Cardiovascular genetics: are we there yet?

A C Sturm

Cardiovascular genetics

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.¹

CORONARY ARTERY DISEASE

Coronary artery disease has a complex aetiology, involving multiple genetic and environmental influences and interactions. In a recent review, Lusis estimates the total number of genes involved in cardiovascular disease by considering the risk factors for cardiovascular disease that are under genetic control.³ Some of these risk factors with a significant heritability include cholesterol levels, triglyceride levels, hypertension, obesity, diabetes, and the metabolic syndrome, all of which themselves have many genes involved in their susceptibility.^{3,4} Therefore, at least hundreds of genes are involved in the susceptibility to cardiovascular disease.³ The identification and characterisation of these genes has been a major undertaking and challenge for researchers. A recent call to arms from Sing et al⁵ makes recommendations for what needs to be done to cope with these complexities, including developing new statistical methods to analyse the exponentially increasing amount of data

generated by contemporary technology as well as training scientists for a "biocomplex future" and encouraging collaborations between researchers that help to study "how the parts are put together" in complex traits.

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 al⁶ 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.⁷ 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 a *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.⁸ A susceptibility locus for coronary artery disease on chromosome 16p13.3 was identified in Indo-Mauritians.⁹ A whole genome scan in 513 western European families found evidence for linkage to myocardial infarction in a region on chromosome 14q11.2–12.¹⁰ And the acute coronary syndrome, consisting of myocardial infarction and unstable angina, has been linked to 2q36–q37.3.¹¹

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 al⁶ 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

replicated, and that these studies should be restricted to the study of polymorphisms that have been shown to have a direct effect on gene function.¹² In fact, in a recent review of genetic association studies, Hirschhorn et al¹³ found that over 600 positive associations between common gene variants and disease have been reported. Of the putative associations which have been studied three or more times, only 3.6% have been consistently replicated.

Many have offered their assessment of association studies, and of why type I errors (false positives) seem to abound.^{13–15} There are several reasons for the inability to replicate genetic associations. These include population stratification, modification of the association by other genetic or environmental factors that vary between groups studied, genotyping error rates that differ between cases and controls, absence of power leading to false negative results, failure to exclude chance as an explanation for association in some studies, and publication bias, where several studies are undertaken but only positive results are reported, which some claim is the most likely reason for failure to replicate.¹⁴

GENOME WIDE CASE CONTROL STUDIES

While there are definite reasons to be pessimistic about association studies, a number of them have provided strong evidence that genome wide case control studies are powerful tools in the identification of genes related to common diseases such as coronary artery disease.

One recent large scale case control study identified a candidate gene, lymphotoxin- α , associated with susceptibility to myocardial infarction and two genetic variants within this gene were proven to affect its expression level and biological function.¹⁶ Further, a number of other genes have exhibited consistent evidence of association with coronary artery disease or its known risk factors. Now, in this issue of *Journal of Medical Genetics*, McCarthy et al¹⁷ extend these findings with an analysis of 111 candidate genes underlying premature coronary artery disease, currently the only large scale association study using a case control design for coronary artery disease in white Americans. Before now, there have been three other similar studies, one an interim report on 62 candidate genes by this group¹⁸ and two others, which assessed Japanese individuals with myocardial infarction.^{16, 19} To improve the power of their study, McCarthy et al¹⁷ selected cases that were genetically loaded, having early onset coronary artery disease (onset <45 in men and <50 in women) and coming

from high risk families with hypertension, high body mass index, and diabetes.

The strongest association found was with the A387P variant in thrombospondin-4, conferring a greater than twofold increase in the odds of myocardial infarction. This association was also reported in this group's previous report¹⁸ and has since been replicated in a number of studies.^{19–22} Further, functional genomic studies performed by this group (currently in press) have shown that this variant is a gain of function mutation that interferes with endothelial cell adhesion and proliferation, and is therefore biologically relevant.²³

While McCarthy et al¹⁷ 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 replicative studies. So, while criticisms of association studies are ever present, the authors' conclusion that case control association studies are effective in generating hypotheses for candidate genes in coronary artery disease rings true.

THE PURPOSE OF GENETIC EVALUATION

Finally, then, the question remains: are we there yet? The answer to this question depends on what your own definition of "there" is. No, not every susceptibility gene or single nucleotide polymorphism within these susceptibility genes associated with coronary artery disease is known. However, we must ask ourselves, what is the primary goal of all this genomic research? And we must answer this question by stating that the reason for this research is to reduce the morbidity and mortality caused by the disease in question. Importantly, 50% of men and 63% of women who died suddenly from coronary artery disease had no previous symptoms of this disease.¹ For this reason alone, the utility of cardiovascular genetics clinics whose main goals are identification of those at increased risk, subsequent modification of this risk, and therefore prevention of coronary artery disease, must be recognised. Obtaining a comprehensive family history should be an integral component of any disease prevention programme.²⁴ The significance of the familial occurrence of coronary artery disease has been a focus of research for at least 50 years,²⁶ with a positive family history of coronary artery disease emerging as an independent predictor of risk in the development of coronary artery disease even when other risk factors are considered.^{27–30}

Applying what we know now about cardiovascular genetics in a clinical

setting to prevent this leading cause of death is a reality. In a recent review from Scheuner,³¹ the components that should be included in a genetic evaluation for coronary artery disease are recommended, including genetic risk assessment, risk factor modification, early detection strategies, and genetic counselling and education. Risk assessment includes pedigree analysis, personal medical history, physical examination, laboratory testing, and early detection techniques, which not only serve to help in risk stratification, but also serve to screen those individuals already in greater than average risk categories based on family history.

These techniques will allow aggressive risk factor modification when necessary or, when unnecessary, may lead to patient reassurance, comparative to colonoscopy in screening for colon cancer.³¹ The goal of the genetic evaluation for coronary artery disease then is to provide the patient, through genetic counselling and education, with an individualised strategy for early detection and prevention that fits with their preferences, and potentially includes both lifestyle changes involving diet, weight control, exercise, and smoking cessation, as well as targeted drug therapy in appropriate cases.

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. 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.

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Correspondence to: Mrs A C Sturm, Division of Human Genetics, Department of Internal Medicine, The Ohio State University, 2050 Kenny Road, 8th Floor Tower, Columbus, OH 43210; sturm-1@medctr.osu.edu

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PTEN hamartoma tumour syndrome

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 commentary¹ 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,^{3,4} 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

Table 1 International Cowden Consortium operational criteria for the diagnosis of Cowden syndrome. Ver 2000		
Pathognomonic criteria	Major criteria	Minor criteria
Mucocutaneous lesions: Trichilemmomas, facial Acral keratoses Papillomatous lesions Mucosal lesions	Breast cancer Thyroid cancer, especially follicular thyroid cancer Macrocephaly (occipital frontal circumference ≥97th percentile) Lhermitte-Duclos disease, defined as presence of a cerebellar dysplastic gangliocytoma Endometrial carcinoma	Other thyroid lesions (for example, goitre) Mental retardation (IQ ≤ 75) Hamartomatous intestinal polyps Fibrocystic disease of the breast Lipomas Fibromas Genito-urinary tumours (for example, uterine fibroids, renal cell carcinoma) or genito-urinary malformation
An operational diagnosis of Cowden syndrome is made if an individual meets any one of the following criteria:		
1) Pathognomic mucocutaneous lesions alone if there are: <div>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</div>		
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		

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.^{2–5} 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%.^{1–2} (Eng, unpublished observations). Germline mutations in *PTEN* are associated with Cowden syndrome.^{6–7} The frequency of germline *PTEN* mutations in Cowden syndrome probands ascertained by the strict operational diagnostic criteria for Cowden syndrome^{1–8} 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).¹⁰ 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.¹³ 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 hemimegacephaly 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 presentation 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

have increased Akt phosphorylation which reflects downstream dysfunction leading to inability to undergo apoptosis and G1 cell cycle arrest.²¹ 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 and should routinely be offered *PTEN* gene testing, even in the absence of other signs of *PTEN* hamartoma tumour syndrome. While these data are almost compelling to move Lhermitte-Duclos disease to the pathognomonic category in the operational diagnostic criteria, we have left it in the major criteria section for now (table 1).

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.^{7–10} Whether the remaining patients have undetected *PTEN* mutations or mutations in other, unidentified, genes is not definitively known, although it is believed that Cowden syndrome is not genetically heterogeneous.⁸ 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.²⁴ 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; one 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.²⁴ 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*.^{10, 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.²⁶ 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, all 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 or Cowden syndrome/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.

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Authors' affiliations

R Pilarski, Clinical Cancer Genetics Program, Comprehensive Cancer Center, Division of Human Genetics, Department of Internal Medicine, The Ohio State University, Columbus, OH, USA

C Eng, Clinical Cancer Genetics Program and Human Cancer Genetics Program, Comprehensive Cancer Center, Division of Human Genetics, Department of Internal Medicine, The Ohio State University, Columbus, OH, USA

C Eng, Cancer Research UK Human Cancer Genetics Research Group, University of Cambridge, UK

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Correspondence to: Professor C Eng, Human Cancer Genetics Program, The Ohio State University, 420 W 12th Avenue, Ste 690 TMRF, Columbus, OH 43210, USA; eng-1@medctr.osu.edu

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