111	2	194	83	78.7	1.5	Rule Out	P:I42; F:13,14
FH2062	2	111	87	81.9	3.2	Rule Out	P:II13
FH3359	2	416	98	92.8	10.9	Rule Out	P:III25
C02.342	2	68	100	96.4	3.6	Rule Out	P:II03,23; F:15
C02.864.A	2	29	n/a	n/a	n/a	Uninformative	P:I32
AHT132	2	31	n/a	n/a	n/a	Rule Out	P:I33
FH2087U	2	112	n/a	n/a	n/a	Rule Out	P:II13
Terminus	2			99	2.6		

CFA 3

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
FH3396	3	417	3	3.8	3.8	Rule Out	P:III25
FH3115	3	229	4	8.1	4.3	Rule Out	P:III09
FH2895	3	307	13	15	6.9	No Data	P:III18,III27
FH2976	3	274	14	16.1	1.1	Rule Out	P:III15
FH2131	3	R	24	23.8	7.7	Rule Out	P:I16
FH2984	3	275	37	33.2	9.4	Rule Out	P:III15
FH2980	3	67	49	43.2	10	Rule Out	P:II03; F:16
FH3464	3	308	53	46.5	3.3	Rule Out	P:III18
REN273H17	3	276	55	51.2	4.7	Rule Out	P:III15; F:23
REN47O24	3	309	60	54.5	3.3	Rule Out	P:III19
REN260I04	3	277	64	58.5	4	Rule Out	P:III15; F:17, 23
FH2316	3	16	74	67.7	9.2	Rule Out	P:I28
REN216N05	3	230	84	76.4	8.7	Rule Out	P:III09
FH4076	3	278	91	83.9	7.5	Rule Out	P:III15
FH2107	3	G	97	91	7.1	Rule Out	P:I12
FH2302	3	103	104	99.5	8.5	Rule Out	P:II12
C03.895	3	28	n/a	n/a	n/a	Rule Out	P:I32,40
FH2531	3	82	n/a	n/a	n/a	Rule Out	P:II05
Terminus	3			105	5.5		

CFA 4

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
REN298N18	4	279	4	8.9	8.9	Rule Out	P:III15
REN303C04	4	280	9	15.4	6.5	No Data	P:III15; F:23,48,72
FH2773	4	310	14	20.9	5.5	Rule Out	P:III19
FH2732	4	281	17	25.5	4.6	Rule Out	P:III15
FH3310	4	231	24	34.5	9	Rule Out	P:III09
FH2399	4	354	27	37.6	3.1	Uninformative	P:III22; F:48,72
FH2776	4	282	34	49.8	12.2	Rule Out	P:III15
FH2412	4	3	39	53.3	3.5	Rule Out	P:I23
FH2142	4	79	49	64	10.7	Rule Out	P:II05
FH4018	4	232	57	65.8	1.8	Rule Out	P:III09
PEZ17	4	165	75	76.5	10.7	Rule Out	P:II37
FH2097	4	355	79	79.4	2.9	Rule Out	F:49
REN126G20	4	195	82	87.9	8.5	Uninformative	P:II42; F:13,14
AHT103	4	123	85	89.8	1.9	No Data	P:II03,28; F:18,19,20,21
FH2457	4	65	92	93.7	3.9	Rule Out	P:I50
G07704	4	283	100	99.8	6.1	Rule Out	P:III16
FH2534	4	102	n/a	n/a		Rule Out	P:II12
AHT128	4	124	n/a	n/a		Rule Out	P:II15
Terminus	4			100	0.2		

CFA 5

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
AHTH68REN	5	286	3	4.4	4.4	Rule Out	P:III16; F:17
REN283H21	5	198	6	8.6	4.2	Uninformative	P:II42; F:24
FH3928	5	311	8	10.4	1.8	Rule Out	P:III19
AHTH248	5	197	10	12.6	2.2	Rule Out	P:II42; F:13,14,25
FH2140	5	F	14	16.3	3.7	Rule Out	P:I11
REN78M01	5	287	23	25.1	8.8	Rule Out	P:III16
AHT141	5	196	32	31.8	6.7	Rule Out	P:II42; F:13,14
ZUBECA6	5	107	36	34.4	2.6	Uninformative	P:II14; F:26,27
REN285I23	5	233	41	37	2.6	No Data	P:III21; F:10,49,72
REN241A23	5	453	43	38.7	1.7	Uninformative	P:III28
FH3702	5	288	48	45.9	7.2	Rule Out	P:III16
DTR05.8	5	289	50	50.6	4.7	Rule Out	P:III16
REN262G24	5	290	57	59.1	8.5	Rule Out	P:III16
FH3278	5	418	62	66.4	7.3	Rule Out	P:III25
REN162F12	5	191	67	70.2	3.8	Uninformative	P:II40; F:28
FH3978	5	291	68	70.7	0.5	Rule Out	P:III17,III21; F:49
CPH18	5	46	69	71.2	0.5	Rule Out	P:I46; F:29,30
REN137C07	5	199	74	74.6	3.4	Uninformative	P:II42; F:24
C05.414	5	154	79	78.8	4.2	Rule Out	P:II36
REN175P10	5	284	84	82.4	3.6	Rule Out	P:III16
REN05D05	5	234	89	85	2.6	Uninformative	P:III21; F:49
REN12P17	5	285	90	85.5	0.5	Uninformative	P:III16; F:17
REN67D03	5	312	91	85.8	0.3	Rule Out	P:III29
C05.377	5	M	94	88.9	3.1	No Data	P:I13,I14,I33
REN287B11	5	174	100	91.6	2.7	Rule Out	P:II38
CPH14	5	163	110	98.2	6.6	Rule Out	P:II37; F:31,32,33
FH2383	5	83	n/a	n/a		Rule Out	P:II5
FH2594	5	108	n/a	n/a		Rule Out	P:II13,II23,II41; F:1
GLUT4	5	130	n/a	n/a		Rule Out	P:II16; F:3
Terminus	5			99	0.8		

CFA 6

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
REN285H12	6	292	2	3.4	3.4	Rule Out	P:III17,III21; F:49,69,73
FH2525	6	80	6	10.8	7.4	Rule Out	P:II05
FH2576	6	jj	9	17.5	6.7	Rule Out	P:I20
REN206A12	6	293	17	29.1	11.6	Rule Out	P:III17
REN172A18	6	294	22	37.1	8	Rule Out	P:III17; F:17
AHT109	6	169	29	44.3	7.2	Rule Out	P:II38
FH3933	6	235	37	51.2	6.9	Rule Out	F:10
FH2956	6	295	39	52.1	0.9	Rule Out	P:II17
FH2164	6	cc	51	58.5	6.4	Rule Out	P:I18; F:31,32,33,34
FH2119	6	139	62	66.7	8.2	Rule Out	P:II20, F:34
FH2370	6	296	69	70.3	3.6	Rule Out	P:III17
FH3303	6	236	74	76.8	6.5	Rule Out	F:50
REN65K24	6	297	78	79	2.2	Rule Out	P:III17; F:17,50
REN111L07	6	298	82	85.6	6.6	Rule Out	P:III17; F:35
REN287L04	6	447	82	85.6	0	Uninformative	P:III28
C06.636	6	63	n/a	n/a		Rule Out	P:I50
CPH3	6	129	n/a	n/a		Rule Out	P:II15
Terminus	6			87	1.4		

CFA 7

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
AHTH289REN	7	356	1	0	0	Rule Out	P:III22; F:50
REN97M11	7	317	6	11.1	11.1	Rule Out	P:III19
FH2226	7	bb	10	16.7	5.6	Rule Out	P:I18
FH3972	7	314	18	26.5	9.8	Rule Out	P:III19
VIASD10	7	237	23	34.4	7.9	Rule Out	P:III10
REN162C04	7	316	39	43.7	9.3	Rule Out	P:III19
C07.620	7	170	47	48.5	4.8	Rule Out	P:II38; F:5,6,7
FH2174	7	84	58	55	6.5	Rule Out	P:I05
FH3970	7	313	69	62.3	7.3	Uninformative	P:III19
FH2201	7	P	80	70.6	8.3	Rule Out	P:I15
FH2581	7	106	90	76.8	6.2	Rule Out	P:II13
FH2860	7	357	99	81.5	4.7	Rule Out	P:III22; F:50
FH3042	7	238	110	89.5	8	Rule Out	P:III10
REN116E14	7	315	115	94	4.5	Uninformative	P:III19; F:50
REN109O15	7	200	116	94.9	0.9	Uninformative	P:II42; F:20,21
C07.1000a	7	136	n/a	n/a		No Data	P:II28
FH2301	7	69	n/a	n/a		Rule Out	P:I03
FH2396	7	105	n/a	n/a		Rule Out	P:I12
Terminus	7			94.9	0		

CFA 8

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
FH3241	8	320	2	2.4	2.4	Rule Out	P:III19
AHTH240REN	8	318	8	11.6	9.2	Rule Out	P:III19; F:36,37
REN204K13	8	322	13	19.5	7.9	Rule Out	P:III19
FH3425	8	239	25	30.4	10.9	Rule Out	P:III10
FH4003	8	358	29	34.6	4.2	Rule Out	P:III22
C08.410	8	88	37	41.9	7.3	Rule Out	P:II7,II23
FH2144	8	66	46	49.8	7.9	Rule Out	P:II3
REN288F11	8	323	55	57	7.2	Rule Out	P:III19
REN178J05	8	321	63	64.1	7.1	Rule Out	P:III19
C08.618	8	92	75	74.4	10.3	Rule Out	P:II11
FH2989	8	319	82	79.6	5.2	Rule Out	P:III19
FH2138	8	dd	n/a	n/a		Rule Out	P:I18
FH2149	8	K	n/a	n/a		Rule Out	P:I13
Terminus				86	6.4		

CFA 9

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
GALK1	9	73	2	1	1	Rule Out	P:II4
FH2263	9	O	11	9	8	Rule Out	P:I14
C09.173	9	324	17	13.6	4.6	Rule Out	P:III19
REN54L20	9	362	25	21.1	7.5	No Data	P:III23; F:75
REN75M10	9	240	33	24.8	3.7	Uninformative	P:III10
FH4059	9	361	44	30.7	5.9	Uninformative	P:III23
FH2186	9	J	52	35.9	5.2	Rule Out	P:I12
REN145P07	9	326	62	42.8	6.9	Rule Out	P:III20; F:36,37
REN42F01	9	327	66	49.3	6.5	Uninformative	P:III20
FH3235	9	360	73	54.6	5.3	Rule Out	P:III22
LEI2D2	9	33	80	60.6	6	Uninformative	P:I42
REN73K24	9	359	85	64.4	3.8	No Data	P:III22; F:51
FH2885	9	325	92	73.4	9	Uninformative	P:III20
C09.250	9	146	n/a	n/a		Rule Out	P:II34
Terminus	9			77	3.6		

CFA 10

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
FH2537	10	81	6	9.5	9.5	Uninformative	P:II5
FH4081	10	329	13	18.6	9.1	Rule Out	P:III20; F:51,67
C10.781	10	241	19	27.5	8.9	Rule Out	P:III10; F:38
REN06H21	10	330	28	35.8	8.3	Rule Out	P:III20,III21; F:51,72
FH2293	10	H	35	43.6	7.8	Rule Out	P:I12
AHT101	10	145	53	54	10.4	Rule Out	P:II34
C10.16	10	128	61	60.5	6.5	Uninformative	P:II15; F:18
FH2422	10	ii	65	66.6	6.1	Rule Out	P:I20; F:51
DTR10.5	10	328	72	71.8	5.2	Rule Out	P:III20
REN154O19	10	419	83	77.3	5.5	Rule Out	P:III25
C10.865	10	155	n/a	n/a		Rule Out	P:II36
FH2339	10	64	n/a	n/a		Rule Out	P:I50
Terminus	10			80	2.7		

CFA 11

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
AHT137	11	121	2	1.4	1.4	Rule Out	P:II14; F:18
FH3203	11	332	13	11.7	10.3	Rule Out	P:III20
FH2096	11	100	23	22.2	10.5	Rule Out	P:II12
REN242K04	11	334	30	28.5	6.3	No Data	P:III20; F:51
REN54C20	11	242	34	32	3.5	Uninformative	P:III10; F:38,75
REN89J24	11	335	38	35.5	3.5	Rule Out	P:III20
FH2004	11	331	45	43.9	8.4	Uninformative	P:III20
FH2319	11	122	53	49.7	5.8	Rule Out	P:II15
REN174P22	11	333	69	59.5	9.8	No Data	P:III20; F:52,75
FH2019	11	166	79	69.3	9.8	Rule Out	P:II37
REN249L05	11	363	87	74.3	5	Uninformative	P:III23
C11.873	11	89	95	80.4	6.1	No Data	P:II7,II28
LEI001	11	11	100	83.9	3.5	Rule Out	P:I22; F:39,40
DGN13	11	201	102	85.7	1.8	Rule Out	P:II42; F:24,52
FH2018	11	27	n/a	n/a		No Data	P:I32
C11.750	11	37	n/a	n/a		Rule Out	P:I43
Terminus				86	0.3		

CFA 12

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
FH2200	12	45	2	0.7	0.7	Uninformative	P:II45
FH2202	12	364	7	5.7	5	Rule Out	P:III23
FH2975	12	337	15	12.8	7.1	Rule Out	P:III20,III21
REN153O12	12	365	20	19.7	6.9	Rule Out	P:III23
FH2152	12	Q	25	24	4.3	Rule Out	P:I15
REN194P02	12	339	39	34.2	10.2	Rule Out	P:III20
FH2054	12	243	56	44.8	10.6	Rule Out	P:III11
FH2223	12	85	66	49.4	4.6	Rule Out	P:II5
FH3591	12	244	87	59.5	10.1	Rule Out	P:III11
G01811	12	338	97	65.7	6.2	No Data	P:III20; F:41
FH1040	12	336	100	70.3	4.6	Rule Out	P:III20; F:41
PEZ5	12	167	104	75.5	5.2	Rule Out	P:II37
FH2347	12	A	107	77.2	1.7	Rule Out	P:I6,I9; F:67,72
C12.406	12	171	116	82.8	5.6	Rule Out	P:II38
C12.852	12	93	117	84.2	1.4	Rule Out	P:II9,II11,II28
Terminus				85	0.8		

CFA 13

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
C13.391	13	36	1	0	0	Rule Out	P:I42
FH3494	13	340	3	5.8	5.8	Rule Out	P:III20
REN98K18	13	366	6	14.2	8.4	Rule Out	P:III23
REN120P21	13	342	8	17.4	3.2	Uninformative	P:III20; F:67
FH3986	13	341	13	26.3	8.9	Rule Out	P:III20
FH3503	13	245	14	28.1	1.8	Rule Out	P:III11; F:38
REN65A19	13	343	22	36.3	8.2	Uninformative	P:III20; F:60
REN13N11	13	443	23	38.2	1.9	Uninformative	P:III28; F:75
REN286P03	13	202	27	42.2	4	Rule Out	P:II42; F:26,27,67
FH2348	13	168	33	47.1	4.9	Rule Out	P:II37; F:42,43,44
REN65L04	13	344	38	55	7.9	Rule Out	P:III20; F:60
AHT121	13	173	49	64.4	9.4	Rule Out	P:II38
C13.900	13	149	55	70.5	6.1	Rule Out	P:II34
FH2394	13	4	n/a	n/a		Rule Out	P:I23
Terminus	13			75	4.5		

CFA 14

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
FH2978	14	345	5	5.9	5.9	Rule Out	P:III21
FH3951	14	346	11	14.2	8.3	Rule Out	P:III21
FH2600	14	97	16	22.5	8.3	Rule Out	P:II11; F:15
CXX.866	14	148	17	23.1	0.6	Rule Out	P:II34; F:12
FH2060	14	18	26	31.3	8.2	Rule Out	P:I29
REN235M05	14	347	40	41.3	10	Uninformative	P:III21; F:60,68
REN289L09	14	348	50	50.4	9.1	Rule Out	P:III21
FH2258	14	160	59	58.9	8.5	Rule Out	P:II36; F:12,31,32,33
AHTK207	14	203	76	69.6	10.7	Rule Out	P:II42
PEZ10	14	133	79	71.2	1.6	Uninformative	P:II16; F:3
C14.390.2	14	39	n/a	n/a		Rule Out	P:I43; F:30
FH2547	14	71	n/a	n/a		Rule Out	P:II4
Terminus	14			72	0.8		

CFA 15

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
REN219H03	15	300	4	5.8	5.8	Rule Out	P:III18
FH3802	15	246	5	6.9	1.1	No Data	P:III11; F:60
REN297D17	15	299	11	14.2	7.3	Rule Out	P:III18; F:35
FH4012	15	367	17	25.1	10.9	Uninformative	P:III23; F:68
AHTH257	15	204	18	26.3	1.2	Rule Out	P:II42; F:24
FH3888	15	368	21	29	2.7	Uninformative	P:III23
REN06C11	15	119	28	35.4	6.4	Rule Out	P:II14; F:46
FH3813	15	349	29	38.9	3.5	Uninformative	P:III21
FH2535	15	10	36	43.3	4.4	Rule Out	P:I25
FH2171	15	E	40	45.1	1.8	No Data	P:I11,I33
FH2017	15	62	43	47.9	2.8	Rule Out	P:I50
FH2295	15	N	53	56.2	8.3	Rule Out	P:I14,25,26,27,28,30,36
REN123N11	15	350	63	66.1	9.9	Uninformative	P:III21
FH2278	15	15	75	71.1	5	Rule Out	P:I28
AHT139	15	120	79	73.8	2.7	Rule Out	P:II14; F:18
C15.608	15	90	n/a	n/a		Rule Out	P:I8,I28; F:16
Terminus	15			75	1.2		

CFA 16

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
REN238J02	16	351	4	3.9	3.9	Rule Out	P:III21; F:60,72
REN199I06	16	444	7	8	4.1	Uninformative	P:III28
REN206C13	16	371	9	10.1	2.1	Rule Out	P:III23; F:69
REN73O19	16	352	13	15.1	5	Uninformative	P:III21
REN85M08	16	192	20	25.9	10.8	Rule Out	P:II41
REN176D05	16	206	22	28.4	2.5	Rule Out	P:II43; F:26,28,61,63
AHTH260Ren	16	370	28	35.1	6.7	Uninformative	P:III23,III27; F:71
FH3592	16	369	32	45.2	10.1	Rule Out	P:III23,III27
FH2990	16	247	36	49.3	4.1	Rule Out	P:III11,III21
FH2155	16	61	39	51.7	2.4	No Data	P:I49; F:45
REN124F09	16	205	42	54.7	3	Rule Out	P:II43; F:61,63
REN44k22	16	175	49	59.5	4.8	Uninformative	P:II38; F:5,6,7,8,9,25,47
FH2175	16	24	57	65.4	5.9	Rule Out	P:I31
REN210K18	16	372	60	68.5	3.1	Rule Out	P:III23,III27
AHT131	16	32	n/a	n/a		No Data	P:I33,I34
Terminus	16			73	4.5		

CFA 17

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
COS15	17	59	3	7.1	7.1	Rule Out	P:I49
DTR17.1	17	373	7	14.9	7.8	Rule Out	P:III23
FH2321	17	17	11	21.5	6.6	Rule Out	P:I29
FH3349	17	374	17	26.7	5.2	Rule Out	P:III23
CPH5	17	127	25	36.1	9.4	Rule Out	P:II15
AHTH265	17	177	27	37.4	1.3	Rule Out	P:II39
REN02C03	17	375	35	41.9	4.5	Rule Out	P:III23
REN164F06	17	376	46	50.9	9	Rule Out	P:III23;F:69,74
FH2843	17	248	56	59.4	8.5	Rule Out	P:III11,III21; F:61,63,68
TSHB	17	II	66	66.4	7	Uninformative	P:I20,I38; F:19,43,44,47
FH3775	17	377	67	66.9	0.5	Rule Out	P:III23
CPH10	17	22	77	73.8	6.9	Rule Out	P:I31,I39
FH2869	17	378	85	80	6.2	Rule Out	P:III23
Terminus	17			80	0		

CFA 18

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
FH4060	18	379	1	0	0	Rule Out	P:III23
FH3944	18	380	5	8.4	8.4	Rule Out	P:III23
FH2834	18	381	15	17.3	8.9	Uninformative	P:III24,III27; F:71
REN106H23	18	449	16	18.7	1.4	Uninformative	P:III28
REN249N22	18	249	21	24.2	5.5	Rule Out	P:III11
C18.156	18	150	29	32.8	8.6	Rule Out	P:II34
FH3815	18	382	37	38.3	5.5	Rule Out	P:III24,III27
WILMS-TF	18	7	47	45.7	7.4	Rule Out	P:I24
FH3010	18	75	57	51.6	5.9	Rule Out	P:II4
FH2429	18	2	73	62.3	10.7	Rule Out	P:I22
FH2429	18	hh	73	62.3	0	Rule Out	P:I19
AHT130	18	53	82	65.7	3.4	Rule Out	P:I47
FH2356	18	B	n/a	n/a		Rule Out	P:I9
Terminus	18			66	0.3		

CFA 19

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
FH3299	19	383	1	0	0	Rule Out	P:III24
FH2783	19	251	9	6.9	6.9	Rule Out	P:III12; F:38
REN212E22	19	452	10	8.1	1.2	Uninformative	P:III28
FH3830	19	420	11	8.9	0.8	No Data	P:III25; F:71
PEZ3	19	164	17	12.4	3.5	Rule Out	P:II37
AHT124	19	ff	25	22.3	9.9	Rule Out	P:I19.I37; F:43,44,61,64
FH3100	19	384	32	26.3	4	Rule Out	P:III24
FH3940	19	250	41	31.5	5.2	Rule Out	P:III11
FH3834	19	385	46	41.3	9.8	Rule Out	P:III24
FH2206	19	V	50	46	4.7	No Data	P:I17
FH2380	19	386	57	55.5	9.5	Rule Out	P:III24
FH2279	19	44	n/a	n/a		Rule Out	P:I45
FH3969	19	387	60	65.7	10.2	Rule Out	P:III24
Terminus	19			66	0.3		

CFA 20

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
Pez19	20	388	2	1	1	Rule Out	P:III24
REN55P21	20	140	9	9.4	8.4	Rule Out	P:II20; F:34
C20.610	20	S	18	17.2	7.8	Rule Out	P:I16,25,27,28,30,36,II30
FH2951	20	301	23	20.4	3.2	Rule Out	P:III18
REN124F16	20	252	30	24.3	3.9	Rule Out	P:III12
REN100J13	20	302	37	30.5	6.2	Rule Out	P:III18
CPH16	20	25	45	35.7	5.2	Rule Out	P:I31
PRKCD	20	86	64	43.1	7.4	Rule Out	P:II06
FH2158	20	W	75	51.9	8.8	Rule Out	P:I17
REN249D14	20	389	86	57.8	5.9	Rule Out	P:III24
AHTK209	20	60	97	64.2	6.4	Rule Out	P:I49
FH2528	20	72	n/a	n/a		Rule Out	P:II04; F:19,39,40
FH2536	20	161	n/a	n/a		Uninformative	P:II37,II41
C20.446	20	207	n/a	n/a		No Data	P:II43; F:26,27
Terminus	20			66	1.8		

CFA 21

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
FH3803	21	390	4	5.1	5.1	Rule Out	P:III24;F:69
FH2233	21	D	9	8.9	3.8	Rule Out	P:I10; F:61,64,71
REN107L03	21	391	25	19.5	10.6	Uninformative	P:III24,III27
FH2441	21	gg	41	29.7	10.2	Rule Out	P:I19,I37; F:43,44
REN37A15	21	253	56	36.8	7.1	Rule Out	P:III12; F:38,62,68,72
REN108G11	21	392	79	47.1	10.3	Rule Out	P:III24,III27
FH2312	21	58	91	57.6	10.5	Rule Out	P:I49
AHT123	21	172	n/a	n/a		Rule Out	P:II38
Terminus				61	3.4		

CFA 22

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
REN49F22	22	8	3	2.8	2.8	Rule Out	P:I24,I38
AHTH211	22	178	10	11.6	8.8	Uninformative	P:II39; F:62,70
CXX.763	22	151	11	12.8	1.2	Rule Out	P:II34; F:12
CXX.768	22	152	13	15.4	2.6	Rule Out	P:II34
REN257M23	22	393	21	22.8	7.4	Uninformative	P:III24; F:72,75
FH4048	22	454	23	24.5	1.7	Uninformative	P:III28
REN49C08	22	94	30	31.6	7.1	Rule Out	P:II9,11,28; F:16
REN196G10	22	394	39	39	7.4	Rule Out	P:III24; F:71
C22.279	22	38	52	47.4	8.4	Rule Out	P:I43
FH2538	22	76	64	57.1	9.7	Rule Out	P:II04
Terminus	22			61	3.9		

CFA 23

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
FH2508	23	397	3	5.6	5.6	Uninformative	P:III24
AHTK253	23	179	9	14.1	8.5	Rule Out	P:II39
CPH6	23	396	13	17.8	3.7	Rule Out	P:III24
C23.277	23	Y	21	25.2	7.4	Rule Out	P:I17,III24; F:62
REN264K20	23	395	31	35.2	10	Uninformative	P:III24; F:71
REN02P03	23	208	42	45.9	10.7	Rule Out	P:I43; F:26,27
FH2227	23	52	44	47.9	2	Rule Out	P:I41
FH2001	23	26	56	58.5	10.6	Rule Out	P:I31
FH2325	23	77	n/a	n/a		Rule Out	P:II04
C23.745	23	138	n/a	n/a		Rule Out	P:II20; F:8,9
FH2283	23	aa	n/a	n/a		Rule Out	P:I18
Terminus	23			61	2.5		

CFA 24

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
FH3750	24	398	2	2.1	2.1	Rule Out	P:III24
FH3023	24	399	6	12.2	10.1	Rule Out	P:III24
FH2159	24	20	15	22.3	10.1	Rule Out	P:I29
REN106I06	24	400	22	31.8	9.5	Rule Out	P:III24; F:71
FH2261	24	74	29	39.8	8	Rule Out	P:II04
AHT125	24	153	39	50.8	11	Rule Out	P:II36,II41
REN170K23	24	209	45	58.9	8.1	Rule Out	P:II43
REN272I16	24	176	46	60.4	1.5	Uninformative	P:II39; F:2,4,5,6,7,62
FH2079	24	51	47	61.4	1	No Data	P:I47,II28
FH3287	24	264	51	66.1	4.7	Rule Out	P:III13
REN228J19	24	422	55	71.6	5.5	Rule Out	P:III26,III27
Terminus	24			73	1.4		

CFA 25

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
FH3977	25	401	3	3.6	3.6	Rule Out	P:III25
FH3245	25	402	9	11.9	8.3	Rule Out	P:III25
FH2324	25	254	19	20.1	8.2	Rule Out	P:III12
FH3923	25	445	27	27.1	7	Rule Out	P:III28
FH1004	25	403	30	30.4	3.3	Uninformative	P:III25; F:71
FH3327	25	446	32	32.1	1.7	Rule Out	P:III28
FH2141	25	19	42	38.9	6.8	Rule Out	P:I29
FH3627	25	404	50	46.9	8	Rule Out	P:III25
C25.213	25	91	55	50.9	4	Rule Out	P:II09
FH4027	25	405	63	55.1	4.2	Rule Out	P:III25
AHT140	25	43	n/a	n/a		No Data	P:I45,II32
FH2526	25	87	n/a	n/a		Uninformative	P:II06
FH2087L	25	162	n/a	n/a		Rule Out	P:II37
Terminus	25			60	4.9		

CFA 26

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
REN62M06	26	421	1	0	0	Rule Out	P:III26; F:72
REN02C11	26	212	10	7.5	7.5	Rule Out	P:I48;F:13,14
AHTK211	26	180	18	16.1	8.6	Rule Out	P:II39,II41; F:53,54
C02911	26	181	25	22.8	6.7	Rule Out	P:II39; F:53,54
REN01023	26	95	32	27	4.2	Rule Out	P:II09,II11; F:68
AHTK200	26	211	40	35	8	Rule Out	P:II42; F:20,21,69
REN88N03	26	303	41	35.6	0.6	Rule Out	P:III18
FH2130	26	L	46	39.3	3.7	Rule Out	P:I13,I14,II44;F:69
REN160C23	26	210	49	41.8	2.5	Rule Out	P:II42; F:20,21
REN304J03	26	304	50	42.1	0.3	Rule Out	P:III18; F:36,37,72
REN48E01	26	57	51	42.7	0.6	Rule Out	P:I48
C26.733	26	96	51	42.7	0	Uninformative	P:II11,28; F:18,39,40,55
REN111A03	26	224	52	42.8	0.1	Rule Out	P:II44; F:71
N41	26	137	n/a	n/a		Rule Out	P:II20; F:8,9
FH2566	26	kk	n/a	n/a		Rule Out	P:I20
Terminus	26			48	5.2		

CFA 27

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
FH2289	27	C	3	3.1	3.1	Rule Out	P:I10
FH4079	27	406	9	7.8	4.7	Rule Out	P:III25
PEZ16	27	159	17	14.9	7.1	Rule Out	P:II36
LEI002	27	12	25	24.1	9.2	Rule Out	P:I26
C27.502	27	255	33	30.4	6.3	Rule Out	P:III12
FH2346	27	Z	43	37.5	7.1	Rule Out	P:I18,I44
FH3924	27	407	47	42.5	5	Rule Out	P:III25
PEZ6	27	56	58	50	7.5	Rule Out	P:I48
REN181L14	27	408	63	54.5	4.5	Rule Out	P:III25
RVCE	27	127	n/a	n/a		Rule Out	P:II15
Terminus				57	2.5		

CFA 28

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
C28.176	28	117	1	0	0	Rule Out	P:II14
REN205C12	28	409	6	8.6	8.6	Rule Out	P:III25
FH3963	28	256	10	12.8	4.2	Rule Out	P:III12; F:56,57
REN136E14	28	410	17	20	7.2	Rule Out	P:III25
FH2208	28	49	23	24.9	4.9	Uninformative	P:I46,II22,III27
FH2668	28	411	29	29.1	4.2	Uninformative	P:III25
FH2585	28	98	36	36.4	7.3	Rule Out	P:II11
LEI006	28	1	44	45.2	8.8	Uninformative	P:I22; F:29,58,59,70
REN51i12	28	113	54	52.1	6.9	Rule Out	P:II13
Terminus	28			55	2.9		

CFA 29

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
FH2952	29	413	3	2	2	Rule Out	P:III25
FH2364	29	6	8	7.5	5.5	Rule Out	P:I23
REN170M10	29	412	12	13.5	6	Rule Out	P:III25, F:412
FH2328	29	158	20	21.4	7.9	Rule Out	P:II36
REN45F03	29	47	27	29	7.6	Rule Out	P:I46,II22,III27
FH2385	29	101	38	36.9	7.9	Rule Out	P:II12
C29.002	29	182	43	39.5	2.6	Rule Out	P:II39; F:53,54
REN74A15	29	213	53	48.1	8.6	Rule Out	P:II43
Terminus	29			51	2.9		

CFA 30

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
FH3489	30	414	3	2.9	2.9	Rule Out	P:III25
FH2050	30	ee	8	8.9	6	Rule Out	P:I19
REN89K14	30	257	15	16.2	7.3	Rule Out	P:III12
C02806	30	415	19	21.2	5	Rule Out	P:III25
FH2290	30	104	27	30.8	9.6	Rule Out	P:II12
REN50N18	30	220	33	37.9	7.1	Uninformative	P:II43,III27; F:71
REN245M07	30	183	39	42.4	4.5	Uninformative	P:II39; F:53,54
LEI-1F11	30	41	42	45.1	2.7	Rule Out	P:I44,II32
F8C	30	135	n/a	n/a		Rule Out	P:II16
FH2305	30	T	n/a	n/a		Rule Out	P:I16
Terminus	30			47	1.9		

CFA 31

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
RVC11	31	423	2	3.1	3.1	Rule Out	P:III26
REN43H24	31	215	9	13.8	10.7	Rule Out	P:II43
REN109B10	31	424	15	21.4	7.6	Rule Out	P:III26
C01003	31	184	20	27.5	6.1	No Data	P:II40; F:53,54
FH2239	31	13	24	31.4	3.9	Rule Out	P:I27
REN110K04	31	425	27	36.2	4.8	Rule Out	P:III26
REN50I04	31	214	37	49.6	13.4	Rule Out	P:II43
FH2199	31	42	n/a	n/a		Rule Out	P:I45,II22
C31.642	31	134	n/a	n/a		Uninformative	P:II16
FH2540	31	157	n/a	n/a		Rule Out	P:II36
Terminus	31			50	0.4		

CFA 32

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
REN244E04	32	440	4	10	10	Rule Out	P:III26
CPH2	32	23	6	11.7	1.7	Uninformative	P:I39,I41;F:58,59,65,70
REN41D20	32	115	7	12.8	1.1	Rule Out	P:I14
FH2875	32	426	10	19.8	7	Uninformative	P:III26; F:72
FH3744	32	455	12	23.6	3.8	Uninformative	P:III28
FH3635	32	258	15	27.9	4.3	Rule Out	P:III12;F:56,57,70
REN111K07	32	427	18	33.7	5.8	Rule Out	P:III26
UOR0421	32	456	21	39.2	5.5	Uninformative	P:III28
AHT127	32	116	24	44.6	5.4	Uninformative	P:II14,III27; F:71,73
FH4036	32	457	25	47	2.4	Uninformative	P:III28
FH3294	32	428	27	51.4	4.4	Rule Out	P:III26
FH2238	32	55	n/a	n/a		Uninformative	P:I48
Terminus	32			55.7	4.3		

CFA 33

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
FH2790	33	429	1	0	0	Uninformative	P:III26;F:75
REN112D03	33	217	7	6.4	6.4	Rule Out	P:II43; F:26,27
FH3608	33	259	12	13.3	6.9	Rule Out	P:III13; F:56,57,70
FH2361	33	430	21	20.4	7.1	Uninformative	P:III26; F:75
REN147E03	33	185	30	27.7	7.3	Rule Out	P:II40
REN291M20	33	216	34	31.6	3.9	Rule Out	P:II43
FH2165	33	21	41	38.5	6.9	Rule Out	P:I31
FH2507	33	54	n/a	n/a		Rule Out	P:I47
Terminus	33			41.1	2.6		

CFA 34

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
REN160M18	34	186	3	3.4	3.4	Rule Out	P:II40
REN125M11	34	431	8	12.7	9.3	Rule Out	P:III26,III27
REN174M24	34	432	13	16.5	3.8	Uninformative	P:III26; F:75
FH2377	34	5	16	23.9	7.4	Rule Out	P:I23; F:58,59,65,66,39,40
REN229C09	34	448	21	27.6	3.7	Uninformative	P:III28
REN243O23	34	260	24	31.6	4	No Data	P:III13; F:56,57,70
REN85F20	34	450	30	36.1	4.5	Uninformative	P:III28
DTRCN11	34	187	35	39.3	3.2	Rule Out	P:II40
REN44K21	34	34	44	46.1	6.8	No Data	P:I42,II31; F:58,59,65,66,71
REN314H10	34	451	49	49.8	3.7	Uninformative	P:III28
Terminus	34			50	3.9		

CFA 35

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
REN126G10	35	433	4	5.5	5.5	Uninformative	P:III26,III27
RENO1G01	35	35	7	9.8	4.3	Uninformative	P:I42,II31; F:31,66
REN282I22	35	219	11	14.9	5.1	Rule Out	P:II43
REN103G02	35	189	15	18.7	3.8	Uninformative	P:II40; F:25,28
REN166C14	35	218	21	26.8	8.1	Rule Out	P:II43,III27
REN112C08	35	188	27	34.4	7.6	Rule Out	P:II40; F:25,28
				38	3.6		

CFA 36

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
FH3090	36	434	4	4.2	4.2	Uninformative	P:III26; F:75
FH2611	36	156	11	12.6	8.4	Rule Out	P:II36,II41
REN179H15	36	262	20	21.1	8.5	Rule Out	P:III13
FH3865	36	435	30	30	8.9	Uninformative	P:III26
DTR36.3	36	261	40	37.2	7.2	Uninformative	P:III13
FH2516	36	9	n/a	n/a		Uninformative	P:I25,I39
AHTH130	36	40	n/a	n/a		Uninformative	P:I43
C36.672	36	114	n/a	n/a		Rule Out	P:II14
Terminus	36			41	3.8		

CFA 37

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
C37.172	37	118	6	4.3	4.3	Rule Out	P:II14; F:46
H10101	37	263	17	12.2	7.9	Rule Out	P:III13,III27
AHT133	37	30	28	18.5	6.3	Rule Out	P:II32,II41;58,59,65
FH2387	37	436	37	29.3	10.8	Rule Out	P:III26
FH2532	37	50	47	38.8	9.5	Rule Out	P:I47
FH2587	37	99	n/a	n/a		Uninformative	P:II11
Terminus	37			40			

CFA 38

Locus	Chr #	ID	Position #	Position (Mb)	DNM Up (Mb)	Status	Reference
FH2766	38	437	5	14.7	14.7	Uninformative	P:III26; F:75
D03821	38	438	11	25	10.3	Uninformative	P:III26; F:75
REN02C20	38	48	14	29.6	4.6	Rule Out	P:I46; F:45
REN164E17	38	439	20	38.7	9.1	Rule Out	P:III26
REN109O13	38	190	23	42.9	4.2	Rule Out	P:II40
FH2244	38	14	n/a	n/a		Rule Out	P:I27
Terminus	38			44.2	1.3		