Published in final edited form as:

Mol Genet Metab. 2012 June ; 106(2): 241–243. doi:10.1016/j.ymgme.2012.03.008.

Molecular Analysis of the Noggin (*NOG*) Gene in Holoprosencephaly Patients

Kshitij Srivastava¹, Ping Hu¹, Benjamin D. Solomon¹, Jeffrey E. Ming², Erich Roessler¹, and Maximilian Muenke^{1,*}

¹Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland ²The Children's Hospital of Philadelphia, Division of Human Genetics and Molecular Biology, Philadelphia, Pennsylvania

Abstract

Holoprosencephaly (HPE) is the most common structural anomaly of the human forebrain. Various genetic and teratogenic causes have been implicated in its pathogenesis. A recent report in mice described *Noggin* (*NOG*) as a candidate gene involved in the etiology of microform HPE. Here, we present for the first time genetic analysis of a large HPE cohort for sequence variations in *NOG*. On the basis of our study, we conclude that mutations in the coding region of *NOG* are rare, and play at most an uncommon role in human HPE.

Keywords

Holoprosencephaly (HPE); *Noggin* (*NOG*); forebrain; mutation; modifier

1. Introduction

Holoprosencephaly (HPE, MIM: #236100), the most common structural anomaly of the developing forebrain in humans, occurs due to insults that affect the fetus between the third and fourth week of gestation [1]. The prevalence of HPE is estimated at 1/250 conceptuses and 1/16,000 live births [2]. HPE is a genetically heterogeneous disease with incomplete penetrance and highly variable expressivity. The severity ranges from severe craniofacial and neuroanatomical malformations incompatible with postnatal life to isolated, mild midline craniofacial anomalies (such as hypotelorism, a sharp and narrow or flattened nasal bridge, and a single central maxillary incisor), often termed “microform” HPE [3]. Nonsyndromic HPE is inherited in an autosomal dominant manner with mutations in 4 genes (*SHH*, *ZIC2*, *SIX3* and *TGIF*) accounting for a large proportion of the cases; mutations in a number of other genes have also been identified as less frequent causes [2,4,5,6].

The partial penetrance and variable expressivity of HPE has been ascribed to several possible hypotheses including genetic and/or environmental modifiers. In attempting to account for intrafamilial variability in HPE patients, Ming and Muenke [7] proposed the

*Correspondence: Maximilian Muenke, MD, Chief, Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, 35 Convent Drive, Building 35, Room 1B-203, Bethesda, MD-20892, mamuenke@mail.nih.gov, Phone: 301-402-8167, FAX: 301-496-7184.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

“multiple hit hypothesis” in which various genes act synergistically to generate or modify the HPE phenotype. However, a recent article by Roessler et al. [8] suggests the role of modifying genetic variants of individually small effect, as well as the influence of environmental factors, plays a critical role in generating phenotypic variability among HPE cases, as opposed to simpler digenic gene-gene interactions. Multiple studies in mouse models have also shown strain-specific responses in terms of HPE severity due to the knockout of key developmental genes [9,10,11], further supporting the involvement of genetic modifiers in HPE disease variability [12].

The human Noggin gene (*NOG*; MIM# 602991), spanning ~1.9 kb of genomic DNA on chromosome 17q22 (NM_005450.4), comprises a single coding exon, and encodes a 1.9 kb mRNA, which is translated into a 232-amino acid N-glycosylated protein (NP_005441) with a molecular mass of 25.7 kDa (UniProt (NOGG_HUMAN, # Q13253). It can be further classified by gene Ontology (GO) categories (www.geneontology.org) of biological processes of the BMP signaling pathway, cartilage development, embryonic digit and skeletal joint morphogenesis, and various regulatory pathways. Noggin acts as a negative modulator of the Bone morphogenetic protein (Bmp) signaling pathway by sequestering Bmps 2, 4, 6, and 7 in an inactive complex. It plays a critical role in early embryogenesis by inducing differentiation and development of neural tissue, skeletal muscles, cartilage and hair follicles [13,14,15] and also has a role in the development of the head structures, including the telencephalon and eyes [16,17].

Noggin null mice display increased Bmp signaling, which results in growth and patterning defects in the neural tube and somites [13,18,19,20]. Removing one copy of Noggin in chordin null allele mice, which is also a Bmp inhibitor, (*Chrd*^{-/-}; *Nog*^{+/-}) results in a spectrum of HPE phenotypes including multiple craniofacial anomalies and deficient patterning of forebrain, while mice with double mutants (*Chrd*^{-/-}; *Nog*^{-/-}) show severe HPE and are not viable [18,21,22]. A recent article by Lana-Elola et al. [23] demonstrated craniofacial abnormalities including solitary median maxillary incisor, midfacial narrowing and abnormalities of developing hyoid bone, pituitary gland and vomeronasal organ in *Noggin*^{-/-} mice embryos consistent with a microform of HPE. These findings in mice suggest *NOG* to be a plausible candidate gene for HPE in humans.

In humans, HPE-type anomalies have not been described as associated with *NOG* mutations. Mutations in the gene encoding the NOGGIN protein have been associated with symphalangism proximal syndrome (SYM1) [MIM #185800], multiple synostoses syndrome type 1 (SYNS1) [MIM #186500], tarsal-carpal coalition syndrome (TCC) [MIM#186570], stapes ankylosis with broad thumb and toes (SABTS) [MIM #184460] and brachydactyly type B2 (BDB2) [MIM #611377].

In the present study, we hypothesized that sequence variants of *NOG* gene could contribute to an HPE phenotype in humans, perhaps acting as principal susceptibility factors, or as coding variants that modify the function of pathway related factors. To test these hypotheses, we performed an in depth study to analyze the coding region of *NOG* gene for potential pathogenic genetic variations in a large cohort of patients comprising the entire spectrum of HPE severity.

2. Materials and Methods

2.1 Study subjects

The study comprised 384 unrelated control subjects [Sigma-Aldrich Corporation and the European Collection of Cell Cultures (Human Random Control Panels 1, 3, and 4) of Caucasian healthy citizens of the United Kingdom] and 443 patients representing the entire

clinical spectrum of HPE. Studied individuals included 21 (4.7%) individuals with microform HPE. Although full phenotypic data was not uniformly available, there were no reports of limb anomalies consistent with previous studies of human *NOG* mutations [24]. All subjects provided informed consent for research participation and clinical data was collected in accordance with NHGRI IRB-approved protocols.

2.2 Bi-directional Sequence Analysis

Bi-directional sequence analysis was performed with primers previously described [25]. PCR products were sequenced through the DNA Sequencing Facility, National Institute of Neurological Disorders and Stroke (NINDS), NIH. DNA sequences were manually annotated and analyzed using Sequencher 4.7 software package (Gene Codes Corporation). Sequences were compared with the indicated reference sequences. All variations are described according to current mutation nomenclature guidelines, ascribing the A of the first ATG translational initiation codon as nucleotide +1 [26]. Nucleotide changes detected by sequencing were classified as novel alterations or known likely neutral polymorphisms [SNPs (single nucleotide polymorphisms)] if found in dbSNP, Build 132, (www.ncbi.nlm.nih.gov/projects/SNPs).

3. Results

Our HPE cohort of 443 unrelated individuals, including 21 individuals with microform HPE, represented the entire HPE phenotypic spectrum. Family-specific mutations and their frequencies in the four major HPE genes *SHH*, *ZIC2*, *SIX3* and *TGIF* in our HPE cohort are summarized in by Roessler et al. [27]. Apart from these, novel and known variations were also identified in genes such as *GAS1* [28], *CDON* [29], *TGIF* [30] and *BOC* (Srivastava et al., unpublished data). Among the studied cohort, there were no reports of limb anomalies consistent with previous reports of the clinical manifestations of human *NOG* mutations. However, due to limited available clinical information comprehensive clinical details were not available in all cases.

Bidirectional sequencing of our 443 HPE cases and 384 control subjects identified two known variations (c.251C>A, Pro84His rs138481449 and c.582G>A, Pro194Pro rs76347008) in the *NOG* gene (Table 1). Novel *NOG* variations were not found in any of the HPE patients or control subjects investigated in the present study.

4. Discussion

Variability in genetic background has been linked to variability in the penetrance and expressivity of mutations in mouse models of HPE [9,10,11]. This phenotypic variability in differing genetic backgrounds might potentially help to identify modifier loci that have an epistatic relationship with a mutation in the major HPE genes. Recent studies in mice support Noggin's role as a candidate gene in human HPE pathogenesis [23]. However, on the basis of the present study, mutations in the *NOG* gene do not appear to be a common cause of HPE, or a modifier of clinical severity, in our cohort of affected (including microform) HPE patients.

The results may be explained on the basis of following three possibilities; 1) experimental generation of gene-targeted *NOG*^{-/-} mice demonstrating microform HPE does not imply that the *NOG* gene is also an important player in human HPE pathogenesis; 2) The BMP antagonist Chordin compensates for Noggin during early development [22] resulting in normal phenotypic individuals not represented in our HPE cohort; 3) variations outside the coding regions (regulatory regions) may be more frequently involved than exonic regions. Finally, due to the difficulties in identifying and analyzing highly pathogenic mutations that

lead to a high rate of intrauterine embryonic lethality in HPE [2], we cannot exclude *NOG* as a potential major factor in its etiology.

The full landscape of the prevalence of mutations as well as their functional consequences in HPE pathogenesis will not be recognized until thousands of HPE genomes have been sequenced. Future studies using high-throughput genomic sequencing and disease-network analysis may help in the identification of mutations and potential candidate modifier genes in HPE pathogenesis.

Acknowledgments

We appreciate the many participating families who have enabled physicians and researchers to advance our understanding of holoprosencephaly. We would also like to thank the NINDS DNA Sequencing Facility for assistance with DNA sequencing. This research was supported by the Division of Intramural Research (DIR), National Human Genome Research Institute, National Institutes of Health, Department of Health and Human Services, USA. The funding body had no role in study design, collection, analysis and interpretation of data, writing of the report and in the decision to submit the article for publication.

References

1. Golden JA. Towards a greater understanding of the pathogenesis of holoprosencephaly. *Brain Dev.* 1999; 21:513–521. [PubMed: 10598051]
2. Dubourg C, Bendavid C, Pasquier L, Henry C, Odent S, et al. Holoprosencephaly. *Orphanet J Rare Dis.* 2007; 2:8. [PubMed: 17274816]
3. Solomon BD, Mercier S, Velez JI, Pineda-Alvarez DE, Wyllie A, et al. Analysis of genotype-phenotype correlations in human holoprosencephaly. *Am J Med Genet C Semin Med Genet.* 2010; 154C:133–141. [PubMed: 20104608]
4. Bellone S, De Rienzo F, Prodam F, Savastio S, Busti A, et al. Etiopathogenetic advances and management of holoprosencephaly: from bench to bedside. *Panminerva Med.* 2010; 52:345–354. [PubMed: 21183895]
5. Roessler E, Muenke M. The molecular genetics of holoprosencephaly. *Am J Med Genet C Semin Med Genet.* 2010; 154C:52–61. [PubMed: 20104595]
6. Geng X, Oliver G. Pathogenesis of holoprosencephaly. *J Clin Invest.* 2009; 119:1403–1413. [PubMed: 19487816]
7. Ming JE, Muenke M. Multiple hits during early embryonic development: digenic diseases and holoprosencephaly. *Am J Hum Genet.* 2002; 71:1017–1032. [PubMed: 12395298]
8. Roessler E, Vélez JI, Zhou N, Muenke M. Utilizing prospective sequence analysis of *SHH*, *ZIC2*, *SIX3* and *TGIF* in holoprosencephaly probands to describe the parameters limiting the observed frequency of mutant gene×gene interactions. *Molecular Genetics and Metabolism.*
9. Cole F, Krauss RS. Microform Holoprosencephaly in Mice that Lack the Ig Superfamily Member *Cdon*. *Current biology: CB.* 2003; 13:411–415. [PubMed: 12620190]
10. Zhang W, Kang J-S, Cole F, Yi M-J, Krauss RS. *Cdo* Functions at Multiple Points in the Sonic Hedgehog Pathway, and *Cdo*-Deficient Mice Accurately Model Human Holoprosencephaly. *Developmental cell.* 2006; 10:657–665. [PubMed: 16647303]
11. Petryk A, Anderson RM, Jarcho MP, Leaf I, Carlson CS, et al. The mammalian twisted gastrulation gene functions in foregut and craniofacial development. *Developmental biology.* 2004; 267:374–386. [PubMed: 15013800]
12. Krauss RS. Holoprosencephaly: new models, new insights. *Expert Reviews in Molecular Medicine.* 2007; 9:1–17. [PubMed: 17888203]
13. Brunet LJ, McMahon JA, McMahon AP, Harland RM. Noggin, cartilage morphogenesis, and joint formation in the mammalian skeleton. *Science.* 1998; 280:1455–1457. [PubMed: 9603738]
14. Smith WC, Harland RM. Expression cloning of noggin, a new dorsalizing factor localized to the Spemann organizer in *Xenopus* embryos. *Cell.* 1992; 70:829–840. [PubMed: 1339313]

15. Botchkarev VA, Botchkareva NV, Roth W, Nakamura M, Chen L-H, et al. Noggin is a mesenchymally derived stimulator of hair-follicle induction. *Nat Cell Biol.* 1999; 1:158–164. [PubMed: 10559902]
16. Christof N. Head in the WNT: the molecular nature of Spemann's head organizer. *Trends in Genetics.* 1999; 15:314–319. [PubMed: 10431193]
17. Piccolo S, Agius E, Leyns L, Bhattacharyya S, Grunz H, et al. The head inducer Cerberus is a multifunctional antagonist of Nodal, BMP and Wnt signals. *Nature.* 1999; 397:707–710. [PubMed: 10067895]
18. Anderson RM, Lawrence AR, Stottmann RW, Bachiller D, Klingensmith J. Chordin and noggin promote organizing centers of forebrain development in the mouse. *Development.* 2002; 129:4975–4987. [PubMed: 12397106]
19. McMahon JA, Takada S, Zimmerman LB, Fan C-M, Harland RM, et al. Noggin-mediated antagonism of BMP signaling is required for growth and patterning of the neural tube and somite. *Genes & Development.* 1998; 12:1438–1452. [PubMed: 9585504]
20. Wijgerde M, Karp S, McMahon J, McMahon AP. Noggin antagonism of BMP4 signaling controls development of the axial skeleton in the mouse. *Developmental Biology.* 2005; 286:149–157. [PubMed: 16122729]
21. Yang YP, Anderson RM, Klingensmith J. BMP antagonism protects Nodal signaling in the gastrula to promote the tissue interactions underlying mammalian forebrain and craniofacial patterning. *Hum Mol Genet.* 2010; 19:3030–3042. [PubMed: 20508035]
22. Bachiller D, Klingensmith J, Kemp C, Belo JA, Anderson RM, et al. The organizer factors Chordin and Noggin are required for mouse forebrain development. *Nature.* 2000; 403:658–661. [PubMed: 10688202]
23. Lana-Elola E, Tylzanowski P, Takatalo M, Alakurtti K, Veistinen L, et al. Noggin null allele mice exhibit a microform of holoprosencephaly. *Human Molecular Genetics.* 2011; 20:4005–4015. [PubMed: 21821669]
24. Potti TA, Petty EM, Lesperance MM. A comprehensive review of reported heritable noggin-associated syndromes and proposed clinical utility of one broadly inclusive diagnostic term: NOG-related-symphalangism spectrum disorder (NOG-SSD). *Human Mutation.* 2011; 32:877–886. [PubMed: 21538686]
25. Takahashi T, Takahashi I, Komatsu M, Sawaishi Y, Higashi K, et al. Mutations of the NOG gene in individuals with proximal symphalangism and multiple synostosis syndrome. *Clinical Genetics.* 2001; 60:447–451. [PubMed: 11846737]
26. Wildeman M, van Ophuizen E, den Dunnen JT, Taschner PEM. Improving sequence variant descriptions in mutation databases and literature using the Mutalyzer sequence variation nomenclature checker. *Human Mutation.* 2008; 29:6–13. [PubMed: 18000842]
27. Roessler E, Velez JI, Zhou N, Muenke M. Utilizing prospective sequence analysis of SHH, ZIC2, SIX3 and TGIF in holoprosencephaly probands to describe the parameters limiting the observed frequency of mutant gene $\times$ gene interactions. *Mol Genet Metab.*
28. Pineda-Alvarez D, Roessler E, Hu P, Srivastava K, Solomon B, et al. Missense substitutions in the GAS1 protein present in holoprosencephaly patients reduce the affinity for its ligand, SHH. *Human Genetics.* 2012; 131:301–310. [PubMed: 21842183]
29. Bae GU, Domene S, Roessler E, Schachter K, Kang JS, et al. Mutations in CDON, encoding a hedgehog receptor, result in holoprosencephaly and defective interactions with other hedgehog receptors. *Am J Hum Genet.* 2011; 89:231–240. [PubMed: 21802063]
30. Keaton AA, Solomon BD, Kauvar EF, El-Jaick KB, Gropman AL, et al. TGIF Mutations in Human Holoprosencephaly: Correlation between Genotype and Phenotype. *Mol Syndromol.* 2010; 1:211–222. [PubMed: 22125506]

Highlights

- Holoprosencephaly (HPE): the most common anomaly of the human forebrain
- Genetic analysis for sequence variations in *NOG* in an HPE cohort
- Patients representing the entire clinical spectrum of HPE
- Bi-directional sequence analysis did not find any novel variation
- *NOG* is not a major player in HPE

Table 1List of variations identified in *NOG* gene.

Sample	Variation	Protein	dbSNP	dbSNP frequency (Caucasians)
Controls				
HRC2 – H3	c.251C>A	p.Pro84His	rs138481449	<0.001
HRC2 – F7	c.251C>A	p.Pro84His	rs138481449	<0.001
HRC4-F11	c.251C>A	p.Pro84His	rs138481449	<0.001
HRC1-A6	c.251C>A	p.Pro84His	rs138481449	<0.001
HRC2 – B12	c.582G>A	p.Pro194Pro	rs76347008	0.009
HPE patients				
BL10063	c.251C>A	p.Pro84His	rs138481449	<0.001