Research in canine and human genetic disease

This was a Wellcome Trust Meeting which took place at Selsdon Park Hotel, Sanderstead, South Croydon on 28–31 March 1995. Between 60 and 70 scientists working in both veterinary and human medical backgrounds participated in an extremely wide ranging meeting. Eight sessions covered topics as diverse as skeletal malformations, autoimmunity, tumourigenesis, sensory deficiencies, heart disease, behaviour, neuromuscular degenerations, and gene therapy.

Dogs were probably the first animals to be domesticated by man, and have certainly had a close association with human settlements since palaeolithic times. They have been bred for particular anatomical or behavioural traits for many thousands of years so that already by between 4000 and 5000 years ago many distinct types were kept by the civilisations of Egypt and subsequently Babylon and Assyria. During this long association with man, and especially in the last 150 years in which formal breed standards have been recognised, the breeding of dogs in genetically isolated pools has allowed the emergence of a large number (>400) of inherited diseases. The meeting had the twin objectives of familiarising human geneticists with the wealth of resources presented by the many inherited diseases described in the dog, and familiarising those working in canine disease with the most recent developments in the analysis of human genetic diseases.

In keeping with these themes, the meeting began with a session surveying the genetics of connective tissue and skeletal abnormalities. In the mouse the full panoply of transgenic methodologies and in the human the power of modern positional cloning have been brought to bear. In the dog, in which the diversity of skeletal phenotypes considered normal by breed societies is probably greater than in any other species, little if any molecular work has yet been done. Malcolm Willis (Newcastle) reported on hip dysplasia (HD) which is the most common skeletal abnormality recognised by veterinarians. The heritability for this trait when measured by radiological assessment is 40%, but this is probably still too low for conventional genetic mapping to isolate the genes responsible. Matthew Warman (Case Western Reserve University, Cleveland) then spoke about the insights that studies of osteochondrodysplasias in mouse and humans can provide into skeletal morphogenesis. The role of the burgeoning family of collagen genes received special attention as Dr Warman described the differences in effect of col10α1 mutation in man and mouse (col10α1 null causes spondylometaphyseal dysplasia in man, but in mouse is phenotypically silent). Dr Warman described mutations in two further collagens which give rise to skeletal and joint abnormalities in both species. The role of growth factors and their receptors in skeletal morphogenesis came to the fore as Robin Winter (Institute of Child Health, London) reviewed the known causes of craniosynostosis (early fusion of the cranial sutures) in man. Mutations in fibroblast growth factor receptors (FGFRs) have recently been shown to cause different types of craniosynostosis as well as forms of bone dysplasia. A base change in FGFR3 found in achondroplasia appears to have the highest de novo mutation rate of any codon yet measured. The extremely high mutation rate of this codon has not yet been explained. The use of achondroplastic dogs as models was discussed, but these models are not yet sufficiently characterised. The findings in human and mouse may offer candidate genes for mutations causing the range of form seen in the dog.

The dog provides naturally occurring models of systemic lupus erythematosus (SLE) and of myasthenia gravis as well as a large number of immunodeficiencies. These diseases were explored in a session on autoimmunity and errors of immune function. For SLE, described by William Ollier (University of Manchester), genetic associations with the MHC have been known in human patients for some time, and similar associations are found in dogs; the same associations are important in myasthenia and RA in humans (studied by Jean-Francois Bach, INSERM, Paris and Alan Silman, University of Manchester), although in the former case the canine genetics have not been studied while no canine model of RA exists. In each disorder, disease development is associated with particular alleles at additional loci. Most recently, Professor Bach has shown strong associations between particular haplotypes at the acetylcholine receptor α chain locus and risk of myasthenia. Immune deficiency syndromes with simpler (largely single locus) genetics were the theme of a talk by Urs Giger (University of Pennsylvania). Many of the deficiencies studied in the dog have exact human homologues: examples are leucocyte adhesion deficiency in the Irish Setter (caused by absence of β integrin) and severe combined immunodeficiency in the Bassett Hound (caused by a frameshift in the IL-2Rγ gene).

A session on the genetics and inheritance of tumours allied the rapid increase in our
understanding of this subject in human medicine with the evidence for dog breed specific predispositions to particular tumours. Michael Steel (St Andrew's) reviewed the inheritance of cancer in humans, a topic at an exciting stage of rapid evolution, but a research area notable for the lack of appropriate (long lived) animal models. The contribution inheritance makes to cancers is not clear. Heritability of all cancer (any tumour at any site) is low, but for cancer at a single site heritability is about 30%. Part of this is the contribution of a small number of high penetrance loci (for instance, in the breast these loci account for about 5% of all cancers) but a substantial proportion is attributable to mutations at much lower penetrance loci. Molecular studies of canine tumours have in their infancy, as Gerard Rutteman (Utrecht) made clear. George Padgett (Michigan State University), who has studied histiocytosis and mast cell tumours in the Bernese Mountain Dog, provided an example of how breed specific predispositions to a particular tumour may shed light on the same tumour in humans, but also of the difficulties involved in studying the genetics of a partially penetrative trait without performing a deliberate outcross. Olyvind Bruland (Oslo) reported his studies of clinical and therapeutic aspects of osteosarcoma. In humans this is a rare tumour, so the availability of giant canine breeds, in which the same tumour is common, has contributed greatly to his studies of monoclonal antibody based therapy.

The second day opened with a discussion of the comparative genetics of sensory deficiencies in mouse, dog, and man, an area where monogenic disorders predominate, and where developments in the dog have sometimes led those in human medicine. Karen Steel (MRC, Nottingham) divided inherited deafness in the mouse into morphogenetic defects of the inner ear, linked syndromically with other skeletal defects, cochleosaccular defects often associated with abnormalities of pigmentation, and neuroepithelial defects where morphogenesis is normal but subsequently degeneration and dysfunction occur. For all three forms some of the responsible genes have been isolated (for morphogenetic defects, pax-3 in the splotch mouse; for cochleosaccular defects (as well as pax-3), c-kit, mgf, mi, and trp-1, and most recently she and Steve Brown (St Mary's, London) have shown the involvement of myosin 7 in neuroepithelial deafness in the mouse and in Usher's syndrome type Ib in man). Sheldon Steinberg (University of Pennsylvania) and Vilma Yusbasayan-Gurkan (Michigan State University) then reported research progress on a neuroepithelial deafness syndrome in Pointer dogs and a cochleosaccular defect in the Dalmatian. The latter shows interesting associations with coat patterning. The presence of dark patches as well as spots inversely correlates with risk of deafness. Deafness in this breed is much less common in Norway, where dark ear patches are allowed by dog show judges, than in the US, where they are not.

Inherited forms of blindness were dealt with in the second half of the session. Mutation in the gene encoding cGMP-PDE β subunit (PDEB) causes an early onset blindness with retinoschisis in mice, dog, and man. These diseases were described in detail by Debora Farber (UCLA), Gus Aquirre (Cornell), and Peter Clements (University of Cambridge). Correction of the defect has been achieved in transgenic mice by introduction of a bovine PDEB gene linked to the opsin promoter. In the Irish Setter, mutation in this gene is responsible both for an early onset retinal degeneration, and for rare atypical late onset degenerations. The extraordinary genetic heterogeneity seen in retinal degenerations was touched on for other breeds of dog by Dr Aquirre who pointed out that X linked as well as several non-allelic autosomal loci are being studied in this species, and was the major theme of Shomi Bhattacharya's (Institute of Ophthalmology, London) talk on retinitis pigmentosa in man. In man over 40 separate loci have been implicated in retinal degenerations, and at one of these alone (opsin), more than 100 mutations have been recorded.

Heart disease has conventionally been considered a disease of age, in which the environment interacts strongly with many genetic factors. John Burn (Newcastle) reassessed human data to show that strong contributions to congenital heart disease can be made by just a few genes although these have not yet been mapped. Donald Patterson (University of Pennsylvania) then showed the utility of breeding experiments in the dog. In his work on conotruncal defects in Keeshond dogs, these dogs were selected over 10 generations for severe defects and then outcrossed to normal Beagles. These experiments indicated a single locus defect owing to a gene acting in an autosomal recessive manner with incomplete penetrance. He is now working to isolate the gene responsible through both candidate gene and positional cloning approaches using F1 double backcross matings. Human hyperlipidaemia was addressed by Ian Day (UC Medical School, London). As with the inheritance of tumours, individual phenotypes may arise through rare single gene mutations that have a major impact on plasma lipid concentrations (for example, some LDL receptor mutations) or more common mutations of small effect. Dr Day described methods to screen large populations for a number of mutations. Tim Watson (Waltham) described hyperlipidaemia in dogs where, in contrast to the human situation, most cases are the result of metabolic or endocrine diseases. Peter O'Brien (Guelph) has analysed the biochemical and molecular basis for idiopathic dilated cardiomyopathy in Doberman Pinschers. Studies of gene expression showed mRNA instability owing to an increase in mitochondrial RNase activity and there are hints that the defect might be mitochondrially inherited.

Behavioural genetics have long been a contentious area: the idea that complex behaviours in man can be genetically programmed is unattractive to many. An exciting session on this topic was kicked off by Elaine Ostrander (Fred Sargent)
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Hutchison Cancer Research Institute, Seattle) pointing out that the dog, with its behavioural traits which breed true, offers several advantages for elucidating the genes which underlie behaviour. Dr Ostrander’s research, in collaboration with Professor Jasper Rine (Berkeley) is focusing on the distinct behaviours of Newfoundland dogs, which are attracted to water but lack a herding instinct, and Border Collies which have the opposite characteristics. An interbreeding programme has established F1 and F2 crosses of these breeds, and a video was shown illustrating methods of assaying these behaviours. Dr Ostrander also reported rapid progress made in her laboratory to create a canine genetic map; 100 polymorphic microsatellite markers have been located in 12 syntenic groups so far. Professor Jo Takahashi (Northwestern University, Evanston) reported his studies of the genetic control of mammalian circadian rhythms. A mouse generated by chemical mutagenesis had an internal circadian rhythm lengthened from 23.7 hours to 24.6 hours in heterozygotes. Homozygotes have circadian periods of about 28 hours, but circadian rhythm quickly breaks down completely. This mutation, termed Clock, was localised to a subregion of chromosome 5 and is now being mapped using YACs. Han Brunner (Utrecht) presented studies of a Dutch family in which some male members shared abnormal impulsive and aggressive behaviour. Mapping of the X chromosome implicated a locus near to monoamine oxidase A (MAOA). Biochemical analysis implicated a deficiency in this enzyme, and DNA sequencing showed a nonsense mutation in the MAOA gene in affected subjects. Brunner was at pains to put this work into a wider context. Increased aggression would not be predicted from the known effects of modulating MAOA and neurotransmitter levels, although changes in serotonin levels in the fetus might influence cerebral architecture. Behaviour results from interactions of many different brains systems so seeing this gene as a master gene for aggression is a massive over-simplification of a very complex biological system. Michael Owen (University of Wales College of Medicine, Cardiff) discussed genetic aspects of schizophrenia. This disorder is clearly familial but no evidence for linkage to a marker has been found although chromosomal abnormalities point to specific candidate regions, including 22q12–13.1. Bruce Cattanach (MRC, Dicodot) drew attention to the existence of behavioural problems in dogs, such as rage syndrome, which could provide potential models of human conditions. Linda Cork (Stanford) pointed out that the brain of primates develops much more slowly than that of dogs and that the behavioural repertoire of dogs is limited.

The final day was devoted to muscular dystrophies and gene therapy, two sessions that provide a complementary perspective on genetic disease and therapeutic strategies since muscular dystrophies have been an ongoing target in the development of gene therapy approaches. Terry Partridge (Hammersmith Hospital, London) began by discussing the mdx mouse mutant and its use as a model for human Duchenne muscular dystrophy and the study of pathological processes involved in muscle degeneration. He focused on extensive studies of the fates of myogenic cells grafted into mdx muscles. Several important considerations of this approach for therapeutic purposes include the age of the donor in relation to the proliferative potential of transplanted myogenic cells as well as the viability of this approach in larger mammals, for example, dog or human where therapeutic potential may be compromised by the ability of transplanted cells to disperse and terminatation. Nick Sharp (University of North Carolina) subsequently introduced the canine and feline models of DMD, including Golden Retriever muscular dystrophy (GRMD) emphasising some of the pathological similarities and differences. The advantages of studying the same or similar mutations in different mammalian species were emphasised throughout the discussion. GRMD results from a splice site mutation that leads to skipping of the seventh exon of the dystrophin gene and predictable premature termination. Surprisingly, GRMD dogs show very low levels of a near normal sized dystrophin protein. Even small increases in dystrophin expression can have a beneficial effect and GRMD dogs may represent an interesting system to assess routes to ameliorate the condition by increasing production of the near normal dystrophin. Eric Hoffman (Pittsburgh) discussed the American Quarter horse, a breed that carries a mutation for hyperkalaemic periodic paralysis. Hyperkalaemic periodic paralysis occurs in the human population and has been shown to be the result of mutations in the skeletal muscle voltage sensitive sodium channel gene. A phenylalanine to valine mutation in the homologous horse gene is responsible for periodic paralysis in American Quarter horses and accurate molecular diagnosis is now available to the horse breeding community. Linda Cork introduced a large pedigree of dogs inheriting the autosomal dominant hereditary canine spinal muscular atrophy (HCSMA) locus. A DNA marker near to the HCSMA gene was mapped in the dog genome and microsatellites and gene markers, is being used to identify a genetic map linkage for HCSMA. Keith Johnson (Charing Cross and Westminister Medical School, London) concluded the session with a detailed summary of work to date on the molecular basis for the myotonic dystrophy (DM) gene. There are no known animal models of DM and indeed the basis of the DM mutation, a triplet repeat expansion, has not been shown to underlie a genetic disease in any other species. Dominic Wells (Royal Veterinary College, London) continued the morning’s theme by discussing his experiments on muscle gene therapy also using the mdx mouse. He described three approaches towards the introduction of constructs: (1) the use of retroviral vectors; (2) adenoviral vectors; and (3) injection of naked plasmid. The most successful system in the mouse was plasmid DNA injection, retroviral approaches being compounded by immuno-
logical problems with the endogenous proviruses present in some strains of mice. Adenoviral systems were also compromised by immunological problems as mouse muscle matures. However, direct DNA injection is confounded by problems of scale and it is clearly important that plasmid be introduced when the target tissue is still growing or following regeneration. There are few results yet on similar approaches in the GRMD dog. John Wolfe (University of Pennsylvania) focused on gene therapy for lysosomal storage disease using the dog model for mucopolysaccharidosis type VII. The strategy used is cross correction; the normal lysosomal enzyme can be synthesised by one cell and exported to enzyme negative mutant cells. Introduction of the gene into bone marrow cells by retroviral vectors and their implantation in vivo resulted in increased life span of the dogs and improvements in both bone and cornea. Finally, Charles Coutelle (St Mary's, London) presented his latest perspective on the well advanced programme for gene therapy for cystic fibrosis, a chloride channel defect for which a mouse model was produced by gene targeting of the homologous mouse gene. Following gene therapy experiments in mice, Phase I trials have been undertaken in man using both adenovirus and liposomes as delivery systems. The adenovirus trials were terminated following problems with inflammation of increased doses, but transient electrophysiological correction was observed at lower doses. The liposome trials showed low toxicity and a transient but minimal improvement in nasal epithelium electrophysiology. During questions, Charles Coutelle expressed the view that a dog model would not have been used had one been available, particularly since the efficacy of the therapy was being tested in nasal epithelium for which the mouse is a suitable model. In wider discussions, the dangers and limitations of taking therapy trials straight from the mouse to human were remarked upon. Lessons might be learned from the problems encountered with myoblast transfer therapy for DMD where, although good results had been achieved in the mouse, the same success was not achieved in human trials. The ideal scenario may be one where trials move from small to large animals allowing the gene therapist to assess the problems of scale up that may present in larger organisms.

From the conference as a whole several lessons were learned: opportunities for understanding human disease and the normal human condition are presented by the dog which have not yet been fully exploited. This was particularly clear in the sessions on skeletal abnormalities, on inherited predisposition to tumours, and on the genetics of behaviour. In all these fields the pedigree breed resource is enormous, but with a few exceptions the outcrosses needed to exploit it have not been performed. The possibility may exist to tease out the "common mutations of small effect" which are important in the complex inheritance of heart disease, cancer, and many of the other syndromes tackled at the conference. There is enormous value in breeding lines carrying mutations with well defined pathology and high penetrance. Such mutations should at an early stage be established on a canine linkage map even if at this time syntenic relationships with mouse and humans are not well founded. It will be possible to identify candidate genes from the mouse and human maps as the maps grow and syntenic regions are filled in and, in addition, the mapped mutations have veterinary importance in allowing the production of robust diagnostic tests for genetic disease. Attention was drawn to the distinction between true species homologues, where the same gene is defective, and models where the disease appears similar but may have a different cause. In many situations true homologues may offer greater insight, but exceptions exist, and this is especially noticeable when a small, short lived organism such as a mouse is compared with a large, long lived one. Thus, the importance of the dog as an intermediate model became the dominant theme of the later sessions of the conference. Its full exploitation will depend both on the production of outcrosses to simplify genetic studies and on the development of the canine genetic map. Both of these topics were major themes of the Second Dogmap conference in Cambridge, which immediately followed the Wellcome Trust meeting.

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