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REVIEW

NELL-1 in Genome-Wide Association Studies across Human Diseases

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Neural epidermal growth factor—like (EGFL)-like protein (NELL)-1 is a potent and key osteogenic factor in the development and regeneration of skeletal tissues. Intriguingly, accumulative data from genome-wide association studies (GWASs) have started unveiling potential broader roles of NELL-1 beyond its functions in bone and cartilage. With exploration of the genetic variants of the entire genome in large-scale disease cohorts, GWASs have been used for establishing the connection between specific single-nucleotide polymorphisms of *NELL1*, in addition to osteoporosis, metabolic diseases, inflammatory conditions, neuropsychiatric diseases, neurodegenerative disorders, and malignant tumors. This review summarizes the findings from GWASs on the manifestation, significance level, implications on function, and correlation of specific *NELL1* single-nucleotide polymorphisms in various disorders in humans. By offering a unique and comprehensive correlation between genetic variants and plausible functions of *NELL1* in GWASs, this review illustrates the wide range of potential effects of a single gene on the pathogenesis of multiple disorders in humans. *(Am J Pathol 2022, 192: 395—405; https://doi.org/10.1016/j.ajpath.2021.11.006)*

The genome-wide association study (GWAS) has been a useful method in medical and complex trait genomics for >2 decades.[1] By probing into large-scale genetic variants across the genomes of many individuals, GWASs can shed light on novel genotype—phenotype associations.[2] Results from GWASs contribute to an expanded knowledge of certain diseases, including, but not limited to, their causes, pathophysiology, and clinical treatment.[3] Current interpretation of data from GWASs is typically focused on a single disease or trait and its multiple associated genes. However, studies focused on one specific gene of high frequency in multiple diseases or traits are lacking. This article reviews one specific gene, the gene that encodes neural epidermal growth factor—like (EGFL)-like protein (NELL)-1, the osteogenic capacity of which has been studied for >2 decades, by compilation of its diverging roles beyond its well-known osteochondrogenic properties.[4,5] This unique perspective can illustrate the potential complex implications of the functions of a single gene on multiple systems of the body, and shed light on the importance of a comprehensive understanding of genes and diseases at a systemic level. Clearly, NELL-1 is just one key element of the functional molecules in the extremely sophisticated networks involved in multiple conditions and treatments. The potential role of

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NELL-1 is indeed worthy of further exploration, given the broader involvement of *NELL1* in diseases and treatments revealed by GWASs.

NELL1 Gene and the Identified Functions of the NELL-1 Protein

NELL1 was named for its similarity to the gene *NEL*, which encodes EGFL protein.[4] In humans, *NELL1* has been mapped to chromosome 11 at 11p15.1-p15.2, spanning around 906 kb, with 20 coding exons.[4] *NELL1* is highly conserved in humans and mice, with 95% nucleotide homology.[6] *NELL1* encodes a cytoplasmic protein that has five EGFL repeats, in addition to a thrombospondin N-terminal domain, several von Willebrand factors, as well as histidine-rich and cysteine-rich domains.[7] NELL-1 protein has been reported to have several binding partners, such as protein kinase C (PKC)-β_1, apoptosis-related protein 3, and roundabout homolog 2.[5] These binding proteins are either nonspecific or not naturally present on the cell surface.[8,9] However, recently, the physical high-affinity ligand receptor–like binding between NELL-1 and contactin-associated protein–like protein (Cntnap)-4 was identified, indicating Cntnap-4 as a specific NELL-1 receptor.[10] Notably, NELL-1 protein has complex quaternary structures and several isoforms with distinct functions,[5] potentially accounting for its functional diversity.

The functional role of NELL-1 began to be unraveled when the connection between NELL-1 expression and unilateral coronal craniosynostosis, a pathologic condition in humans, was established.[7] Since then, a myriad of animal studies have explored the functions of NELL-1, starting with disease-model simulations of human nonsyndromic CS in Nell-1–overexpression transgenic mice.[11] Continuous research efforts have attributed multiple roles to NELL-1 in physiologic and pathologic processes, which can be summarized as follows: i) NELL-1 is necessary in normal craniofacial and appendicular skeletogenesis and is a potent pro-osteogenic factor for osteochondral tissue regeneration[12–14]; ii) NELL-1 exhibits anti-adipogenic effects when applied in bone-regeneration conditions[14–16]; iii) NELL-1 has therapeutic potential in osteoarthritis due to its pro-osteogenic and anti-inflammatory effects[5,17]; iv) *NELL1* is a potential tumor-suppressing gene, on account of its involvement in promoter hypermethylation[18,19]; v) NELL-1 could play an important regulatory role in the nervous system due to its newly discovered receptor, Cntnap-4, which is crucial in synapse development[5,10]; and vi) NELL-1 serves as a podocyte antigen marker to define a distinct type of membranous nephropathy.[20,21]

Genome-Wide Association Study of *NELL1*

NELL1 gene has been a frequent hit in GWASs in a wide range of human diseases such as metabolic, neuropsychiatric, neurodegenerative, and inflammatory diseases, and cancers with susceptible gene loci spread throughout the *NELL1* sequence (Figure 1).[22–25] Some of these association studies have offered novel perspectives on the genuine susceptibility gene loci.[22,23] Other association studies and corresponding function studies have verified the role of NELL-1 by highlighting the potential for combating the relevant disease using therapy.[24,25] These gene loci are of genome-wide significance ($P < 5 \times 10^{-8}$),[3] suggestive significance ($P < 5 \times 10^{-5}$),[26] or nominal significance[27] (Table 1).

Metabolic Diseases

Given the established link of NELL-1 to craniosynostosis, the functional role of NELL-1 in bone-related disease has been thoroughly investigated. Despite the large number of animal studies in which the potential role of NELL-1 in bone regeneration was verified,[10,11,13,14,17] a definite association is lacking in human studies. Nonetheless, some promising results have emerged from the connection between *NELL1* and other metabolic diseases, such as lipid-metabolism disorders.

Osteoporosis

Osteoporosis is an aging-related bone-degenerative disease characterized by compromised bone strength and an increased risk for fracture.[40,41] Genetic determinants in the progression of osteoporosis have been identified.[40] Bone mineral density (BMD) is frequently used to predict osteoporotic fracture and remains the single best trait for the analysis of its prognosis.[42] Previous GWASs have revealed >20 susceptible gene loci associated with a low BMD.[40,42]

In the Framingham study, performed by Karasik et al,[28] analysis of data from 2073 US patients showed that rs10766761, located in intron 12 of the *NELL1* gene, was nominally associated with low BMD of both the femoral neck ($P = 7.9 \times 10^{-4}$) and the lumbar spine ($P = 3.3 \times 10^{-4}$). The study brought into perspective the different traits of osteoporosis and investigated the relationship between phenotype and the percentage of shared SNPs. *NELL1* was among the 10 associated genes identified by more than one proxy for osteoporosis.

Acute lymphoblastic leukemia leads to bone loss in some affected patients,[43] which can result either from the disease itself or from concomitant physical conditions.[43] The BMD of lumbar vertebrae L1/L2 investigated in a study of the genetic risk factors for BMD loss in patients with acute lymphoblastic leukemia[29] suggested NELL-1 as being significant for a decrease in BMD.[29] Similarly, an SNP in intron 12 of *NELL1*, rs11025915, was found to be positively associated with BMD loss in patients with acute lymphoblastic leukemia.

Studies of the functions of NELL-1 in modulating osteogenesis have been underway for decades.[14,44–46] Systemic delivery of NELL-1 to mice with ovariectomy-induced

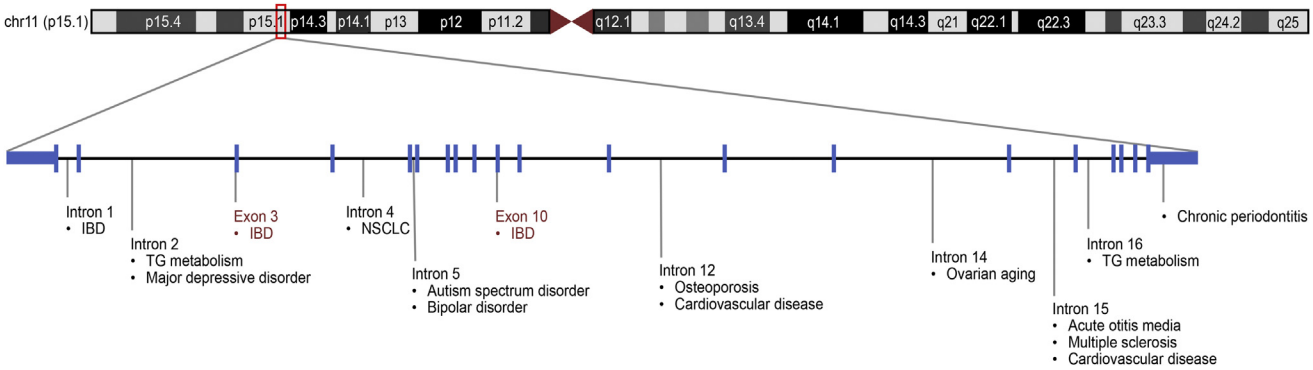

Figure 1 *NELL1*-related susceptible gene loci in human disorders. **Boxed** region indicates the region of *NELL1* gene on chromosome 11. IBD, inflammatory bowel disease; NSCLC, non—small cell lung cancer; TG, triglyceride.

osteoporosis is associated with increased BMD, possibly by enhancement of the osteoblast differentiation and inhibition of the osteoclast-directed bone resorption.[14] Similarly, in a sheep model of osteoporosis, local delivery of NELL-1 was associated with a significant increase in bone formation, as evidenced by increased BMD, bone volume, and mean cortical length.[14,46] Furthermore, NELL-1 is associated with counteraction of the adverse effect of adipose-filled, poor-quality bone in bone morphogenetic protein (BMP)-2—induced skeletal repair, possibly by directing BMP-2—treated cells away from adipogenesis and toward osteogenesis.[44]

Triglyceride-Related Metabolic Diseases

Triglycerides (TGs) are circulating lipoproteins, the serum concentration of which reflects lipid status in clinical practice.[47] Apart from serving as structural components,

Table 1 *NELL1* Manifested in GWASs of Human Disorders

Classification of disorders	Publication	Sample size	Ethnicity	Evidence of association	SNPs	Location on *NELL1*
Metabolic disease						
Osteoporosis	Karasik et al (2010)[28]	—	—	Nominal	rs10766761	Intron 12
ALL BMD loss	Inaba et al (2018)[29]	393	American	Suggestive	rs11025915	Intron 12
TG metabolism	Del-Aguila et al (2014)[30]	767	American	Genome-wide	rs12279250; rs4319515	Intron 16; intron16
TG metabolism	Rudkowska et al (2014)[22]	141	Canadian	Suggestive	rs752088	Intron 2
TG metabolism	Aslibekyan et al (2013)[31]	793	American	Suggestive	25 SNPs*	—
Neuropsychiatric and neurodegenerative disorder						
Autism	Connolly et al (2013)[32]	2165	American	Genome-wide	rs1429793	Intron 5
Bipolar	Mathieu et al (2015)[33]	291	French	Suggestive	rs10766743	Intron 5
MDD	Lin et al (2018)[34]	455	Taiwanese	Suggestive	rs2139423	Intron 2
Multiple sclerosis	Tiwari et al (2015)[35]	608	Caucasian	Suggestive	rs7130553	Intron 15
Inflammatory diseases						
IBD	Franke et al (2007)[23]	792	German	Suggestive	rs1793004; rs8176785; rs8176786	Intron 1; exon 3; exon 10
Chronic periodontitis	Sanders et al (2017)[36]	10,935	Hispanic	Suggestive	rs75715012	Intergenic region
Otitis media	van Ingen et al (2016)[27]	8790	American	Nominally	rs11026076	Intron 15
Cancer						
NSCLC	Wu et al (2013)[37]	620	American	Suggestive	rs10766739	Intron 4
Other disorders						
Cardiovascular mortality	Figarska (2014)[38]	1546	Caucasian	Suggestive	rs11026076	Intron 15
QT prolongation	Seyerle et al (2018)[25]	78,199	European	Suggestive	rs12225793	Intron 12
Ovarian aging	Voorhuis (2013)[39]	791	European	Suggestive	rs7939346; rs10833509	Intron 14

*The specific loci of the 25 SNPs were not mentioned by the author.

—, not available; ALL, acute lymphoblastic leukemia; BMD, bone mineral density; GWAS, genome-wide association study; IBD, inflammatory bowel disease; MDD, major depressive disorder; NSCLC, non—small cell lung cancer; SNP, single-nucleotide polymorphism; TG, triglyceride.

TGs are the energy reservoir that provide fuel for the body.[47] In a genetic study, individuals with a weaker TG response to a treatment drug had a genetic profile different from those with a normal response.[48]

Del-Aguila et al[30] conducted a GWAS of the TG response to hydrochlorothiazide, a drug widely prescribed for use in the treatment of patients with hypertension, which can cause hypertriglyceridemia. That study was performed in two independent populations composed of 425 European Americans and 342 African Americans.[30] Two SNPs, rs12279250 and rs4319515, both in intron 16 of *NELL1*, showed genome-wide significance, while 77 SNPs in 27 genomic regions exhibited suggestive significance in the African-American population. The investigators conjectured that hydrochlorothiazide might regulate adipocyte differentiation through NELL-1, as NELL-1 was found to repress adipogenic differentiation.[30]

In another study, Rudkowska et al[22] probed the effects of supplementation with long-chain Ω-3 polyunsaturated fatty acids on the risk-related loci of inadequate plasma TG response. The study included 141 Canadians classified as responders or nonresponders. *NELL1*, among 13 loci, had suggestive significant association; rs752088, located in intron 2 of the *NELL1* gene, was more frequent in nonresponders than in responders.[22]

Similarly, Aslibekyan et al[31] performed a study of the changes in circulating adiponectin levels in response to fenofibrate therapy in 793 individuals from the United States. Several SNPs located on *NELL1* were of suggestive significant association with baseline adiponectin level.

In an *in vitro* study, *NELL1* was associated with reduced adipogenic differentiation in a human pre-adipocyte cell line, as evidenced by decreases in lipid droplet formation and expression of all of the adipogenic genes examined.[16] In a study in a rodent model of femoral segmental defect, NELL-1 was reported to guide target cells away from adipogenesis.[44] Considering the well-established genome-wide association of *NELL1* with lipid metabolism, and the valid function testing of NELL-1 in animal studies, the development NELL-1–related drugs to combat excessive fat production in diseases in humans is foreseeable.

Neuropsychiatric and Neurodegenerative Disorders

NELL-1 exhibits the highest expression in human and mouse brain tissues[49] and plays an important role in skull morphogenesis.[11] For these reasons, there is speculation that NELL-1 might play a role in neuropsychiatric and neurodegenerative disorders. Significantly, the discovery of Cntnap-4 as the NELL-1–specific receptor[10] has prompted exploration of the potential regulatory role of NELL-1 in the nervous system in animal models. GWASs have revealed *NELL1* to be one of the genes frequently associated with neural disorders.[50–52]

Autism Spectrum Disorder

Autism spectrum disorder (ASD) is a constellation of diseases characterized by repetitive, unusual sensorimotor behaviors and deficits in social communication.[50] It has been reported that 74% to 93% of ASD risk is heritable.[50] Evidence from a study in twins indicates that 16p11.2 deletions and *CHD8* are among the top genetic variants associated with ASD.[53]

Connolly et al[32] performed a GWAS to detect susceptible genes associated with ASD. They selected endophenotypes from the commonly used behavioral assessments and included 2165 patients of European, Asian, or African descent. Among several of the candidate genes, *NELL1* was found to be associated with endophenotypes of fainting, fits, or blackouts, and rs1429793, located in intron 5 of *NELL1*, showed genome-wide significance.

The investigators proposed that the association of NELL-1 with ASD might be a result of the comorbidity of ASD and other conditions, such as epilepsy.[32] The NELL-1 binding partner, PKCβ,[5] has been proposed as a target for anticonvulsant drugs.[32] Notably, the NELL-1 receptor Cntnap-4 has been implicated in ASD.[54] Cntnap-4 normally acts to attenuate dopamine release through a presynaptic mechanism. *Cntnap4*-knockout mice exhibit perseverative behaviors, such as over-grooming, which remains a common behavior abnormality in ASD mice.[54] In addition, during human-gene analysis, patients diagnosed with ASD harbored exon deletion in the *CNTNAP4* gene.[54] Investigative work to understand the role of NELL-1 in ASD is ongoing.

Bipolar Disorder

Bipolar disorder, or manic depression, is characterized by episodic and recurrent mood transition from extreme elation to severe depression.[51] Inheritable factors play an important role in the pathogenesis of the disease, and family history is a clinical predictor.[55] The diagnosis of bipolar disorder is primarily based on clinical features.[51] The inclusion of a quantitative trait, emotional reactivity (the threshold and magnitude to which one responds in emotion-eliciting situations), has largely contributed to the identification of susceptible genes in bipolar disorder.

Mathieu et al[33] performed a study of the genetic background of 281 patients from France who met the criteria for bipolar disorder. They selected emotional reactivity to quantify the severity of bipolar disorder. On GWAS, rs10766743, located in *NELL1* intron 5, remained the single SNP with suggestive significance after adjustment for multiple comparisons using the Bonferroni correction.

Similar to that in other diseases of the nervous system, the functional role of NELL-1 in bipolar disorder has not yet been tested. More functional experiments are required to verify the role of NELL-1 in bipolar disorder.

Major Depressive Disorder

Major depressive disorder is a group of psychosocial-dysfunction diseases manifested by a combination of

emotional, neurovegetative, and neurocognitive symptoms.[56] In genetic studies, major depressive disorder has been reported as moderately inheritable through multiple genes.[57]

Lin et al[34] implemented a study in 455 Taiwanese patients diagnosed with major depressive disorder to hone in a predictive model for an antidepressant response. The investigators first performed a GWAS to select the candidate gene loci. They then built a predictive model with a combination of patients' clinical and genetic biomarkers. In the association study, *NELL1* was among the top 10 genes predictive of treatment response. The SNP located in intron 2 of *NELL1*, rs2139423, was detected to be of suggestive significance.

As current studies have not implicated the functional role of NELL-1 in mediating psychiatric disorders, the antidepressant role of NELL-1 requires further elucidation.

Multiple Sclerosis

Multiple sclerosis is a degenerative disease of the central nervous system caused by neuroinflammation. The clinical manifestation of multiple sclerosis is dependent on the location of the affected central nervous system region and the extent of the inflammatory process.[52,58] Genetic factors play a prominent role in the development of the disease.[52] An association between a multiple sclerosis subtype and human leukocyte antigen DR isotype 15 and 16 has long been known and consistently replicated.[52]

In a study of 608 Caucasians screened for the candidate genes that contribute to the outcomes of patients with multiple-sclerosis relapse, *NELL1* was found to be of suggestive significance. The SNP rs7130553, located in intron 15, was suspected.[35] The fact that NELL-1 is highly expressed in brain tissue may have accounted for this observation. Studies of the function of NELL-1 in multiple sclerosis have not yet been performed.

Inflammatory Diseases

The first association of *NELL1* with diseases in GWASs appeared in a 2007 study of the susceptibility of the gene in inflammatory bowel disease (IBD).[23] Indeed, *NELL1* has been linked to other inflammatory diseases, including periodontitis and otitis media.

Inflammatory Bowel Disease

IBD is a group of chronic inflammatory disorders of the gastrointestinal tract, featured by two subtypes, Crohn disease, and ulcerative colitis.[59] The onset and progression of IBD are attributed to the dysregulated immune response of the resident microbial communities in a genetically susceptible host. Genetic studies have revealed >240 loci that confer a risk for IBD, but the contribution of these genetic variants to the disease remains largely unknown.[59] Among these genes, *NELL1* has been shown by GWASs to be susceptible.[23,24]

Franke et al[23] performed a multistage genome-wide scan in different panels. They first conducted the screening in a German panel of 393 cases of Crohn disease and 399 controls and identified the 200 most significant SNPs. In the subsequent replication study in an independent German panel, rs1793004, located in intron 1 of *NELL1*, showed a consistent association of suggestive significance with Crohn disease. The German panel comprised 942 patients, 1082 controls, and 375 trios, and rs1793004 was also associated with the ulcerative colitis case-control panel, highlighting *NELL1* as a ubiquitous IBD-susceptibility gene. In the replication study, two additional SNPs, rs8176785 and rs8176786, located on exons 3 and 10 respectively, showed an association of suggestive significance with Crohn disease. The SNPs in the two exons both were missense mutations. Since the initial identification of *NELL1* as a susceptible gene in IBD, several replication studies from other research centers have ensued. Studies in a Dutch-Belgian cohort and in a Canadian population failed to show statistical association with *NELL1*.[24,60] The fact that the German study included stringent criteria for Crohn disease, in which only patients with the severe phenotype[23] were included in the analysis, might account for the discrepancy among the studies.

Apart from association studies, histologic and molecular studies in IBD patients have indicated interesting results. Substantial *NELL1* transcript levels were detected in the colon and small intestine. However, no significant differences between normal and affected tissue were revealed by quantitative PCR. Localization of NELL-1 in colonic mucosa by immunohistochemistry analysis showed a confined expression in inflammatory cells in the lamina propria.[23]

Although the association between IBD and *NELL1* was susceptible to population stratification, interestingly, osteopenia and osteoporosis were among the top comorbidities in IBD patients.[61,62] Sophisticated diagnostic modalities also demonstrated that young IBD patients experienced more bone fragility.[63] It was suggested that BMD and bone microstructure could serve as early diagnostic criteria to better identify IBD patients.[63] Thus, it is reasonable to infer that NELL-1 may be associated with comorbidities of IBD rather than susceptibility to IBD *per se*.[23] Moreover, so far, two SNPs, rs8176785 and rs8176786, are the only *NELL1* SNPs in the coding exons identified and associated in GWAS reports across many disorders in humans. In this case, the actual roles of the identified SNPs in the development of IBD could be pursued by the construction of the corresponding point-mutation vectors or by the use of the *CRISPR/CAS9* gene-editing technique in both *in vitro* and *in vivo* models.[64] The verification and validation of the functions of *NELL1* SNPs may be assayed as suggested elsewhere[65–69] (Figure 2).

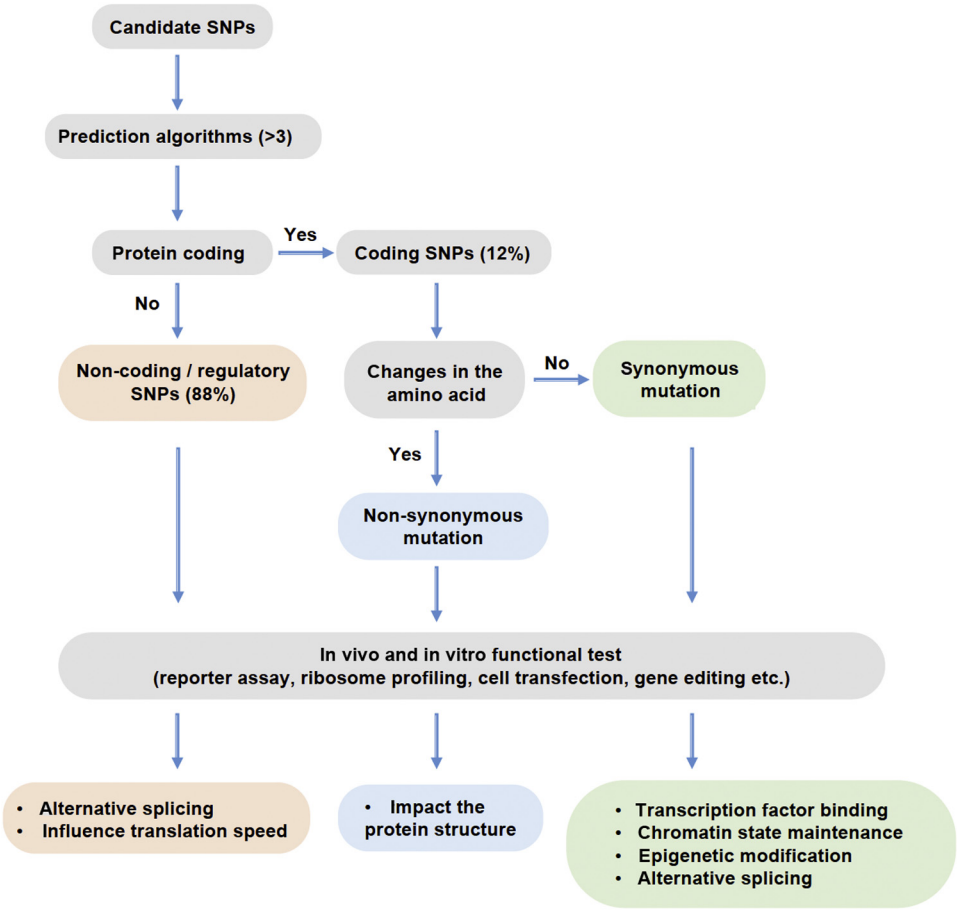

Figure 2 Functional validation of candidate SNPs. For candidate SNPs of statistical significance revealed by GWASs, further bioinformatics analysis is applied to categorize them as coding or noncoding. Usually, a combination of multiple servers (more than three) with complementary algorithms was used to minimize errors.[66] In the GWASs in the literature, up to 88% of the SNPs screened are noncoding.[68] Coding SNPs were subclassified as nonsynonymous mutations, in which the amino acid and consequently the encoded protein is changed, and synonymous mutations.[69] While nonsynonymous SNPs could have a direct impact on the protein structure, synonymous SNPs could lead to alternative splicing, or limit the speed of the translation.[67] Noncoding SNPs can also be called regulatory SNPs, which can be located in the promoter, enhancer, 5′-untranslated region (UTR), 3′-UTR, or intronic region. First, GWAS, linkage analysis, and quantitative trait locus mapping are frequently used to detect causal regulatory SNPs.[68] Then, functional testing can be applied to determine the role of targeted SNPs to be transcription-factor binding, chromatin state maintenance, epigenetic modification,[68] or alternative splicing.[65] The general framework of functional validation of noncoding SNPs is suggested elsewhere.[68]

Chronic Periodontitis

Chronic periodontitis is a progressive inflammatory disease characterized by gradual detachment of the periodontal tissue from the tooth and a loss of alveolar bone.[70,71] The cause of chronic periodontitis involves a dynamic interaction between the periodontal microbiota and the host-defense system, which is under strong genetic control.[70] Studies in twins have verified the heritability of chronic periodontitis, and a handful of GWASs have begun to unravel the genetic susceptibility of the disease.[71–73] To date, no gene has been found to be of genome-wide significance, although several bear suggestive significance.[72,73]

Five GWASs of chronic periodontitis were conducted in Caucasian and Asian populations.[26,72–75] However, none of them found a gene locus of genome-wide significant association.[36,71] Then Sanders et al[36] performed a GWAS in 10,395 Hispanic/Latino participants, followed by a replication study in 4402 European Americans and 908 African Americans. They selected interproximal clinical attachment levels as the measurement of chronic periodontitis and reported one gene locus of genome-wide significance and four others of suggestive significance. The *NELL1* gene, with its nearby SNP rs75715012, showed suggestive significance.

Apart from association studies, *in vitro* and *in vivo* animal studies have also revealed the functional role of NELL-1 in tooth and periodontal tissues.[52] NELL-1 may promote osteogenic differentiation in human periodontal ligament stem cells.[76] Moreover, NELL-1 demonstrated osteoinductive capacity to promote bone formation in alveolar bone areas within rhesus-monkey and rat models.[77,78]

Acute Otitis Media

Otitis media remains a highly prevalent childhood disease worldwide, of which acute otitis media (AOM) accounts for the largest percentage.[27,79] AOM is characterized by the presence of fluid in the middle-ear cavity, frequently

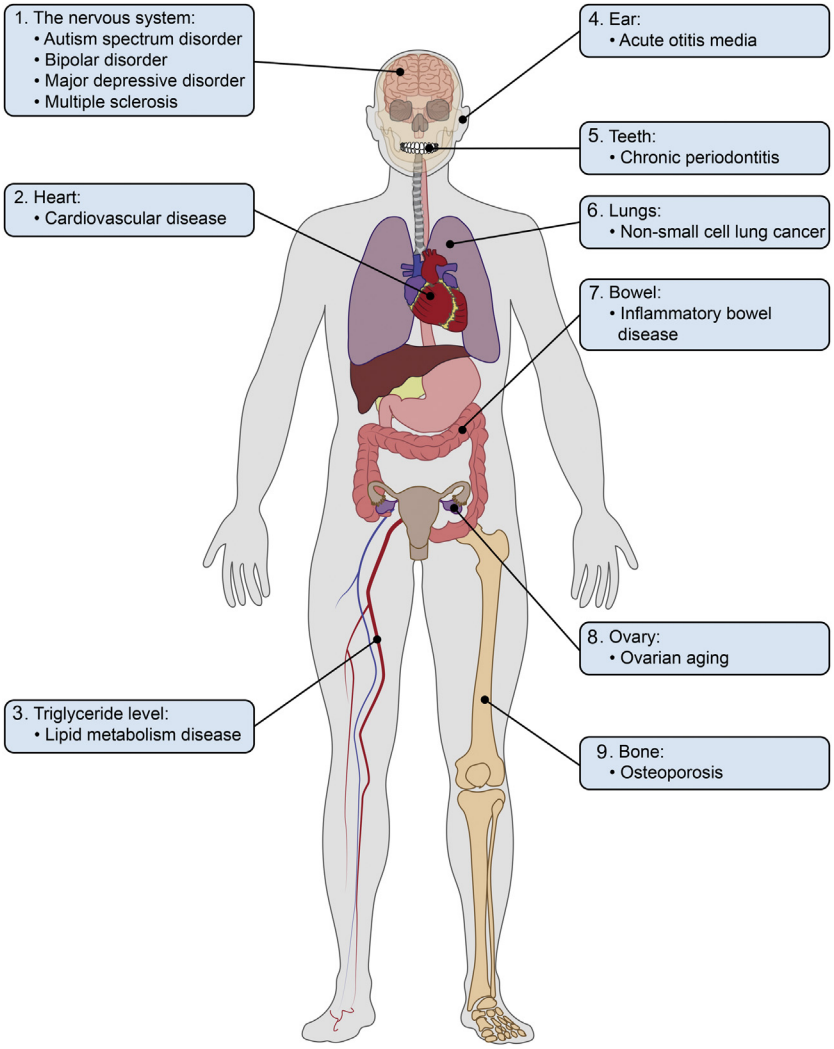

Figure 3 Human disorders with *NELL1* manifestation in GWASs.

accompanied by earache, fever, and upper respiratory tract infection.[80] The cause of AOM involves multiple aspects of the pathogen, host, and genetic factors.[80,81] The heritability of AOM is well established, and *FNDC1* has been identified as a disease-contributing gene.[27] Furthermore, GWASs have unraveled several other susceptible gene loci in AOM.[27,79,82]

Van Ingen et al[27] performed a GWAS in AOM in an American population with 825 cases and 7936 controls. They discovered 1 gene of genome-wide significance and 8 genes of suggestive significance. In addition, they found that 45 of 82 genes demonstrated nominally significant association. The *NELL1* gene, with its SNP rs11026076 in intron 15, fell into the latter category.

Evidence of the association between NELL-1 and AOM is still at its preliminary stage. Further studies are needed to verify this association and to illustrate the possible functional role of NELL-1 in AOM.

Non–Small Cell Lung Cancer

Non–small cell lung cancer (NSCLC) is the most common type of lung cancer and accounts for nearly 80% of cases.[83] Although smoking is an important risk factor for NSCLC, a notable amount of NSCLC patients have never smoked.[37] These observations led to the discovery that nonsmokers with NSCLC bear a distinct genetic profile in comparison to smokers with NSCLC.[84] The *ALK* gene ranked top among the NSCLC oncogenes.[83]

In order to investigate the genetic variants of NSCLC in nonsmokers, Wu et al[37] conducted a GWAS. The study included as a screening panel 620 American patients and normal controls from two medical centers. The top 25 gene candidates generated from this study were replicated in 1256 Taiwanese patients. Only two genes were still significant after the replication analysis. The SNP in intron 4 of *NELL1*, rs10766739, ranked among the top candidate loci in the screening panel ($P = 3.66 \times 10^{-7}$) and was of borderline significance ($P = 0.051$) in the replication panel. Therefore, *NELL1* emerged as a candidate tumor-suppressor gene. This is supported by its potentially protective role in other types of tumors, such as esophageal adenocarcinoma and colon cancer,[18,19] although more validation studies are needed.

In parallel to GWAS, studies of the function of NELL-1 in tumorigenesis have yielded corroborating results. *NELL1*

promoter region is hypermethylated, a hallmark of gene inactivation, in colon cancer tissue compared to normal colon tissue.[19] Similarly, in esophageal adenocarcinoma, *NELL1* exhibits a loss of heterozygosity and promoter hypermethylation, two mechanisms of gene inactivation.[18] The application of demethylation agents could decrease promoter methylation and up-regulate NELL-1 expression in both colon cancer and esophageal adenocarcinoma cell lines.[18,19] Furthermore, in a recent study in lung cancer stem-like cells,[85] overexpression of NELL-1 in a 95-D human cell line, a highly invasive and metastatic lung carcinoma cell line, was associated with cell differentiation and thus a reduction in metastasis. Together, these results support the promising tumor-suppressing role of NELL-1.

Other Human Disorders

In GWASs, NELL-1 has also been implicated in other human disorders that are not categorized readily, such as cardiovascular diseases and ovarian aging.[38,39] The SNP rs11026076, located in intron 15 of *NELL1* gene, is positively associated with cardiovascular mortality with suggestive significance.[38] In a human functional study by Chen et al,[86] NELL-1 protected the mitral valve from inflammatory injury. Another GWAS explored thiazide diuretics–induced QT-interval prolongation. A total of 78,199 Europeans, African Americans, and Hispanics were enrolled in that study, in which rs12225793 in intron 12 of *NELL1* was of suggestive association with QT prolongation.[25] Two SNPs, rs7939346 and rs10833509, both in intron 14 of *NELL1*, were suggestively significantly associated with ovarian aging.[39] However, currently, no studies of the function of NELL-1 in ovarian aging are available.

Conclusions and Future Perspectives

In the relevant GWASs of *NELL1*, a spectrum of diseases, including bone-related metabolic diseases, lipid-metabolic diseases, inflammatory conditions, neuropsychiatric diseases, neurodegenerative disorders, and cancers have been implicated (Figure 3). Taking into account the high replicability of GWASs and verifications of the relevant function studies, *NELL1* gene polymorphisms are likely to have significant associations with a wide variety of disorders in humans.

On the other hand, it is also apparent that these GWASs have some limitations. First, the heritability explained by GWASs is relatively low, and the biological significance and GWAS-based association of a certain gene could be disproportionate. So, the contribution of NELL-1 to the onset and progression of these diseases or to the responsiveness to specific treatments is uncertain. Second, the GWAS-based data on *NELL1* presented in this review are all directly drawn from primary individual studies, which do not include preliminary results from indirect meta-analyses.[87–89] Third, GWAS-based signals could be spurious because of cryptic population stratification. Thus, a sober perception of the exact role of NELL-1 in all of the aforementioned diseases should be further interrogated in post-GWAS function studies.

While GWASs offer new perspectives on the genetic architecture of diseases, the results should be interpreted conservatively. The practical realization of whole-genome sequencing will tremendously improve studies on SNPs in the noncoding regions of *NELL1*. With a better understanding of multiple potential roles of the *NELL1* gene in disorders in humans, this review highlights the future perspectives for research on NELL-1, shifting from investigations on associations to those on function and causation.

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Author Contributions

X.C. contributed to the investigation and drafted the manuscript; J.S. drafted the manuscript; Z.J. provided genetic interpretation and drafted the manuscript; P.H., C.S., and K.T. supervised the work; A.W.J. and B.S. supervised the work and critically revised the manuscript; X.Z. conceptualized and supervised the work and critically revised the manuscript; X.C., C.S., T.K., B.S., and X.Z. acquired funding. All of the authors approved the manuscript and are accountable for all aspects of the work.

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