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Hypertensive cardiac hypertrophy—is genetic variance the missing link?

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1 Hypertensive cardiac hypertrophy is a major independent predictor of adverse cardiovascular events. In man the cardiac response to increased afterload is very variable, even when ambulatory blood pressure monitoring is used. Analysis of breeding experiments using normotensive and hypertensive rat strains, human twin studies and other data indicate that genetic factors play a significant role in regulating cardiac mass; in other words, a large component of total variability is accounted for by genetic variance.

2 The observation that some patients with only mild-to-moderate hypertension exhibit *gross* left ventricular hypertrophy (LVH) similar to the inherited hypertrophic cardiomyopathies such as familial hypertrophic cardiomyopathy (FHC) and Friedreich's ataxia (FA) has prompted us to investigate the hypothesis that genetic factors associated with excessive myocardial hypertrophy, *viz.* mutations in FHC and FA genes alter the hypertrophic response of the heart to pressure overload. Here we review briefly three lines of study: (i) association analysis to test whether the allele frequencies differ in hypertensive patients with or without left ventricular hypertrophy; (ii) characterization of the cardiac manifestations of FA to understand the mechanism by which the heart is affected in a disease associated with pathology in a subgroup of neurons, and (iii) creation of transgenic models to facilitate the investigation of the interaction between hypertrophic stimuli and underlying genetic predisposition.

3 Information on the nature of the cardiac-mass-modifying genes involved may be useful not only for selecting high risk patients in strategies aimed at *preventing* the development of LVH, but also in opening new avenues of research on the reprogramming of cardiac myocytes to encourage them to hypertrophy in situations where cardiac muscle has been damaged or is hypoplastic.

Keywords genetics heart hypertension transgenic Friedreich's ataxia hypertrophic cardiomyopathy

The importance of left ventricular hypertrophy (LVH)

Epidemiological studies such as the Framingham Heart Study have established that the presence of LVH in hypertensives is a major risk factor for cardiovascular morbidity and mortality [1–3]. Indeed, it may be a more potent predictor of strokes and coronary artery disease than high blood pressure itself, hyperlipidaemia and cigarette smoking. It is not clear whether this risk is directly attributable to the cardiac hypertrophy itself or whether the latter is a surrogate marker for some pathogenetic process common to the various complications. Nevertheless, approaches have been advocated to modify the hypertrophic response of the myocardium to pressure overload in the expectation that these will make a major impact on cardiovascular morbidity and mortality. Although in animals hypertensive LVH can be prevented with drug therapy, it would seem from

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published data that in man LVH can be reduced only to a limited extent (<15%) by pharmacological means [4–6], perhaps because some of the studies have been too short [7]. In any case, there is no firm evidence that the excess adverse risk which accompanies LVH is abolished by reducing the hypertrophy once it is established. Thus, there may be advantages in promoting a strategy aimed at early identification of high-risk individuals and *prevention* of the development of LVH, perhaps by employing lower target blood pressure levels.

For such a preventative programme to be effective robust techniques are needed which can stratify patients according to risk. Clinical methods such as echocardiography are only useful once the hypertrophy is established, and even then there is considerable intra- and inter-observer variation in the measurement of cardiac mass. An alternative approach, that we favour, would be to employ molecular genetic methods which have the distinct advantage that they can be used at a time when the clinical techniques would not be expected to yield positive results. Clearly, it will be a large task to prove the validity of such a strategy as long-term outcome data for stratified individuals would be required for the assessment. Nevertheless, this may be justified if the cost–benefit and risk–benefit ratios of anti-hypertensive treatment can be improved.

One aim of our work is to define the genetic factors controlling the hypertrophic response(s) to hypertension, as this will permit us to produce genetic risk profiles with attendant risk ratios. First we discuss the evidence supporting the genetic regulation of cardiac mass; then we present a brief outline of our research.

Cardiac mass is highly variable

It is well-known that cardiac mass increases when afterload is elevated for a sufficient period of time. The pressure load on the left ventricle has been evaluated by single blood pressure readings and by repeated measurements involving ambulatory monitoring over full wake–sleep periods, including episodes of physical work or exercise [8]. What is less often appreciated is that the many positive correlations, sometimes highly significant, between cardiac mass and afterload underemphasize the substantial degree of variability in cardiac phenotype observed for any level of afterload. Indeed, in hypertensives, much of the variance in cardiac mass remains unexplained even when factors such as age, weight, physical activity, glucose intolerance and alcohol intake are also taken into consideration [8, 9]. Similarly, in patients with aortic stenosis there is only a weak relationship between LV mass and the degree of stenosis as assessed by aortic valve area [10–13]. This unexplained variance in the response to pressure overload is likely to include an element of unsuspected 'environmental' variance which has not been controlled for, as well as a considerable effect due to heritable factors; more specifically, 'cardiac-mass-modifying' genes.

The distribution of cardiac mass within populations of unselected normotensive or hypertensive individuals appears to be continuous for the most part [14], supporting the view that any heritable effects are likely to be polygenic. In addition, there is a sub-group, discussed further below, who have gross cardiac hypertrophy which is out of proportion to the level of identifiable 'causal' secondary factors. Some of these have monogenic diseases associated with hypertrophic cardiomyopathy and therefore have 'primary' cardiac hypertrophy, but the majority are hypertensives or athletes, and they are much more difficult to classify.

Evidence supporting the genetic determination of cardiac mass

A search through an electronic database for papers on cardiac hypertrophy produces a flood of titles on the subject. However, there is a startling lack of robust data on the extent and importance of inherited variability in the response of the heart to hypertrophic stimuli. For example, there are no extensive family studies documenting the segregation of cardiac phenotype in relatives of patients with 'secondary' LVH which might provide evidence for or against the polygenic nature of the response. This is not surprising; large families with hypertension have been studied with respect to blood pressure rather than cardiac phenotype.

Animal studies

Animal studies provide the most persuasive evidence for a significant genetic contribution to the total variance of cardiac mass. Tanase *et al.* have examined the relationship between blood pressure and cardiac mass in 23 strains of normotensive and hypertensive rats [15]. They found that heart weight increased in proportion to blood pressure only in hypertensive animals and that genetic variance accounted for 65–75% of the total variability of cardiac mass. When these strains were crossed, three blood pressure phenotypes were observed (normotensive, intermediate and hypertensive groups) within which there was a large variability in heart weight. From analyses of these crosses, the genetic variance of cardiac mass was estimated as 45–65%. The authors concluded that heart weight was a highly heritable trait; the effect of genetic factors on cardiac mass being greater than that of blood pressure. Others have arrived at similar conclusions based on crosses between spontaneously hypertensive (SHR) and normotensive (BN.1× or Wistar Kyoto, WKY) rats [16, 17]. An extensive survey of the hearts from normotensive first generation (F1) offspring of WKY/NCrj rats demonstrated increased heart weight in about 10%, disproportionate septal thickness in 50%, right ventricular hypertrophy in 40% and myocardial fibrosis in 70% [18–20]. Also, SHRs exhibit LVH before the blood pressure is elevated [21, 22]; one variant, the Wistar Kyoto-Hyperactive (WK-HA) strain is normotensive, but displays LVH [23, 24]. One strain of rats develops biventricular hypertrophy without

hypertension [25], whilst others, such as the F344 strain, appear to be relatively resistant to hypertrophic stimuli [15, 26]. Importantly, the breeding studies indicate that the 'cardiac-mass-determining' loci are distinct from those involved in the genesis of the hypertension itself [15, 27]. Undoubtedly, combined segregation and linkage studies using inbred rat strains and modern highly-polymorphic markers spread across the whole genome will define some of the LVH-susceptibility loci contributing to the strain differences. These loci will then have to be tested in man to determine their importance.

Human studies

In man, the most convincing data on the heritability of cardiac mass comes from various twin and ancillary studies [28–31], the largest of which is that collated by the Medical College of Virginia [32]. In this study 341 11-year-old twins pairs were recruited, from which 254 white pairs were analysed. The authors found that genetic variance accounted for 63% of the total variance of cardiac mass in boys and 71% in girls. They found that genes which control LV mass include those which regulate LV mass alone and others which affect both LV mass and weight (but not ponderosity). In boys the genetic effects were, for the most part, due to the latter, whereas in girls the former predominated. Similar results were obtained when data were analysed from one twin randomly selected from each of 243 white twin pairs [33]. Boys had a greater LV mass than girls, but both had similar anthropometric determinants. Interestingly, the data indicated that body fat was negatively associated with LV mass. In a smaller study of twins aged 18 to 31 years, Fagard *et al.* also found a significant genetic contribution to left ventricular mass, which they attributed mainly to the inheritance of body size [34]. Harshfield *et al.* measured LV mass index in seven monozygotic and 15 dizygotic normotensive young adult black twins [34]. When adjusted for gender, blood pressure and age, the intraclass correlation for monozygotic twins was 0.9 and for dizygotic twins 0.33 (before and after adjustment for body size). They concluded that in black people there is a substantial genetic influence determining LV mass independent of sex, systolic blood pressure and age. On the other hand, Adams *et al.* studied 31 monozygotic twins, 10 dizygotic twins, 6 sib pairs and 15 pairs of unrelated subjects, before and after a 14-week training programme, and concluded that familial influences (common environmental + genetic factors) are more important determinants of cardiac size than non-familial or purely genetic influences [35].

Other evidence comes from the well-known differences in racial susceptibility to develop LVH [14, 36, 37]. This has been documented most comprehensively in two large studies, one of which compared whites and blacks (The Evans County Heart Study [38, 39]), and the other light- and dark-skinned Puerto Rican men (The Puerto Rico Heart Health Program [40]). Electrocardiographical LVH was more prevalent in dark-skinned than in lighter-skinned men, after adjusting for blood pressure, body mass index, physical activity, cigarette smoking and skin-fold thickness. There was a higher prevalence of LVH in dark-skinned men even when blood pressure was normal. These data and the abnormal LV response to hypertension in blacks may represent an altered genetic susceptibility of the myocardium to pressure overload [41–48].

In matched groups of *normotensive* offspring of hypertensive and normotensive subjects, LV mass index and interventricular septum/posterior wall ratio are greater in the children of hypertensives than in those of normotensive controls [49–54]. It is of considerable interest that in normotensive children and adults there is evidence that the relationship between blood pressure and LVH may be the reverse of that expected, inasmuch as differences in LV mass have been found to pre-date, and be predictive of, elevation in blood pressure over the next 4–5 years [55–62]. Clearly, these data raise many interesting issues which cannot be discussed in this short review.

The data presented above and the exuberant skeletal muscle hypertrophy that black males develop in response to exercise imply the existence of modifying genes which affect not only cardiac structure, but also skeletal and smooth muscle cell biology.

Gross LVH associated with hypertension

In general individuals exhibiting extremes of a phenotype are most valuable when genetic influences are being investigated. At Hammersmith Hospital we have identified a group of patients with gross LVH and only mild-to-moderate hypertension, who are unlike the majority of our hypertensives. For example, in one of these patients, now aged 57 years, cardiac mass has increased progressively over 14 years despite having clinic blood pressure readings at or below 160/90 mmHg over this period. Others have recognized similar patients in whom cardiac mass is increased out of proportion to the pressure load on the left ventricle [63–72]. These patients are, in general, elderly, predominantly female, and mostly black. They differ from patients with classical inherited hypertrophic cardiomyopathy in their age, racial origin, low incidence of early sudden death and the absence of a history of familial segregation as reported by the patients. Although the cardiac phenotype has not been defined formally in family members of most of these patients, there is a report of greater-than-expected cardiac mass in first degree relatives of patients with disproportionate septal hypertrophy and hypertension [63].

Opinions have differed as to the pathogenesis of the hypertrophy in these patients. One group [71] believes that they represent an exaggerated response to hypertension, whilst another [73] proposes that the patients have hypertrophic cardiomyopathy and hypertension. We believe that both explanations may be correct; these patients having inherited a spectrum of LVH-susceptibility genes which sensitises their hearts to hypertrophic

stimuli. Similarities in histological features (including the presence of variable amounts of myocyte disarray [74–76]) also emphasize the overlap between severe hypertensive LVH and the inherited hypertrophic cardiomyopathies.

Genetic syndromes associated with cardiac hypertrophy

This overlap between disproportionately-severe hypertensive LVH and inherited hypertrophic cardiomyopathies raises the question whether alterations in the genes responsible for the latter play a role in determining the hypertrophic response to hypertension. Although this hypothesis has been criticized because there is no clear family history of cardiac hypertrophy in hypertensives, the hypothesis should not be dismissed outright. As Goodfellow & Schmitt [77] have commented 'the gene variants contributing to complex (polygenic) diseases are likely to be common polymorphisms which, in the absence of other risk factors, do not cause disease' in other words, a heart which has the right genetic priming only hypertrophies when a number of circumstances, one of which is pressure overload, coincide in the same patient. This would complicate the interpretation of the segregation of phenotype, particularly when the latter has not been rigorously and systematically assessed.

A number of primary inherited disorders are associated with cardiac hypertrophy independently of each other, and of secondary stimuli such as hypertension and valvular heart disease. These include familial hypertrophic cardiomyopathy (FHC), Friedreich's ataxia (FA), the Ullrich-Noonan syndrome, mutations in mitochondrial DNA, the Moynihan-Polani syndrome and perhaps adult polycystic kidney disease [78–83]. Fabry's disease is an X-linked recessive disorder resulting from the deficiency of α-galactosidase and accumulation of glycosphingolipids in which LVH occurs in hemizygous men as part of the generalized storage disease. However, an atypical variant has been reported in which the heart is the main site of pathology (the hypertrophy can be indistinguishable from hypertrophic cardiomyopathy) [84]. Although the full disease is very rare, Nakao and colleagues have reported that of 230 unselected general cardiology patients with LVH, seven (3%) had hemizygous Fabry's disease. The prevalence rose to 10% in those patients in whom no other underlying cause of LVH was found, i.e. 1:10 of the patients who would have been labelled as idiopathic hypertrophic cardiomyopathy had Fabry's disease.

Our working hypothesis is that polymorphisms in the genes causing FHC and FA sensitize the heart to hypertrophic stimuli such as hypertension; the variation in cardiac phenotype reflecting the inheritance of a particular combination of susceptibility factors. We have focused on two primary cardiomyopathies, but the approach is general and can be used to investigate the role of other genes.

Familial hypertrophic cardiomyopathy (FHC)

FHC is a genetically heterogeneous autosomal dominant disorder which has been linked to loci on chromosomes 1 (the cardiac troponin T gene), 11 (the cardiac myosin binding protein-C gene), 7 (associated with Wolf-Parkinson-White syndrome; gene unknown), 14 (the cardiac β-myosin heavy chain (β-MHC) gene) and 15 (the α-tropomyosin gene) [85]. In some families, recombination between the trait and markers for all these loci indicates that there are likely to be other causative genes. So far, all the causative mutations in these genes have been in exons, but there has been no systematic analysis of the role of polymorphisms in regulatory regions which may exist in linkage disequilibrium with exonic mutations. Classical FHC is a disease with a malignant prognosis causing death at an early age. However, the molecular genetic studies have shown that some FHC-causing mutations are compatible with a long lifespan [86, 87]. Thus, carriers of mutations of the FHC-type may be more common in hypertensives with exuberant cardiac hypertrophy than in the general population.

There is considerable phenotypic variation in FHC [88–94]. This holds true within and between FHC families with the same β-MHC gene mutations [95, 96], so-much-so that some obligate carriers have no abnormalities on conventional electrocardiography or echocardiography at an age when the disease should have manifested itself clinically, whilst others in the same family have gross hypertrophy. There appears to be a similar spectrum in adults who carry 'causative' troponin T and α-tropomyosin mutations although the number of these individuals is smaller [97]. How this variability arises is unknown, but it seems logical to suppose that there must be a complex interaction of 'environmental' and epistatic genetic effects. Some between-family variability may be explained by polymorphic changes in strong linkage disequilibrium with the causative mutation. However, this cannot explain the striking variation observed within some pedigrees; in this case, the genetic changes must be present far from the causative genes in order to permit independent segregation within a single pedigree.

The four genes producing FHC encode components of the myofibril. It has been proposed [97] that sarcomere disorganization by 'poison peptides' may play a role in the pathogenesis of the disease in a manner analogous to that suggested for mutated UNC-54 in *Caenorhabditis elegans* [98, 99]; perhaps the abnormal protein impairs the correct packaging of proteins into functional macromolecular structures. When the adult human heart hypertrophies the amount of β-MHC and other myofibrillar proteins increases (in adult rodents there is a switch from α-MHC to β-MHC) [100]. Inherited differences in the transcriptional response of these genes to hypertrophic stimuli may affect the composition of the myofibril by altering the relative proportions of the encoded proteins in different individuals. These inherited differences in the stoichiometry of structural sarcomeric proteins would amplify the effects of mutations which produce subtle disturbances in their

structure and function. We hypothesize that these two processes, acting in concert, contribute to the variability of cardiac mass seen in hypertensives. There is a precedent for this: different levels of expression of a mutated dystrophin gene are associated with distinct phenotypes of dilated cardiomyopathy ([101, 102] and Dr F. Mutoni, RPMS, personal communication). An ancillary question we are addressing relates to the role of other genes encoding structural myofibrillar proteins to determine whether they may act as LVH-susceptibility genes (even when some, such as the cardiac actins, have been excluded as causes of FHC). Transgenic Balb/c mice have increased expression of actin in the heart because duplication of the promotor region enhances transcription [103, 104]. These mice do not appear to have significant cardiac hypertrophy, but the cardiac response to stimuli such as exercise and hypertension has not been compared with that of other strains.

Maternally inherited forms of hypertrophic cardiomyopathy, often occurring with neurodegenerative and neuromuscular problems, have been associated with defects in oxidative metabolism that are due to mutations in mitochondrial DNA, most often in tRNA genes (without changes in nuclear DNA) [83]. Recently, Merante *et al.* have proposed a unifying hypothesis to explain FHC and mitochondrial hypertrophic cardiomyopathy [105]. This is based on an inherited incapacity to generate sufficient ATP, in the case of mitochondrial disorders, or to an inability to use it efficiently when the contractile proteins, such as β-MHC, are defective and muscle contraction abnormal.

Friedreich's ataxia (FA)

Friedreich's ataxia (FA), an autosomal recessive disorder, is the most common of the hereditary ataxias, with a carrier frequency in the United Kingdom of approximately 1 in 110. Despite the extensive neurological disability, it is the concomitant, predominantly hypertrophic, cardiomyopathy which is the principal cause of death in these patients. All cases are caused by mutation in a single gene locus (*FRDA*) which maps to the long arm of chromosome 9 [106]. An exciting recent development in this field has been the cloning of the FA gene, X25, which encodes a protein, frataxin, of unknown function. This disease appears to be caused by a very unusual intronic GAA triplet repeat expansion [107].

Despite its prognostic importance the cardiac pathology has been incompletely characterized in this disorder. The existence of multiple mutations within the *FRDA* locus has been postulated to explain the variability of the neurological phenotype observed between families, although in general the age of onset, severity and rate of progression are concordant between affected sibs [108–112]. In most families, affected individuals have similar ECG changes, but this is a very insensitive measure of cardiac involvement. As with FHC, there is evidence for within- and between-family variation in the cardiac phenotype exhibited by homozygous individuals. In one study discordance of cardiac phenotype has been noted in some kindreds suggesting that the non-neurological aspects of FA may be controlled by factors other than differences in the 'causative' mutations within the *FRDA* locus [113]. Indeed, others have reported a lack of correlation between the severity and duration of the neurological component and cardiomyopathy within a family [109, 114–116]. We conclude that additional hypertrophic factors (genetic ± environmental) modify expression of the cardiac pathology in this disorder.

There is very little information on the cardiac status of heterozygotes, whose prevalence is considerably greater than that of the homozygote-affected individuals. In the small series reported to date, no neurological or cardiac abnormalities have been found in these subjects although only clinical examination and ECG analysis has been performed [113, 117]. It remains to be established whether carriers of *FRDA* mutations contribute to the 'pool' of primary hypertrophic cardiomyopathy and secondary hypertrophy in the general population.

Aspects of cardiac hypertrophy research at Hammersmith Hospital

Hammersmith Hospital has a long-standing interest in the clinical evaluation of heart muscle disease. Clinicians and scientists in the Departments of Clinical Pharmacology and Cardiology are investigating and evaluating myocardial hypertrophy, employing an integrated approach which spans the spectrum from basic science to clinical practice.

Genetic factors modifying hypertensive LVH

There are two approaches to unravel this problem. The first is the candidate gene approach which requires prior knowledge of the biology of a particular factor or system indicating that it may be involved in the pathophysiological process of interest. We have applied this strategy in various ways, for example, to study the expression of trophic (eg. endothelin [118]) and antitrophic (e.g. natriuretic peptides [119, 120]) factors in animal hearts, and now to study hypertensive LVH in man using association analysis to test the contribution of candidate gene polymorphisms. Secondly, there is the anonymous screening approach which makes no prior assumptions of the factors involved. We are employing it to compare (by differential-display reverse transcription polymerase chain reaction [121]) gene expression in myocardial tissue which has been perturbed in different ways. In the future it may be feasible to screen for hypertrophic loci using genome-wide sets of anonymous microsatellite markers when the consortia investigating the genetic basis of hypertension have assembled large groups of affected-sib pairs.

Mutations in the structural myofibrillar genes causing FHC We are using single strand conformational polymorphism (SSCP) and heteroduplex analysis to

screen for mutations in amplified DNA from these genes [122]. In SSCP analysis, DNA generated by the polymerase chain reaction (PCR) is first melted by heating with formamide and then electrophoresed through a non-denaturing polyacrylamide gel. The two strands of DNA form conformers with different mobility because of the difference in nucleotide composition. DNA samples from patients with a mutation within the amplified region may produce conformers distinguishable from wild-type. For heteroduplex analysis, amplified DNA is melted with wild-type DNA and then allowed to anneal slowly. If there is a mutation in the test DNA, heteroduplexes will be formed with wild-type DNA which can be distinguished from homoduplexes by electrophoresis through a non-denaturing polyacrylamide gel. We are using both techniques because each has a false negative rate of $\sim$20%, and the same mutation is unlikely to be missed by both. Any abnormal DNA samples detected are then sequenced using a PCR-based method with fluorescently-labelled dideoxynucleotides, the dideoxy-terminated products being resolved on a semi-automated DNA sequencer.

To date we have screened exons 9, 13, 16, 19, 20 and 23 of the β-MHC gene (the exons in which FHC mutations have been found most commonly) in over 40 patients with excessive LVH. We have not detected mutations known to cause FHC. In marked contrast, we have found considerable polymorphism in other β-MHC exons in the same group of patients. We have also observed substantial variation in sequences very close to exon-intron boundaries. These may be important given the strict sequence requirements in these areas for efficient splicing and processing to the mature mRNA. Differences in transcript size and prevalence would be reflected at the protein level, ultimately affecting protein stoichiometry and packaging within the myofibril.

Association studies We will employ association analysis to establish whether structural myofibrillar genes act as LVH-susceptibility genes. Association studies rely on the presence of linkage disequilibrium between the polymorphism and the true 'causative' mutation, and compare the frequencies of the polymorphism alleles in unrelated controls and affected individuals from the same population [123]. We will use polymorphisms within the genes as well as microsatellites close-to or within them; the demonstration of linkage disequilibrium with the latter should increase the likelihood of finding phenotype-associated mutations within the candidate gene, although it is possible that the association relates to other regions of DNA.

Association methods have been employed successfully to elucidate susceptibility loci in polygenic disorders such as diabetes mellitus [124] and hyperlipidaemia [125], but there is a measurable false-positive rate which we will have to accept in order that true susceptibility loci are not overlooked [126]. Because of the danger of producing spurious associations it is imperative to ensure that control and experimental groups are as homogeneous and as well-matched as possible. For example, we have found that blacks and whites have strikingly different frequencies for a particular allele of the *Pst I* polymorphism in intron 7 of the angiotensin converting enzyme gene (blacks 19% and whites 49%) [127]. It is not difficult to see how inadvertant population admixture might generate very different associations between this allele and the LVH phenotype depending on the relative proportion of blacks and whites in the study group.

It may be possible to minimize the importance of unknown confounding factors by comparing groups which differ significantly in the phenotype of interest. To do this we plan to determine the distribution of FHC and candidate gene alleles in two groups of unrelated hypertensives, one with exuberant LVH and the other with minimal LVH, defined as the top and bottom quintiles, respectively, of a cumulative frequency distribution plot of cardiac mass obtained from our study population. By choosing to compare the top and bottom 20% we expect drug treatment (irrespective of the type of drug) to have only a small confounding effect because of the relatively small degree of regression of LVH observed in published reports (60).

Transgenic models of hypertrophic cardiomyopathy

Transgenic mice are being generated in the expectation that the models will offer unprecedented insights into the natural history of this disorder and the nature of the underlying mechanisms by which mutations in some structural myofibrillar genes lead to exuberant cardiac hypertrophy. Two groups have reported, but only in abstract form, the generation of transgenic mouse lines based on the introduction of mutations into the α-MHC gene at the same positions as those in the human β-MHC gene known to cause FHC. This was done because α-MHC, rather than β-MHC, is the predominant heavy chain form in adult rodents; a switch from β-MHC occurs in the perinatal period [100]. We are aware that others are generating transgenic animals with troponin T and myosin binding protein C mutations. The transgenic mouse lines with α-MHC mutations were described at the 68th Scientific Sessions of the American Heart Association (13–16 Nov. 1995, Anaheim, CA). In one line (#131), reported by Leinwand's group, intra-cavity pressure gradients were produced by atrial pacing or infusion of dobutamine, but in another line (#140) with a more severe LVH no intra-cavity gradients were observed in response to these stimuli. The Seidman group have reported a model with a point mutation in codon 403 of α-MHC leading to conversion of arginine to glutamine. This mutation was lethal in the homozygous state. Surprisingly, heterozygotes from this line exhibited striking *atrial* hypertrophy, but *minimal ventricular* hypertrophy. The interpretation of these experiments is complicated by the fact that the functions of α-MHC and β-MHC are not identical. For example, α-MHC has higher ATPase activity than β-MHC and the genes have different regulatory elements. Thus mutations at equivalent positions in the α-MHC and β-MHC genes need not produce the same consequences in rodents and man,

respectively. Clearly, a satisfactory model for the human disease has not been produced yet. In this context it is important to note that the transgenic cystic fibrosis models have shown that even subtle differences in the way the target gene is altered may have a profound effect on the overall phenotype.

We propose to generate a transgenic mouse model by expressing a mutated human α-tropomyosin gene, introduced using yeast artificial chromosome (YAC)-based technology and pronuclear injection of fertilized oocytes. Although mutations in the human α-tropomyosin gene do not constitute the most common cause of this disorder, we have elected to introduce this particular mutation to circumvent problems arising from differential expression of β-MHC in adult human and rodent hearts.

If clinically relevant models can be generated they will facilitate the assessment of the efficacy and potential toxicity of any future pharmacological or gene therapies.

Pathogenesis of hypertrophic cardiomyopathy in Friedreich's ataxia

We are working in close collaboration with Dr S. Chamberlain and her colleagues at St Mary's Hospital Medical School, London, on a project to study the spectrum of cardiac disease in affected, carrier and normal individuals. As part of a study to determine the chromosomal assignment of the FA gene, a large scale collection of multiply-affected nuclear pedigrees was instigated by Dr Chamberlain with the help of the Friedreich's Ataxia Association. This pedigree resource is one of the largest available in the world and comprises more than 200 families with two or more affected individuals. Although the neurological manifestations have been well characterized in these patients by the late Professor A. Harding and her colleagues, cardiac evaluation with modern equipment is not available for these individuals. We aim to achieve the systematic and comprehensive documentation of cardiac phenotype by echocardiography, radionucleide blood-pool imaging, Holter monitoring and signal-averaged electrocardiography in up to 140 multiplex pedigrees from the UK with two or more affected individuals. In addition, more than 650 singly-affected families will also be available for the study. This information on cardiac phenotype will be invaluable to understand the mechanisms by which the heart is affected by mutations in a sequence normally associated with pathology in a sub-group of neurons, will be critical in the design of a mouse transgenic model to evaluate any therapeutic strategies which result from this molecular genetic work.

There is only limited microscopical data on the cardiomyopathy of FA. At post-mortem, there is often a considerable increase in the heart weight and LVH [128]. Histology reveals myocyte hypertrophy and extensive fibroelastosis (without fatty infiltration) which on occasion coalesces to form small scars. In the coronary arteries a degree of intimal thickening has been observed, with some atheromatous plaques, but no gross obstructive lesions. However, the interpretation of these results is confounded by the fact that over 25% of patients with FA have glucose intolerance [128]. We are fortunate to have access to post-mortem cardiac tissue from many patients with FA confirmed by the Harding criteria [113]. These samples are being evaluated with modern microscopical techniques to establish the pattern and degree of fibrosis and myofibril disarray. Furthermore, cloning of the gene for FA has opened new lines of histochemical investigation, for example, using antibodies against peptides from different parts of the predicted protein.

Because patients with FA develop a variable cardiomyopathy in association with a mutation in a single locus, our comprehensive information on cardiac phenotype will provide a unique opportunity to exploit this human genetic model to determine what epistatic susceptibility genes alter cardiac growth in these patients. This will be considerably facilitated by the fact that a large portion of the human genome has already been screened in these patients for the linkage studies to define the FA locus.

Epilogue

Most patients with systemic hypertension die from cardiovascular events, and the risk is substantially increased by the presence of LVH. The appreciation of the great diversity in cardiac size in hypertensives with similar levels of blood pressure has prompted our search for some of the genetic factors contributing to the cardiac hypertrophy. In a staged approach, we envisage the elucidation of susceptibility (and protective) loci scattered across the genome leading to the definition of the actual genes by positional cloning methods.

Information on the nature of the cardiac-mass-modifying genes involved may be useful not only for selecting high risk patients in strategies aimed at *preventing* the development LVH, but also in opening new avenues of research on the reprogramming of cardiac myocytes to encourage them to hypertrophy in situations where cardiac muscle has been damaged or is hypoplastic.

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