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EDITORIAL

PTGIR, a susceptibility gene for fibromuscular dysplasia?

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This editorial refers to 'Rare loss-of-function mutations of PTGIR are enriched in fibromuscular dysplasia' by A. Georges *et al.*, pp. 1154–1165.

Fibromuscular dysplasia (FMD) is a relatively common but underestimated cause of renovascular hypertension, carotid dissection, and stroke in young-middle aged women. It may also present with mesenteric ischaemia, renal, and myocardial infarction.[1] Furthermore, patients with spontaneous coronary arterial dissection (SCAD), another condition occurring predominantly in women with few cardiovascular risk factors, display typical FMD lesions in extra-coronary arterial beds in at least 30–40% of cases.[1] Although historical cohorts suggested relatively frequent positive family histories, more recent estimates were between 2.8% and 5.4%.[1,2]

We have made incremental progress in the understanding of FMD, SCAD, and related diseases during the past 5 years.[3] From the geneticist's perspective, these mostly include (i) identification of an association between an intronic variant of the *PHACTR1* gene, FMD,[4] and SCAD;[5] (ii) documentation of increased TGF-beta levels in patients with severe FMD[6] and, more recently, of an increased burden of Loeys–Dietz Syndrome gene variants in patients with SCAD;[7] (iii) identification of a tentative proteogenomic signature of FMD,[8] and finally (iv), identification of rare variants in *COL5A1*,[9] *TSR1*,[10] and *TLN1*[11] associated with FMD and SCAD, respectively.

These progresses were made possible by the implementation of wide-scale national and international registries such as ARCADIA, ARCADIA-POL, the US Registry for FMD, and the European/International FMD Registry and Initiative (FEIRI), encompassing several thousands of patients;[3] (ii) the establishment of genetic networks and parallel biobanks; and (iii) a major intellectual, technical and financial investment taking advantage of the most up-to-date approaches in genomic research.

Georges *et al.*[12] report the results of analysis of 29 exomes from patients with both familial and sporadic FMD. Following a prioritization pipeline partly based on machine learning, a stop codon mutation in *PTGIR* (Q163X), the gene encoding the prostaglandin I2 receptor, was identified in two sisters with FMD. Exome or Targeted re-sequencing of *PTGIR* ($n = 1135$ FMD patients) revealed the Q163X variant in two additional patients, a rare frameshift variant (P17RfsX6) in two patients and rare missense variants (A2T, L67P, M107V, and R137C) in four additional subjects. Production of cAMP after exposure to iloprost, an analogue of prostacyclin, was abolished in HEK293 cells transfected with either one of the two loss-of-function mutations (Q163X, P17RfsX6) and reduced by 100-fold in cells harbouring the rare missense variant L67P. The impact of the three other variants remains unsubstantiated. In the absence of arterial samples (one of the major drawbacks in FMD research), the influence of the variants on vascular architecture and expression of the prostacyclin receptor could not be evaluated *in vivo*. The eight patients harbouring variants with documented functional impact (Q163X, P17RfsX6, and L67P) were all women, all but one had multifocal FMD, six out of eight were diagnosed after 50 years, the presentation of the disease was renal in four and cerebrovascular in three, none had documented arterial dissection and only one (one of the two 'index' siblings) had a documented aneurysm.

Similarly, genetic analysis of 843 SCAD patients disclosed two patients harbouring variants already identified in FMD patients (Q163X and L67P, respectively), and the third one within a splicing site (c.768 + 1G>C) likely resulting in loss of function. All three patients were women without documented FMD lesions in extra-coronary arteries. Finally, an analysis of the frequency of variants in *PTGIR* at large—i.e. irrespective of documented or suspected functional impact—showed an enrichment in *PTGIR* variants in patients with FMD ($n = 1335$, $P = 4 \times 10^{-4}$)—but not in SCAD patients ($n = 843$, $P = 0.12$)—compared to controls from the publicly available gnomAD database ($n = 71\,702$).

The authors propose an attractive explanation for the tentative role of prostacyclin receptor mutations in the pathophysiology of FMD.[12] Prostacyclin is a well-established vasodilator, known to repress proliferation and migration of smooth muscle cells, as well as fibrosis, in different tissues. The effects involve cAMP signalling downstream of the prostacyclin receptor. As such, it is hypothesized that the *PTGIR* loss-of-function leads to increased fibrosis of arterial media and relative depletion in vascular smooth muscle cells, as observed in patients with FMD of the medial subtype-roughly corresponding to the predominant, multifocal form with its iconic string of beads. Further, the authors speculate that the tentative benefits of aspirin in FMD may result from restoration of a supposedly altered prostacyclin/thromboxane balance, at least in patients with

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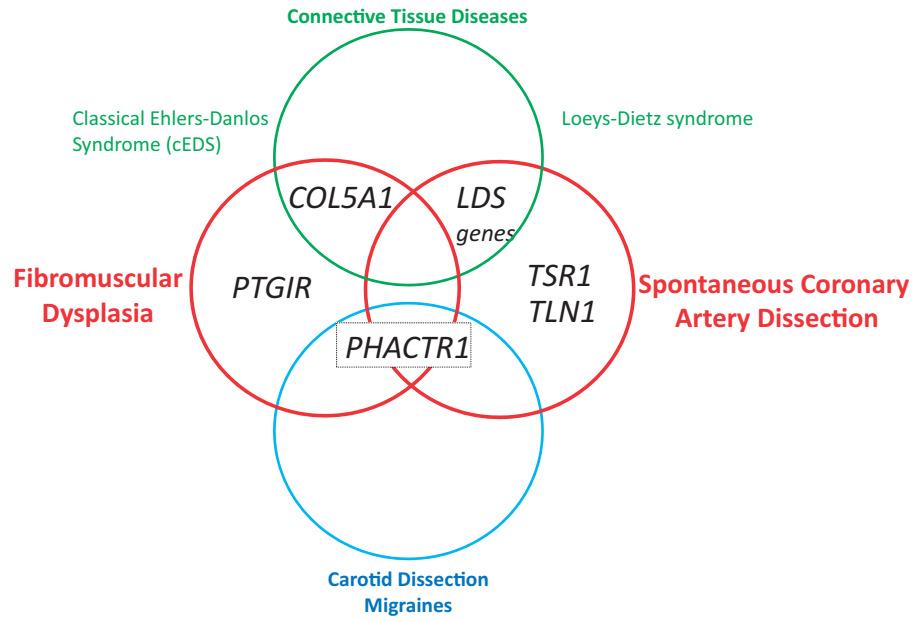

Figure 1 Venn diagram showing the main genes involved so far in FMD, SCAD or both, and overlap with other vascular diseases.

PTGIR mutations, thus supporting the recommendation to consider the use of aspirin in patients with FMD.[1]

The work of George *et al.*[12] is interesting in many aspects. Nevertheless, definitive evidence of a substantial implication of *PTGIR* in the aetiology of FMD remains open for discussion. First, proven functional variants were found in less than <5/1000 patients with FMD. Second, from the abolition of sensitivity to an analogue of prostacyclin in embryonic cultured renal cells to the demonstration of a causative role in the pathogenesis of FMD there is still a long way to go. Third, the pLI-value, commonly used as a reference to evaluate if loss-of-function variants have been negatively selected for in the human population indicating that they likely cause a severe disease, is 0 for *PTGIR*, indicating that the number of loss of function variants in the database does not significantly differ from expected hazard frequency. While the authors point out that, in view of the frequency of silent renal FMD in the population, some of the corresponding controls may have undetected FMD, which is certainly possible, it may also be hypothesized that *PTGIR* variants identified in a small minority of patients with FMD are 'innocent bystanders' or disease modifiers rather than causal mutations. The latter is most likely for the three missense variants without functional impact after transfection in HEK293 cells. Of notice, patients with FMD harbouring the loss-of-function variants were mostly diagnosed after 50 years, had no dissection or other severe manifestations/complications of FMD and, with the exception of the index sibship, no known family history of FMD.

The genetic evidence supporting a role of *PTGIR* variants in SCAD patients is less solid, as only 3 cases out of 834 were found to harbour credible mutations, none of them had FMD (yet patients with FMD harbouring *PTGIR* variants had no reported arterial dissection either) and, despite a higher number, the burden of *PTGIR* variants in SCAD patients was not increased compared to gnomAD controls. Finally, as shown by the initially negative results of exome sequencing in 16 out of the 29 subjects included in the current exome analysis,[13] the results of such genetic screenings may be critically oriented by the mode of filtering of variants and underlying hypothesis. It is relatively obvious—though we would have done the same—that based on a list of genes involved in arterial abnormalities, artificial intelligence will end up selecting a gene with these properties. On the contrary, Genomewide Association Studies, while they may miss rare mutations with strong effects, have the advantage of being fully non-hypothesis driven, at times leading to important discoveries such as the association of *PHACTR1* with FMD[4]—a major breakthrough made by the same leading group of investigators.

While these limitations may apply to other, recently identified genes at the origin of rare cases of FMD or SCAD, the genetic make-up of FMD as well as the contribution of genetics to its pathogenesis remain largely elusive. Is it really possible that variants in genes involved in a wide spectrum of diseases may all lead to the same, unique elementary lesion: a string of beads, either in the context of primary FMD or SCAD? (*Figure 1*). And are all strings of beads the same? We feel that imaging of FMD patients harbouring tentative causative mutations should be systematically provided at least as a data supplement to allow comparison of different published cases, and identify possible distinctive characteristics which may allow prioritizing genetic screens. It would also be of interest to systematically track suspected genetic variants within pedigrees, and to look for cosegregation with FMD lesions. This would also allow to obtain better penetrance estimates. In the near future, high-resolution echo-tracking and ultra-high resolution ultrasonography may become reasonable non-invasive, non-irradiating alternatives to angiographic imaging techniques to explore asymptomatic relatives of patients with FMD. Furthermore, they may allow detecting the signature of the disease before the development of overt FMD lesions, making these approaches even more attractive.[14]

Finally, it is generally admitted that with the exception of a small subset, the development of FMD results from the interplay of genetic and environmental factors. Smoking has been suggested as a potentially aggravating if not a causative factor in FMD.[1,3] Interestingly, all but one FMD patient with a *PTGIR* mutation in the current study[12] were smokers

or former smokers. While this may be due to chance, it draws our attention towards the importance of standardized, detailed phenotypical descriptions of patients with the whole spectrum of FMD manifestations, and inclusion in wide-scale registries with centralized quality control. We are convinced that the authors of the present article would agree with the fact that this is neither the easiest nor the least important part of successful genetic studies.

Conflict of interest: none declared.

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