Genetics of neuropathic pain

Impact paragraph

Pain is a complex sensory and emotional experience, in some cases lasting over a life-time with no improvement despite medication used [1]. Chronic pain affects approximately one fourth of adults and it is often associated with numerous complications, including anxiety and depression dramatically influencing patients' quality of life [1]. Moreover, it has huge economic and societal impact [2]. In 2020, the the health care costs of patients with painful neuropathic disorders in US were estimated at $17,355, which is 3-fold higher comparing to age-matched controls without this condition [2]. Neuropathic pain management includes non-pharmacological, pharmacological (mainly anticonvulsants and antidepressants), and interventional therapies focusing largely on symptomatic treatment [3]. This might be the reason of poor therapy outcome, resulting in a significant number of patients struggling with pain during their lifespan [4]. This creates an urgent need for novel treatments, targeting mechanistically the specific causes of pain in an individual patient. For example, different specific blockers have been used to treat dysfunctional VGSC channels as a pain source, but they can be applied only in patients carrying VGSC mutation, and they also have variable efficacy for instance between Nav1.7- mutations-carrying SFN individuals [5]. Therefore, it is of medical and societal importance to fully characterize the molecular and genetic mechanisms underlying NeP and understand the individual-to-individual differences.

Therefore, in **Chapter 3,** we investigated 15 ion channel genes for variants that could influence channel function and explain pain features. Screening of two patients group, painful- and painless-Diabetic Neuropathy (DN), resolved a number of candidate variants in novel genes. Moreover, our findings revealed that painful-DN patients with ICG variants had more pain than painful-DN without ICG variant, indicating these patients might benefit from ion channel targeted treatment. These results were confirmed in **Chapter 4**, where we have shown that patients with SFN and ion-channel gene variants reported more severe pain compared to patients with SFN and without ICG variants, expanding the relevance of this work for SFN patients. Most of the potentially causative variants have been localized in the TRP genes, remaining promising group of therapeutic targets especially in the light of developing TRP channel pharmacology [6].

Although VSCG are known to be associated with NeP and screening of ICG extended the list of potential gene candidates, there is still a large number of individuals with unresolved pain pathogenicity. Therefore, we screened 592 pain-related genes in SFN patients, not only including ion channels, but involved in a

broad variety of pain-associated processes. In **Chapter 5** we identified pathogenic and likely pathogenic variants and VUS in multiple genes; n=17, 42.5% variants in genes involved in neurotransmission, n=16, 40% in ion channel genes, n=6, 15% in metabolism related genes and n=1, 2.5% variant in gene involved in immune response. This work provided new insight into pain-related genetic markers and pathways associated with NeP and revealed novel gene candidates for further investigation.

Undoubtedly, NGS is a valuable tool in pain diagnostics and research, providing large numbers of potentially relevant variant, however the NGS outcome cannot be validated by bioinformatics only, and should be confirmed in system mimicking physiological conditions, especially in the case of VUS and novel genes. Unfortunately, models for functional read out are limited, costly and highly laborious, requiring novel systems with a higher throughput at lower cost. In **Chapter 6** we explored a novel cell model to functionally test ANO3 variants. ANO3 protein expression was analysed in several cell lines, as creating a model overexpressing wild-type and mutant *ANO3* gene would bring new information about gene function and its role in pain pathophysiology.

In conclusion, this work provides more knowledge about genetic landscape of neuropathic pain, highlighting important role of ion channels and novel pain related genes identified in this study. Findings presented in this thesis may contribute in a future perspective to improve diagnostics of NeP, disease prognosis and development of new therapeutic interventions and more effective pain-targeting personalized treatment.

References

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