I want to discuss with you today the molecular biology of hypertrophic cardiomyopathy and how advances in this science have helped in the diagnosis and prognosis of this disease. To begin, I’m going to give you a brief review of some of the clinical features of this entity. Hypertrophic cardiomyopathy has a prevalence of three per 10,000 of population; it can present at any age, but most often manifests in the third to fourth decade, and there is an annual mortality rate of 3.5%.

Here is a gross pathological specimen cut in transverse section. The disease is characterized by this extreme thickening of the ventricular septum. This is the septum, this is the left ventricular free wall. The left ventricular chamber with the outflow tract, which is narrowed by this extensive hypertrophy, the aorta, the left atrium. And indeed, the right ventricular cavity is also almost entirely obliterated by this extreme ventricular septal hypertrophy.

There are variant subtypes of hypertrophic cardiomyopathy, the most frequent being the asymmetric septal variety, which accounts for over 90% of the different subtypes. The frequency of these different types really depends on the population that one is studying. For example, in the Japanese population, the apical variety accounts for the majority of the subtypes.

The pathophysiology of hypertrophic cardiomyopathy can be explained by these diagrams. With early systolic contraction of the left ventricle there is an acceleration of blood flow through the cavities, through the outflow tract. Because of the septal hypertrophy of this level, there is acceleration of the blood flow which sets up forces, pulling on the anterior mitral valve leaflet. This causes obstruction at that level, and as well, gives rise to what is known as SAM, or systolic anterior motion of the mitral valve. The now open mitral valve also causes mitral regurgitation.

Now hypertrophic cardiomyopathy can present as both systolic dysfunction and diastolic dysfunction. Patients may have angina, dyspnea, syncope, or pre-syncope. They can present with arrhythmias or even sudden death. Hypertrophic cardiomyopathy is the most common cause of sudden death in young athletes.

Pathologically, hypertrophic cardiomyopathy is characterized by myocardial fiber disarray and interstitial pipsosis as illustrated on this trichrome stain. Myocardial fiber disarray is not pathognomonic of the disease. There is some proportion that if the myocardial fiber disarray accounts for greater than 30% of the myocardial impairment, then the likelihood that this is hypertrophic cardiomyopathy increases. Myocardial fiber disarray, however, can also be seen in other overload states, as well as in about 5% of normal myocardial tissue.

More pathognomonic and less well known is the finding of septal interdigitations between the myocardial fibers, as seen here on this staining. In addition, another classical feature is the finding of capping fibrosis of vessels, usually eccentric, in the case of hypertrophic cardiomyopathy.

Now traditionally, the diagnosis of hypertrophic cardiomyopathy has been made by echocardiography. Different institutions use different criteria for this diagnosis. The MIH, for example, have put forward the criteria that the septal wall thickness must be greater than or equal to 13mm. Other groups have added that the septal to posterior wall thickness ratio must be greater than or equal to 1.3. In Toronto, Dr. Weisel has put forth criteria which are much more stringent and require that the septal wall thickness be greater than or equal to 15mm and that the septal to posterior wall thickness ratio be greater than or equal to 1.5.

Now these various criteria come from studies which have looked at the degree of hypertrophy in different states, including other cardiac states, normal individuals, and individuals with hypertrophic cardiomyopathy. In this slide, which was taken from a study
by Maren in the 1980's, one can see that the separation of hypertrophic cardiomyopathy, as indicated by these open circles, from people who are normal and individuals with other cardiac states starts to occur at a level of 1.3 in terms of the ventricular septum to posterior wall ratio and is maximal at the level of 1.5. This is where the other criteria that I mentioned come from.

As good as these criteria are, there are shortcomings. In a study that we performed several years ago on one large Canadian family with hypertrophic cardiomyopathy, which is probably the largest family with this disease, we classified individuals by echo as being effected if they had septal wall thicknesses greater than or equal to 15mm as well as the ratio greater than or equal to 1.5. Individuals were non-effected if they had wall thicknesses less than 13mm and ratios less than 1.3. And we found a group of individuals who fell into this borderline category, having values that were somewhere in between. Ten of these individuals had wall thicknesses of 15mm or 14mm, as well as ratios of 1.3 or 1.4. One individual had a wall thickness ratio of greater than 1.5 and had a septal wall thickness of 12mm. So in a family at risk for hypertrophic cardiomyopathy there were 8.6, almost 10%, of the people in this family who could not be clearly classified.

Here are the echocardiograms; there is an illustration of each of these different categories; this is a 2D power strain long axis view, with left atrium here, left ventricle, aorta, right ventricle. In the unaffected individuals, you can see that the ventricular septum is of normal thickness. By comparison, in the affected individuals, the ventricular septum is largely thickened; in this case measuring almost 30 mm. And the borderline individuals fall somewhere in between; in this case this individual has some hypertrophy, which measured about 14mm.

We're dating back to 1958 in Tiers original description of cases of hypertrophic cardiomyopathy. In an addendum to his paper, he suggested that the disease might in fact be familial in origin. Holman looked at this family of Tiers' and indeed did show that the disease was familial and suggested that the inheritance was autosomal dominant. In fact, other people have looked at the familial and suggested that the inheritance was autosomal recessive heredity of this disease, and it is believed that the disease is not sex-linked, that the familial form accounts for at least 55% of cases. There is some controversy over whether sporadic cases do exist, and that maybe all cases of hypertrophic cardiomyopathy are familial. The disease is most often characterized by autosomal dominant inheritance. There a few case reports, a few family reports in the literature, suggesting autosomal recessive inheritance, as well.

Before I go into the molecular biology of this disease, I want to briefly review some aspects of molecular genetics. The DNA is contained in all of our cells, coding for the 100,000 genes of the human genome. The genes, however, are not expressed in all cells, the tissue-specific expression depends on regulatory factors within each gene. DNA is composed of the purines, adenine and guanine, as well as thymidine, cytosine, and thymidine. The DNA is arranged in two strands; the nucleotides are held together by a backbone of phosphates and sugars, and they align themselves in a complimentary fashion, thiamine with adenine, cytosine with guanine, with the linkages being done by hydrogen bonding. The two strands of DNA run in anti-sense directions: one runs from the five-prime end to the three-prime end of the phosphate, and one runs from the three-prime end to the five-prime end.

Each gene is composed of exons and introns. Exons are the DNA sequences that are transcribed into messenger RNA and subsequently translated into proteins. The introns are DNA sequences that are not expressed in the formation of a protein.

When looking for a disease-associated gene, one can take two approaches. One is the classical genetics approach, which looks at an abnormal protein associated with the disease and works back from the protein to the messenger RNA, then to the gene, and then to identify mutations within that gene. For most inherited diseases, and hypertrophic cardiomyopathy is one example, reverse genetics is used. One looks first for the gene; one can then extrapolate to the messenger RNA and then identify the protein that is abnormal. Identifying the gene first requires the identification of the chromosome where that gene is. That's done by a process called linkage analysis, which is the search for cosegregation of a marker with a disease. I've illustrated here exactly what linkage is. One can take a chromosome that has two markers, A and B, as well as the disease locus, C. Here are the two alleles, one of maternal, one of paternal origin. And as the disease is autosomal dominant, then the disease locus is located on only one of these alleles. If a marker is close to a disease locus, as in the case of this marker A, then during myosis, these travel together and are then said to be linked. By comparison, if a marker locus is far from a disease locus, then during myosis, there is a chance for recombination or a crossover event to occur. These two markers then travel independently and are said not to be linked.

Once one identifies on what chromosome a disease gene is located, one can narrow down to identify the gene and then subsequently the mutations within that gene. One technique that I would like to briefly describe to you is...
polymerase chain reaction, which is a very powerful molecular biological technique, and has really allowed for the rapid advances made in molecular biology. In theory, one can start with one DNA molecule and amplify it to millions of copies. One denatures the DNA into the two separate strands, and by adding short complimentary sequences into the original DNA, as well as adding a stable polymerase enzyme, one causes the synthesis of new strands of DNA. So that one has a duplication in one cycle of the initial DNA into two copies. Repeating this about 30 times allows one to obtain millions of copies of the DNA, which can then serve for other molecular biological reactions, including direct sequencing.

Now I'm going to briefly review for you in the next couple of slides work that has been done in molecular biology of hypertrophic cardiomyopathy in the last four years. The first linkage of this disease was reported by Christine Sideman's group in Boston in 1989 and showed that hypertrophic cardiomyopathy links to chromosome 14. The cardiac myosin heavy chain genes were known to lie on this chromosome, and she was able to show therefore that hypertrophic cardiomyopathy links to the cardiac beta myosin heavy chain gene. In fact, she went further to identify two initial mutations in two families with hypertrophic cardiomyopathy. One was characterized by a fusion gene defect at the level of intron 27 of the cardiac beta myosin heavy chain gene...

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..DNA. Here in the gel where DNA is electrophoresed according to its charge and its molecular weight, and you see here that the radio label band is at this 2.8 kilobase fragment.

She also showed that there was another mutation which was a missense mutation, where one base pair within the beta myosin heavy chain was substituted by another base pair, resulting in one amino acid substitution in exon-13, and this was characterized by a 385-base pair fragment on DD1 digest.

We and others looked for these mutations in other families with hypertrophic cardiomyopathy. Here is an example of some of the work we did when there was the absence of the 2.8 kilobase fragment, the characteristic of the fusion gene. So these families who were positive here did not have the fusion gene defect. And using some slightly different techniques using PCR we showed that the exon-13 defect was not present in these families.

We were therefore one of the first independent groups to confirm that hypertrophic cardiomyopathy is genetically heterogeneous and suggested that there were either mutations elsewhere within the cardiac beta myosin heavy chain gene or, alternatively, on other genes that were associated with this disease.

Just to remind you the myosin heavy chain gene exists in two iso forms, alpha and beta. The cardiac beta myosin heavy chain gene is 30,000 base pairs long, of which 6,000 code for the 1939 amino acids of this protein. So it was very likely that other mutations within this gene would be responsible for hypertrophic cardiomyopathy in other families. And in point of fact, today twenty mutations are known to exist within the human cardiac beta myosin heavy chain gene in association with hypertrophic cardiomyopathy. Here is a list of only a few of these. The majority of them are due to single base pair mutations, resulting in single amino acid substitutions within the myosin molecule. Most of these mutations exist in the head and hinge region of the myosin protein. The two which are not myosin missense mutations are the exon-27 fusion gene which I described to you earlier. And in fact, at this point in time, this is not believed to be a disease-associated gene, in that the family having this fusion gene also had a missense mutation in exon-14, which is believed to be the cause of the mutation. In addition, exon 40 has been shown to have a deletion of 2400 base pairs, and this is the only exon so far that has been identified in the tail region of the molecule to be associated with hypertrophic cardiomyopathy.

Work from our laboratory, as well as others, has screened the entire myosin gene for mutations in other families with hypertrophic cardiomyopathy, and the consensus at present is that hypertrophic cardiomyopathy results from mutations in myosin in only 20-30% of cases. So in 70% of cases of hypertrophic cardiomyopathy the disease is due to other genes.

In the last four months, other chromosomal linkages have been found for hypertrophic cardiomyopathy. The 14Q1 is where the myosin is, and now there are linkages shown to chromosome 15Q2, 1Q3, and 11P13Q13. In these situations, only the chromosome has been identified, and active research is ongoing to identify the actual genes involved in hypertrophic cardiomyopathy at each of these loci. I can also tell you that there is at least on other locus to be identified in the family that I've been involved in, that is not linked to any of these previous chromosomal locations.

It's well known that there is a lot of phenotypic variation between different families. Sideman has looked at some
of the mutations within myosin in different families and has shown that some of the mutations are associated with a better prognosis than are some of the other mutations. It has been suggested that the mutations resulting in a charge change of the myosin molecule are associated with a worse prognosis than are those mutations leaving a neutral charge.

This work is being confirmed by Epstein's group, looking at other mutations within myosin, and has shown that some mutations are associated with less sudden cardiac death and fewer events than other mutations. In fact, the exon-13 mutation seems to be especially lethal.

The two families that have been identified on chromosome 1Q have been compared to the myosin mutations and have been shown to have a bad prognosis similar to that of the exon-13 mutation.

Now, as well as phenotypic variation between families, there is also phenotypic variation within a given family. There are individuals in the family who might have the septal type of hypertrophy, others who may have the apical form, others with mid-ventricular type; as well, there are individuals with obstruction to the left ventricular outflow and others with no obstruction.

I'd like to show you one of the pedigrees of a family that I was studying. The males are indicated by the squares, the females are indicated by the circles, and the solid figures are those representing affected individuals. I'd like to bring your attention to generation two and this individual in particular, this male, who is the offspring of an affected male parent, and himself has an offspring who is affected. By his position in the pedigree, he must be an obligate carrier of the abnormal genotype, despite the fact that by all clinical criteria he does not have hypertrophic cardiomyopathy. This has been seen in pedigrees of other families, as studied by other groups, an affected individual whose maternal great grandfather is affected, therefore in these two generations there must be individuals, this father and this mother, who have the abnormal genotype.

Interestingly, as well, there is this set of identical twins where one individual has been identified as having hypertrophic cardiomyopathy, and this individual by all clinical criteria is negative for the disease.

To make matters more complicated, there are individuals who seem to have electrical abnormalities without actually having any evidence of hypertrophy.

To date, the results I've been showing you are based on linkage studies which are not practical in the clinical sense. There are, however, several techniques, a few of which I've listed here, which can be applied to the screening for actual gene mutations. Now the key word here is "gene," such that at the present time only the myosin mutations can be screened for. We hope that in the near future, when the genes for the other loci are identified, these techniques will be applicable to screening those genes, as well.

These techniques can be done on DNA that is derived from lymphocytes, obtained through a peripheral venipuncture. The techniques are based on the ectopic transcription, in the case of myosin which is found in these lymphocytes, to be expressed. Although the techniques are not 100% sensitive, varying laboratory conditions under which they're done, as well as combining several of the techniques together, leads to a high yield of sensitivity for detecting the different myosin mutations.

Now could this be applied clinically? Well, in the setting of a family with a known myosin mutation, one could test individuals at risk within the family and identify individuals that also have this mutation. These may be young individuals who by echo criteria have not yet manifested any hypertrophy; it can identify individuals in a preclinical stage. This may lead the way to the preclinical intervention therapies, which might be helpful in this disorder. In addition, as we gain more experience we might find that certain mutations respond preferentially to certain specific treatments, and therefore we may be able to target our specific therapies to specific gene mutations.

We can provide more precise genetic counselling, as well as career counselling. For instance, if individuals in a family with a known mutation that has a high incidence of sudden death, we may counsel young individuals not to go into professional sports. And we might be able to target individuals for follow-up; individuals who are shown to have the abnormal genotype would require close follow-up, while those who do not have the abnormal genotype do not necessarily need to be followed any longer, as they won't develop the disease and neither will their progeny. In the cases of individuals who fall into that borderline category, we could clearly classify them as affected or unaffected on the basis of their genotype.

Lastly, there are certain clinical situations which pose a diagnostic dilemma for the clinician. These may be well-trained athletes, some elderly, as well as some hypertensive individuals who might have left ventricular hypertrophy, which seems out of proportion to what one normally sees under these situations. And these individuals might have concomitant hypertrophic
cardiomyopathy. We can look at the genotype in these individuals and see if, indeed, they do have hypertrophic disease.

In conclusion, advances have been made to further understanding of hypertrophic cardiomyopathy. We're just beginning to see the clinical impact that this information will have. Thank you.