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Predictive precision medicine efforts for voltage-gated sodium channel genetic variants

This scientific commentary refers to ‘Gene variant effects across sodium channelopathies predict function and guide precision therapy’ by Brunklaus *et al.* (doi:10.1093/XXXXXX)

We are all aware of the numerous issues that arise when a pathogenic gene variant is identified in an individual. We can imagine the concerns of the patient and, in particular for paediatric cases, of parents and relatives. After discovering the probable cause of the condition, they would like to know more about the disease and of course to receive the most effective therapy. Physicians are frequently confronted with this scenario and strive to provide precision medicine approaches. However, predicting the effect of variants and using this as a basis for the subsequent rational selection of therapies is often challenging, notably for variants associated with pathologies of cellular excitability. In this issue of *Brain*, Brunklaus and co-workers focus specifically on published pathogenic variants of the nine subtypes of voltage-gated sodium channels (named *SCNx* for the genes, Nav1.x for the proteins), comparing the relationships between variant type, location in the protein and functional effects on channel properties.¹ Their results suggest that experimental identification of the functional properties of variants in one subtype can be used to predict the effect (gain of function vs loss of function: GOF vs LOF) of variants in other subtypes, when experimental functional data are not available. Using data from functional studies, they propose a predictive precision medicine approach that can be used to inform clinical practice and

guide therapy, and integrate the approach into the SCN-viewer webtool to assist functional genetic variant interpretation across sodium channelopathies: <http://SCN-viewer.broadinstitute.org>.

The goal of precision medicine is to provide personalized therapeutic interventions that take account of underlying aetiologies, environmental factors, and lifestyle habits.² The integration of genetic information has the potential to precisely identify the molecular causes of disease and of drug effects. Its use has seen a steep acceleration in recent years, in particular in cancer therapy, in which a number of mechanism-based treatments have been successfully implemented.³ Successful predictive approaches are still limited in other fields, although it has been suggested that epilepsy could also be a suitable target.⁴ One frequent complication is that pathogenic missense variants can cause either GOF or LOF in most genes, unlike variants that cause protein truncations which have a clear LOF effect. The predictive tools currently used are in general limited to uncertain prediction of pathogenicity, and fail to provide information about detailed effects on the function of the protein.⁵

In particular, *SCNx/Nav1.x* variants have been implicated in numerous pathologies including epilepsies, neurodevelopmental disorders, skeletal muscle diseases, peripheral neuropathies and cardiac arrhythmias. *SCNx/Nav1.x* GOF can be treated with sodium channel blockers, which are common drugs used in numerous clinical therapies. However, their use should be avoided for variants causing *SCNx/Nav1.x* LOF, for example the *SCN1A/Nav1.1* variants that give rise to the developmental and epileptic encephalopathy (DEE) Dravet syndrome⁶ or *SCN2A/Nav1.2* variants that cause other DEEs or neurodevelopmental diseases.⁷ In these cases, approaches aiming to

decrease the expression of the mutant protein (e.g. antisense oligonucleotides, ASOs, which can be effective against GOF variants⁸) should also be avoided. Furthermore, gene therapy approaches that increase the expression of the mutant protein should potentially also be avoided for GOF variants, such as recently identified *SCN1A*/*Nav1.1* variants that cause phenotypes even more severe than Dravet syndrome.⁹

The gold standard for functional characterization of ion channel variants is patch-clamp electrophysiological recording in transfected mammalian cells. By comparing the properties of heterologously expressed wild type and mutant channels, patch clamping can reveal in detail the effects on the function of the targeted protein, and on cellular excitability, and allow investigation of the underlying mechanisms. However, functional characterizations are time consuming and resource intensive. Thus, they are not normally included in routine clinical diagnostic practice. The quest for alternative fast and reliable methods that can reveal functional effects is therefore ongoing and has already yielded some results. For instance, machine learning-based statistical models have been used to generate an in silico prediction tool (funNCion) for estimating the functional consequences (LOF versus GOF, neutral versus pathogenic) of *SCNx/Nav1.x* variants (<http://funNCion.broadinstitute.org>).¹⁰ This tool does not consider evidence from functional characterization, but infers channel (dys)function from a large dataset of clinical disease phenotypes. However, agreement between funNCion predictions and functional evaluation with patch-clamp recordings ranged between 50-96%¹, showing that predictions were not accurate for a considerable number of variants.

Brunklaus and co-workers hypothesized that inferring function only from clinical datasets may not be sufficient to achieve high-precision predictions, and therefore developed a method based on evidence from functional characterizations. They compared the functional effects of all the *SCNx/Nav1.x* missense variants reported in the literature, considering those that had been electrophysiologically characterized in mammalian cells with patch-clamp recordings in whole-cell configuration. After an initial triage and performing an alignment of linear protein sequences of all sodium channel genes, correlating variants by their effect on functional properties, they included a total of 435 variants and found 38 variant pairs with an identical disease-associated variant in a different sodium channel gene. Overall, they propose that biophysical characterization of variants in one *SCNx/Nav1.x* type can predict channel (dys)function across different *SCNx/Nav1.x* subtypes, providing the basis for a fast predictive tool, implemented in the SCN-viewer webtool.

They suggest that, when a new voltage-gated sodium channel missense variant is identified and pathogenicity is probable according to American College of Medical Genetics (ACMG) criteria, the following key indicators should be used to rapidly estimate its likely functional effect (GOF vs LOF): (i) when an identical missense variant in a paralogue sodium channel has already been functionally characterized (to be checked on <http://SCN-viewer.broadinstitute.org>), there is up to 92% likelihood that reported findings apply to the new variant; (ii) in silico functional prediction tools should be used as a second step (e.g. <http://funNCionbroadinstitute.org>, with 58–96% likelihood that the prediction applies to the new variant, mainly depending on its location in the protein); (iii) when a similar missense variant at an equivalent position in a paralogue sodium channel has been reported in a related sodium channel disorder, for which characteristic GOF or

LOF have been observed (to be checked on <http://per.broadinstitute.org>), it is likely that the functional effect of the new variant is consistent with that of the reported phenotypes.

This method is novel and interesting, and can be helpful in rapidly inferring the effect of a newly discovered sodium channel variant. Moreover, it may be in principle extended to other gene families. Efforts towards this goal are extremely valuable for variant interpretation within the standard clinical workup and for precision medicine approaches.

However, the reliability of the prediction is not perfect and, as highlighted by Brunklaus and co-workers,¹ functional electrophysiological investigations should still be considered the gold standard, and may be the only method for reliably disclosing functional effects of some variants. Notably, thus far this is the only method available for gaining insights into the complex biophysical properties of ion channels, thereby providing a basis for targeting specific dysfunctions with drugs in order to rescue the function of mutants. Functional tests have been extensively used in basic research, but efforts are needed to integrate them into the standard clinical workup, where they can complement predictive tools. Towards this goal, it will be important to standardize experimental conditions (which may depend on the specific channel investigated^{1,6}) and data interpretation, and optimize the pipeline to minimize the complexity of functional tests and the time needed to execute them.

Moreover, an additional level of complexity has to be taken into account. In fact, although the simple GOF-LOF dichotomy has practical utility, it may not always be sufficient to understand detailed pathological mechanisms and identify effective therapies, which may depend on complex cellular/network dysfunctions that are not easily identified in reduced systems. Additionally, although the genetic variant is the initial pathogenic cause, it may induce complex homeostatic and pro-pathologic responses during the natural history of the disease (e.g. several have already been identified in models of Dravet syndrome⁶), the identification of which will require model organisms.

Precision medicine has already yielded important results in sodium channel diseases. The integration of predictive tools, like the one developed by Brunklaus and co-workers¹, of efficient methods of functional analysis adapted to the standard clinical workup, and of model organisms able to reproduce correctly pathological mechanisms and complex homeostatic/pro-pathologic responses may lead to even more effective precision medicine approaches, which could be extended to numerous genes and pathologies.

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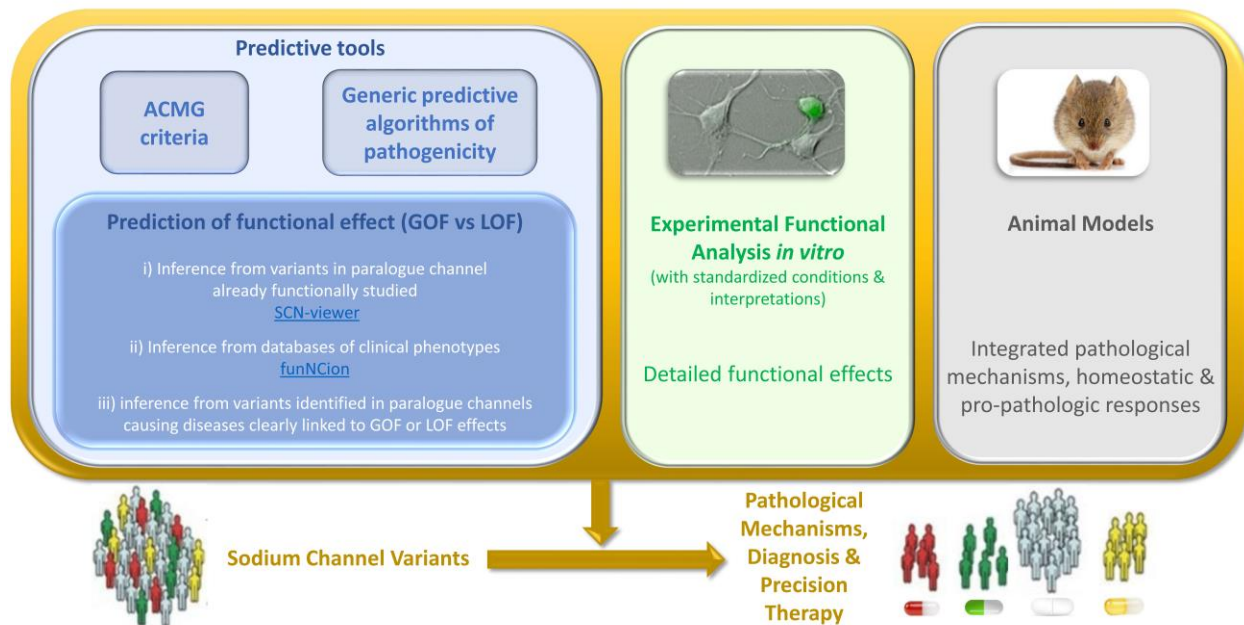
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Competing interests

The authors report no competing interests



Predictive tools within an integrated pipeline for identifying functional effects of sodium channel genetic variants in precision medicine approaches. The method proposed by Brunklaus and co-workers¹ (light blue box) is a fast and simple approach for predicting functional effects (gain of function vs loss of function: GOF vs LOF) of voltage-gated sodium channel variants with good reliability, implemented in the SCN-viewer webtool. Predictive tools, in particular the SCN-viewer, can be very useful for the standard clinical workup, because they provide rapid inference of functional effects. For difficult to solve variants and for obtaining more precise insights into the effect of a variant, predictive tools should be complemented by experimental functional analysis *in vitro* (green box), the gold standard for identifying detailed functional effects, which should be optimized and included in routine clinical workups. Investigations in animal models (grey box)

are too time-consuming, intricate and expensive to be included in routine clinical workups, but are necessary for identifying integrated pathological mechanisms, homeostatic responses and pro-pathologic remodelling. These methods should be integrated in precision medicine pipelines to reveal pathological mechanisms, improve diagnosis and develop effective precision therapies.

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