Circulation: Genomic and Precision Medicine

EDITORIAL

PCSK9 (Proprotein Convertase Subtilisin/Kexin 9) Status and Protection Against Ischemic Stroke

PheWAS, TreWAS, and More

See Article by Rao et al

David Sullivan, MBBS, FRACP, FRCPA
Paul Bonnitcha, MBBS, PhD
Catherine Spinks, MGC
Tony Keech, MBBS, FRACP, MSc Epi

The large-scale PheWAS (phenome-wide association study) reported by Rao et al[1] provides an important complementary perspective on the likely safety and efficacy of antiatherosclerotic treatment to lower PCSK9 (proprotein convertase subtilisin/kexin 9) levels (eg, with PCSK9 inhibitors). Two recent randomized controlled clinical trials using this class of therapy demonstrated significant reductions in atherothrombotic cardiovascular outcomes[2,3] while achieving hitherto unheard-of reductions in on-trial LDL (low-density lipoprotein) cholesterol levels. This did not seem to be associated with serious adverse outcomes, and total mortality was reduced in the longer of these 2 trials. However, the duration of these large, well-powered trials was relatively short, and the number of potential users of anti-PCSK9 therapy is very large by comparison, with the intention for long-term use. Sporadic reports of adverse outcomes during smaller developmental trials, together with the limited ability of postmarketing surveillance to detect side effects, have prompted this elegant analysis.

Leveraging not only the size but also the genetic and clinical detail associated with the UK Biobank, Rao et al[1] have confirmed coronary protection against atherothrombotic cardiovascular disease in combination with a *PCSK9* gene variant (rs11591147) that has been associated with lower plasma LDL cholesterol levels. Lower lifetime exposure to LDL cholesterol was inferred from a lower likelihood of the need for cholesterol-lowering therapy, and this was proportional to the number of variant alleles. The study also reports a novel association of the *PCSK9* variant (or an expanded *PCSK9* genetic score) with protection against ischemic cerebrovascular disease, presumably on the basis of reduced occlusive atherosclerotic plaque. Embolic cerebrovascular disease may have been a component; however, atrial fibrillation was not associated with *PCSK9* genotype. It is interesting to note that the proportional protection against ischemic stroke approximated, or even exceeded, that against coronary disease. Cholesterol reduction has traditionally been thought to impact sooner, or more favorably, on the natural history of coronary disease as compared with ischemic cerebrovascular disease. The findings in this study suggest that the natural history of these 2 conditions may be more closely aligned when lifelong risk factor profile is taken into account.

The targeted PheWAS component of the study provided helpful reassurance concerning another neurological aspect, namely cognitive function. Despite convincing evidence of reduced rates of atherothrombotic stroke in response to cholesterol-lowering statin therapy[4] concern has been expressed about a possible predisposition toward nonsustained impairment of cognitive function.[5] The results of the recent Ebbinghaus trial[6] also provide reassurance in that short-term use of PCSK9 inhibitors is not associated with neurocognitive decline. The absence

Key Words: Editorials ■ atrial fibrillation ■ cataract ■ epilepsy ■ prediabetic state ■ uncertainty

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of a detrimental association in this PheWAS study was judged in terms of performance of a trail test. The reassuring conclusion is important because *PCSK9* is expressed in neuronal tissue and is detectable in cerebrospinal fluid. It may play a role in neuronal development, and indeed it is thought to increase in response to cerebral ischemia.[7] The risks of epilepsy and cataract were also unrelated to *PCSK9* genetic status.

Controversy persists as to the associations between LDL cholesterol, cholesterol-lowering therapy, and the risk of new-onset type 2 diabetes mellitus.[8] Reproducible associations have been observed, but it is debated as to whether the risk of new-onset type 2 diabetes mellitus is proportional to the degree of LDL cholesterol reduction or the dose and potency of statin treatment. There is also uncertainty as to whether or not the risk is confined to subjects who are in a prediabetic state. New-onset diabetes mellitus has been noted in association with *PCSK9* gene variants,[9] but it has not been widely reported in relatively short-term trials of PCSK9 inhibitors.[10] This suggests that the risk of diabetes mellitus may be dependent on the LDL cholesterol level itself rather than the medications that influence that level. Rao et al[1] do not report a significant association on initial analysis, but after adjustment for lipid-lowering therapy, the *PCSK9* variants' association with type 2 diabetes mellitus becomes apparent. These results are difficult to interpret because there is an underlying assumption concerning the relationship between the history of individual use of routine lipid-lowering drugs and lifelong LDL cholesterol level.

There are some instructive and groundbreaking aspects to the methods used in this study. A large number of participants in the UK Biobank permitted the designation of both the discovery and validation subgroups from the same cohort. This facilitates some aspects of the comparison but might limit the generalizability of the findings beyond subjects of British ancestry who comprised most of the cohort. It is interesting that the *PCSK9* variant was rarely detected in individuals of black or South Asian heritage. Relatively premature onset of coronary disease in South Asian populations has been attributed to earlier and more severe onset of traditional risk factors rather than the presence of novel risk factors.[11] The rarity of a *PCSK9* variant associated with lower cholesterol may be a contributor in this regard.

Analyses in which the discovery and validation cohorts were combined were used to increase the power of detection, but this did little to alter the pattern of protection against occlusive and ischemic stroke or the absence of side effects. The study did not directly explore the relationship between the *PCSK9* genetic variant and its putative biomarker, LDL cholesterol. One of the unfortunate consequences is that it was not possible to translate small degrees of lifelong reduction of LDL or other lipoproteins into an associated estimate of cardiovascular disease protection, as has been possible in the case of Mendelian randomization studies of *NCP1L1*,[12] apolipoprotein (a),[13] apolipoprotein C3,[14] *ANGPTL3*,[15] or indeed *PCSK9* itself.[16] The study was unable to identify and report on the relationship between the *PCSK9* variant and peripheral vascular disease. Patients with peripheral vascular disease have been reported to gain significantly greater benefit from anti-PCSK9 therapy,[17] but they were subsumed within the category of diseases of the circulatory system in the phenotypic data.

Indeed, the use of PheWAS and TreWAS (Trenome-wide association study) hypothesis-free approaches demonstrates some important issues. First, technology now favors the accumulation of genetic data, and this tends to numerically outweigh the clinical data that populate PheWAS and TreWAS analyses. Coordination and curation of the underlying data are very challenging, and the bioinformatics and data-linkage aspects are formidable. Second, and understandably, the clinical components of PheWAS may not necessarily align with clinical end points and side effects as they may have been reported in randomized controlled trials. Third, the International Classification of Diseases, Tenth Revision system is used to try to establish standardization of the phenotypic data. This involves data compression and manipulation. Although this has some positive benefits, it also represents a possible source of misinterpretation. For example, it seems unclear whether the fundamental categories of hyperlipidemia and lipid-lowering therapy included hypertriglyceridemia and its therapy. Finally, the statistical analysis clearly compensates for multiple comparisons, but there is scope for massive expansion in the amount of phenotypic and other information. For example, a vast array of environmental factors impinging on disease could be considered in parallel. This will test the skills of biostatisticians, and their ability to succinctly communicate their findings to clinicians who are unfamiliar with these analyses.

The hypothesis-free PheWAS component provides an appealing way to identify unanticipated side effects of PCSK9 inhibition. The authors acknowledge that this does not extend to off-target effects of these agents, but in the absence of detailed mechanistic insight, it is difficult to distinguish between treatment-related and off-target side effects. One important question that may be amenable to these techniques is the issue of the relationship between lipid-lowering therapies and coronary calcification. Statins and other protective interventions have been associated with an increase in coronary calcium score, possibly as a consequence of biological processes involved in plaque stabilization.[18] Cautious interpretation of the relationship between genetic variants (including *PCSK9*) associated with low LDL cholesterol and age-adjusted coronary calcium scores could provide important information in this regard.

The study reported in this issue[1] raises the profile of one end of the spectrum of hypothesis-free genetic studies. Whereas Mendelian randomization studies established a tradition of studying a targeted hypothesis by examining the associations between a biomarker, its genetic determinants and the observed clinical outcomes,[19] GWAS (Genome-Wide Association Study) studies took an hypothesis-free approach to the relationship between a disease or phenotype and a wide array of genetic polymorphisms to identify candidate relationships.[20] In the hypothesis-free component of this report, the balance between the number of genetic polymorphisms and the extent of the phenotypic information is reversed. A single polymorphism (or a score comprising cholesterol-lowering polymorphisms of the same gene) is examined against a wide array of phenotypic features to identify favorable and unfavorable associations of potential therapeutic significance. Together with a Mendelian randomization component investigating targeted hypotheses, the findings strongly support the role of PCSK9 lowering agents, including PCSK9 inhibitors, for the prevention of atherothrombotic cardiovascular disease. In the process, the potential for stroke prevention is emphasized. At a more fundamental level, the article highlights the potential for large-scale data analysis, but in doing so, it reveals an urgent need to invest in the standardized recording of phenotypic data so that it can keep pace with the explosion in genetic information.

ARTICLE INFORMATION

Correspondence

David Sullivan, MBBS, FRACP, FRCPA, Department of Chemical Pathology, Royal Prince Alfred Hospital, Missenden Rd, Camperdown, NSW 2050, Australia. E-mail david.sullivan1@health.nsw.gov.au

Affiliations

Central Clinical School, Sydney Medical School, University of Sydney, Camperdown, NSW, Australia (D.S., P.B., T.K.). Department of Chemical Pathology, NSW Health Pathology (D.S., P.B., C.S.) and Cardiology Department (T.K.), Royal Prince Alfred Hospital, Camperdown, NSW, Australia.

Disclosures

D. Sullivan has received research program support from Amgen, Sanofi, Regeneron, and educational program support from Sanofi and Amgen. T. Keech received speakers' honoraria, advisory board fees, or research support and educational program support funding from Abbott, Amgen, Astra-Zeneca, Bayer, Eli Lilly, MerckMylan, Novartis, Pfizer, Roche Diagnostics, Sanofi, Regeneron, and the Juvenile Diabetes Research Foundation and Solvay the National Health and Medical Research Council of Australia. The other authors report no conflicts.

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Circ Genom Precis Med. 2018;11:
doi: 10.1161/CIRCGEN.118.002247
Circulation: Cardiovascular Genetics is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 1942-325X. Online ISSN: 1942-3268