Cardiovascular genetics

Coronary artery disease

Since 1900, cardiovascular disease has been the number one killer in the United States every year except 1918, the year of the great influenza pandemic. Cardiovascular disease claims more lives each year than the next five leading causes of death combined, which are cancer, chronic lower respiratory diseases, accidents, diabetes mellitus, influenza and pneumonia.1 Cardiovascular disease is also the leading cause of death in Europe, accounting for over 4 million deaths each year and, according to World Health Organization estimates, 16.6 million people around the globe die of cardiovascular disease each year.2 Based on the United States National Heart, Lung, and Blood Institute Family Heart Study, in its 44 year follow up of participants and a 20 year follow up of their offspring, coronary artery disease accounts for more than half of all cardiovascular events in men and women under the age of 75. Further, coronary artery disease is the single largest killer of men and women in America.3

MEF2A

Much about the genetic basis of coronary artery disease remains unknown and the search for genes that cause major susceptibility in the development of coronary artery disease has been frustrating. In a recent Science report, Wang et al4 described a large family segregating an autosomal dominant form of coronary artery disease. Thirteen individuals in this family displayed coronary artery disease and nine of these thirteen patients developed acute myocardial infarction. A genome wide linkage scan identified a significant linkage to chromosome 15q26, a region containing approximately 93 genes, 43 of which were known genes. A member of the myocyte enhancer factor 2 transcription factors, MEF2A, became a strong candidate, since its mRNA has been detected in blood vessels during early mouse development.5 In all 10 living affected family members, a 21 base pair deletion in exon 11 of MEF2A was identified. Further, this deletion was not present in family members with normal phenotypes nor was it present in 119 individuals with normal angiograms. Wang et al6 also showed evidence that the deletion is a functional mutation that probably acts by a dominant negative mechanism, and immunostaining revealed a strong MEF2A protein signal within the endothelial cell layer of coronary arteries. However, Wang and his colleagues also reported that three other large families with coronary artery disease or myocardial infarction are not linked to this locus, and 50 patients, of unknown ages, with sporadic coronary artery disease or myocardial infarction did not have an MEF2A mutation. Therefore, MEF2A may only be a rare cause of coronary artery disease and myocardial infarction, accounting for only a small percentage of incidences of the disease overall.

Optimists would like to think that MEF2A may be as important as the BRCA1 and BRCA2 genes in the development of the hereditary breast and ovarian cancer syndromes. Pessimists, on the other hand, will counter that this may only be a private mutation found in just one single family. Possibly, MEF2A will fall somewhere in between these two extremes. Future studies will be necessary to determine the prevalence of patients having coronary artery disease or myocardial infarction with MEF2A mutations as well as whether single nucleotide polymorphisms within this gene with smaller effects may be associated with common coronary artery disease and myocardial infarction.

Other loci linked to cardiovascular disease

Whether you are an optimist or a pessimist, the discovery of the first autosomal dominant gene for coronary artery disease and myocardial infarction is truly exciting. To date, there have been just four other published studies that have identified loci linked to cardiovascular disease. Linkage to two loci likely to contribute to premature coronary artery disease, one on chromosome 2q21.1–22 and another on Xq23–26, were found using a Finnish population.7 A susceptibility locus for coronary artery disease on chromosome 16p13.3 was identified in Indo-Mauritians.8 A whole genome scan in 513 western European families found evidence for linkage to myocardial infarction in a region on chromosome 1q41.1–2.9 And the acute coronary syndrome, consisting of myocardial infarction and unstable angina, has been linked to 2q36–q37.3.10

Problems in gene identification

Although the above linkage reports provide a definitive starting place for the identification of potential candidate genes, and the availability of the genomic sequence for these starting places should facilitate gene identification, the specific genes have yet to be found. In fact, up until the Wang et al10 report, the majority of genes known to be involved in coronary artery disease have been identified using association studies, either using a candidate gene approach or by conducting whole genome association studies that take advantage of the hundreds of thousands of single nucleotide polymorphisms that span our genome.

Critics have conceded that findings from many genetic association studies are inconsistent and cannot be...
From high risk families with hypertension, high body mass index, and diabetes.

The strongest association found was with the A387P variant in thrombomodulin-4, conferring a greater than twofold increase in the odds of myocardial infarction. This association was also replicated in a recent review of genetic association studies of coronary artery disease rings true.

While McCarthy et al also report on novel genetic associations with coronary artery disease in this manuscript, the validity of these associations remains to be seen and will require future replication studies. So, while criticisms of association studies are ever present, the authors' conclusion that case control association studies are ever present, are "there" yet in terms of understanding cardiovascular genetics clinics.

Because of the high prevalence of cardiovascular disease, the moment we are "there", genetic counsellors, cardiovascular geneticists, and cardiologists should expect a tidal wave of demand, one that would dwarf that for even clinical cancer genetics. Now would therefore be the time to think of viable strategies to involve primary caregivers in helping with the 21st century practice of molecular based cardiovascular risk assessment and management.

CONCLUSION

So, while we may not be completely "there" yet in terms of understanding the complex genetics of cardiovascular disease, there are definite ways in which morbidity and mortality due to cardiovascular disease can be reduced by genetic counselling of at risk individuals in cardiovascular genetics clinics.

REFERENCES

COMMENTARY

Will the real Cowden syndrome please stand up (again)? Expanding mutational and clinical spectra of the PTEN hamartoma tumour syndrome

R Pilarski, C Eng

PTEN hamartoma tumour syndrome

Since consensus operational diagnostic criteria for Cowden syndrome (MIM 158350) were first established in 1995, our understanding of this complex disease—and the spectrum of disorders related to it by virtue of also having germline mutations in the PTEN tumour suppressor gene—has continued to evolve. This was reflected in a commentary[1] in this journal in 2000 in which it was proposed that endometrial cancer and renal cell carcinoma be added to the operational diagnostic criteria for Cowden syndrome (table 1). This updated commentary is intended to provide a review of significant changes in our understanding of the growing group of disorders, which are known to be caused by germline mutations in PTEN on 10q23.3, and which have been termed the PTEN hamartoma tumour syndrome.

THE CLINICAL SPECTRUM OF THE PTEN HAMARTOMA TUMOUR SYNDROME

Cowden syndrome is a complex disorder with malignant and benign (hamartomatous) lesions affecting derivatives of all three germ cell layers. Major organs involved include the breast, thyroid, uterus, brain, and mucocutaneous tissues.[2] It has been estimated to affect about 1 in 200 000 individuals,[3] although this is probably an underestimate given the difficulty in diagnosis presented by this highly variable disease and the fact that many component features in and of themselves can occur in the general population. Penetrance is related to age, with most patients presenting by their late twenties with at least the mucocutaneous lesions of this disorder, which are reportedly seen in 99% of affected individuals. The
lifetime risk for breast cancer in Cowden syndrome is estimated to be 25–50%, with an average age of diagnosis between 38 and 46 years old.2 3 The risk for thyroid cancer (typically follicular, but occasionally papillary) is approximately 10%, while the risk for endometrial cancer, although not well established, has been estimated to be 5–10%.2 4 The frequency of germline PTEN mutations in Cowden syndrome are associated with Cowden syndrome.7 The frequency of germline PTEN mutations in Cowden syndrome probands ascertained by the strict operational diagnostic criteria for Cowden syndrome1,4 was 80%.7 These data demonstrated that the operational clinical diagnostic criteria are robust even at the molecular level. Nonetheless, it became obvious that other types of alterations in PTEN might account for the remainder of Cowden syndrome.

Bannayan-Riley-Ruvalcaba syndrome (MIM 153480) is a congenital disorder characterised by macrocephaly, lipomatosis, haemangiomatosis, and pigmented macules of the glans penis.9 Bannayan-Riley-Ruvalcaba syndrome was shown to be allelic to Cowden syndrome when germline mutations of PTEN were found in approximately 50–60% of individuals with Bannayan-Riley-Ruvalcaba syndrome.10 Supporting this is the fact that identical PTEN mutations have been seen in individuals with Cowden syndrome and with Bannayan-Riley-Ruvalcaba syndrome, and indeed families have been reported in which some affected individuals have Cowden syndrome and others Bannayan-Riley-Ruvalcaba syndrome.10 11 Over 90% of these Cowden syndrome/Bannayan-Riley-Ruvalcaba syndrome overlap families are found to have germline PTEN mutations (Eng, unpublished observations).14 While Bannayan-Riley-Ruvalcaba syndrome has, in the past, not been considered to have increased risks for cancer, the identification of germline mutations in PTEN in over 50% of cases suggests that at least these patients with Bannayan-Riley-Ruvalcaba syndrome should be considered to be at risk for cancers related to Cowden syndrome.

Proteus syndrome (MIM 176920) is a highly variable disorder involving congenital malformations, hamartomatous overgrowth of multiple tissues, connective tissue and epidermal naevi, and hyperostoses which affect patients in a mosaic pattern. Consensus diagnostic criteria have been published.12 While initially thought to be unrelated to Cowden syndrome or Bannayan-Riley-Ruvalcaba syndrome, at least two independent reports have identified germline PTEN mutations in approximately 20% of patients with Proteus syndrome.13 14 Furthermore, approximately 50% of patients with a Proteus-like syndrome (with significant features of Proteus syndrome, but not meeting diagnostic criteria) were also found to have germline PTEN mutations.11 Thus, at least a subset of Proteus syndrome and Proteus syndrome-like conditions are allelic to Cowden syndrome and Bannayan-Riley-Ruvalcaba syndrome, and are part of the PTEN hamartoma tumour syndrome spectrum.

In addition, several reports have now identified PTEN mutations in patients with macrocephaly and autistic features.15 16 At least one child with VATER association and macrocephaly has been found to have a germline PTEN mutation.17 Recently, a child with Bannayan-Riley-Ruvalcaba syndrome-like features and hemimegencephaly was shown to carry a germline PTEN IVS5+1delG mutation.18 However, the true contribution of PTEN mutations to the aetiology of these types of presentations remains to be determined.

COWDEN SYNDROME COMPONENT NEOPLASIAS

Lhermitte-Duclos disease, or dysplastic gangliocytoma of the cerebellum, is a hamartomatous overgrowth believed to be a component feature of Cowden syndrome.19 20 Clinically, patients with Lhermitte-Duclos disease may present with ataxia, increased intracranial pressure, and seizures. Although familial cases are known, Lhermitte-Duclos disease is usually sporadic, and the exact contribution of Cowden syndrome to the overall incidence of Lhermitte-Duclos disease is unknown. In a recent report, PTEN mutations were identified in tissues from Lhermitte-Duclos disease lesions from 15 (83%) of 18 unselected patients.21 Immunostaining also showed reduced or absent PTEN expression in 11(78%) of 14 samples analysed and, more importantly, they were shown to

### Table 1 International Cowden Consortium operational criteria for the diagnosis of Cowden syndrome. Ver 2000

<table>
<thead>
<tr>
<th>Pathognomonic criteria</th>
<th>Major criteria</th>
<th>Minor criteria</th>
</tr>
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<tbody>
<tr>
<td>Mucocutaneous lesions:</td>
<td>Breast cancer</td>
<td>Other thyroid lesions (for example, goitre)</td>
</tr>
<tr>
<td>Trichilemmomas, facial</td>
<td>Thyroid cancer, especially follicular thyroid cancer</td>
<td>Thyroid cancer, especially follicular thyroid cancer</td>
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<tr>
<td>Acral keratoses</td>
<td>Macrocephaly (occipital frontal circumference</td>
<td>Mental retardation (IQ &lt; 75)</td>
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<tr>
<td>Papillomatous lesions</td>
<td>&gt;97th percentile</td>
<td>Hamartomatous intestinal polyps</td>
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<tr>
<td>Mucosal lesions</td>
<td>Lhermitte-Duclos disease, defined as presence of a cerebellar dysplastic gangliocytoma</td>
<td>Fibroconnective disease of the breast</td>
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<tr>
<td></td>
<td>Endometrial carcinoma</td>
<td>Lipomas</td>
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<td>Fibromas</td>
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<td></td>
<td></td>
<td>Genito-urinary tumours (for example, uterine fibroids, renal cell carcinoma) or genito-urinary malformation</td>
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An operational diagnosis of Cowden syndrome is made if an individual meets any one of the following criteria:

1. Pathognomonic mucocutaneous lesions alone if there are:
   - Six or more facial papules, of which three or more must be trichilemmoma, or
   - Cutaneous facial papules and oral mucosal papillomatosis, or
   - Oral mucosal papillomatosis and acral keratoses, or
   - Six or more palmo plantar keratoses

2. Two major criteria but one must be either macrocephaly or Lhermitte-Duclos disease

3. One major and three minor criteria

4. Four minor criteria

In a family in which one individual meets the diagnostic criteria for Cowden syndrome, other relatives are considered to have a diagnosis of Cowden syndrome if they meet any of the following criteria:

1. A pathognomonic mucocutaneous lesion
2. Any one major criterion with or without minor criteria
3. Two minor criteria
have increased Akt phosphorylation which reflects downstream dysfunction leading to inability to undergo apoptosis and G1 cell cycle arrest. All individuals with mutations had adult onset Lhermitte-Duclos disease, while the three patients without mutations had the childhood onset disease. This corroborates two previous reports from the literature in which germline PTEN mutations and signs of Cowden syndrome and Bannayan-Riley-Ruvalcaba syndrome were absent in patients with childhood onset Lhermitte-Duclos disease.22 23 Germline DNA was available from six of the adult patients, and testing confirmed the presence of a germline mutation in each. Two of the six had features of Cowden syndrome, one did not, and three did not have available clinical information. These results confirm that adult onset Lhermitte-Duclos disease is a major component of Cowden syndrome, and that individuals with adult onset Lhermitte-Duclos disease should be evaluated carefully for signs of Cowden syndrome.24

GERMLINE PTEN MUTATION FREQUENCY AND SPECTRUM

Promoter and deletion mutations. Germline PTEN mutations have been identified in 80% of individuals meeting diagnostic criteria for Cowden syndrome and in 50–60% of patients with a diagnosis of Bannayan-Riley-Ruvalcaba syndrome, using PCR based mutation analysis of the coding and flanking intronic regions of the gene. Whether the remaining patients have undetected PTEN mutations or are unidentifiable genes is not definitively known, although it is believed that Cowden syndrome is not genetically heterogeneous. Scanning the 600 base pair full promoter region has recently revealed germline PTEN promoter mutations in 9 (10%) of 95 Cowden syndrome patients who had previously been found to be mutation negative on testing of the coding and flanking intronic regions. None of these mutations were found among 372 control alleles studied. All nine patients had breast cancer or benign breast disease, or both, but otherwise relatively few other organs were involved. Protein lysates from peripheral blood lymphoblastoid cells from five of these nine patients were found to have decreased levels of normal PTEN protein and concordant increase or laddering of alternate bands on western blotting which were not observed in 32 control samples or 23 mutation negative samples. Reflecting PTEN dysfunction, germline protein from individuals with promoter mutations had increased phosphorylated Akt. Further, large germline PTEN deletions encompassing all or part of the gene were noted in 3 (11%) of 27 Bannayan-Riley-Ruvalcaba syndrome patients studied (two Bannayan-Riley-Ruvalcaba syndrome/Bannayan-Riley-Ruvalcaba syndrome/Cowden syndrome overlap). Protein analysis from one of these patients found a 50% decrease in PTEN protein level and concomitant increase in phosphorylated Akt.

Of note, only Cowden syndrome probands were found to harbour germline PTEN promoter mutations. No promoter mutations have been found in Bannayan-Riley-Ruvalcaba syndrome to date although the work is ongoing. In contrast, only Bannayan-Riley-Ruvalcaba syndrome (or Cowden syndrome/Bannayan-Riley-Ruvalcaba syndrome overlap) patients have been found to have large deletions or rearrangements involving PTEN.24 25

Finally, a de novo case of Cowden syndrome presenting with Lhermitte-Duclos disease in adulthood was found to have the de novo germline PTEN mutation c.179delA arise on the paternal chromosome. Whether the majority of cases of Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome, and PTEN hamartoma tumour syndrome arise on paternal chromosomes is unknown and is a question which needs to be investigated.

MANAGEMENT OF PTEN HAMARTOMA TUMOUR SYNDROME

Management for Cowden syndrome is primarily focused on the cancer risks. Clinical breast examinations are recommended annually for women beginning at age 25, and annual mammography starting at age 30–35. Breast cancer has been reported in men with Cowden syndrome, and it is thus recommended that they practise regular breast self-examinations. Men and women should have an annual physical examination beginning at age 18, with careful attention paid to the skin and neck region. A baseline thyroid ultrasound is also recommended at age 18 (with consideration of annual thyroid ultrasound thereafter). Women with Cowden syndrome should also undergo endometrial screening involving annual blind (repel) biopsies starting at age 35–40, and annual endometrial ultrasound after menopause, with biopsy of suspicious lesions. Annual urine analysis for the detection of renal cell carcinoma is also recommended along with annual urine cytology and renal ultrasound if there is a family history of renal cancer.27

While cancer risks in Bannayan-Riley-Ruvalcaba syndrome were initially felt to be similar to those of the general population, confirmation that >60% of Bannayan-Riley-Ruvalcaba syndrome is allelic to Cowden syndrome and due to PTEN gene mutations has led to the recommendation that Bannayan-Riley-Ruvalcaba syndrome patients should conservatively be managed according to Cowden syndrome guidelines. While the gastrointestinal hamartomatous polyposis seen in Bannayan-Riley-Ruvalcaba syndrome does not predispose to significantly increased cancer risks, patients should be monitored for complications of the polyps themselves, such as intussusception.

It has only recently been shown that a subset of patients with Proteus syndrome and Proteus-like syndrome have PTEN mutations, and at this point it is not clear whether cancer risks associated with Cowden syndrome pertain to these patients as well. To be conservative, it might be prudent to follow Cowden syndrome screening guidelines for patients with PTEN mutation positive Proteus syndrome and Proteus-like syndrome as well.

CONCLUSIONS

The recent identification of germline PTEN deletions and promoter mutations indicates that these types of mutation account for approximately 10% each of patients with Cowden syndrome and patients with Bannayan-Riley-Ruvalcaba syndrome who are mutation-negative on PCR based analysis, even using direct sequencing of exons 1–9 and flanking intronic regions. Thus, in all, the germline PTEN mutation frequencies for Cowden syndrome and Bannayan-Riley-Ruvalcaba syndrome would approach 85–90% and 65%, respectively. Because promoter mutations have only been noted in Cowden syndrome up until now, it is possible that promoter analysis should be limited to Cowden syndrome. Similarly, should large deletions continue to be observed only in Bannayan-Riley-Ruvalcaba syndrome/Cowden syndrome and Bannayan-Riley-Ruvalcaba syndrome overlap, and not Cowden syndrome, perhaps deletion analysis should only be offered in Bannayan-Riley-Ruvalcaba syndrome.

In addition to being aetiologic for classic Cowden syndrome and Bannayan-Riley-Ruvalcaba syndrome, germline PTEN mutations are being shown to be responsible for an increasing spectrum of clinical disorders. PTEN...
mutations, which are responsible for the majority of cases of Cowden syndrome and Bannayan-Riley-Ruvalcaba syndrome, have now been clearly shown to contribute to a subset of cases of Proteus syndrome and Proteus-like syndrome. In addition, germline PTEN mutations may account for the majority of patients with adult onset Lhermitte-Duclos disease, with or without the presence of other signs of Cowden syndrome in the patient. Thus, PTEN gene testing may be indicated in all cases of adult onset Lhermitte-Duclos disease. The contribution of PTEN mutations to cases with both macrocephaly and autism remains open to investigation, as do other aspects of this fascinating and evolving spectrum of disorders.

ACKNOWLEDGEMENTS

We would like to thank the patients and families who have participated in our studies, and all of the other clinic personnel, the biochemistry laboratory, collaborators, colleagues, and genetic counselors who have also contributed, in one way or another, to our research.

doi: 10.1136/jmg.2004.018036

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J Med Genet 2004 41: 323-326
doi: 10.1136/jmg.2004.018036