

Is there a link between *COQ6* and schwannomatosis?

To the Editor: Schwannomatosis (OMIM 162091) is a late-onset tumor-predisposition syndrome characterized by the development of multiple nonvestibular, nonintra-dermal schwannomas. These tumors typically display a molecular signature characterized by biallelic somatic mutations of the *NF2* gene in the absence of *NF2* germline mutations.

Constitutional heterozygous loss-of-function mutations in *SMARCB1* have been reported in less than 10% of sporadic and in 50% of familial schwannomatosis cases. The *LZTR1* gene was recently found to be mutated in about 80% of patients with *SMARCB1*-negative Schwannomatosis.¹ Nevertheless, the remaining cases still lack a genetic diagnosis, suggesting the existence of other loci predisposing to schwannomatosis.

The *SMARCB1/SNF5/INI1* gene encodes a core subunit of the SWI/SNF adenosine triphosphate-dependent chromatin remodeling complex. The product encoded by the *LZTR1* gene belongs to a superfamily of BTB/POZ proteins involved in fundamental cellular processes, ranging from the regulation of cytoskeleton dynamics to the control of the cell cycle.

The article “A Germline Missense Mutation in *COQ6* Is Associated with Susceptibility to Familial Schwannomatosis,” by Zhang et al.,² described a novel mutation in the coenzyme Q10 (CoQ) biosynthetic gene *COQ6* associated with susceptibility to familial schwannomatosis. They performed whole-exome/genome sequencing on genomic DNA from affected and healthy members of a large family with schwannomatosis without constitutional mutations in *SMARCB1* and *LZTR1*. They identified the novel heterozygous missense mutation c.622G>C p.(Asp208His) in the *COQ6* gene, segregating with the disease, and demonstrated that it abolishes the ability of the human *COQ6* gene to complement a *coq6*-deficient yeast strain. We believe that these data should be interpreted with caution and that appropriate experimental models are necessary to validate novel disease associations.

The pedigree of the family reported by Zhang et al.² clearly suggests dominant inheritance, which has been reported in most forms of familial schwannomatosis described so far. The probability that the *COQ6* variant in this family is segregating just by chance is 1:128, and the number of variants identified by exome sequencing is so large that the analysis of a small family may yield several such variants. In fact, the authors identified a total of 12 heterozygous variants, including 9 missense variants, all segregating in the affected members and absent in the healthy individuals. A possible pathogenetic role of the other variants was not excluded.

Importantly, both the genetic and the immunohistochemical analyses of schwannomas from the affected patients did not reveal any alteration in *NF2*. Moreover, Zhang et al.² did not provide any evidence of possible biologically plausible mechanism(s) linking the haploinsufficiency of *COQ6* to oncogenesis in Schwann cells. *COQ6* encodes a monooxygenase required for the C5-hydroxylation of the quinone ring of CoQ. We previously characterized the human *COQ6* gene, showing that its function is highly conserved, as indicated by its ability to complement the defective respiratory phenotype of a *coq6*-deleted strain.³

The yeast model used by Zhang et al.² simply proved that the p.Asp208His substitution causes a loss of function in the *COQ6* gene, as revealed by the lack of complementation in the defective *coq6*-yeast strain. However, all known mutations in the *COQ6* gene are inactivating and have been reported so far in patients affected by an autosomal recessive multisystemic syndrome characterized by nephrotic syndrome and sensorineural deafness.³ Conversely, to date there is no evidence that haploinsufficiency of *COQ6* causes significant CoQ deficiency; accordingly, none of the patients or their heterozygous parents, even those carrying null alleles, develop schwannomas. Among genes required for CoQ biosynthesis, the only one whose haploinsufficiency has been related to a human disorder is *COQ4*; however, this gene does not seem to have known enzymatic functions. It seems instead to be involved in the assembly and regulation of the CoQ biosynthetic complex.

Furthermore, knockdown of *COQ6* in a cellular model does not reproduce the situation observed in cells of heterozygous patients. In fact, *COQ6* is not a limiting factor in CoQ biosynthesis, and Zhang et al.² observed an increase in reactive oxygen species only when knockdown reduced residual messenger RNA (and possibly protein) concentrations to values significantly inferior to 50% of the wild type, a situation resembling that in cells with homozygous hypomorphic alleles.³

A possible dominant-negative effect or a toxic gain of function of the missense *COQ6* variant, which would explain the dominant inheritance, was not demonstrated by the experimental model used by Zhang et al.² Moreover, none of the affected members they described showed any symptoms of CoQ deficiency.

Mitochondrial enzymes may act as antioncogenes (complex II subunits or fumarate hydratase are classical examples), but in these cases there is always loss of heterozygosity in the tumor cells. In this case molecular analysis of schwannoma tissues from the affected individuals excluded loss of heterozygosity, and other alterations of the *COQ6* gene were not identified. This is in contrast with what it is usually found in schwannomatosis tumors related to *SMARCB1* or *LZTR1*. In fact, both genes act as oncosuppressors; accordingly, tumors from patients with schwannomatosis typically show biallelic loss of the *NF2* gene and loss of heterozygosity of *SMARCB1* or *LZTR1* with retention of the germline mutation.¹

Overall, these data only demonstrate that the p.Asp208His substitution is a “typical” loss-of-function *COQ6* mutation; they do not provide any explanation of why it is associated with schwannomatosis, and it is not possible to exclude that this variant is just an incidental finding unrelated to the disease is not possible.

Schwannomatosis requires adequate follow-up to promptly detect novel schwannomas and possible complications. The identification of a novel gene associated with schwannomatosis has important implications in genetic counseling because it allows both the prenatal and the presymptomatic diagnosis of this adult-onset condition in at-risk family members.

Unless further experimental evidence explaining the link between the *COQ6* heterozygous missense mutation and the susceptibility to schwannomatosis is provided, we are skeptical about the opportunity of screening for the *COQ6* gene in patients affected by schwannomatosis (and screening for schwannomas in individuals with heterozygous mutations in *COQ6*).

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Response to Trevisson *et al*.

To the Editor: First, we thank Trevisson *et al*.¹ for their valuable contribution to this subject and their comments on our brief report. In our study, we reported a familial schwannomatosis without mutations of *SMARCB1/INI1/hSNF5* and *LZTR*, the two known causative genes for schwannomatosis. Using genome and exome sequencing, we found a heterozygous loss-of-function mutation of the coenzyme Q10 (CoQ10) biosynthesis monooxygenase 6 (*COQ6*) gene in patients affected by disease.² CoQ10 is an electron carrier in the mitochondrial respiratory chain, as well as a lipid-soluble antioxidant implicated in protecting cells from damage by reactive oxygen species. Its biosynthesis is still not well characterized in human cells. Mutations in CoQ10 biosynthesis genes, including *COQ4* and *COQ6*, cause primary CoQ10 deficiency. The manifestations of primary CoQ10 deficiency are very heterogeneous, and CoQ10 deficiency has been involved in many common disorders with increased oxidative stress, such as neurodegenerative diseases, cancer, cardiovascular diseases, diabetes mellitus, aging, and Alzheimer disease. It is well known that chronic increases in oxidative stress may trigger transformation and contribute to cancer progression by amplifying genomic instability. We proposed that the haploinsufficiency of *COQ6* monooxygenase due to a loss-of-function mutation may lead to CoQ10 deficiency and chronic overproduction of reactive oxygen species in Schwann cells, thereby predisposing to schwannomatosis.

A critical issue of the hypothesis is whether heterozygous loss-of-function *COQ6* causes haploinsufficiency. A previous study of yeast found that haploinsufficiency of *COQ6* resulted in a mild reduction of fitness in a medium containing glucose.³ Moreover, we assumed that the haploinsufficiency of the *COQ6* gene and CoQ10 deficiency may be conditional, dynamic, and tissue/cell specific. For example, one study found that cellular

DISCLOSURE

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and tissue concentrations of CoQ10 decrease with age, and cellular concentrations below a critical threshold are incompatible with life. Furthermore, carcinogenesis is determined not only by genetic alterations but also gene–environment interaction, as well as metabolism, and not all transgenic mice with the exactly same genetic background and alterations may develop cancers. In addition, germ-line abnormalities associated with cancer may be detected in every cell in the body or only in the tumor cells. Interestingly, despite the presence of a constitutional genetic abnormality that might affect growth regulatory pathways in all cells, people are generally predisposed to only certain tumor types. The average age at disease onset was ~40 years for this familial schwannomatosis, indicating a chronic disease progression. In this particular family, we considered that the loss-of-function *COQ6* allele may lead to a chronic or conditional haploinsufficiency of *COQ6* in a cell/tissue-specific manner.

So far, the roles of mutations of CoQ10 biosynthetic genes in cancers are completely unknown. The association is only now coming of age, for example, the most recent cancer genomic studies identify *COQ2* gene mutations in human melanoma, colon and rectal cancers, ovarian carcinoma, and glioblastoma multiforme. In addition, more than 48 missense mutations of the *COQ6* gene have been identified in various human cancers (<http://cancer.sanger.ac.uk/cancergenome/projects/cosmic>). Furthermore, abnormally low plasma concentrations of CoQ10 have been found in a number of cancer types, including cervical cancer, breast cancer, and melanoma.⁴ Although intensive research is needed to further explore the underlying mechanisms, increasing data have strongly indicated an undetermined implication of CoQ10 biosynthesis gene mutations in carcinogenesis.

Indeed, the exact oncogenic mechanism of the loss-of-function *COQ6* gene in disease remains a challenging question to be elucidated. Cancer is a complex multigenic disease