A mini-review: Bridging the gap between autism spectrum disorder and pain comorbidities

Chad O. Brown\textsuperscript{a,b,c}, Jarryll Uy\textsuperscript{a,b}, and Karun K. Singh\textsuperscript{a,b}

\textsuperscript{a}Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada; \textsuperscript{b}Stem Cell and Cancer Research Institute, McMaster University, Hamilton, Ontario, Canada; \textsuperscript{c}Michael G. DeGroote Institute for Pain Research and Care, McMaster University, Hamilton, Ontario, Canada

\textbf{ABSTRACT}

\textbf{Background:} Pain is a complex neurobiological response with a multitude of causes; however, patients with autism spectrum disorder (ASD) often report chronic pain with no known etiology. Recent research has been aimed toward identifying the causal mechanisms of pain in mouse and human models of ASD. In recent years, efforts have been made to better document and explore secondary phenotypes observed in ASD patients in the clinic. As new sequencing studies have become more powered with larger cohorts within ASD, specific genes and their variants are often left uncharacterized or validated. In this review we highlight ASD risk genes often presented with pain comorbidities.

\textbf{Aims:} This mini-review bridges the gap between two fields of literature, neurodevelopmental disorders and pain research. We discuss the importance of the genetic landscape of ASD and its links to pain phenotypes.

\textbf{Results:} Among the numerous genes implicated in ASD, few have been implicated with varying severities of pain comorbidity. Mutations in these genes, such as SCN9A, SHANK3, and CNTNAP2, lead to altered neuronal function that produce different responses to pain, shown in both mouse and human models.

\textbf{Conclusion:} There is a necessity to use new technologies to advance the current understanding of ASD risk genes and their contributions to pain. Secondly, there is a need to power future ASD risk genes associated with pain with their own cohort, because a better understanding is needed of this subpopulation.

\textbf{RÉSUMÉ}

\textbf{Contexte:} La douleur est une réponse neurobiologique complexe dont les causes sont multiples ; cependant, les patients atteints de troubles du spectre de l’autisme (TSA) rapportent souvent une douleur chronique sans étiologie connue. Des recherches récentes ont visé à identifier les mécanismes causaux de la douleur chez des modèles murins et humains.

Ces dernières années, des efforts ont été faits pour mieux documenter et étudier les phénomènes secondaires observés chez les patients atteints de TSA en clinique. Étant donné que les nouvelles études de séquençage sont devenues plus puissantes et se réalisent avec des cohortes plus importantes au sein des TSA, des gènes spécifiques et leurs variants demeurent souvent non caractérisés ou validés. Dans cette revue, nous mettons en évidence les gènes de risque de TSA qui se présentent souvent avec des comorbidités douloureuses.

\textbf{Objectifs:} Cette mini-revue comble le fossé entre deux domaines de la littérature, les troubles neurodéveloppementaux et la recherche sur la douleur. Nous discutons de l’importance du paysage génétique des TSA et de ses liens avec les phénomènes de la douleur.

\textbf{Résultats:} Parmi les nombreux gènes impliqués dans les TSA, peu ont été impliqués avec divers de degrés de sévérité de la comorbidité de la douleur. Des mutations dans ces gènes, tels que SCN9A, SHANK3 et CNTNAP2, conduisent à une fonction neuronale altérée qui produit des réponses différentes à la douleur, que l’on retrouve à la fois chez le modèles murins et humains.

\textbf{Conclusion:} Il est nécessaire d’utiliser les nouvelles technologies pour faire progresser la compréhension actuelle des gènes de risque de TSA et leurs contributions à la douleur. Deuxièmement, il est nécessaire d’augmenter la puissance des futurs gènes de risque de TSA associés à la douleur avec leur propre cohorte, car une meilleure compréhension de cette sous-population est nécessaire.
**Introduction**

Pain is often described as a complex neurobiological response to an unpleasant sensory or emotional experience associated with actual or potential tissue damage. In recent years, an increased focus has been devoted to pain research because many individuals will experience subtypes of pain, whether chronic, which can last several months, or acute, which typically has a shorter duration, over their lifetime. In 2011, chronic pain in Canada was estimated to have affected 6 million adults (~19% of the population). Approximately half of all reports documented that patients suffered from chronic pain for more than 10 years, with one-third of all cases reporting the intensity to be very severe. Unfortunately, epidemiological systematic reviews that aim to examine the prevalence of chronic pain have reported an alarming importance of age range within population statistics. This was highlighted by the frequency of chronic pain peaking at 45 to 65 years of age found in 17 epidemiological studies ending in the year 2000. Prior to this most recent Canadian chronic pain prevalence estimate, many researchers highlighted an age-dependent frequency increase in chronic pain by 33% to 39% at 55 years of age and older. Additionally, reports suggested that females may experience higher severity of chronic pain than males. In upcoming years these estimates will only increase as the baby boomer generation, the largest subset of the Canadian population determined by Statistics Canada (~29% in 2011), continues to age into the major Canadian cohorts that experience chronic pain. A recent study estimated a weighted annual incremental direct cost to manage chronic pain across Canada of US$7.2 billion, which does not include additional expenses paid by the individual or indirect costs; taking these into account, the approximate total would range from US$56 to US$60 billion per year.

Individuals with multiple neurological conditions have been diagnosed with chronic pain, including those with spinal cord injury, stroke, multiple sclerosis, and Parkinson’s disease. The prevalence of pain in people with neurological conditions is estimated to be twice that of the general population at 15% to 19%. Within this subcategory, approximately 84% of patients with traumatic spinal cord damage report chronic pain. In addition, the overarching theme of altered neural connectivity, which may present as central and peripheral neuropathic pain (42%) or nociceptive musculoskeletal pain (71%), emphasizes the importance of investigating all neurological conditions and their association with chronic pain.

Unfortunately, neurological conditions such as neurodevelopmental disorders have not garnered much attention within the field of pain. One of the major neurodevelopmental disorders that presents comorbidity with chronic pain is autism spectrum disorder (ASD). ASD is defined by the core characteristics impaired social interaction and communication and restricted and repetitive behaviors. ASD’s prevalence has continued to increase over the last 50 years: in 2018 the Canadian Public Health Agency determined the prevalence to be 1 in 66 children. A large number of studies have primarily focused on potential environmental risk factors as contributors for ASD, such as maternal diet, gut microbiota, prenatal exposures to toxins, and cesarean sections. Genetic and neuropathological studies have aimed to identify the causal mechanisms of ASD.

In this review, we aim to provide an overview of the literature highlighting the importance of genetic studies in neurodevelopmental and pain research. One gene that has recently appeared in both bodies of literature without researchers merging their efforts to gain a better molecular and cellular understanding is the voltage-gated sodium channel, named SCN9A. We hope to bridge these fields toward a common goal of improving patient outcomes while also elucidating potential functional characterization approaches to provide targeted therapeutic approaches to patients with ASD and pain comorbidities.

**Genetics of Autism Spectrum Disorder**

Among the determinants of ASD, genetics are largely known to play a key role in the disease pathophysiology. Previous monozygotic twin studies revealed a large genetic component of ASD, with heritability rates between 50% and 90%. Recently, a large population-based multinational study estimated the general heritability of ASD as approximately 80%. Genome-wide association studies were used initially to identify common genetic variants in the form of single nucleotide variations or copy number variations that might be enriched in populations with ASD versus their neurotypical counterparts. However, these studies have found few potential common variants and thus researchers have turned toward rare inherited variants (present in <1% of the population) and de novo mutations (present only in the affected child). Recent large-scale whole-genome and exome sequencing studies have implicated numerous genes as associated with ASD. These studies have found multiple rare inherited and de novo single nucleotide variations and copy number variations that underlie the complex genetic landscape of ASD. Many of these genetic variants are categorized in the SFARI gene database, the foremost accepted database of ASD risk genes, using scores based on sequencing evidence and studies that look at the neurodevelopmental impact caused by disruptions of those genes. Based on the number of variants and their correlation to clinical and cellular phenotypes related to pain, the gene SCN9A, which encodes a voltage-gated sodium channel
within the peripheral nervous system (PNS), has been designated as a category 2 gene in relation to ASD. This gene has started to garner attention due to its high penetrance and distinct pain phenotypes observed in patients with ASD with pain comorbidities. Thus, this gene has been the most studied ASD risk gene in relation to pain.

**SCN9A the Pain Gene**

Pain response is a complex process that commonly involves transduction of nerve signals from the PNS to the autonomic and central nervous system (CNS), known as nociception. In addition to processing of nociceptive inputs, neuropathic pain conditions have their own unique origins of pain and processing. Neuropathic pain can be further divided into central or peripheral, where central is commonly a consequence of a previous disease or injury such as stroke, multiple sclerosis, and spinal cord injury that damages the CNS. More commonly observed is peripheral neuropathic pain, which originates from impairment to PNS pathways, including complex regional pain syndrome and postherpetic neuralgia. A key contributor to nociceptive neuron excitability that has emerged within recent years is the gene SCN9A, which encodes a voltage-gated sodium channel (Na,1.7). Na,1.7 is an ion channel subtype of the voltage-gated sodium family that is primarily expressed in the PNS, specifically in dorsal root ganglion, specialized sensory neurons known as nociceptive neurons, and other ganglion neurons. These neurons are crucial for signal transduction, which is important for pain. In addition to Na,1.7 expression in specialized sensory neurons, limited but emerging evidence has highlighted rare variant expression that decreases firing of specific inhibitory GABAergic neurons. Dorsal root ganglia are sites of clustering sensory neurons that propagate signals from the periphery, where nociceptors detect the threat of damaging stimuli, to the spinal cord. Nociceptors are highly modular, whether they take on more mechanosensitive functions responding to temperature, pressure, or chemosensitive to chemicals.

Biophysical studies of Na,1.7 have implicated its function in action potential initiation and its regulation of subthreshold stimuli. Recent studies have highlighted efforts to sequence patients with pain phenotypes to determine a genetic etiology of pain syndromes such as erythromelalgia, paroxysmal extreme pain disorder, small fiber neuropathy, and channelopathy-associated congenital indifference to pain. A common observation was a convergence of unknown variants accumulating in SCN9A. This provided initial necessary observations, but further functional validation or phenotyping is needed to ensure that these variants are the cause of the pain syndromes observed in patients. Further evidence suggested that gain-of-function variants in SCN9A enhanced neuronal excitability, which was observed in erythromelalgia, paroxysmal extreme pain disorder, and small fiber neuropathy; loss-of-function variants recapitulated a hypoxecitable neuronal phenotype that exhibited as channelopathy-associated congenital indifference to pain. These experiments were primarily performed in HEK293 cells and, more recently, in human-derived sensory neurons with electrophysiology measuring both sodium currents and inactivation and activation properties of the currents. Many authors have highlighted the convergence of mutations in SCN9A, but a plethora of variants are still uncharacterized in patients with pain syndromes.

**The Autism Spectrum Disorder Risk Gene SCN9A and Pain Comorbidities**

It is well known that ASD is a genetically heterogeneous disorder, where multiple genes contribute to a phenotype. Patients express the core characteristics of ASD but may also have secondary behavioral contributions or comorbidities to other diseases and disorders. Secondary or comorbid conditions that have been observed in patients with ASD include altered sensory sensitivity and pain. A recent investigation using data from 48,591 children without ASD, 1158 children with ASD, and 314 children with ASD and one other comorbid disorder found the prevalence of pain per group to be 8.2%, 15.6%, and 19.9%, respectively. This was on average double the prevalence observed in children without ASD. ASD is often conceptualized to involve disruption in neural signaling and, more specifically, caused by an imbalance in excitation or inhibition. Many sensory abnormalities found in the clinic align with the potential convergence at the cellular level of atypical neural connectivity. It has been shown that individuals with ASD exhibit an altered neural response to pain as demonstrated by functional magnetic resonance imaging. In addition, it has been reported that 95% of individuals with ASD have aberrant reactivity to sensory stimuli, including tactile stimuli, and varying severities of acute and chronic pain. Previous cases in the literature showed an overlap between ASD and chronic pain, particularly among adolescents. As previously mentioned, ASD has a strong genetic contribution, and advances of arrays and sequencing technologies have provided tools to elucidate both de novo single nucleotide variants and copy number variations and other genetic variants that contribute to approximately 10% to 40% of cases. Sodium channels in ASD research have gained significant traction, with SCN2A, a gene that encodes a voltage-gated sodium channel subtype 2 alpha protein (Na,1.2), becoming a front runner due to the sheer
amount of de novo single nucleotide variants that occur in patients. The less studied SCN9A gene is important in the PNS and regulates sensory neuronal excitability. When looking outside of ASD research, SCN9A has been highly characterized in pain research. To date, there has only been one direct investigation that has linked all three components: SCN9A, ASD, and pain. In this study, researchers were able to identify two families from the National Institute of Mental Health repository and whole-exome sequence analyses of family members. SCN9A was chosen as one of the candidate genes based on the abundance of variants. One variant, Na\textsubscript{v}1.7\textsuperscript{C1143F}, was validated and it was determined that there were changes in recovery of fast inactivation of the current. Furthermore, other biophysical neuronal properties such as input resistance and rheobase were altered, such that input resistance was lowered and rheobase increased, requiring a larger magnitude of current to evoke action potentials. A second variant was also further investigated, Na\textsubscript{v}1.7\textsuperscript{M932L/V991I}, which has been previously implicated in neuropathic pain syndromes and was associated with partial deletion of pain perception. Biophysical properties of this variant remained unchanged but, similar to Na\textsubscript{v}1.7\textsuperscript{C1143F} cortical neurons, they fired fewer action potentials, indicating a reduced excitability phenotype. This finding highlighted the convoluted nature of predicting pain outcomes based on past clinically relevant information, without functional cellular validation. Previous investigations of Na\textsubscript{v}1.7\textsuperscript{M932L/V991I} suggest that this variant has the capacity to induce increased and decreased neuronal firing, raising the possibility that two different patients with the same mutation can exhibit varying pain phenotypes. Lastly, it is necessary to consider the contributions of the neuron subtype, the auxiliary subunits, and the distinction between Na\textsubscript{v}1.7 expression in CNS and PNS, because ASD risk genes have had an underappreciated role in the PNS surrounding pain phenotypes. In summary, the authors were able to functionally validate known variants in pain research, Na\textsubscript{v}1.7\textsuperscript{C1143F} and Na\textsubscript{v}1.7\textsuperscript{M932L/V991I}, and continue to probe other unknown variant functions discovered from their sequencing results of patients with ASD.

Among the genes in the SFARI database, multiple genes have been identified as being implicated in pain phenotypes. One such gene is SHANK3, a gene mutated in Phelan-McDermid syndrome, where primary symptoms include ASD as well as blunted pain sensitivity. Furthermore, in a study evaluating 201 patients with Phelan-McDermid syndrome, it was found that nearly 80% demonstrated insensitivity to pain. Mouse lacking one or both copies of Shank3 have been shown to have an insensitivity to pain. Han et al. showed that deletion of Shank3 in peripheral sensory neurons of mice led to a decrease in inflammatory pain sensitivity. It was further shown that the specific deletion of Shank3 in sensory neurons recapitulated pain deficits in Shank3 global knockout mice. This finding suggested that insensitivity to pain seen in patients with SHANK3 mutations may be attributed not solely to dysfunction in the brain but within the PNS. Mutations in the mouse model of ASD harboring deletions in Cntnap2 have been shown to display hypersensitivity to pain. Dawes et al. demonstrated that sensory neurons of Cntnap2\textsuperscript{−/−} mice had impaired K\textsubscript{v} channel function that produced unregulated neuronal excitability. They identified the key role of an ASD-associated gene in pain sensitivity and examined potential mechanisms by which it occurs. Additionally, Sener et al. examined expression of genes related to aggression and pain sensitivity in whole-blood samples of patients with ASD. They found altered expression of several genes such as SCN9A and OPRM1, which are genes related to pain as well as significantly higher expression of the aggression-related TACR1 gene in patients with ASD. This study provided novel insight into potential biomarkers of the consequent behavioral phenotypes of individuals diagnosed with ASD. Understanding of the links between ASD and pain remains poor and thus highlights the need to further identify the underlying causal mechanisms for future therapeutic intervention.

**Concluding Remarks**

It is evident that there is a wealth of knowledge within each of the respective fields of ASD and pain, but to increase the success of patient outcomes, more will need to be done to draw parallels between fields and to allow future experiments to emphasize this bridge. As both fields evolve, more human-relevant experiments will be needed. Most current neuroscience experiments use mouse models to investigate the human condition. As the field is starting to note, pathways, proteins, and overall cellular dynamics are not always recapitulated. To further highlight the relevance of SCN9A variants in clinically relevant settings, models such as induced pluripotent stem cells, which can be reprogrammed from healthy or patient somatic cells, can be used as a powerful tool. These induced pluripotent stem cells have been well characterized, with advancements in protocols to finely tune differentiation targeted toward cell and tissue types like cortical neurons, sensory neurons, cardiomyocytes, gastrointestinal cells, and more. Furthermore, these protocols allow for improved scalability for experiments that may help expedite the process of functionally characterizing specific variants in human-derived tissue. In tandem with new technologies such as microelectrode arrays, this may provide a functional platform that may be used in the future for screening patient mutations.
and allow for drug screens to occur on a large scale. Targeted patient therapeutics would thus be enhanced and allow for preliminary screening to occur within the clinic without compromising patient safety (e.g., toxic symptoms or drug treatments that would produce no benefit).

Acknowledgments

This work was supported by a Michael G. DeGroote Institute for Pain Research and Care graduate studentship to C.B. K.K. S. was supported by funding from CIHR, NSERC, and the Ontario Brain Institute.

Author contributions

C.B. and J.U. wrote the paper, and K.K.S. provided supervision.

Disclosure Statement

Chad O. Brown received a studentship from Michael G. DeGroote Institute for Pain Research and Care graduate studentship. Jarryll Uy has no conflicts of interest. Karun K. Singh is supported by funding from CIHR, NSERC and the Ontario Brain Institute.

References

1. Garland EL. Pain processing in the human nervous system: a selective review of nociceptive and biobehavioral pathways. Prim Care. 2012;39(3):561–71. doi:10.1016/j.pop.2012.06.013.
2. Merskey H, Bogduk N. Classification of chronic pain. Seattle: IASP Press; 1994.
3. Schopflocher D, Taenzer P, Jovey R. The prevalence of chronic pain in Canada. Pain Res Manag. 2011;16:445–50. doi:10.1155/2011/876306.
4. Boulanger A, Clark AJ, Squire P, Cui E, Horbay GLA. Chronic pain in Canada: have we improved our management of chronic noncancer pain? Pain Res Manag. 2007;12(1):39–47. doi:10.1155/2007/762180.
5. Moulin DE, Clark AJ, Speechley M, Morley-Forster PK. Chronic pain in Canada - Prevalence, treatment, impact, and the role of opioid analgesia. Pain Res Manag. 2002;7(4):179–84. doi:10.1155/2002/323085.
6. Millar WJ. Chronic pain. Holist Nurs Pract. 1991;6:47–53.
7. Generations in Canada. [Accessed 2019 Nov 19]. https://www12.statcan.gc.ca/census-recensement/2011/as-sa/98-311-x/98-311-x2011003_2-eng.cfm.
8. Wilson MG, Lavis JN, Ellen ME. Supporting chronic pain management across provincial and territorial health systems in Canada: findings from two stakeholder dialogues. Pain Res Manage. 2015;20(5):269–79. doi:10.1155/2015/918976.
9. Hogan M-E, Taddio A, Katz J, Shah V, Krahn M. Incremental health care costs for chronic pain in Ontario, Canada. Pain. 2016;157:1626–33.
10. Finnerup NB, et al. Phenotypes and predictors of pain following traumatic spinal cord injury: A prospective study. J Pain. 2014;15:40–48.
11. Turner JA, Cardenas DD, Warms CA, McClellan CB. Chronic pain associated with spinal cord injuries: A community survey. Arch Phys Med Rehabil. 2001;82(4):501–08. doi:10.1053/apmr.2001.21855.
12. Drulovic J, Basic-Kes V, Grgic S, Vojinovic S, Dincic E, Toncev G, Kezic MG, Ksic-Tepavecvec D, Dujmovic I, Mesaros S. The prevalence of pain in adults with multiple sclerosis: a multicenter cross-sectional survey. Pain Med. 2015;16(8):1597–602. doi:10.1111/pme.12731.
13. Beiske AG, Loge JH, Rønningen A, Svensson E. Pain in Parkinson’s disease: prevalence and characteristics. Pain. 2009;141(1):73–77. doi:10.1016/j.pain.2008.12.004.
14. Broen MPG, Braaksma MM, Patijn J, Weber WEJ. Prevalence of pain in Parkinson’s disease: A systematic review using the modified QUADAS tool. Mov Disord. 2012;27(4):840–84. doi:10.1002/mds.24054.
15. Hanagasi HA, Akat S, Gurvit H, Yazici J, Emre M. Pain is common in Parkinson’s disease. Clin Neurol Neurosurg. 2011;113(1):11–13. doi:10.1016/j.clineuro.2010.07.024.
16. Siddall PJ, McClelland JM, Rutkowski SB, Cousins MJ. A longitudinal study of the prevalence and characteristics of pain in the first 5 years following spinal cord injury. Pain. 2003;103(3):249–57. doi:10.1016/S0304-3959(02)00452-9.
17. Appelros P. Prevalence and predictors of pain and fatigue after stroke: a population-based study. Int J Rehabil Res. 2006;29(4):329–33. doi:10.1097/MRR.0b013e328010c7b8.
18. Klitt H, Finnerup NB, Overvad K, Andersen G, Jensen TS. Pain following stroke: a population-based follow-up study. PLoS One. 2011;6(11):e27607. doi:10.1371/journal.pone.0027607.
19. Kong KH, Woon VC, Yang SY. Prevalence of chronic pain and its impact on health-related quality of life in stroke survivors. Arch Phys Med Rehabil. 2004;85(1):35–40. doi:10.1007/S00099-003-00369-1.
20. Foley PL, Vesterinen HM, Laird BJ, Sena ES, Colvin LA, Chandran S, MacLeod MR, Fallon MT. Prevalence and natural history of pain in adults with multiple sclerosis: systematic review and meta-analysis. Pain. 2013;154(5):632–42. doi:10.1016/j.pain.2012.12.002.
21. Truini A, Galeotti F, Laesa S, Di Rezzè S, Biasiotta A, Di Stefano G, Tinelli E, Millefiorini E, Gatti A, Crucchi G. Mechanisms of pain in multiple sclerosis: A combined clinical and neurophysiological study. Pain. 2012;153(10):2048–54. doi:10.1016/j.pain.2012.05.024.
22. Vandenkerkhof E. The prevalence of chronic pain and pain-related interference in the Canadian population from 1994 to 2008. 2011:31.
23. Burke D, Fullen BM, Stokes D, Lennon O. Neuropathic pain prevalence following spinal cord injury: A systematic review and meta-analysis. Eur J Pain. 2017;21(1):29–44. doi:10.1002/ejp.905.
24. Müller R, Brinkhof MWG, Arnet U, Hinrichs T, Landmann G, Jordan X, Béchir M. Prevalence and associated factors of pain in the Swiss spinal cord injury population. Spinal Cord. 2017;55(4):346–54. doi:10.1038/sc.2016.157.
25. Diagnostic and statistical manual of mental disorders (DSM-5) - American psychiatric association - Google books; [Accessed 2019 Nov 28]. https://books.google.ca/books?hl=en&id=JivBAAQBAJ&oi=fnd&pg=PT18rq&dq=Diagnostic+and+statistical+manual+of+mental+disorders&ots=ceUPl3KNs8&sig=LniKa9ygLRxKvWu87alZXFFWA#v=onepage&q=

26. Ofner M, Coles A, Decou MI, Do MT, Bienek A, Snider J, Ugnat A-M Autism spectrum disorder among children and youth in Canada 2018 - Canada.ca. A Report of the National Autism Spectrum Disorder Surveillance System; [Accessed 2018 Dec 14]. https://www.canada.ca/en/public-health/services/publications/diseases-conditions/autism-spectrum-disorder-children-youth-canada-2018.html.

27. Sealey LA, Hughes BW, Sriskanda AN, Guest JR, Gibson AD, Johnson-Williams L, Pace DG, Bagasra O. Environmental factors in the development of autism spectrum disorders. Environ Int. 2016;88:288–98. doi:10.1016/j.envint.2015.12.021.

28. Landrigan PJ. What causes autism? Exploring the environmental contribution. Curr Opin Pediatr. 2010;22(2):11–13. doi:10.1097/MOP.0b013e328336eb9a.

29. Curran EA, O’Neill SM, Cryan JF, Kenny LC, Dinan TG, Khashan AS, Kearney PM. Research review: birth by caesarean section and development of autism spectrum disorder and attention-deficit/hyperactivity disorder: a systematic review and meta-analysis. J Child Psychol Psychiatry. 2015;56(5):500–8. doi:10.1111/jcpp.12351.

30. Strati F, Cavalieri D, Albanese D, De Felice C, Donati C, Hayek J, Joussein O, Leoncini S, Renzi D, Calabrò A. New evidences on the altered gut microbiota in autism spectrum disorders. Microbiome. 2017;5(1):24. doi:10.1186/s40168-017-0242-1.

31. Mangiola F, Ianiro G, Franceschi F, Fagiuoli S, Gasbarrini G, Gasbarrini A. Gut microbiota in autism and mood disorders. World J Gastroenterol. 2016;22(1):361–68. doi:10.3748/wjg.v22.i1.361.

32. Edlow AG. Maternal obesity and neurodevelopmental and psychiatric disorders in offspring. Prenatal Diagnosis. 2017;37(1):95–110. doi:10.1002/pd.4932.

33. Krakowiak P, Walker CK, Bremer AA, Baker AS, Ozonoff S, Hansen RL, Hertz-Picciotto I. Maternal metabolic conditions and risk for autism and other neurodevelopmental disorders. Pediatrics. 2012;129(5):e1121–e1128. doi:10.1542/peds.2011-2583.

34. Reynolds LC, Inder TE, Neil JJ, Pineda RG, Rogers CE. Maternal obesity and increased risk for autism and developmental delay among very preterm infants. J Perinatol. 2014;34(9):688–92. doi:10.1038/jp.2014.80.

35. Hallmayer J. Genetic heritability and shared environmental factors among twin pairs with autism. Archiv General Psychiatry. 2011;68(11):1095–102. doi:10.1001/archgenpsychiatry.2011.76.

36. Freitag CM, Staal W, Klauck SM, Duketis E, Waltes R. Genetics of autistic disorders: review and clinical implications. European Child Adolescent Psychiatry. 2010;19(3):169–78. doi:10.1007/s00787-009-0076-x.

37. Bai D, Yip BHK, Windham GC, Sourander A, Francis R, Yoffe R, Glasson E, Mahjani B, Suominen A, Leonard H. Association of genetic and environmental factors with autism in a 5-country cohort. JAMA Psychiatry. 2019;76(10):1035–43. doi:10.1001/jamapsychiatry.2019.1411.

38. Yuen RKC, Thiruvahindrapuram B, Merico D, Walker S, Tammimies K, Hoang N, Chrysler C, Nalpathamkalam T, Pellechia G, Liu Y. Whole-genome sequencing of quartet families with autism spectrum disorder. Nat Med. 2015;21(2):185–91. doi:10.1038/nm.3792.

39. Yuen RKC, Merico D, Bookman M, I. Howe J, Thiruvahindrapuram B, Patel RV, Whitney J, Deflaux N, Bingham J, Wang Z. Whole genome sequencing resource identifies 18 new candidate genes for autism spectrum disorder. Nat Neurosci. 2017;20(4):602–11. doi:10.1038/nm.4524.

40. Jossifov I, O’Roak BJ, Sanders SJ, Ronemus M, Krumm N, Levy D, Stessman HA, Witherspoon KT, Vives L, Patterson KE. The contribution of de novo coding mutations to autism spectrum disorder. Nature. 2014;515(7526):216–21. doi:10.1038/nature13908.

41. Sanders SJ, Murtha MT, Gupta AR, Murdoch JD, Raubeson MJ, Willsey AJ, Erkan-Sencicek AG, DiLullo NM, Parikhshak NN, Stein JL. De novo mutations revealed by whole-exome sequencing are strongly associated with autism. Nature. 2012;485(7397):237–41. doi:10.1038/nature10945.

42. Banerjee-Basu, S. & Packer, A. SFARI gene: an evolving database for the autism research community. Dis Models Mech. 2010;3:133–35.

43. Watson JC, Sandroni P. Central neuropathic pain syndromes. Mayo Clinic Proc. 2016;91(3):372–85. doi:10.1016/j.mayocp.2016.01.017.

44. McDermott LA, Weir GA, Themistocleous AC, Segerdahl AR, Blesneac I, Baskozos G, Clark AJ, Millar V, Peck IJ, Ebner D. Defining the functional role of Na V 1.7 in human nociceptionSilos-Santiago. Neuron. 2019;101(5):905–919.e8. doi:10.1016/j.neuron.2019.01.047.

45. Dih-Hajj SD, Cummins TR, Black JA, Waxman SG. From genes to pain: Nav1.7 and human pain disorders. Trends Neurosci. 2007;30(11):555–63. doi:10.1016/j.tins.2007.08.004.

46. Yu FH, Catterall WA. Overview of the voltage-gated sodium channel family. Genome Biol. 2003;4:207.

47. Sanders SJ, Campbell AJ, Cottrell JR, Moller RS, Wagner FF, Aldridge AL, Bernier RA, Catterall WA, Chung WK, Empfield JR. Progress in understanding and treating SCN2A-mediated disorders. Trends Neurosci. 2018;41(7):442–56. doi:10.1016/j.tins.2018.03.011.

48. Toledo-Aral JJ, Moss BL, He ZJ, Koszowski AG, Whisenand T, Levinson SR, Wolf JJSilos-Santiago, I, Halegoua S, Mandel G. Identification of PNI, a predominant voltage-dependent sodium channel expressed principally in peripheral neurons. Proc National Acad Sci U S A. 1997;94(4):1527–32. doi:10.1073/pnas.94.4.1527.
49. Rubinstein M, Patowary A, Stanaway IB, McCord E, Nesbitt RR, Archer M, Scheuer T, Nickerson D, Raskind WH, Wijsman EM. Association of rare missense variants in the second intracellular loop of Na v 1.7 sodium channels with familial autism. Mol Psychiatry. 2018;23(2):231–39. doi:10.1038/mp.2016.222.

50. Moutal A, Dustrude ET, Largent-Milnes TM, Vanderah TW, Khanna M, Khanna R. Blocking CRMP2 SUMoylation reverses neuropathic pain. Mol Psychiatry. 2018;23(11):2119–21. doi:10.1038/mp.2017.117.

51. Dubin AE, Patapoutian A. Nociceptors: the sensors of the pain pathway. J Clin Invest. 2010;120(11):3760–72. doi:10.1172/JCI42843.

52. Momin A, Wood JN. Sensory neuron voltage-gated sodium channels as analgesic drug targets. Curr Opin Neuropathol. 2008;18(4):383–88. doi:10.1016/j.conb.2008.08.017.

53. Minett MS, et al. Distinct Nav1.7-dependent pain sensitizations require different sets of sensory and sympathetic neurons. Nat Commun. 2012;3.

54. Dib-Hajj SD, Yang Y, Black JA, Waxman SG. The Na v 1.7 sodium channel: from molecule to man. Nat Rev Neurosci. 2013;14(1):49–62. doi:10.1038/nrn3404.

55. Cox JJ, Reimann F, Nicholas AK, Thornton G, Roberts E, Springell K, Karbani G, Jafari H, Mannan J, Raashid Y. An SCN9A channelopathy causes congenital inability to experience pain. Nature. 2006;444(7121):894–98. doi:10.1038/nature05413.

56. Eberhardt M, Nakajima J, Klinger AB, Neacsu C, HühneG K, O’Reilly AO, Kist AM, Lampe AK, Fischer K, Gibson J. Inherited pain sodium channel nav1.7 A1632T mutation causes erythromelalgia due to a shift of fast inactivation. J Biol Chem. 2014;289(4):1971–80. doi:10.1074/jbc.M113.502211.

57. Wu MT, Huang PY, Yen CT, Chen CC, Lee MJ. A novel SCN9A mutation responsible for primary erythromelalgia and is resistant to the treatment of sodium channel blockers. PLoS One. 2013;8:e55212.

58. Drenth JPH, Te Morsche RHJ, Guillette G, Taieb A, Lee Kirby R, Jansen JBMJ. SCN9A mutations define primary erythromelalgia as a neuropathic disorder of voltage gated sodium channels. J Invest Dermatol. 2005;124(6):1333–38. doi:10.1111/j.0022-202X.2005.23737.x.

59. Estacion M, Dib-Hajj SD, Benke PJ, Te Morsche RHJ, Eastman EM, Macala LJ, Drenth JPH, Waxman SG. NaV1.7 gain-of-function mutations as a continuum: A1632E displays physiological changes associated with erythromelalgia and paroxysmal extreme pain disorder mutations and produces symptoms of both disorders. J Neurosci. 2008;28(43):11079–88. doi:10.1523/JNEUROSCI.3443-08.2008.

60. Faber CG, Hoeijmakers JGJ, Ahn H-S, Cheng X, Han C, Choi J-S, Estacion M, Lauria G, Vanhoutte EK, Gerrits MM. Gain of function Na V 1.7 mutations in idiopathic small fiber neuropathy. Ann Neurol. 2012;71(1):26–39. doi:10.1002/ana.22485.

61. Mannion A, Leader G. Comorbidity in autism spectrum disorder: A literature review. Res Autism Spectr Disord. 2013;7(12):1595–616. doi:10.1016/j.rasd.2013.09.006.

62. Black C, Kaye JA, Jick H. Relation of childhood gastrointestinal disorders to autism: nested case-control study using data from the UK General Practice Research Database. Br Med J. 2002;325(7361):419–21. doi:10.1136/bmj.325.7361.419.

63. Davis TE, Hess JA, Moree BN, Fodstad JC, Dempsey T, Jenkins WS, Matson JL. Anxiety symptoms across the lifespan in people diagnosed with Autistic Disorder. Res Autism Spectr Disord. 2011;5(1):112–18. doi:10.1016/j.rasd.2010.02.006.

64. Coury DL, et al. Gastrointestinal conditions in children with autism spectrum disorder: developing a research agenda. Pediatrics. 2012;130:916178.

65. Allely CS. Pain sensitivity and observer perception of pain in individuals with autistic spectrum disorder. Sci World J. 2013;173(12):1203–05.

66. Whitney DG, Shapiro DN. National prevalence of pain among children and adolescents with autism spectrum disorders. JAMA Pediatr. 2019. doi:10.1001/jamapediatrics.2019.3826.

67. Yasuda Y, et al. Sensory cognitive abnormalities of pain in autism spectrum disorder: A case-control study. Ann Gen Psychiatry. 2016;15.

68. Failla MD, Moana-Filho EJ, Essick GK, Baranek GT, Rogers BP, Cascio CJ. Initially intact neural responses to pain in autism are diminished during sustained pain. Autism. 2018;22(6):669–83. doi:10.1177/1362361317706043.

69. Moore DJ. Acute pain experience in individuals with autism spectrum disorders: A review. Autism. 2015;19(4):387–99. doi:10.1177/1362361314527839.

70. Wiwe Lipsker C, von Heijne M, Bölte S, Wickerskell R. A case report and literature review of autism and attention deficit hyperactivity disorder in a pediatric chronic pain. Acta Paediatr. 2018;107(5):753–58. doi:10.1111/apa.14220.

71. Bursch B, Ingman K, Vitti L, Hyman P, Zelter LK Chronic pain in individuals with previously undiagnosed autistic spectrum disorders. J Pain 5, 290–95 (2004).

72. Loades ME. Evidence-based practice in the face of complexity and comorbidity: a case study of an adolescent with asperger’s syndrome, anxiety, depression, and chronic pain. J Child Adolesc Psychiatr Nurs. 2015;28:73–83.

73. Thier MC, et al. Identification of embryonic neural plate border stem cells and their generation by direct reprogramming from adult human blood cells. Cell Stem Cell. 2019;24:166–182.e13.

74. Pinto D, et al. Convergence of genes and cellular pathways dysregulated in autism spectrum disorders. Am J Hum Genet. 2014;94:677–94.

75. Constantino JN, et al. Autism recurrence in half siblings: strong support for genetic mechanisms of transmission in ASD. Mol Psychiatry. 2013;18:137–38.

76. Yin J, Oleson D, Schaaf CP. Next generation sequencing in autism spectrum disorder. OBM Genet. 2018;2:1–1.

77. Yuan R, et al. Two novel SCN9A gene heterozygous mutations may cause partial deletion of pain perception. Pain Med. 2011;12:1510–14.
78. Oberman LM, Boccuto L, Cascio L, Sarasua S, Kaufmann WE. Autism spectrum disorder in Phelan-McDermid syndrome: initial characterization and genotype-phenotype correlations. Orphanet J Rare Dis. 2015;10:105.
79. Sarasua SM, et al. Clinical and genomic evaluation of 201 patients with Phelan-McDermid syndrome. Hum Genet. 2014;133:847–59.
80. Han Q, et al. SHANK3 deficiency impairs heat hyperalgesia and TRPV1 signaling in primary sensory neurons. Neuron. 2016;92:1279–93.
81. Dawes JM, et al. Immune or genetic-mediated disruption of CASPR2 causes pain hypersensitivity due to enhanced primary afferent excitability. Neuron. 2018;97:806–822.e10.
82. Sener EF, et al. Altered global mRNA expressions of pain and aggression related genes in the blood of children with autism spectrum disorders. J Mol Neurosci. 2019;67:89–96.
83. Takahashi K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007;131:861–72.
84. Spence JR, et al. Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. Nature. 2011;470:105–10.
85. Masumoto H, et al. Human iPS cell-engineered cardiac tissue sheets with cardiomyocytes and vascular cells for cardiac regeneration. Sci Rep. 2014;8:603.
86. Alshawaf AJ, et al. Phenotypic and functional characterization of peripheral sensory neurons derived from human embryonic stem cells. Sci Rep. 2018;8:603.
87. Zhang Y, et al. Rapid single-step induction of functional neurons from human pluripotent stem cells. Neuron. 2013;78:785–98.