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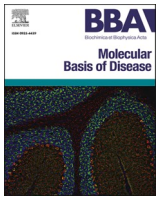

Review

A possible genetic predisposition to suspected hypoxic-ischaemic encephalopathy

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ABSTRACT

Within the last decade, several studies have explored whether there might be a genetic component in hypoxic-ischaemic encephalopathy (HIE) that influences susceptibility to or outcomes following hypoxic-ischaemic injury. This review provides a comprehensive overview of the findings to date from published studies investigating the genetics of HIE. It also highlights some of the challenges faced by researchers, as well as recommendations for future research.

1. Introduction

Neonatal encephalopathy (NE) is a heterogeneous disorder occurring in newborns born from 35 weeks gestation, characterised by signs of altered neurological function. A leading cause of NE is hypoxic-ischaemic encephalopathy (HIE), which is a form of brain injury resulting from reduced oxygenation and cerebral blood flow (CBF) around the time of birth, also termed perinatal asphyxia [1]. The incidence of HIE in high-income countries (HICs) is approximately 1–2 cases per 1000 live births [2]. The incidence in low- to middle-income countries (LMICs) is several-fold higher ranging from approximately 1.5 to 35.2 cases per 1000 live births [3,4]. Possible reasons for the higher incidence of HIE in LMICs include delays in seeking medical care, inadequate monitoring during pregnancy and labour, and reduced or unavailable hospital equipment and staff resources in certain settings [5,6]. Additionally, it is conceivable that genetic factors play a role in differences in incidence between specific population groups, although this remains unexplored.

HIE is diagnosed if (i) a neonate of 35 weeks gestational age or greater presents with clinical signs of brain injury, including reduced consciousness, reduced muscle tone, and seizures, and (ii) if there is evidence of perinatal asphyxia, including the need for prolonged resuscitation, low Apgar scores, and metabolic acidosis either in the cord blood or within one hour of birth. However, HIE can only be diagnosed definitively once other possible causes of NE have been ruled out [7]. It is challenging to entirely rule out all potential causes of NE, especially in rural settings where medical resources necessary to do so, may be unavailable [8,9]. Thus, patients are often diagnosed with suspected HIE at birth. To avoid possible causal misattribution, this review will use the term NE with suspected HIE (NESHIE) instead of HIE from this point forward.

Infants who experience perinatal asphyxia are impacted to varying degrees and show varying degrees of response to treatment [10]. To identify additional factors predictive of outcomes, researchers have begun to explore whether there might be a genetic predisposition to NESHIE or the consequences thereof, including response to treatment [11–14]. Identifying genetic factors associated with NESHIE may (i) provide opportunities for prevention, (ii) identify prognostic information about the likely progression of the disorder, and (iii) inform potential treatment methods [15].

In this review, we provide a brief overview of the pathogenesis of NESHIE, along with its associated risk factors and outcomes. Additionally, we delve into why a genetic predisposition to NESHIE is suspected, providing a comprehensive review of the existing literature on the genetics of NESHIE and suggesting areas for future investigation.

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2. Pathogenesis overview

Hypoxic-ischaemic brain injury occurs in phases of energy failure, termed primary, secondary and tertiary, with a latent recovery phase between the primary and secondary energy phase [16]. During the primary phase of energy failure, decreased blood flow and oxygen delivery to the neonatal brain trigger a switch from aerobic to anaerobic metabolism [17]. This metabolic switch leads to oxidative stress, cellular pathway disruption, mitochondrial dysfunction, neuronal cell death, and vascular reperfusion [18–21]. A brief recovery period, termed the latent phase, ensues after primary energy failure [16]. This phase is characterised by (i) partial restoration of mitochondrial function, (ii) activation of cellular repair mechanisms, and (iii) ongoing inflammation due to proinflammatory signals released in response to injury. Following the latency period, the secondary energy failure phase begins in some infants [22]. During this phase, intracellular pH and cardiorespiratory functioning are normal; however, the arrival of inflammatory cells and mediators, mitochondrial dysfunction, continual oxidative stress, and excitatory neurotoxicity contribute to neuronal cell death [18,20,23–33]. The final phase, tertiary energy failure, may occur in the weeks and months following secondary energy failure. During this time, neuronal repair, remodelling, and regeneration processes are initiated [34]. In infants with severe NESHIE, chronic inflammation hinders regeneration and repair and causes additional neuronal cell death [34].

3. Outcomes

The severity of NESHIE is determined by evaluating clinical signs associated with neurological injury using Thompson and modified Sarnat scoring systems. Infants diagnosed with mild NESHIE, as per the Sarnat scoring system, can recover without facing any long-term consequences after the latent recovery phase. However, up to 25 % of infants with mild NESHIE may have neurological impairment [35]. For infants with moderate to severe NESHIE, the prognosis is often unfavourable. These infants are at high risk of long-term sequelae such as sensory disruption, developmental disorders, epilepsy, cerebral palsy (CP), coma, and death [36]. Patients with physical and/or intellectual disability resulting from NESHIE require a significant amount of medical and supportive care throughout childhood and adulthood, with estimated lifetime medical costs being as high as $ 900,000 [37]. The consequences of NESHIE, therefore, place a considerable psychological, social, and financial burden on patients, their families, and society.

4. Risk factors

Risk factors for NESHIE can be grouped as pre-conceptual, antepartum and intrapartum. Pre-conceptual maternal risk factors include advanced maternal age, diabetes mellitus, obesity, hypertension, and a history of previous foetal death or stillbirth [38,39]. Antepartum risk factors include maternal infection, maternal obesity and gestational weight gain, alcohol and/or drug use, foetal malposition or malpresentation, macrosomia, nuchal umbilical cord, and gestational diabetes [38,39]. Placental abnormalities, including placental insufficiency, funisitis, or vascular abnormalities, are increasingly being recognised as causes of NESHIE. Intrapartum sentinel events, such as umbilical cord prolapse, placental abruption, maternal seizure at birth, prolonged labour, uterine rupture, caesarean section, and intrapartum haemorrhage can contribute to NESHIE [2,39].

5. A possible genetic predisposition

To date, few studies have been published on the genetics of NESHIE, and our understanding of a possible genetic component lags behind that of other neurodevelopmental disorders, such as CP and epilepsy. It is hypothesised that for some neonates, genetic factors may be present, increasing their vulnerability to an environmentally-triggered perinatal asphyxia event [15]. Alternatively, genetic factors may influence the extent of brain injury following asphyxia and the eventual outcomes. A genetic influence may explain why patients exposed to similar insults develop different levels of motor impairment, or why some patients respond well to treatment while others do not.

If NESHIE does have a genetic component, it is likely to involve the contribution of multiple, relatively common genetic variants with a low individual phenotypic effect, but which predispose to NESHIE when combined with environmental risk factors. These genetic factors may influence underlying pathological mechanisms, such as the extent of neuroinflammation, neuronal recovery following vascular reperfusion, and the response to oxidative stress. For instance, genetic variants resulting in increased production of pro-inflammatory cytokines may exacerbate the extent of neuroinflammation experienced during energy failure, reducing the protective effect of therapeutic hypothermia and increasing the risk of severe outcomes such as CP [60].

6. Findings from candidate-gene association studies

Within the last decade, several investigations have been performed to identify genetic factors underlying a predisposition to NESHIE. Most of these have focused on identifying statistically significant associations between variants within pre-determined candidate genes and NESHIE. Table 1 lists the genes and variants investigated in these studies. The candidate genes examined were chosen by the investigators based on their known involvement in processes important to the pathogenesis of NESHIE, including blood flow regulation, inflammation, and oxidative stress [61].

When interpreting the findings of the candidate-gene association studies performed thus far, it is important to consider the limitations of this approach. For example, the sample populations included in the studies have been small, ranging from 11 to 226 patients [62,63]. Such limited sample sizes significantly decrease the statistical power to detect meaningful associations between genetic variants and disease. The studies also differed with respect to population demographics, inclusion/exclusion criteria, patient gestational age, and whether or not the patients had undergone therapeutic hypothermia. Additionally, the techniques used in these studies, such as TaqMan single nucleotide polymorphism (SNP), fluorescent allele-specific polymerase chain reaction (FAS-PCR), and restriction fragment length polymorphism (RFLP) assays, limited the researchers to examine only a few well-known variants within these genes, due to the limited sequence coverage.

Due to the limitations outlined above, independent replication of association study findings is vital to validate the results, ensure consistency across different populations and across various conditions, and guide future research. Genes that have been investigated in at least two NESHIE association studies include caspase recruitment domain family member 8 (*CARD8*), catalase (*CAT*), coagulation factor V (*FV*), glutathione peroxidase 1 (*GPX1*), interleukin 1-beta (*IL1*β), methylenetetrahydrofolate reductase (*MTHFR*), NLR family pyrin domain containing 3 (*NLRP3*), nitric oxide synthase 3 (*NOS3*), prothrombin (*FII*), superoxide dismutase 2 (*SOD2*), and tumour necrosis factor-alpha (*TNF*α) [12–14,62–70].

Studies on the genes of blood coagulation factors *FV* (factor V) and *FII* (prothrombin), the inflammasome gene *NLRP3*, and antioxidant genes *GPX1* and *SOD2*, have not yielded statistically significant associations with NESHIE. In contrast, statistically significant associations (p-value ≤ 0.05) were found between NESHIE and SNPs in other genes, including the inflammasome gene *CARD8*, the antioxidant gene *CAT*, inflammatory cytokine genes *IL1*β and *TNF*α, the folate metabolism gene *MTHFR*, and the gene for the vasodilatory factor gene *NOS3*. However, as described below, these genetic associations have not been replicated consistently across different studies. One reason might be variations in the outcomes examined. While some studies explored susceptibility to NESHIE as a whole, others examined associations of genetic variants

Table 1
Genes and variants investigated in NESHIE genetic association studies.

Gene	Variant/s	Statistically significant association	Studied association/s	Citation
AGT	rs2067853	Yes (risk of NESHIE, *P*-value: 0.043)	NESHIE	[11]
APOE	rs429358 and rs7412 (e2, e3, e4 genotypes)	No	Disability following NESHIE	[101]
AP4B1	rs1217398	No	CP following NESHIE	[86]
	rs1217401	Yes (risk of CP following NESHIE, P-value: 0.042)	CP following NESHIE	
	rs7523862	No	CP following NESHIE	
AP4E1	rs11070824	No	CP following NESHIE	
	rs2249535	No	CP following NESHIE	
	rs2306331	No	CP following NESHIE	
	rs2306335	No	CP following NESHIE	
	rs3825799	No	CP following NESHIE	
AP4M1	rs13309	No	CP following NESHIE	
	rs1534310	No	CP following NESHIE	
	rs2293479	No	CP following NESHIE	
AP4M1	rs4729577	No	CP following NESHIE	
AP4S1	rs11624437	No	CP following NESHIE	
	rs3742920	No	CP following NESHIE	
	rs3784165	No	CP following NESHIE	
	rs7155228	No	CP following NESHIE	
CARD8	rs2043211	Yes (protective against brain damage following NESHIE, *P*-values $\leq$ 0.048)	MRI severity and findings following NESHIE	[12]
		No[†]	CP or epilepsy following NESHIE	[13]
CAT	rs1001179	Yes (risk of CP following NESHIE, P-value: 0.026)	CP following NESHIE	[64]
		Yes (protective against brain damage following NESHIE, P-value: 0.034)	MRI severity and findings following NESHIE	[12]
		No	Epilepsy following NESHIE	[65]
		No	CP or epilepsy following NESHIE	[13]
FII	rs1799963	No	NESHIE, and MRI and US findings following NESHIE	[102]
		No	Sarnat staging, EEG, trans fontanelle US, survival and neurological findings following NESHIE	[66]
		No	MRI or cUS severity and findings following NESHIE	[67]
FV	rs6025	No	NESHIE, and MRI and US findings following NESHIE	[102]
		No	Sarnat staging, EEG, transfontanelle US, survival and neurological findings following NESHIE	[66]
		No	MRI or cUS severity and findings following NESHIE	[67]
FVII	rs6046	No	NESHIE, and MRI and US findings following NESHIE	[102]
GPX1	rs1050450	No	MRI severity and findings following NESHIE	[12]
		No	CP following NESHIE	[64]
		No	Epilepsy following NESHIE	[65]
		No	CP or epilepsy following NESHIE	[13]
HIF1A	rs11549465	No	CP or epilepsy following NESHIE	[103]
	rs11549467	No	CP or epilepsy following NESHIE	
IL10	rs1800896	Yes (protective against PVL following NESHIE, P-value $<$ 0.0001)[†]	PVL following NESHIE	[68]
IL1β	rs1071676	Yes (protective against brain damage following NESHIE, *P*-value: 0.011)	MRI severity and findings following NESHIE	[12]
		No	CP or epilepsy following NESHIE	[13]
	rs1143623	Yes (risk of brain damage following NESHIE, *P*-value $\leq$ 0.04)	MRI severity and findings following NESHIE	[12]
		No	CP or epilepsy following NESHIE	[13]
	rs16944	Yes (risk of brain damage following NESHIE, P-value: 0.04)	MRI severity and findings following NESHIE	[12]
		Yes (risk of PVL following NESHIE, P-value: 0.003)[†]	PVL following NESHIE	[68]
		Yes (risk of CP following NESHIE, P-value: 0.018)[†]	CP following NESHIE	[69]
		No[†]	CP or epilepsy following NESHIE	[13]
IL6	rs1800795	Yes (protective against brain	Sarnat staging, EEG, trans	[66]

(*continued on next page*)

Table 1 (*continued*)

Gene	Variant/s	Statistically significant association	Studied association/s	Citation
		damage following NESHIE, P-value: 0.04)	fontanelle US, survival and neurological findings following NESHIE	
MTHFR	rs1801131	No	MRI or cUS severity and findings following NESHIE	[67]
	rs1801133	Yes (risk of brain damage following NESHIE, *P*-value < 0.05)	MRI or cUS severity and findings following NESHIE	
		Yes (risk of brain damage following NESHIE, P-value < 0.05)	MRI severity and findings following NESHIE	[62]
		No	Sarnat staging, EEG, trans fontanelle US, survival and neurological findings following NESHIE	[66]
MMP2	rs243849	Yes (risk of NESHIE, P-value < 0.01)	NESHIE	[88]
NLRP3	rs35829419	No	MRI severity and findings following NESHIE	[12]
		No	CP or epilepsy following NESHIE	[13]
NOS2	rs1137933	Yes (risk of brain damage following NESHIE, P-value: 0.028)	MRI severity and findings following NESHIE	[102]
		No	US findings following NESHIE	
NOS2	rs3833912[a]	No[†]	CP following NESHIE	[69]
NOS3	rs1799983	No	NESHIE	[70]
		No	Gender, gestational age, birth weight, Apgar score, cUS and MRI findings following NESHIE	[14]
		No	NESHIE	[63]
	rs1800779	No[†]	NESHIE	[70]
		No[†]	Gender, gestational age, birth weight, Apgar score, cUS and MRI findings following NESHIE	[14]
		No	NESHIE	[63]
	rs1800783	No[†]	NESHIE	[70]
		No[†]	Gender, gestational age, birth weight, Apgar score, cUS and MRI findings following NESHIE	[14]
	rs1808593	Yes (risk of NESHIE, P-value: 0.008)	NESHIE	[70]
		Yes (risk of brain damage following NESHIE, *P*-values $\leq$ 0.015)	Gender, gestational age, birth weight, Apgar score, cUS and MRI findings following NESHIE	[14]
	rs2070744	Yes (risk of NESHIE, P-value: 0.009)	NESHIE	[63]
		No[†]	Gender, gestational age, birth weight, Apgar score, cUS and MRI findings following NESHIE	[14]
	rs3918186	No	NESHIE	[70]
		No	Gender, gestational age, birth weight, Apgar score, cUS and MRI findings following NESHIE	[14]
	rs3918188	No	NESHIE	[70]
		No	Gender, gestational age, birth weight, Apgar score, cUS and MRI findings following NESHIE	[14]
	rs3918227	No	NESHIE	[70]
	rs3918227	No	Gender, gestational age, birth weight, Apgar score, cUS and MRI findings following NESHIE	[14]
OLIG2	rs1005573	No[†]	CP following NESHIE	[87]
	rs1059004	No	CP following NESHIE	
	rs146665636	No	CP following NESHIE	
	rs6517135	Yes (risk of CP following NESHIE, P-value: 0.003)[†]	CP following NESHIE	
	rs6517137	No[†]	CP following NESHIE	
	rs9653711	No[†]	CP following NESHIE	
SERPINB2 (*PAI2*)	rs6103	Yes (risk of NESHIE, P-value: 0.013)	NESHIE	[102]
		No	NESHIE and US findings following NESHIE	
SOD2	rs4880	No	MRI severity and findings following NESHIE	[12]
		No	CP following NESHIE	[64]
		No	Epilepsy following NESHIE	[65]
		No	CP or epilepsy following NESHIE	[13]
*TNF*α	rs1799964	Yes (risk of PVL following NESHIE, P-values $\leq$ 0.044)[†]	PVL following NESHIE	[68]
	rs1800629	No	MRI severity and findings following NESHIE	[12]
		No	Sarnat staging, EEG, trans fontanelle US, survival and neurological findings following NESHIE	[66]
		No	CP or epilepsy following NESHIE	[13]

Abbreviations: CP – cerebral palsy; cUS – cranial ultrasound; EEG – electroencephalogram; MRI – magnetic resonance imaging; NESHIE – neonatal

encephalopathy with suspected hypoxic ischaemic encephalopathy; PVL – periventricular leukomalacia; US – ultrasonography.

[a] The rs3833912 variant was withdrawn from dbSNP on June 17, 2016.

[†] Variants with this symbol have had statistically significant associations with NESHIE or NESHIE outcomes as part of a haplotype or in interaction with other variants.

with severe outcomes such as epilepsy or CP as a consequence of NESHIE. This lack of consistency in the end points investigated makes it challenging to compare results effectively.

Below, we discuss the findings for genes with significant variant associations with HIE, focusing on those showing strong associations across multiple studies. Fig. 1 highlights the key aspects of energy failure that these genes are likely to contribute to.

6.1. *CARD8*

CARD8 encodes an inflammasome sensor protein, which initiates an inflammatory response to infection- or stress-associated stimuli [71]. The *CARD8* rs2043211 A > T variant, which results in a truncated form of the CARD8 protein, causes reduced CARD8 protein expression and impaired protein structure and function [72]. Two studies by Esih et al. investigated the role of the *CARD8* rs2043211 T allele in NESHIE outcomes. The first study, conducted on 44 neonates with NESHIE born at the University Children's Hospital, Ljubljana, Slovenia between 2007 and 2019, found that the rs2043211 T allele had a protective effect against moderate to severe brain damage (p-values ≤ 0.048) [12]. These results were supported by the second study, which involved 55 children aged two years or older, all of whom were born at the same hospital between 2007 and 2019 [13]. In this study, it was observed that the frequency of the rs2043211 T allele was lower among children with epilepsy, indicative of a potential protective effect, although this was not statistically significant. However, significant associations were observed between rs2043211 and a reduced risk of epilepsy (p-value = 0.019) in HIE patients carrying two *IL1*β rs16944 G alleles, which are associated with lower levels of inflammatory cytokines compared to its variant counterpart. These findings suggest that genetic factors, acting either alone or in unison to decrease the extent of inflammasome-mediated inflammation, may lessen the severity of the outcomes of NESHIE in some patients.

6.2. *CAT*

CAT encodes the antioxidant enzyme, catalase, which is responsible for converting reactive hydrogen peroxide to water and oxygen. Catalase plays an important role in reducing the toxic accumulation of reactive oxygen species (ROS) and restoring tissue oxygen levels following a hypoxic ischaemic event [73]. The *CAT* rs1001179 C > T variant alters the transcription binding site of the *CAT* promotor region, leading to increased transcriptional activity and higher catalase levels [74]. Interestingly, some studies have found that the rs1001179 T allele can have both protective and harmful effects in conditions where excessive production of ROS contributes to disease progression. For example, a study by Gromadzka et al. found that patients homozygous for the rs1001179 T allele were likely to experience early-onset neurological symptoms of Wilson disease [75]. Another study by Quick et al. found that women taking hormone replacement therapy who were also carriers of the rs1001179 T allele had a higher risk of postmenopausal breast cancer [76].

In the case of NESHIE, it seems likely that the rs1001179 T allele would reduce the risk of severe outcomes; however, studies investigating this have produced contradictory results. A study by Esih et al. on 80 NESHIE patients of European descent born at the University Children's Hospital, Ljubljana, Slovenia, between 2011 and 2014, reported a three-fold higher risk of CP in NESHIE patients carrying the *CAT* rs1001179 T allele (p-value = 0.026) [64]. In contrast, the same group later reported a protective effect of this variant on magnetic resonance imaging (MRI) brain injury in NESHIE patients. This was observed in a study of 44 patients born at the same hospital between 2007 and 2019 (p-value = 0.034) [12]. The authors hypothesised that the observed difference in outcomes may be attributed to differences in patient treatment regimes, as the patients recruited for the second study had undergone therapeutic hypothermia. In contrast, those for the initial study had not [12].

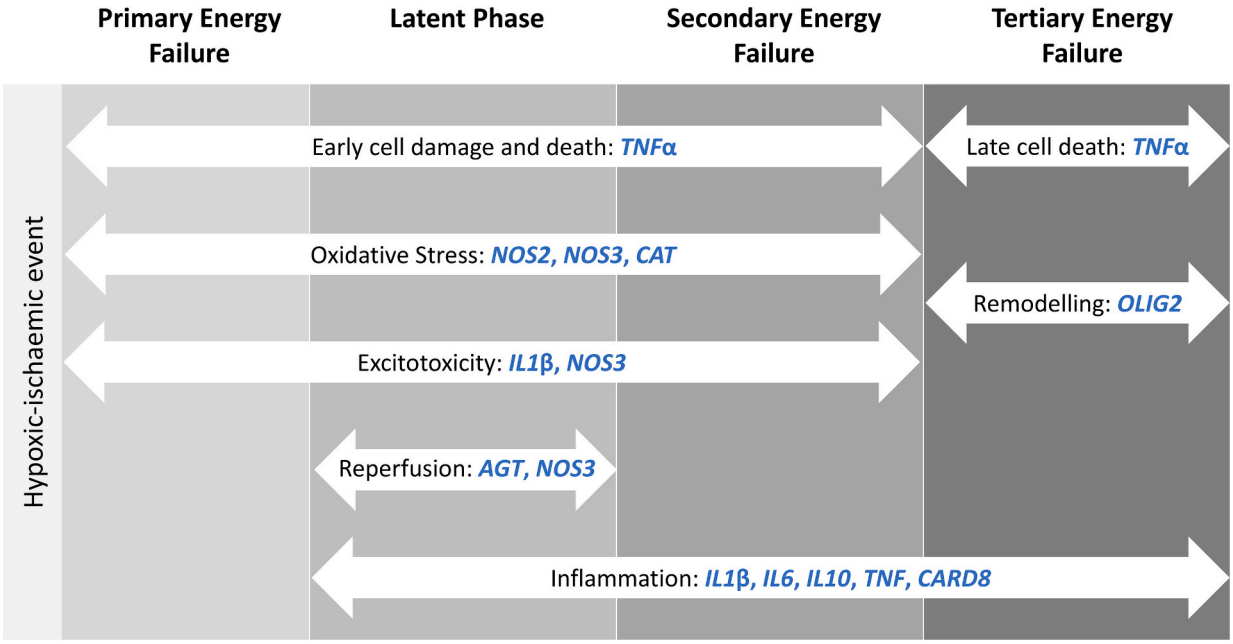

Fig. 1. Energy failure phases connected to genes associated with a genetic predisposition to neonatal encephalopathy with suspected hypoxic ischaemic encephalopathy (NESHIE). Although variants in *AP4B1*, *MMP2*, and *SERPINB2* have also been associated with NESHIE, these genes were not included in the illustration due to uncertainty of the roles of these genes in energy failure.

6.3. *IL1β*

Several *IL1*β SNPs have been investigated for a possible association with NESHIE [12,13,68,69]. Notably, rs16944 yielded reproducible results in several independent investigations, indicating associations with distinct severe outcomes following NESHIE. The rs16944 G > A variant is located upstream of the *IL1*β gene. The A allele of rs16944 is known to result in increased levels of the *IL1*β proinflammatory cytokine [77]. Studies by Esih, Gabriel, Torres-Merino and colleagues found a link between the rs16944 A allele and NESHIE outcomes. Esih et al. conducted a retrospective study on 44 patients from the University Children's Hospital in Ljubljana, Slovenia between 2007 and 2019 [12]. They discovered that two *IL1*β SNPs, rs16944 and rs1143623, were associated with a three-fold higher occurrence of NESHIE brain damage, particularly in the posterior limb of the internal capsule (PLIC) and the basal ganglia (p-value = 0.04). In a follow-up study, the same team reported an association between *IL1*β rs16944 and *CARD8* rs2043211 variants and a 13-fold increased risk of epilepsy after NESHIE (p-value = 0.044) [13]. Similarly, Gabriel et al. recruited 85 newborns with NESHIE from the Children's Hospital and Maternity of the São José do Rio Preto Medical School in 2015 and found that the rs16944 CT and TT genotypes were associated with a 23-fold higher risk of brain damage, specifically periventricular leukomalacia (PVL), in infants with NESHIE (p-value = 0.003) [68]. Lastly, Torres-Merino et al. conducted a study on 48 NESHIE patients diagnosed with CP from the Neuropediatrics Department of the Comprehensive Rehabilitation Centre in Tlalnepantla de Baz, Mexico, and found a link between rs16944 and a two-fold higher risk of CP in these infants (p-value = 0.018) [69].

6.4. *TNFα*

Two variants in the gene of the proinflammatory cytokine *TNF*α have been investigated for potentially conferring a genetic predisposition to NESHIE – rs1799964 T > C and rs1800629 G > A. Although investigations of rs1800629 did not yield statistically significant findings [12,13,66], a study by Gabriel et al. on 50 neonates with NESHIE, recruited at the Children's Hospital and Maternity of the São José do Rio Preto Medical School, São Paulo, Brazil, between April and July 2015, found a significant association between rs1799964 and NESHIE [68]. In this study, carriers of at least one copy of the rs1799964 C allele were reported to have a two-fold higher risk of PVL following NESHIE compared to individuals homozygous for the T allele (p-values ≤ 0.044). PVL, which is a form of white matter brain injury, is a leading cause of CP [78]. Notably, the *TNF*α rs1799964 C allele has been associated with CP in a study of 60 preterm neonates from the Special Hospital for Children with Neurodevelopmental and Motor Difficulties, Zagreb, Croatia, of which 35 had PVL [79]. These findings suggest a possible role for *TNF*α variants in the predisposition to CP due to NESHIE. Additional studies with larger sample numbers are necessary to confirm this.

6.5. *MTHFR*

MTHFR encodes methylenetetrahydrofolate reductase, an enzyme necessary for the folate-mediated conversion of homocysteine into methionine. *MTHFR* rs1801133 G > A is associated with decreased enzymatic activity and impaired homocysteine metabolism, especially in individuals with a folate deficiency [80]; this can lead to hyperhomocysteinaemia, secondary risk of blood clot formation and endothelial damage [81]. Studies investigating the influence of the *MTHFR* rs1801133 G > A variant on NESHIE have yielded inconsistent results. A study by de Kremer and Grosso on 11 NESHIE infants with multicystic encephalomalacia and severe brain vasculopathy and their respective mothers, recruited between 1999 and 2002, reported an association between NESHIE outcomes and rs1801133 (p-value < 0.05) [62]. The authors found that all patients and their mothers were either heterozygous or homozygous for the rs1801133 A allele. Notably, the mothers of all patients had hyperhomocysteinaemia. A later study by Harteman et al. produced similar findings. These authors studied 118 NESHIE infants from the Wilhelmina Children's Hospital in Utrecht, The Netherlands, recruited between 2006 and 2012 [67]. They reported independent associations between rs1801133, plasma homocysteine, and white matter/watershed (WM/WS) injury in NESHIE infants (p-values < 0.05). More specifically, individuals with the GA and AA genotypes were found to have a three- and nine-fold higher risk of WM/WS injury, respectively. However, surprisingly, the authors found no association between the rs1801133 genotype and increased levels of plasma homocysteine. The authors hypothesised that elevated maternal homocysteine may be a key predictor of high neonatal homocysteine levels [82], independent of the rs1801133 genotype. This suggests a possible multifactorial role for rs1801133 in the predisposition to NESHIE outcomes that is yet to be established.

In contrast to the findings of de Kremer and Grosso, and Harteman and colleagues, Calkavur et al. found no association between rs1801133 and NESHIE outcomes in patients recruited from the Department of Neonatology at Ege University and Dr. Behçet Uz Children's Hospital in Izmir, Turkey, between 2006 and 2009 [66]. The authors proposed that the absence of an association may be due to genetic heterogeneity between population groups on disease outcomes. It is important to note that the differences in results observed in individual studies may have been influenced by additional confounding factors, such as the intake of supplementary folate during pregnancy, which is known to lower plasma homocysteine levels [83]. The de Kremer and Grosso study reported that the mothers had not taken supplementary folate. Information on the mothers' supplementary folate intake was not provided by Harteman et al. and Calkavur et al.

6.6. *NOS3*

The *NOS3* gene encodes endothelial nitric oxide synthase (NOS), an enzyme involved in the synthesis of nitric oxide (NO). Nitric oxide is involved in several biological processes including the regulation of endothelial vascular tone and neurotransmission [84,85], which are relevant to the pathogenesis of NESHIE. In total, eight *NOS3* variants were studied for an association with NESHIE [14,63,70]. Of these, two variants, rs1808593 G > T and rs2070744 C > T, showed statistically significant associations.

Two studies conducted by Samija et al. reported an association of rs1808593 with NESHIE. The first found an association between the rs1808593 T allele and susceptibility to NESHIE in 110 neonates admitted to the Clinical Hospital in Split, Croatia, between 1992 and 2008 (p-value = 0.008, odds ratio: 9.625) [70]. The second study reported an increased risk of moderate to severe brain injury with the T allele in the same group of NESHIE patients (p-values ≤ 0.015) [14]. The group also observed an association between the rs1800783 T, rs1800779 G, and rs2070744 T haplotype and premature NESHIE infants with severe brain injury [14]. In addition to the findings of Samija and colleagues, Wu et al. reported an association of the T allele of another *NOS3* variant, rs2070744, with susceptibility to NESHIE in a study of 226 patients admitted to the Xinxiang Central Hospital in China between 2008 and 2013 (p-value = 0.009, odds ratio: 8.795) [63].

6.7. *Other potentially important genes*

Individual studies have demonstrated statistically significant SNP associations with NESHIE in several genes, including angiotensinogen (*AGT*), adapter related protein complex 4 beta 1 (*AP4B1*), *IL10*, *IL6*, matrix metallopeptidase 2 (*MMP2*), *NOS2*, oligodendrocyte transcription factor 2 (*OLIG2*) and serpin family B member 2 (*SERPINB2*) [11,66,68,86–88]. However, these findings have not been replicated in other studies. Nevertheless, these genes have potential relevance to NESHIE due to their pivotal roles in its pathogenesis and their association with similar disease phenotypes.

For example, *AGT* is involved in blood pressure modulation and is crucial for restoring cerebral blood flow following hypoxic-ischaemia [89]. Genetic variants in *AGT* have been associated with hypertension and renal tubal dysgenesis, a condition characterised by abnormal foetal kidney development and hypotension [90]. Similarly, *MMP2* has been implicated in blood flow regulation by controlling blood vessel remodelling through the cleavage of type IV collagen, and is associated with diseases characterised by compromised blood flow, such as diabetic retinopathy and stroke [91,92]. Additionally, *SERPINB2* is involved in regulating blood clot formation and fibrinolysis [93]. Although indirectly, it may influence the regulation of blood flow in hypoxic-ischaemia.

IL10 and *IL6* encode anti- and pro-inflammatory cytokines, respectively. Variants in these genes have been associated with neurodevelopmental disorders such as autism and CP [94,95]. The NO product of *NOS2* potentiates the inflammatory cascade, initiated by *IL6* in response to tissue damage and infection [96]. Additionally, NO produced by *NOS2* is involved in both the relaxation of blood vessel smooth muscle cells during vasodilation and neurotransmission, due to its role as a retrograde neurotransmitter [85,97].

AP4B1 encodes a protein subunit that regulates protein trafficking within neurons and is associated with neurodevelopmental disorders such as CP [98]. This raises the possibility that it may contribute to neuronal outcomes in NESHIE. Lastly, *OLIG2*, which encodes a transcription factor essential for oligodendrocyte and motor neuron differentiation, is associated with schizophrenia and obsessive-compulsive disorder [99,100]. It may also play a role in the severity of neurodevelopmental outcomes as a consequence of NESHIE.

7. Findings from clinical panel, microarray, whole exome, whole genome studies

Using gene panels, microarrays, whole exome sequencing (WES), and whole genome sequencing (WGS), clinical studies have identified pathogenic or likely pathogenic variants in NESHIE patients. These studies did not test for the statistical association of these variants and NESHIE but instead hypothesised the involvement of these variants in the clinical phenotype on a case-by-case basis, based on predicted variant effect, gene function, and known involvement of the gene in Mendelian disease. It is important to consider the replication of findings across multiple NESHIE patients when analysing these studies, as some of the identified variants may not be relevant to NESHIE.

Table 2 summarises the findings from these studies. Findings involving patients with the NESHIE phenotype from CP studies are also included since so few NESHIE-specific studies have been published. Thus far, genes/gene families with causative variants reported in more than one patient include the type IV collagen (*COL4*) family of genes (*COL4A1* and *COL4A4*), G protein subunit alpha O1 (*GNAO1*), kinesin family member 1A (*KIF1A*), and the sodium voltage-gated channel (*SCN*) family of genes (*SCN1A* and *SCN9A*) [15,104–106]. The findings reported for these genes are described in more detail below.

7.1. COL4A1 and COL4A4

The *COL4* gene family encodes proteins that make up type IV collagen, an essential component of the vascular endothelial basement membrane [107]. Variants in *COL4* genes have been implicated in conditions pertaining to dysregulated blood flow including stroke and hypertension, as well as CP [108]. Notably, variants in *COL4* genes have also been reported in patients with NESHIE. A de novo variant (c.2263G > A, p.G755R) in *COL4A1* was reported in a patient with spastic quadriplegic CP that had birth asphyxia [105]. Similarly, a variant of unknown inheritance (c.4720C > T, p.Q1574*) was reported in a patient with spastic quadriplegic CP with lactic acidosis and MRI evidence of hypoxic brain injury following MRI, and seizures [104]. The authors deemed these variants to be causative of the observed phenotypes in these patients based on variant pathogenicity. It is plausible that variants in *COL4* genes may disrupt blood flow restoration following hypoxic-ischaemia, increasing the severity of brain injury. This hypothesis is supported by the findings by Zhang et al. described in the previous section, which demonstrate an association between the *MMP2* variant, rs243849, and NESHIE risk [88]. The functions of *MMP2* and *COL4* genes are closely related, as *MMP2* is involved in the enzymatic degradation of type IV collagen as part of the vascular remodelling process [109].

7.2. GNAO1

GNAO1 encodes a subunit of the heterotrimeric G-protein signal transduction complex, which is involved in various transmembrane signalling pathways, including the neuronal system [110]. Variants in *GNAO1* have been causally linked to epileptic encephalopathy, characterised by seizures, developmental delay and abnormal movement [111]. Two independent studies reported de novo variants in *GNAO1* in two NESHIE patients that were deemed causative of the patients' phenotype. In the first, a heterozygous de novo variant (c.118G > A, p.G40R) was identified in a male patient diagnosed with mild NESHIE in the absence of an identifiable sentinel asphyxia event [106]. The second reported a heterozygous de novo variant (c.662C > A, p.A221D) in a patient with hemiplegic CP with birth asphyxia, intraventricular haemorrhage, and developmental delay [105]. These findings suggest that damaging variants in *GNAO1* may predispose to more severe outcomes, such as seizures and encephalopathy, in patients with birth asphyxia. However, additional studies are needed to confirm this. Since birth asphyxia was suspected but not confirmed for the first patient described above, the *GNAO1* variant may have caused a phenotype resembling HIE in the absence of birth asphyxia.

7.3. KIF1A

Proteins encoded by *KIF1A* play crucial roles in neuronal synaptic vesicle transport and neuron survival and development [112]. Pathogenic variants within *KIF1A* are associated with KIF1A-associated neurological disorders (KANDs), which are characterised by a broad phenotypic spectrum including spastic paraplegia, intellectual disability, cerebral and optic atrophy, progressive white matter changes, seizures, and gastrointestinal dysfunction [113,114]. Recent studies have identified potentially disease-causing variants in *KIF1A* that may contribute to the clinical phenotypes of individuals diagnosed with NESHIE. Woodward et al. reported a *KIF1A* missense variant (c.699G > C, p.E233D) of uncertain significance in a NESHIE patient with multiple parenchymal haemorrhages, and periventricular and deep white matter changes. Similarly, Parobek et al. reported a de novo pathogenic variant (NM_004321:c.757G > A, p.E253K) in another NESHIE patient with static encephalopathy, hypertonia, motor delay, patent foramen ovale, gastrointestinal dysmotility, and liver dysfunction. Notably, both NESHIE patients with *KIF1A* variants displayed phenotypes overlapping those observed in patients with KANDs. However, it is unclear at this stage whether the variants predispose to hypoxic-ischaemia or influence subsequent outcomes, or if they instead indicate a condition mimicking HIE [15].

7.4. SCN1A and SCN9A

Similar to *GNAO1*, variants in the *SCN* family of genes play an essential role in the depolarisation of cell membranes during action potentials. These variants have been implicated in neurodevelopmental disorders characterised by seizures, developmental delay, and abnormal movement [115]. Likewise, proposed causative variants in *SCN* genes have been reported in patients with NESHIE. A heterozygous variant (c.2492_2495delGTTC, p.R830Hfs) in *SCN1A* was reported in a patient with CP, birth asphyxia, intraventricular stroke/haemorrhage, seizures,

Table 2
Genes and variants reported in patients from WES and WGS studies whose phenotype includes NESHIE or perinatal asphyxia.

Gene	Variant description (as per study authors)	In-silico variant effect (as per study authors)	Inheritance	Patient clinical features (as per study authors)	Method	Citation
ABCD1	ChrX:15299154; NM_001127660.1: c.824G > A, p.R275Q‡	NS	Unknown	Spastic diplegic cerebral palsy, birth asphyxia, mild degenerative changes of the knees	WES	[105]
ACTA1	NM_001100.4:c.346G > A, p.A116T	Likely pathogenic	De novo	Hypoxic-ischaemic encephalopathy, hypotonia, seizures, decerebrate rigidity, respiratory failure, abnormal electroencephalogram, cerebral haemorrhage, macrocephaly, patent ductus arteriosus, biventricular hypertrophy, arachnodactyly, long bone fractures, posteriorly rotated ears, frontal bossing	WGS/WES	[117]
ADGRG4	ChrX:135427205C > T; p.P447L†	Probably damaging (MetaSVM)	NS	Spastic-dystonic quadriplegic cerebral palsy, possible hypoxic-ischaemic encephalopathy, microcephaly, intellectual disability, epilepsy, bilateral hypodensity of the anterior and posterior periventricular white matter, foramen ovale	WES	[118]
AGAP1	NM_014914.5:c.1462G > A, p.D488N	Deleterious	Not maternal	Cerebral palsy, hypoxic-ischaemic encephalopathy with evidence of diffuse hypoxic injury, postnatal microcephaly, intermittent exotropia, optic nerve atrophy, global developmental delay, failure to thrive, multiple hyperpigmented macules	WES	[119]
CDKL5	c.2388_2404DEL, p.S798V FS*9	NS	NS	Hypoxic-ischaemic encephalopathy, delivery induced for gestational hypertension, foetal bradycardia, global developmental delay, seizure-free (previous infantile spasms), minimal meconium, low tone, born pale, not breathing	Panel	[15]
COL4A1	Chr13:110831699; NM_001845.6: c.2263G > A, p.G755R	Pathogenic	De novo	Spastic quadriplegic cerebral palsy, birth asphyxia	WES	[105]
COL4A4	Chr2:227872823G > A; NM_000092.5: c.4720C > T, p.Q1574*	Pathogenic	Unknown	Spastic quadriplegic cerebral palsy with evidence of dyskinesia, hypoglycaemia, lactic acidosis, apnoea, seizures, stiff abnormal flexion and extension, fine tremor, difficulties maintaining midline orientation, extensive injury of fronto-parietal white matter basal ganglia and thalamic nuclei, symmetric hypoxic brain injury, moderate hearing deficits, squint, cortical visual impairment, cranial nerve palsies, asthma, gastro-oesophageal reflux disease, feeding difficulties, poor weight gain	WGS	[104]
DUOX2	NM_014080.4:c.3616G > A, p.A1206T, NM_014080.4:c.2654G > T, p.R885L	Pathogenic/likely pathogenic	Inherited	Hypoxic-ischaemic encephalopathy, decreased foetal movement, nephropathy, hypoglycaemia, lactic acidosis, hepatomegaly and hepatic failure, hypothyroidism, anaemia	WGS/WES	[117]
EIF4E2	Chr2:233422672C > G; NM_004846: c.214C > G, p.P72A	Deleterious (MutationTaster)	De novo	Cerebral palsy, parasagittal and basal ganglia restricted diffusion suggesting perinatal hypoxic-ischaemic injury, periventricular leukomalacia	WES	[120]
F8	ChrX:154088793 T > C; p.T2272A†	Probably damaging (MetaSVM)	NS	Spastic-dystonic quadriplegic cerebral palsy, possible hypoxic-ischaemic encephalopathy, microcephaly, intellectual disability, epilepsy, bilateral hypodensity of the anterior and posterior periventricular white matter, foramen ovale	WES	[118]
GARS1	Chr7:30671863; NM_002047.4: c.1904C > T, p.S635L	NS	Maternal	Quadriplegic dystonic cerebral palsy, birth asphyxia, psychiatric issues, chronic bilateral low back pain with sciatica	WES	[105]
GBE1	NM_000158.3:c.1693C > T, p.R565W	Likely pathogenic	Inherited	Hypoxic-ischaemic encephalopathy, flexion contractures, hypotonia, hyporeflexia, cephalohematoma, seizure, pulmonic stenosis, foetal growth restriction, polyhydramnios, single palmar crease	WGS/WES	[117]
GNAO1	Chr16:56370711; NM_138736.3: c.662C > A, p.A221N	Likely pathogenic	De novo	Cerebral palsy, birth asphyxia, intraventricular haemorrhage, parkinsonism, developmental delays, right sided hemiparesis, spasticity, difficulty walking, ataxia toe walking, gliosis involving periventricular deep white matter for both cerebral hemispheres	WES	[105]
	c.118G > A, p.G40R	NS	De novo	Neonatal encephalopathy, mild hypoxic-ischaemic encephalopathy, poor tone, apnoea, generalized tonic clonic seizures, bilateral temporal sharp waves, bilateral increased signal in frontal and peritrigonal white matter	WES	[106]
ISY1	c.607 + 1G > A	Uncertain significance	NS	Hypoxic-ischaemic encephalopathy, refractory epilepsy, global developmental delay, foetal bradycardia, meconium, seizures, diffusion restriction in the frontal, parietal, and occipital lobes and basal ganglia	WES	[15]

(*continued on next page*)

Table 2 (*continued*)

Gene	Variant description (as per study authors)	In-silico variant effect (as per study authors)	Inheritance	Patient clinical features (as per study authors)	Method	Citation
KCNQ2	c.821C > T, p.T274M	NS	NS	Hypoxic-ischaemic encephalopathy, emergency caesarean, multifocal discharges and electroclinical seizures, T1 hyperintensities in basal ganglia and cerebral cortex, refractory epilepsy, global developmental delay	Panel	[15]
KIF1A	c.699G > C, p.E233D	Uncertain significance	NS	Hypoxic-ischaemic encephalopathy, induced delivery for gestational hypertension, multiple parenchymal haemorrhages, repeat showed periventricular and deep white matter T2 changes	Panel	[15]
	NM_004321:c.757G > A, p.E253K	Pathogenic	De novo	Hypoxic-ischaemic encephalopathy, static encephalopathy, gastrointestinal dysmotility, hypertonia, motor delay, visual impairment, patent foramen ovale, acute lactic acidaemia, liver dysfunction	WGS/WES	[117]
MECP2	ChrX:153297682; NM_001110792.2: c.353G > A, p.G118E	Uncertain significance	NS	Spastic hemiplegic cerebral palsy, birth asphyxia, failure to thrive, global developmental delay, generalized hypotonia, spasticity, metatarsus adductus, mild dysmorphism, hypotonia	WES	[105]
MFF	NM_020194.5:c19_20delAGinsTT, pS7F	Uncertain significance	Maternal and paternal	Spastic cerebral palsy, hypoxic-ischaemic encephalopathy, global developmental delay, defective mitochondrial and peroxisomal fission	WES	[121]
MFN2	Chr1:12069698; NM_001127660.1: c.2119C > T, p.R707W‡	NS	Unknown	Spastic diplegic cerebral palsy, birth asphyxia, mild degenerative changes of the knees	WES	[105]
MTFMT	c.994C > T, p.R332*	NS	NS	Hypoxic-ischaemic encephalopathy, delivery induced for gestational hypertension, surfactant deficiency, intraventricular haemorrhage, refractory epilepsy, global developmental delay	WES	[15]
NPHP1	102 kb deletion in Chr 2q13	NS	NS	Hypoxic-ischaemic encephalopathy, induced delivery, minimal meconium, maternal chorioamnionitis, shoulder dystocia with forceps, bilateral perisylvian polymicrogyria, global developmental delay	Microarray	[15]
NS	Chr15:30971530-32455607del	Pathogenic	NS	Hypoxic-ischaemic encephalopathy, emergency caesarean, abruption, abnormal electroencephalogram, coarctation of the aorta, hypospadias	WGS/WES	[117]
NS	Novel deletion in Chr 19	NS	Novel	Spastic-dystonic cerebral palsy, hypoxic-ischaemic encephalopathy, intellectual disability, seizures	WES	[122]
PANK2	Chr20:3893282; NM_153638.3:c.1412 + 1G > C	Likely pathogenic	Maternal and paternal	Spastic quadriplegic cerebral palsy, birth asphyxia, generalized dystonia, severe scoliosis, developmental delays, hypothyroidism, neurogenic bladder, pantothenate kinase-associated neurodegeneration	WES	[105]
PTPN11	NM_002834.3:c.1507G > A, p.G503R	Pathogenic	De novo	Hypoxic-ischaemic encephalopathy, hypotonia, areflexia, abnormal electroencephalogram, respiratory distress, hydrops foetalis, pleural effusion, decreased foetal movement, patent foramen ovale, patent ductus arteriosus	WGS/WES	[117]
SCN1A	Chr2:166895942; NM_006920.6: c.2492_2495delGTTC, p.R830Hfs	Pathogenic	Evidence of parental mosaicism	Spastic quadriplegic cerebral palsy, birth asphyxia, developmental delays, seizures, foetal tachycardia, intraventricular stroke/haemorrhage, cerebral atrophy and temporal arterial stenosis at 5 yrs. old	WES	[105]
SCN9A	c.4652G > A, p.C1551Y	NS	NS	Hypoxic-ischaemic encephalopathy, refractory epilepsy, global developmental delay, apnoeic, low tone, diffuse regions of acute diffusion restriction in bilateral cortex and deep grey matter	WES	[15]
SMARCB1	Chr22:24176330; NM_003073.5: c.1121G > A, p.R374Q	Likely pathogenic	De novo	Spastic cerebral palsy, birth asphyxia, severe scoliosis, global developmental delay, cortical visual impairment, nystagmus, strabismus, duplicated right collecting system with mild right hydronephrosis, sensorineural hearing loss	WES	[105]
SSX1	ChrX:48125721G > C; c.467-1G > C†	Probably damaging (MetaSVM)	NS	Spastic-dystonic quadriplegic cerebral palsy, possible hypoxic-ischaemic encephalopathy, microcephaly, intellectual disability, epilepsy, bilateral hypodensity of the anterior and posterior periventricular white matter, foramen ovale	WES	[118]
SYT14	Chr1:210056221–210262039dup	NS	Maternal	Spastic-dystonic quadriplegic cerebral palsy, hypoxic-ischaemic encephalopathy, neonatal seizures, foetal bradycardia, periventricular deep white matter gliosis	WGS	[104]
ZMYM2	NM_003453.4:c.2843dup, p. E949RfsTer11	Pathogenic	De novo	Cerebral palsy, hypoxic-ischaemic encephalopathy with no obvious perinatal adverse event, restricted diffusion in thalami and globus pallidum, ascending aortic dilation, poor weight gain, anorectal malformation, perineal fistula, strabismus	WES	[119]

‡,†: Variants with one of these symbols were reported in the same patient.
Abbreviations: A – adenine; Chr – chromosome; C – cytosine; CP – cerebral palsy; cUS – cranial ultrasound; Del – deletion; EEG – electroencephalogram; G – guanine; MRI – magnetic resonance imaging; NESHIE – neonatal encephalopathy with suspected hypoxic ischaemic encephalopathy; NS – not stated; p – protein; PVL – periventricular leukomalacia; US – ultrasonography; WES – whole exome sequencing; WGS – whole genome sequencing.

and developmental delay [105], while another variant (c.4652G > A, p.C1551Y) in *SCN9A* was reported in a patient diagnosed with NESHIE that had epilepsy and global developmental delay [15]. For this patient, an improvement was noted following treatment with sodium channel blockers, strengthening evidence for the variant's involvement. However, it was not clear whether the variants in *SCN* genes were involved in a predisposition to severe NESHIE outcomes, since no risk factors for perinatal asphyxia were identified for the patient. If *SCN* variants can predispose to severe NESHIE outcomes, prompt recognition of contributing *SCN* variants can aid in patient treatment. For instance, sodium channel blockers have shown potential in treating seizures in patients with sodium channel epilepsies [116].

8. Future research directions

As research on a genetic predisposition to NESHIE evolves, the importance of implementing full-genome genetic methodologies becomes increasingly apparent. Although next-generation sequencing (NGS) including WES and WGS techniques have been used to identify variants which could potentially contribute to the NESHIE phenotype, few studies using these techniques have been conducted thus far. Additionally, the studies conducted have not examined the statistical association of variants with NESHIE at a genome-wide scale, but have instead focused on a select few variants hypothesised to be involved in the NESHIE phenotype due to a predicted disease-causing effect and known involvement in other Mendelian diseases. Thus, variants without known disease-causing effects or located in unexplored regions of the genome will go unnoticed.

To resolve this issue, genome-wide association studies (GWAS) are an important next step to unveiling whether genetic factors could predispose to NESHIE or outcomes following NESHIE. GWAS, which involve testing statistical associations between variants and disease phenotypes at a genome-wide scale, have been successful at finding associations between variants and other complex neurological disorders, such as multiple sclerosis and restless leg syndrome [123,124]. Although GWAS resolve the inherent bias and low throughput of candidate-gene studies, they are not without their shortcomings. For instance, failing to account for population stratification, population admixture, and relatedness in GWAS, can produce false-positive or false-negative results [125]. Additionally, due to the high number of genetic variants being tested in GWAS, small but relevant variant effects may go unidentified if a study is underpowered [125]. Thus, to detect relevant associations with NESHIE using GWAS, a study would require adequate statistical power, and genetically-matched, unrelated patient and control groups.

9. Conclusion

Thus far, variants in over a dozen genes have been identified as possible contributors to a genetic predisposition to NESHIE. However, since complex genetic disorders are known to be caused by interactions between many, sometimes even hundreds of genetic, epigenetic, environmental, social, and host factors [126] our understanding of the genetic basis of NESHIE is far from complete.

The search to identify genetic predisposing factors to NESHIE has been complicated by study limitations, including small sample sizes, differences in study methodology, and sample population ethnicities. Notably, a major limitation of the investigations undertaken thus far lies in the challenge of distinguishing whether the identified variants are contributing to the aetiology of NESHIE or are indicative of a separate genetic condition masquerading as NESHIE [15]. To better understand the role of genetic variation in the aetiology of NESHIE, additional studies must be performed with larger sample sizes and control measures for confounding factors such as population stratification.

Fortunately, with recent advances in NGS technologies, researchers can identify previously undiscovered novel and rare variants, which may otherwise have remained undetected when employing candidate gene-based approaches [127,128]. For instance, NGS technologies have helped identify over 500 genes containing one or more predicted deleterious variants in patients with CP [129]. Surprisingly, very few studies have explored a genetic predisposition to NESHIE using these approaches. By using these technologies to investigate a genetic predisposition to NESHIE, additional variants that contribute to susceptibility or severity may be identified.

The discovery of a genetic basis for NESHIE may broaden our current understanding of the condition, and allow for more individualised treatment options, as has been the case for example with cystic fibrosis. By identifying subtypes of NESHIE that respond better or worse to certain treatments, medical professionals can provide more personalised care. Additionally, it may be possible to predict which neonates are likely to (i) develop NESHIE or (ii) have severe outcomes following an environmental trigger. This would allow for early interventions to be prioritised for these patients, potentially preventing the long-term implications of poor outcomes for patients, their families and society as a whole.

CRediT authorship contribution statement

M.A. Holborn: Writing – review & editing, Writing – original draft, Investigation, Data curation, Conceptualization. **J. Mellet:** Writing – review & editing, Supervision. **F. Joubert:** Writing – review & editing, Supervision. **D. Ballot:** Writing – review & editing. **M.S. Pepper:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors have no competing interests to declare.

Data availability

No data was used for the research described in the article.

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