Poudel *et al.*
Egyptian Journal of Medical Human Genetics (2022) 23:95
https://doi.org/10.1186/s43042-022-00307-8

Egyptian Journal of Medical Human Genetics

REVIEW

Open Access

Role of engrailed homeobox 2 *(EN2)* gene in the development of the cerebellum and effects of its altered and ectopic expressions

Phanindra Prasad Poudel[1,2], Chacchu Bhattarai[1,2], Arnab Ghosh[3] and Sneha Guruprasad Kalthur[1*]

Abstract

Background: Morphological organization, folial pattern formation and establishment of the neural circuitry within the cerebellum are the important events taking place during the development of the cerebellum. Expression of engrailed homeobox 2 (*EN2*) gene plays an essential role in taking place of these events in the developing cerebellum.

Main body: A search was performed by following the PRISMA guidelines to review the role of the *EN2* gene in the development of the cerebellum. Human and animal in vivo and in vitro studies showed that expression of the *EN2* gene maintains the normal development of the cerebellum, morphological organization, cerebellar foliation, fissure formation, establishment of the afferent topography, molecular pattern formation and patterned gene expression in the developing cerebellum. Altered expression of the *EN2* gene changes the morphology and folial pattern of the cerebellum, whereas its activation rescues these defects. *EN2* gene polymorphism is reported as a susceptible cause for autism spectrum disorder (ASD). Ectopic expression of *EN2* gene may result cancer and it also may play anti-oncogenic role depending on the organ of its expression.

Conclusion: Expression of the *EN2* gene is essential for the normal development of the cerebellum. Its altered expression results deformed cerebellum, polymorphysm is associated with autism and ectopic expression may results cancer.

Keywords: *EN2* gene, Development of the cerebellum, Morphological organization, Folial pattern formation, Neural circuitry, Cancer, Autism spectrum disorder

Background

Engrailed homeobox 2 (*EN2*) is a member of homeobox containing engrailed gene family. It is located in band 7q36.3 of human chromosome 7 within 155,458,129–155,464,831 forward strand. It consists of 3395 base pairs with 2 exon counts. In mice, it is located on chromosome 5. It is a protein encoding gene and expressed during the development of the cerebellum [1].

The human genome project reveals 16 major classes of more than hundred homeobox, 180 base pairs of DNA sequence, containing genes. Homeobox containing genes encode homeodomain protein, a structural motif that binds to DNA and regulates gene expression [2]. For the induction of the *EN2* gene, there is an expression of zinc finger of the cerebellum protein family member 1 (Zic1). Zic1 is activated after inhibition of bone morphogenetic protein (BMP) signaling by Noggin or Chordin. Zic1 and other zinc finger of the cerebellum (Zic) proteins

*Correspondence: sneha.guruprasad@manipal.edu

[1] Department of Anatomy, Kasturba Medical College, Manipal, Manipal Academy of Higher Education (MAHE), Karnataka 576104, India
Full list of author information is available at the end of the article

synergize with other factors to activate expression of wingless related integration site family member 1 (*Wnt1*) and other wingless related integration site (*Wnt)* genes. Wnt proteins activate the expression of the *EN2* gene. Whereas, wingless related integration site family member 3a (Wnt3a) protein may act via a Zic1-independent pathway. Another alternate pathway, by which *EN2* gene expression is activated, may involve fibroblast growth factor 8 (Fgf8) signaling [3].

Foldings of the outer surface of the cerebellum during its development result in the formation of folia and they are separated by fissures. Folia and fissures are the structural unit of the cerebellar lobules in a mediolateral pattern extended from the vermis to the cerebellar hemispheres. Further, neural connections of the cerebellum are distinct region wise [4]. About 101 billion granule cells, 30.5 million purkinje cells and 4 billion of the rest of the neurons are accommodated within the cerebellum by establishing cerebellar circuitry for maintaining the functional integrity of the cerebellum [5].

The cascades of genes are expressed for the development of the cerebellum and it is essential to understand the role played by each of these genes. Morphological pattern, formation of the folia, fissures and neural circuitry of the cerebellum is regulated by the expression of a certain gene. Engrailed homeobox 1 and 2 (*EN1/2)* are homologs of segmentation gene engrailed homeobox (*EN*) [6]. *EN2* mutation or altered expression may cause abnormal morphology of the cerebellum [4, 7]. This review highlighted the role of the *EN2* gene in the development of the cerebellum and the outcomes of its altered and ectopic expression.

A search was performed in the Pubmed and Google Scholar databases by using the following descriptors: "(Fetus or cerebellum) AND (*EN2* gene) AND (Development or cancer or autism)", without any filters, dated 10 August 2021. Preferred reporting items for systematic reviews and meta-analyses (PRISMA) review protocol was followed for the synthesis of the information as shown in Fig. 1.

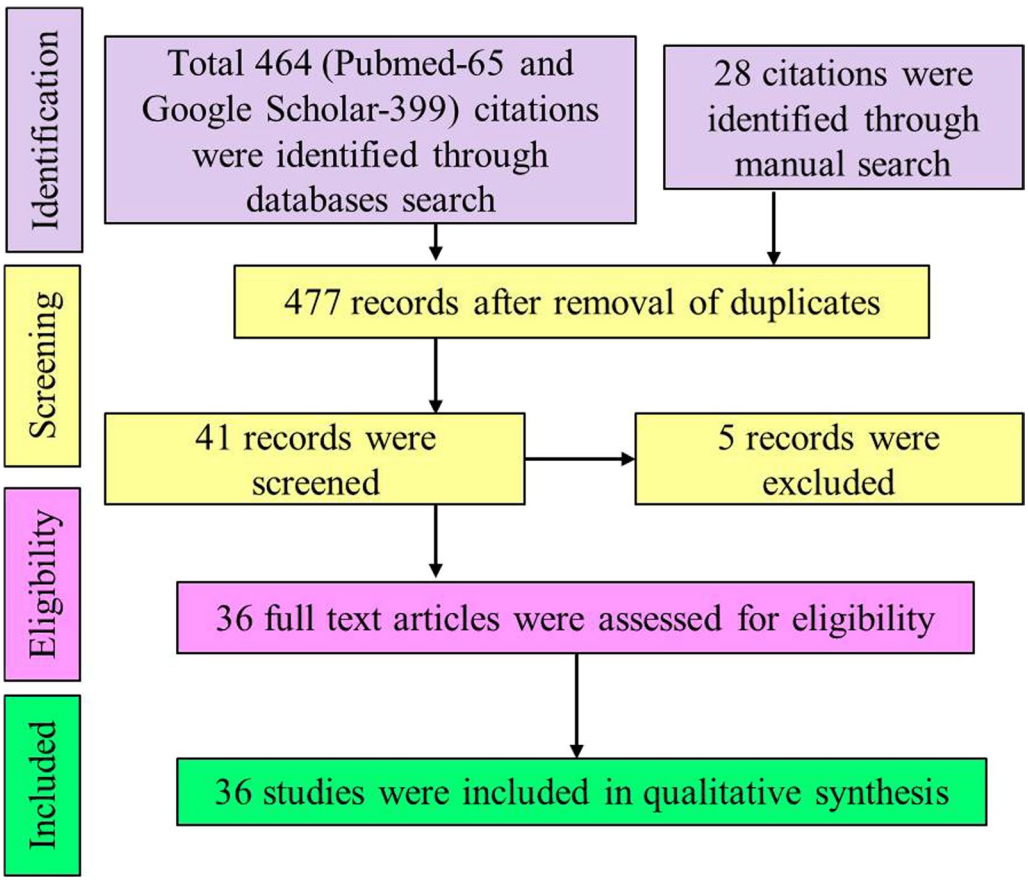

Fig. 1 Flow of information through the different phases of review

Main text

Animals and human in vitro and in vivo studies showed that the *EN2* gene encodes EN2 protein and regulates the development of the cerebellum. The main findings regarding the *EN2* gene and its role in the development of the cerebellum are given in Table 1.

Mutation and ectopic expression of the *EN2* gene result in deformed morphology of the cerebellum and cancer of the involved organ as shown in Table 2.

Expression of *EN2* gene is required for the morphological organization and pattern formation of the cerebellum (Table 1, Fig. 2)

The vertebrate cerebellum is organized into modules evidenced by the pattern of cerebellar development, structure of the cerebellar circuitry, expression of the specific markers in the specific region and phenotype of the mutation. It has three lobes in the anteroposterior (AP) direction. The anterior lobe lies cranial and posterior lobe lies caudal to the primary fissure, flocculonodular lobe lies caudal to the posterolateral fissure. These morphological subdivisions have functional significance. Spinocerebellar somatosensory function is conserved in the vermal region of the anterior and posterior lobes. The motor planning function of the cerebellum is preserved in the hemispherical part of these two lobes. Vestibular function is associated with the flocculonodular lobe. The vermis and hemispheres of the cerebellum are further subdivided into ten lobules in the anteroposterior axis separated by fissures. Some of the cerebellar structures are organized in a mediolateral (ML) pattern. These include the long axis of the cerebellar folia, deep cerebellar nuclei, climbing and mossy fibers of the cerebellum. Similarly, there is a superficial to deep (SD) arrangement of the cerebellar neurons into four laminae in the fetal life and three laminae after birth in the cerebellar cortex [13].

Animal model studies showed that after the closure of the neural tube, the isthmus (mesencephalic/metencephalic junction) acts as an organizing center for the development of the midbrain and cerebellum. Fgf8 is a main organizing molecule expressed in the isthmus and strong Fgf8 signal activates the Ras-ERK signaling pathway for the development of the cerebellum [8]. Expression of the *Wnt1* gene at isthmus stabilizes the expression of the *EN2* gene in the metencephalon. *EN2* gene is expressed in most of the parts of the developing

Table 1 Role of *EN2* gene in the development of the cerebellum

Main findings	Study models	References
EN2 (engrailed homeobox 2) gene encodes homeodomain containing transcription factor EN2	In vitro, Human	[1, 2]
Mesencephalon-metencephalon junction consists of Fgf8. It activates rat sarcoma virus- extracellular signal regulated kinase (Ras-ERK) signaling pathway that regulates the expression of the *EN2* gene	Review	[8]
Zic1 coordinates with Noggin/Chordin for the expression of the *Wnt1* gene and that activates the expression of the *EN2* gene	In vitro, Xenopus and Drosophila	[3]
Expression of the *EN2* gene is essential for the normal development of the cerebellum, morphological organization, cerebellar foliation, fissure formation, establishment of the afferent topography, molecular pattern formation and patterned gene expression in the cerebellum	In vivo, in vitro, Mouse	[4, 6, 7, 9–11]
Cerebellar neurons and glia are estimated to be 105 billion in number and they are organized into superficial to deep layers in the cortex. Similarly, there are distinct sites of projection of afferent fibers in the cerebellum. These sites express distinct proteins	Histology, Human	[5, 12, 13]

Table 2 Effects of mutation and ectopic expression of *EN2* gene

Main findings	Study models	References
EN2 gene mutation results in changes in cerebellar morphology include hypoplasia, abnormal folia and circuitry with a decrease in the number of purkinje cells. These changes are similar to cerebellar changes in the autistic individuals. Overexpression of *EN2* gene is appeared in the autistic cerebellum. Two intronic single nucleotide polymorphisms of *EN2* gene (rs1861972 and rs1861973) are susceptible case for autism. Disruption of *EN2* gene may alter the development of monoamine system and GABAergic interneurons in the brain and may lead to autism	In vivo, in vitro, Mouse, Human	[14–30]
EN2 gene may be expressed ectopically. Ectopic expression of the *EN2* gene may result in prostatic cancer, benign prostatic hyperplasia, ovarian tumor, esophageal squamous cell carcinoma and bladder cancer. Whereas its expression plays an anti-oncogenic role in cases of clear cell renal cell carcinoma and non-small cell lung cancer. EN2 can act as a diagnostic marker or therapeutic target in these cases	In vivo, in vitro, Human	[31–36]

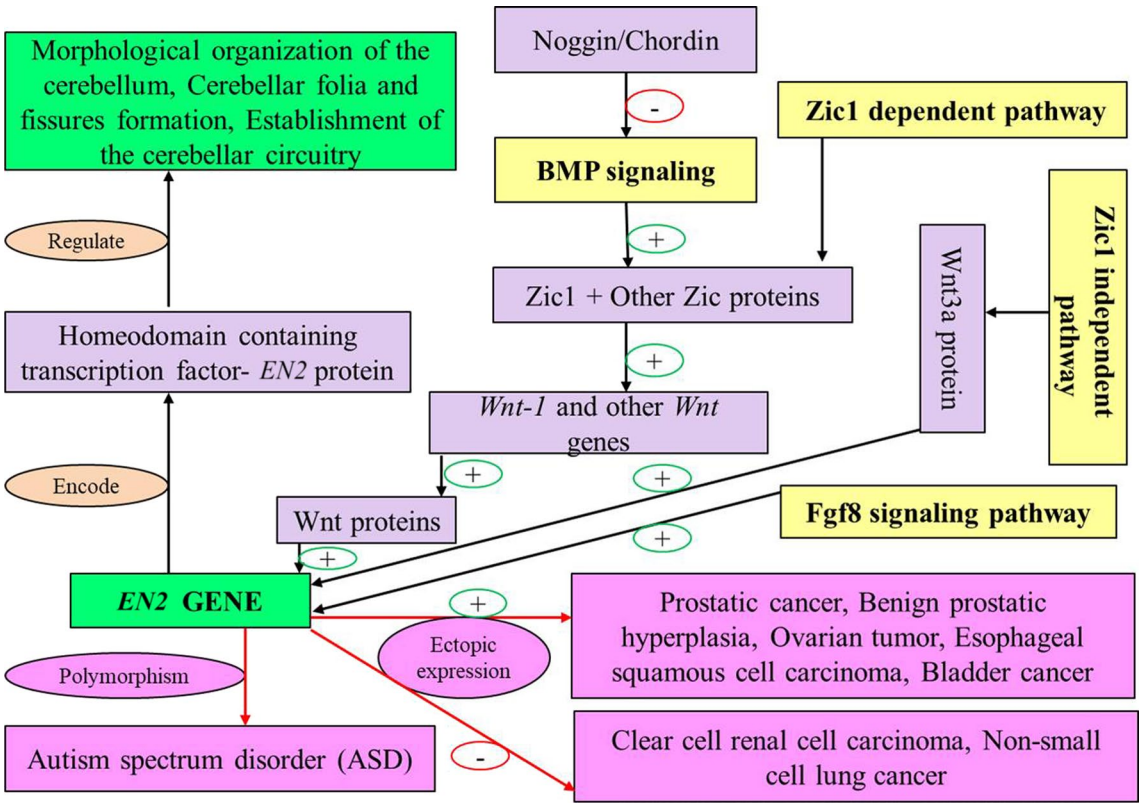

Fig. 2 Normal, ectopic and altered expressions of *EN2* gene and outcomes

cerebellum including the outer cortex, white matter and deep cerebellar nuclei. However, the intensity of its expression is different in the different parts of the developing cerebellum. External granular, purkinje and internal granular layers have high intensity of *EN2* expression, whereas the molecular layer is devoid of its expression.

Animal model studies showed that the inactivation of the *EN2* gene affects the morphology of the lobules of the vermis and cerebellar hemispheres. Some vermal lobules are reduced in size, some are extended more laterally than normal, some are relatively larger than normal and in some regions, hemispheres are fused. Similarly, defective gene results in alteration of positions of fissures in the cerebellar vermis and hemispheres [4, 11]. Purkinje and Bergmann glial fibers form an anchoring unit at the base of each fissure for the proliferation of the granule cells resulting in the formation of folia [9]. Inactivation of the *EN2* gene produces defective foliation in the vermal and hemispherical region of the cerebellum, whereas its activation rescues the foliation defects [4, 11]. These events proved that *EN2* gene expression is essential in the cells of the cerebellar primordium to regulate the cerebellar foliation, patterning and growth of the folia. Furthermore, the *EN2* gene regulates the timing of the formation of the folia and their position along the anteroposterior axis. This gene influences the expression of other genes in the metencephalon to maintain the arrangement and morphology of the cerebellar cortical layers and neurons [4, 7, 11].

EN2 gene expression maintains the cerebellar neural circuitry and morphology of the neurons (Table 1, Fig. 2)

Climbing (olivocerebellar) and mossy (other than olivocerebellar) fibers are the major extrinsic afferents carrying sensory and motor information to the cerebellum. The site of projection of afferent fibers is distinct in the various zones of the cerebellum in the anteroposterior and mediolateral axes and these form distinct parasagittal regions of protein expressions [12]. *EN2* mutant animal models studies showed severe disruption of mediolateral topography of the mossy fibers and ectopic projection of these fibers into the neighboring regions. This proved that normal expression of the *EN2* gene is essential for the targeted termination of the mossy fibers in a specific region of a lobule of the cerebellum [7]. Another animal model study showed a major reduction of the olivocerebellar fibers in the cerebellum of the mutant *EN2* gene. Hence, it can be said that the *EN2* gene is responsible for

the formation of afferent bands of olivocerebellar and mossy fibers circuitry in the cerebellum [7]. Another study revealed that the *EN2* gene is expressed in the precursors of granule cells, deep cerebellar nuclei and neuroglial cells of the cerebellum along with other genes and is responsible for maintaining the shape, number and connections of these neurons [10]. These evidences proved that the expression of the *EN2* gene is essential to maintain the cerebellar neural circuitry and morphology of the neurons.

Ectopic expression of *EN2* gene (Table2, Fig. 2)

Expression of homeobox containing *EN2* gene is a normal phenomenon in the cerebellum, midbrain, medulla, hippocampus and cerebral cortex [24, 26, 27]. However, sometimes it is expressed ectopically in the other body parts. *EN2* gene is ectopically expressed in prostate cancer and benign prostatic hyperplasia (BPH). Expression signals are more in the case of prostate cancer than in BPH [35]. Similarly, *EN2* gene expression is increased in the case of ovarian tumors particularly in high grade serous ovarian cancer and in platinum resistant tumors [34]. Its expression in the esophagus promotes esophageal squamous cell carcinoma by upregulating the expression of other pro-oncogenic genes [36]. It is also expressed in the human bladder cancer cells which is confirmed by overall sensitivity of 82% and specificity of 75% [31]. Whereas in the kidney, its expression may play an anti-oncogenic role in the oncogenesis and development of clear cell renal cell carcinoma [32]. *EN2* gene expression may act as a tumor suppressor gene in non-small cell lung cancer cells and its reduced expression may be associated with the occurrence and development of the non-small cell lung cancer cells [33]. The above studies were confirmed by polymerase chain reaction (PCR), immunofluorescence and immunohistochemical (IHC) tests. The specificity of the antibody used in these studies was validated with western blotting and immunofluorescence tests [32, 34–36]. From these findings, it can be said that the biological function of the *EN2* gene differs in different types of tumors. Quantification studies of EN2 transcription factor in these tumors may act as a novel cancer biomarker for the effective diagnosis and therapy of cancer, however, for its validation still more researches are recommended.

EN2 gene polymorphism is susceptible for autism spectrum disorder (ASD) (Table 2, Fig. 2)

Genetic and environmental factors are responsible for neurodevelopmental-based ASD. The coordination of more than hundred genes regulates the cortical structure and neural synapses in the brain. There may be involvement of five to fifteen genes for the cause of ASD [14]. No such fundamental idea is established in the association between expression of *EN2* gene in cerebellum and ASD. However, studies showed that human *EN2* gene is localized to 7q36, an autism susceptibility locus and *EN2* gene knockout mice displayed hypoplasia of cerebellum, abnormal cerebellar folia, abnormal cerebellar circuitary and a decrease in the number of purkinje cells which are similar to the features observed in the autistic individuals. These findings support that there is an association between *EN2* gene and ASD [14, 16, 28, 29]. Similarly, *EN2* gene knockout studies in mice proved that defective *EN2* gene results in the loss of inhibitory neurons in the dentate gyrus of the hippocampus, a center for learning and memory. This may be one of the causes associated with ASD [15, 24, 27].

Human gene analysis studies identified polymorphism in the *EN2* gene in the case of ASD [23, 25]. Two intronic single nucleotide polymorphisms (rs1861972 and rs1861973) in the *EN2* gene are associated with autism [14, 16, 29]. Disruption of *EN2* gene showed cerebellar neuropathological changes and alteration in the monoamine system development in the brain that results reduction of serotonin, dopamine and norepinephrine related neurons may leads to ASD [17, 19]. Similarly there is partial loss of GABAergic interneurons in the *EN2* knockout cases, accompanied by decrease of brain derived neurotropic factor (BDNF) signaling similar in ASD cases, a crucial determinant of GABAergic differentiation in the brain [16]. From these findings, it can be suspected that defective *EN2* gene or *EN2* gene polymorphism may be associated with ASD.

Conclusion

EN2 gene is expressed in the cerebellar primordium. It plays an important role in the morphological organization, pattern formation of the cerebellar folia and fissures in the anteroposterior, mediolateral and superficial-deep axes. It also plays a role in the establishment of neural circuitry within the cerebellum. Mutation of the *EN2* gene results in deformed cerebellar morphology. Evidence showed that ectopic expression of the *EN2* gene in the prostate, ovary, esophagus and urinary bladder may cause cancer. Further studies are recommended to validate the use of EN2 as a diagnostic biomarker or therapeutic targets in the case of cerebellar morphological disorders and cancer.

Abbreviations

ASD: Autism spectrum disorder; AP: Anteroposterior; BDNF: Brain derived neurotropic factor; BMP: Bone morphogenetic protein; BPH: Benign prostatic hyperplasia; DNA: Deoxyribonucleic acid; EN: Engrailed homeobox; EN1/2: Engrailed homeobox 1 and 2; EN2: Engrailed homeobox 2; GABA: Gamma aminobutyric acid; IHC: Immunohistochemical; ML: Mediolateral; PCR:

Polymerase chain reaction; PRISMA: Preferred reporting items for systematic reviews and meta-analyses; Ras-ERK: Rat sarcoma virus- extracellular signal regulated kinase; SD: Superficial to deep; Wnt: Wingless related integration site; Wnt1: Wnt family member 1; Wnt3a: Wnt family member 3a; Zic: Zinc finger of the cerebellum; Zic1: Zic protein family member 1.

Acknowledgements
We acknowledge DC Agarwal, BM Nagpal, BSS Rao, S Hande, N Nayak, K Iyer, S Singh, G Kalthur and BP Powar for their constant support during this study.

Author contributions
PPP and SGK conceptualized the study, performed the literature search and drafted the original manuscript. CB and SG critically revised it. All authors read and approved the final manuscript.

Funding
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Availability of data and materials
Not applicable.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors have no competing interests to declare.

Author details
[1]Department of Anatomy, Kasturba Medical College, Manipal, Manipal Academy of Higher Education (MAHE), Karnataka 576104, India. [2]Department of Anatomy, Manipal College of Medical Sciences, Pokhara, Nepal. [3]Department of Pathology, Manipal College of Medical Sciences, Pokhara, Nepal.

Received: 2 February 2022 Accepted: 9 May 2022
Published online: 20 May 2022

References

1. EN2 engrailed homeobox 2 [Homo sapiens (human)]—Gene—NCBI n.d. https://www.ncbi.nlm.nih.gov/gene?Db=gene&Cmd=DetailsSearch&Term=2020. Accessed 26 Jan 2022.
2. Bürglin TR, Affolter M (2016) Homeodomain proteins: an update. Chromosoma 125:497–521. https://doi.org/10.1007/s00412-015-0543-8
3. Merzdorf CS, Sive HL (2006) The zic1 gene is an activator of Wnt signaling. Int J Dev Biol 50:611–617. https://doi.org/10.1387/IJDB.052110CM
4. Cheng Y, Sudarov A, Szulc KU, Sgaier SK, Stephen D, Turnbull DH et al (2010) The Engrailed homeobox genes determine the different foliation patterns in the vermis and hemispheres of the mammalian cerebellum. Development 137:519–529. https://doi.org/10.1242/DEV.027045
5. Andersen BB, Korbo L, Pakkenberg B (1992) A quantitative study of the human cerebellum with unbiased stereological techniques. J Comp Neurol 326:549–560. https://doi.org/10.1002/CNE.903260405
6. Liu A, Joyner AL (2001) Early anterior/posterior patterning of the midbrain and cerebellum. Annu Rev Neurosci 24:869–896. https://doi.org/10.1146/annurev.neuro.24.1.869
7. Sillitoe RV, Vogel MW, Joyner AL (2010) Engrailed homeobox genes regulate establishment of the cerebellar afferent circuit map. J Neurosci 30:10015–10024. https://doi.org/10.1523/JNEUROSCI.0653-10.2010
8. Nakamura H, Sato T, Suzuki-Hirano A (2008) Isthmus organizer for mesencephalon and metencephalon. Dev Growth Differ 50(Suppl 1):S113-118. https://doi.org/10.1111/j.1440-169X.2008.00995.x
9. Sudarov A, Joyner AL (2007) Cerebellum morphogenesis: the foliation pattern is orchestrated by multi-cellular anchoring centers. Neural Dev 2:26. https://doi.org/10.1186/1749-8104-2-26
10. Wilson SL, Kalinovsky A, Orvis GD, Joyner AL (2011) Spatially restricted and developmentally dynamic expression of engrailed genes in multiple cerebellar cell types. Cerebellum 10:356–372. https://doi.org/10.1007/S12311-011-0254-5
11. Orvis GD, Hartzell AL, Smith JB, Barraza LH, Wilson SL, Szulc KU et al (2012) The engrailed homeobox genes are required in multiple cell lineages to coordinate sequential formation of fissures and growth of the cerebellum. Dev Biol 367:25–39. https://doi.org/10.1016/J.YDBIO.2012.04.018
12. Apps R, Hawkes R (2009) Cerebellar cortical organization: a one-map hypothesis. Nat Rev Neurosci 10:670–681. https://doi.org/10.1038/nrn2698
13. Haldipur P, Dang D, Millen KJ (2018) Embryology. Handb Clin Neurol 154:29–44. https://doi.org/10.1016/B978-0-444-63956-1.00002-3
14. Gharani N, Benayed R, Mancuso V, Brzustowicz LM, Millonig JH (2004) Association of the homeobox transcription factor, ENGRAILED 2, 3, with autism spectrum disorder. Mol Psychiatry 9:474–484. https://doi.org/10.1038/SJ.MP.4001498
15. Cheh MA, Millonig JH, Roselli LM, Ming X, Jacobsen E, Kamdar S et al (2006) En2 knockout mice display neurobehavioral and neurochemical alterations relevant to autism spectrum disorder. Brain Res 1116:166–176. https://doi.org/10.1016/j.brainres.2006.07.086
16. Boschian C, Messina A, Bozza A, Castellini ME, Provenzano G, Bozzi Y et al (2018) Impaired neuronal differentiation of neural stem cells lacking the Engrailed-2 gene. Neuroscience 386:137–149. https://doi.org/10.1016/J.NEUROSCIENCE.2018.06.032
17. Genestine M, Lin L, Durens M, Yan Y, Jiang Y, Prem S et al (2015) Engrailed-2 (En2) deletion produces multiple neurodevelopmental defects in monoamine systems, forebrain structures and neurogenesis and behavior. Hum Mol Genet 24:5805–5827. https://doi.org/10.1093/HMG/DDV301
18. Behnia F, Parets SE, Kechichian T, Yin H, Dutta EH, Saade GR et al (2015) Fetal DNA methylation of autism spectrum disorders candidate genes: association with spontaneous preterm birth. Am J Obstet Gynecol 212:533.e1-533.e9. https://doi.org/10.1016/J.AJOG.2015.02.011
19. Viaggi C, Gerace C, Pardini C, Corsini GU, Vaglini F (2015) Serotonin abnormalities in Engrailed-2 knockout mice: new insight relevant for a model of Autism Spectrum Disorder. Neurochem Int 87:34–42. https://doi.org/10.1016/J.NEUINT.2015.05.004
20. James SJ, Shpyleva S, Melnyk S, Pavliv O, Pogribny IP (2013) Complex epigenetic regulation of engrailed-2 (EN-2) homeobox gene in the autism cerebellum. Transl Psychiatry 3:e232. https://doi.org/10.1038/TP.2013.8
21. Sgadò P, Provenzano G, Dassi E, Adami V, Zunino G, Genovesi S et al (2013) Transcriptome profiling in engrailed-2 mutant mice reveals common molecular pathways associated with autism spectrum disorders. Mol Autism 4:1–12. https://doi.org/10.1186/2040-2392-4-51
22. Choi J, Ababon MR, Soliman M, Lin Y, Brzustowicz LM, Matteson PG et al (2014) Autism associated gene, engrailed2, and flanking gene levels are altered in post-mortem cerebellum. PLoS ONE 9:e87208. https://doi.org/10.1371/journal.pone.0087208
23. Benayed R, Choi J, Matteson PG, Gharani N, Kamdar S, Brzustowicz LM et al (2009) Autism-associated haplotype affects the regulation of the homeobox gene, ENGRAILED 2. Biol Psychiatry 66:911–917. https://doi.org/10.1016/J.BIOPSYCH.2009.05.027
24. Sgadò P, Genovesi S, Kalinovsky A, Zunino G, Macchi F, Allegra M et al (2013) Loss of GABAergic neurons in the hippocampus and cerebral cortex of Engrailed-2 null mutant mice: implications for autism spectrum disorders. Exp Neurol 247:496–505. https://doi.org/10.1016/J.EXPNEUROL.2013.01.021
25. Hnoonual A, Sripo T, Limprasert P (2016) Whole-exome sequencing identifies a novel heterozygous missense variant of the EN2 gene in two unrelated patients with autism spectrum disorder. Psychiatr Genet 26:297–301. https://doi.org/10.1097/YPG.0000000000000153
26. Zunino G, Messina A, Sgadò P, Baj G, Casarosa S, Bozzi Y (2016) Brain-derived neurotrophic factor signaling is altered in the forebrain of Engrailed-2 knockout mice. Neuroscience 324:252–261. https://doi.org/10.1016/J.NEUROSCIENCE.2016.03.023

27. Ito H, Morishita R, Nagata K (2017) Autism spectrum disorder-associated genes and the development of dentate granule cells. Med Mol Morphol 50:123–129. https://doi.org/10.1007/S00795-017-0161-Z
28. Kuemerle B, Gulden F, Cherosky N, Williams E, Herrup K (2007) The mouse Engrailed genes: a window into autism. Behav Brain Res 176:121–132. https://doi.org/10.1016/J.BBR.2006.09.009
29. Wang L, Jia M, Yue W, Tang F, Qu M, Ruan Y et al (2008) Association of the ENGRAILED 2 (EN2) gene with autism in Chinese Han population. Am J Med Genet B Neuropsychiatr Genet 147B:434–438. https://doi.org/10.1002/AJMG.B.30623
30. James SJ, Shpyleva S, Melnyk S, Pavliv O, Pogribny IP (2014) Elevated 5-hydroxymethylcytosine in the Engrailed-2 (EN-2) promoter is associated with increased gene expression and decreased MeCP2 binding in autism cerebellum. Transl Psychiatry 4:e460. https://doi.org/10.1038/TP.2014.87
31. Morgan R, Bryan RT, Javed S, Launchbury F, Zeegers MP, Cheng KK et al (2013) Expression of Engrailed-2 (EN2) protein in bladder cancer and its potential utility as a urinary diagnostic biomarker. Eur J Cancer 49:2214–2222. https://doi.org/10.1016/J.EJCA.2013.01.019
32. Lai C-Y, Xu Y, Yu G-S, Wu X, Li Y-F, Pan B et al (2016) Engrailed-2 might play an anti-oncogenic role in clear-cell renal cell carcinoma. J Mol Histol 47:229–237. https://doi.org/10.1007/s10735-016-9665-4
33. Lin X, Liu X, Gong C (2018) Expression of engrailed homeobox 2 regulates the proliferation, migration and invasion of non-small cell lung cancer cells. Oncol Lett 16:536–542. https://doi.org/10.3892/OL.2018.8693
34. McGrath SE, Annels N, Madhuri TK, Tailor A, Butler-Manuel SA, Morgan R et al (2018) Engrailed-2 (EN2)—a novel biomarker in epithelial ovarian cancer. BMC Cancer 18:1–11. https://doi.org/10.1186/S12885-018-4816-5
35. Li Q, Shi Y, Sa R, Hao J, Hu J, Xiao M et al (2020) Altered staining patterns and expression level of Engrailed-2 in benign prostatic hyperplasia and prostate Cancer predict prostatic disease progression. BMC Cancer 20:1–13. https://doi.org/10.1186/S12885-020-07049-Z
36. Cao Y, Wang X, Tang L, Li Y, Song X, Liu X et al (2020) Engrailed-2 promotes a malignant phenotype of esophageal squamous cell carcinoma through upregulating the expression of pro-oncogenic genes. PeerJ 8:e8662. https://doi.org/10.7717/peerj.8662

Publisher's Note