The role of primary cilia in the pathophysiology of neural tube defects

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Neural tube defects (NTDs) are a set of disorders that occur from perturbation of normal neural development. They occur in open or closed forms anywhere along the craniospinal axis and often result from a complex interaction between environmental and genetic factors. One burgeoning area of genetics research is the effect of cilia signaling on the developing neural tube and how the disruption of primary cilia leads to the development of NTDs. Recent progress has implicated the hedgehog (Hh), wingless-type integration site family (Wnt), and planar cell polarity (PCP) pathways in primary cilia as involved in normal neural tube patterning. A set of disorders involving cilia function, known as ciliopathies, offers insight into abnormal neural development. In this article, the authors discuss the common ciliopathies, such as Meckel-Gruber and Joubert syndromes, that are associated with NTDs, and review cilia-related signaling cascades responsible for mammalian neural tube development. Understanding the contribution of cilia in the formation of NTDs may provide greater insight into this common set of pediatric neurological disorders. (<http://thejns.org/doi/abs/10.3171/2012.6.FOCUS12222>)

KEY WORDS • cilia • ciliopathy • neural tube defect • sonic hedgehog • development

NEURAL tube disorders are a set of complex neural developmental abnormalities that occur in approximately 1 of 1500 births⁴ and are the second most common birth defect.³² Neural tube defects include both open and closed forms—with open forms resulting in failure of neural tube closure, seen in the cranial region with anencephaly and in the spinal region with spina bifida. Occasionally the entire craniospinal axis can be involved, such as in cases of craniorachischisis. Closed forms can present with more subtle phenotypic changes that are skin covered, including encephalocele, meningocele, and spina bifida occulta.⁴⁷ These disorders are commonly seen in pediatric neurosurgical practices and often require intervention.³⁷

The pathogenesis of NTDs results from a complex contribution of genetic and environmental factors. The folic acid pathway⁵ has been well described in its contribution to the genesis of certain NTDs and significant in-

ternational public health campaigns have helped increase awareness and prevention of NTDs.^{10,74} An understanding of the environmental factors is essential for prevention of these disorders.⁵¹ In addition, genetics^{45,53} is emerging as an essential area to understanding the pathophysiology of NTDs.⁷ Limited size of patient cohorts has made identification of genes associated with NTDs difficult. As a result, investigators have relied on mouse models of NTDs²⁵ to help identify pathways essential in normal neural tube development. The role of cilia⁴⁰ in development is one such area where recent genetics research has improved our understanding of signaling cascades leading to NTDs.²³

Cilia are evolutionarily conserved structures that are present on most vertebrate cells and are involved in essential cellular functions such as signaling.²⁰ Cilia also play an essential role in patterning the developing nervous system²³ by regulating neural stem cell migration,⁴⁴ neural progenitor differentiation,⁶² and CSF movement.^{1,2,19} Disruption of the highly orchestrated processes involved in cilia function leads to CNS phenotypes⁷⁶ seen in disorders known as ciliopathies. Ciliopathies, such as Meckel-Gruber syndrome¹³ (MKS, MIM 249000) and Joubert syndrome³⁶ (JBTS, MIM 213300), are used to

Abbreviations used in this paper: APC = adenomatous polyposis coli; Axin = axis inhibitor; Dsh = disheveled; Frz = frizzled; Hh = hedgehog; JBTS = Joubert syndrome; MKS = Meckel-Gruber syndrome; NTD = neural tube defect; PCP = planar cell polarity; PDGF = platelet-derived growth factor; Ptch = patched; Smo = smoothed; Wnt = wingless-type integration site family.

study a variety of systemic phenotypes and are increasingly associated with CNS abnormalities including cerebellar malformation, hydrocephalus, and NTDs.^{12,36}

In the following sections we discuss the various forms of cilia and how the molecular pathways, proteins, and their respective signaling cascades pattern the developing neural tube. We also present the ciliopathies¹⁶ commonly associated with NTDs and illustrate how these disorders improve insight into the molecular underpinnings of neural tube formation.

Cilia Structure

Cilia are microtubule-based structures with a cytoskeleton called the axoneme (Fig. 1). Two major forms have been distinguished on the basis of ultrastructure: primary cilia and motile cilia. Primary cilia have a 9 + 0 configuration, with 9 microtubule doublets arranged along their periphery. Motile cilia have a 9 + 2 configuration; in addition to the 9 microtubule doublets of the primary cilia, motile cilia also have 2 central microtubules that are capable of generating movement through the interaction of dynein arms.²⁸ Motile cilia are responsible for functions such as CSF movement²² and neural stem migration,⁴⁴ and disruption in these structures leads to a variety of CNS phenotypes.⁷⁶ In this paper, however, we will focus on primary cilia.

Primary cilia are immotile organelles that protrude

Fig. 1. Intraflagellar transport. Cilia are hairlike structures that require transport proteins to function normally. Kinesin and dynein carry cargo proteins via interactions with intraflagellar transport (IFT) particles. These 2 transport proteins “walk” on ciliary axonemes (composed of microtubules) to mediate transport of cargo to the tip of the cilium (anterograde transport, + end, kinesin-mediated) and from the tip of the cilium to the basal body (retrograde transport, – end, dynein-mediated). Based on Goetz and Anderson.²⁰

from the cell surface and serve as signaling centers for various developmental pathways.²⁰ Primary cilia result from interphase of the cell cycle and develop from a mother centriole that fuses with the plasma membrane and gives rise to the microtubule-based axoneme.⁴⁹ The cilia are anchored by a basal body where proteins and protein complexes are recruited to the developing cilium.³⁶ There is a dynamic interaction with surrounding elements³⁹ that regulates protein ingress and egress to the cilia in a process known as intraflagellar transport.¹⁸ Intraflagellar transport allows bidirectional movement of proteins and protein complexes to the distal tip of the cilia for microtubule maintenance, assembly, and signaling.⁵⁰ These same processes occur when material is carried back toward the cell body (Fig. 1). Until recently, primary cilia were not thought to play essential roles in cellular function; however, further investigations have revealed that the flux of material in and out of the cilia plays essential roles in chemosensation²⁰ and signaling cascades.¹⁷ As discussed below, primary cilia are also essential for neural tube patterning.^{29,64}

Signaling in Neural Tube Development

Normal patterning of the dorsal and ventral neural tube results from complex interactions in signaling cascades and concerted changes in cell fate and migration.⁴⁷ Cell fate, the process of cell differentiation to a specific cell type, is determined by the transcriptional regulation of genes, while cell movement relies on signals regulating cytoskeletal components. In vertebrates, neural tube development starts with the flattened neural plate, a thickening of the dorsal ectoderm overlying the mesoderm.³⁴ The neural plate is induced to roll onto itself during neurulation, forming neural crests that then fuse to form the neural tube structure, while the underlying mesoderm also involutes to form the notochord. Closure of the neural tube begins at the boundary near the craniocervical junction and precedes rostrally to the forebrain and caudally along the spine.⁵² The closure of the brain ends in the cranial neuropore, and the spine closure ends in the caudal neuropore.⁷ Dorsal and ventral patterning then follows and leads to the development of the roof plate along the dorsal neural tube and the floor plate along the ventral neural tube²⁹ (Fig. 2). Polarization continues with the development of motor and sensory neurons.

Patterning, differentiation, closure, polarity, fusion, and cell migration in the neural tube are closely regulated by several signaling cascades related to primary cilia. Normal neural tube development⁶⁷ relies on efficient and rapid interaction between protein receptors and complexes. Clustering of this machinery to a highly regulated subcellular location such as primary cilia promotes such resourceful communication between signaling pathways.⁶⁰ The enrichment of proteins in different parts of the cilia is also tightly regulated during development.

Hedgehog Signaling

Neural tube development results from the orchestration of signaling pathways such as the hedgehog (Hh) pathway.¹¹ The Hh pathway relies on the integrity of the primary cilium for signal transduction. Hh proteins and

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Fig. 2. Dorsal-ventral Wnt and Shh gradients in the developing neural tube. Wnt-producing cells are located in dorsal regions and Shh-producing cells are confined to the ventral regions of the neural tube creating a dorsal-ventral gradient with Wnt being expressed at high levels dorsally and Shh being expressed at high levels ventrally. Wnt and Shh act to regulate one another's expression in a negative feedback system. SHH = Shh; WNT = Wnt.

their complexes are localized to the primary cilium either at rest or following activation. During development the notochord generates a gradient of Sonic hedgehog (Shh) to specify the ventral neural tube and regulate neural progenitor development.²⁴ Shh is a morphogen that acts in a concentration-dependent manner to regulate cell fate. Shh is first expressed in the notochord and later expression arises from the floor plate of the ventral neural tube (Fig. 2). Normal dorsal-ventral patterning of the neural tube relies on Shh expression and its interactions with other ciliary proteins.⁴⁷

One set of ciliary proteins crucial for neural tube development is the glioma-associated oncogene homolog (Gli) family members that are zinc-finger transcription factors involved in Hh signaling during embryogenesis.³⁰ The Hh pathway regulates posttranslational modification of Gli proteins and regulates their transcriptional activity. In turn, Gli proteins act as both transcriptional activators and repressors of the Hh pathway.⁴⁷ In mammalian cells during development, the binding of Hh to its receptor patched (Ptch) in the cell membrane inactivates the receptor and releases inhibition of smoothened (Smo), a transmembrane protein. Hh binding to Ptch promotes localization of Smo to primary cilia, and facilitates downstream signaling with Gli proteins relocating to the tips of primary cilia following pathway activation.⁴⁸ Gli function is dependent upon intraflagellar transport for its translocation and function. Without activation, Ptch resides in primary cilia and prevents or limits the localization of Smo. The activation of Smo is, therefore, dependent upon the primary cilia. With Smo activated and present at the tip of the primary cilium, Gli and other transcription regulators can act on their downstream targets for neural tube patterning^{15,30} (Fig. 3A).

Shh regulates Gli and other transcription factors through multiple feedback loops during ventral neural tube patterning. Shh regulates gene expression by restricting dorsally expressed genes and inducing ventrally expressed transcription factors. The resulting Gli gradient regulates ventral neural tube development, and mutations in these proteins lead to abnormal neural progenitor populations.³⁰ The same Shh-dependent mechanisms can be seen in the developing midbrain.

Wnt Signaling

Recent evidence in Wnt signaling is beginning to explain the molecular mechanisms modifying failure of neural tube closure. While Shh expression is limited to a ventral location in the developing neural tube, Wnt expression occurs along the dorsal aspect, regulating neural crest development and patterning of the cells at this location where clinical phenotypes are observed. Along with bone morphogenic proteins, Wnt antagonizes Shh signaling to regulate neural progenitor differentiation at the roof plate.²⁷ Wnt signaling regulates Shh pathways by using, among other ciliary complexes, the Gli proteins to repress Shh expression along the dorsal regions of the neural tube.⁶⁸

Wnt proteins are secreted signaling proteins whose functions are largely dependent upon the receptor bound and the cell type targeted during neural development.⁶⁰ The canonical Wnt pathway is a signaling pathway that involves downstream activation of β -catenin after Wnt ligands bind to a cell-surface receptor in the frizzled (Frz) family. Binding of Wnt to Frz activates a set of dishevelled (Dsh) proteins that function through inhibition of an Axin-Gsk3-APC pathway to prevent degradation of β -catenin as an intracellular signaling molecule.¹⁵ β -catenin then upregulates various transcription factors to promote specific gene expression (Fig. 3C). While the integration of Shh and primary cilia function is well established, the relationship between primary cilia function and Wnt signaling is not as well understood and is an area of ongoing inquiry. There are indications that primary cilia inhibit the canonical Wnt pathway during development and that this signaling cascade is critical for normal CNS patterning.⁴⁰ Further investigation into the role of primary cilia and its influence on Wnt signaling during development is ongoing.¹⁴

The planar cell polarity (PCP) pathway involving Wnt signaling is also a well-established signaling cascade influencing neural tube development, and its interaction with primary cilia⁴³ is an area of significant genetic interest.³¹ The PCP pathway is considered a noncanonical Wnt pathway and does not involve activation of β -catenin.⁵⁴ The PCP pathway regulates the canonical pathway and is involved in patterning and cell orientation. PCP was first identified independent of its role in Wnt signaling; however, as mutant mouse models were developed with genetic knockout of various PCP partners, NTDs were found in the animals,²¹ suggesting Wnt's role in dorsal neural tube development.⁷¹

The PCP pathway is activated by binding of extracellular Wnt to protein receptors such as Frz that associate with the intracellular protein Dsh. There are other trans-

FIG. 3. Ciliary facilitation of cellular signaling. **A:** Shh signaling: Shh binds to its receptor, Ptch1. In the absence of Shh, Ptch1 inhibits Smo. Once Shh binds to Ptch1, Ptch1 inhibition of Smo is removed, and Smo is activated, translocating to the cilium, where it activates Gli transcription factors. These transcription factors translocate to the nucleus and modify gene expression. **B:** PDGFR α signaling: PDGF α activates the receptor, triggering the activation of downstream signaling proteins Akt and MEK/ERK, which promotes cell proliferation and cell survival. **C:** Wnt signaling: Wnt binds to its receptor, Frz (frizzled), leading to inhibition of the “death complex” (Axin, GSK [glycogen synthase kinase], and APC). This results in an increase in the intracellular levels of β -catenin, which translocates to the nucleus and alters gene expression. **D:** Noncanonical Wnt/ Ca^{2+} signaling (a) Intracellular Ca^{2+} release increases expression of inversin, which causes degradation of cytoplasmic Dsh through an APC-dependent mechanism, making it unavailable for canonical WNT signaling (inhibiting canonical WNT signaling). PCP signaling (b) Wnt binding to Frz activates Dsh, triggering activation of JNK, leading to modification of the actin cytoskeleton. Akt = protein kinase B; AXIN = Axin; MEK/ERK = mitogen activated protein kinase signaling pathway; PTCH1 = Ptch1; SMO = Smo. Based

membrane proteins, notably Flamingo (Celsr) and Vangl, which associate with cytoplasmic protein complexes such as prickles. Binding of Wnt ligands leads to activation of the downstream MAPK-JNK and RHO-RAC pathways to influence transcriptional regulation⁵⁹ (Fig. 3D). The transmembrane protein complexes in the PCP pathway accumulate in an asymmetrical manner and establish polarized cell-cell communication³¹ during critical steps in neural development. Neural tube closure is sensitive to loss of function in the PCP pathway,⁷⁵ which leads to adverse effects on neurulation and differentiation of neural progenitors.⁵² The PCP pathway acts upstream of ciliogenesis and regulates the position of basal bodies and cell orientation.⁶⁴ Alterations in PCP signaling in cilia manifest with CNS phenotypes in ependymal cells,²² where the function and morphology of motile cilia are adversely affected, leading to lethal hydrocephalus.⁶⁵

PDGF Signaling

Platelet-derived growth factor is another pathway that is required for normal neural development in the brain and spinal cord. Oligodendrocytic differentiation

and migration as well as neural tube closure are dependent upon this pathway's function. PDGF binds to its receptor, PDGFR, and both the ligand and receptors have homodimers and heterodimers that activate downstream PI3K-AKT and the MEK1-ERK1 pathways¹⁵ regulating cell proliferation and cell death (Fig. 3B). PDGFRs are localized to and upregulated in cilia and have been shown to be essential in oligodendrocytic precursor cell migration³⁵ in the spinal cord⁶⁶ and brain, and PDGFR-knockout mice have NTDs.⁶¹ Primary cilia transduce PDGF signaling, and investigators are exploring the role of this cascade to determine if it is involved in neuron migration.⁴⁰

Ciliopathies as Models of NTDs

There are more than a dozen ciliopathies that have been described in the genetics literature each with a set of genes associated with varying systemic presentations.⁴⁹ The unifying principle behind these disorders, however, is their dysregulation of cilia-related proteins. There are 2 main ciliopathies that are associated with NTDs (Table 1).

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TABLE 1: Ciliopathies commonly associated with NTDs

Ciliopathy	Known Genes	NTD	Systemic Phenotype
Meckel-Gruber syndrome	<i>MKS1-6</i>	occipital encephalocele	polycystic kidney disease, polydactyly, hydrocephalus, Dandy-Walker malformation
Joubert syndrome	<i>NPHP3, NPHP6, NPHP8, AHI1, MKS3, ARL13B, INPP5E, TMEM216, NPHP1</i>	encephalocele	mental retardation, vermian hypoplasia, midbrain malformation, retinal coloboma

Meckel-Gruber Syndrome

Meckel-Gruber syndrome is the most common ciliopathy associated with occipital encephaloceles and meningoceles as NTDs.⁴⁶ The estimated incidence of MKS is 1 in 3000 births,³⁸ and the condition is characterized by polydactyly and renal cystic dysplasia, as well as several CNS phenotypes such as Dandy-Walker malformation, anencephaly, arhinencephaly, holoprosencephaly, and hydrocephalus.⁶³ Meckel-Gruber syndrome is a neonatal lethal pleiotropic autosomal recessive disorder with NTDs resulting from abnormal signaling in primary cilia.³⁸ In MKS patients, NTDs may be diagnosed prenatally through ultrasound investigations, MRI, and elevated alpha-fetoprotein levels.⁵⁷

The pleiotropic phenotypes for MKS patients may result from the different *MKS* genes that are mutated. The *MKS1* protein and its interacting proteins localize to the base of the cilium and are often found in other ciliopathies. *MKS1* is frequently mutated in MKS patients with occipital encephaloceles, with approximately 90% of affected MKS neonates presenting with this NTD.⁵⁵ In mouse models, loss of *Mks1* or other *Mks* gene function alters neural tube patterning, resulting in exencephaly, a severe NTD in mice.¹² *Mks1* interacts with members of the tectonic family, transmembrane proteins that work through Hh pathways to pattern the neural tube in mice.⁵⁶ Altered Hh pathways are also seen in similar defects in human MKS patients.⁷² *MKS1* also interacts with *MKS6*, and *MKS6* mutations are associated with human NTDs.^{46,63} Mutations in *MKS5*, a ciliary gene whose protein product localizes to basal bodies and centrosomes and forms protein complexes with other essential ciliary proteins, are associated with occipital encephaloceles in fetuses.¹² Mutations in the various *MKS* genes,¹³ therefore, lead to abnormalities in proteins localized to primary cilia.⁸ *MKS* proteins are involved in human Hh signaling and are essential for cilia structure and function in maintaining normal neural tube patterning.

Joubert Syndrome

Joubert syndrome⁵⁷ is an autosomal recessive ciliopathy with an estimated prevalence of 1 in 100,000 patients. Patients affected by this disorder present with a variety of phenotypes including hypotonia, hypoplasia of the cerebellum, mental retardation, and abnormal breathing patterns in infancy.^{49,57} Presence of a molar-tooth sign—a widened interpeduncular fossa, thickened superior cerebellar peduncles, and vermian hypoplasia—on MR images is pathognomonic for JBTS.⁷⁰ In addition to these

CNS-related phenotypes, JBTS patients may present with NTDs, including occipital encephaloceles.^{33,69}

The genes associated with JBTS localize to the cilium and the basal body.¹⁴ There is phenotypic overlap seen in MKS and JBTS,⁵⁶ which may result from phenocopies; mutations in 2 different genes presenting with the same phenotype; allelic diversity, in which an affected allele can present with different phenotypes; or modifier genes that regulate or interact with an affected allele and modify its phenotypic expressivity.⁴⁹ The neurological defects seen in JBTS patients do not appear to be degenerative or progressive, and encephaloceles, if they occur with mild presentation, may be repaired with surgery.³⁷

Discussion

Neural tube defects have gained significant attention in the last several years, with the progress made in fetal treatment for selected patients with spina bifida.⁴ As a result of these reports, the prevention and treatment⁹ of these disorders is a growing area of research.⁷⁴ In addition to the therapeutic advances made by neurosurgeons in the fetal treatment of spina bifida, there has been a growing focus on folate supplementation in developing countries,⁷⁷ where access to financial resources and health care is limited. Folate deficiency has been implicated in the genesis of certain NTDs,⁷⁷ and the story is one of success in the medical community's ability to achieve results with preventative measures.¹⁰ Genetic studies⁴⁵ have recently been coupled with this focus on environmental factors⁵¹ and have yielded insight into the various signaling cascades and genes³ necessary for normal neural tube development.

There are several forms of NTDs that are commonly seen in human development and are classified primarily by the level of the CNS that is affected. Anencephaly refers to a defect resulting from failure of closure in the forebrain and midbrain. Craniorachischisis, the most severe NTD, originates from failure of fusion in the areas of both the hindbrain and spinal cord. Spina bifida is the most common NTD and is compatible with postnatal survival; the condition results from partial failure of spinal neural tube closure, usually in the lumbosacral region.⁷¹ Closed forms of NTDs are also seen with encephaloceles and meningoceles (Fig. 4). Genetic knockouts in mice have attempted to model these varying diseases and the anatomical level of neural tube abnormality. Mouse models utilizing PCP-related genes have identified NTDs resulting from abnormal fusion in the spinal cord and hindbrain, suggesting that these are models for cranio-

Fig. 4. Parietooccipital encephalocele—an example of a closed NTD in a newborn. **A:** Axial noncontrast MRI revealing a parietooccipital encephalocele with continuity with the ventricular system. **B:** Axial noncontrast CT scan obtained in the same patient revealing the underlying absence of bone fusion, resulting in a closed NTD.

rachischisis.²⁵ Several other mouse models have identified mutations in proteins required for ciliogenesis and in intraflagellar transport. These mouse models can be used to understand cilia and their essential role in neural tube closure in the cranial and spinal regions.

There is also genetic evidence in human patients with NTDs that primary cilia and the proteins responsible for ciliogenesis play a critical role in cranial and spinal neural development. Both MKS and JBTS are frequently associated with closed forms of NTDs. Studies on open forms of NTDs⁴¹ may be limited by small patient cohorts; however, recent multi-institutional studies⁴² have sought to pool patient sample data to aid in candidate gene identification in spina bifida.⁶ In addition to the MKS and JBTS ciliopathies, there are other ciliary proteins that are mutated in patients with CNS phenotypes resembling NTDs.⁴⁰ Mutations in *GLI2* are associated with human congenital malformations, including holoprosencephaly,²⁵ and a recent study has identified *GLI3* mutations in patients with features of the ciliopathy, oral-facial-digital syndrome³⁰ (OFDS, MIM 311200). PCP genes such as *CELSR1* and *SCRIB1* have been implicated in human cases of craniorachischisis, suggesting a role of Wnt signaling in cilia-related NTDs.⁵² Additional work also has correlated PCP genes *VANGL1*, *VANGL2*, *PRICKLE2*, and *FUZZY*²⁶ with human NTDs.^{58,73}

While genetic mechanisms continue to expand our understanding of neural tube formation, there remain a large percentage of patients with NTDs in whom the etiology of the disease is poorly understood and the genetic contributions remain unclear. Patients presenting for neurosurgical evaluation for treatable forms of NTDs, including meningoceles, encephaloceles, and spina bifida, may provide insight into the evolving role of cilia in the genesis of these disorders. Mutational analysis for cilia-associated genes or related primary-cilia signaling cascades will hopefully elucidate the contributions of these organelles with respect to human neural tube development and disease.

Conclusions

Most recently, fetal treatment of spina bifida has be-

come a reality at specialized fetal care centers in the US. This evolving treatment strategy has brought renewed attention to the treatment, prevention, and genetic study of NTDs. While surgery for NTDs can improve outcomes in affected children, there is hope that understanding the genetic underpinnings of these disorders will lead to non-invasive methods of prevention and treatment. Identifying genetic risk factors is one step toward this goal. Primary cilia are quickly being recognized as major contributors to normal neural development and to the patterning of the neural tube. The Shh pathway is a well-established model implicating these organelles in human disease. Future work on the canonical Wnt and PCP pathways in primary cilia may yield additional insight into the developmental mechanisms underlying neural tube development.

Disclosure

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author contributions to the study and manuscript preparation include the following. Conception and design: Vogel, Carter. Acquisition of data: Vogel, Carter, Abode-Iyamah. Analysis and interpretation of data: all authors. Drafting the article: Vogel. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Vogel. Administrative/technical/material support: Robinson.

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Manuscript submitted June 1, 2012.

Accepted June 18, 2012.

Please include this information when citing this paper: DOI: 10.3171/2012.6.FOCUS12222.

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