SCIENCE BEHIND THE STUDY
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Linking a Neurodevelopmental Disorder with a lncRNA Deletion

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Neurodevelopmental disorders are complex diseases that typically manifest during early childhood. Patients often present with a spectrum of clinical features, such as craniofacial anomalies and impairments in cognitive, behavioral, or motor skills. The current understanding of these disorders and their causes is far from complete. Of interest, then, is a report by Ganesh et al. in this issue of the *Journal*.[1] They describe the cause of a severe neurodevelopmental disorder involving developmental delay, facial dysmorphisms, and cerebral hypomyelination affecting three children: heterozygous de novo deletions in a gene (*CHASERR*) that encodes a long noncoding RNA (lncRNA). This gene lies immediately upstream of the gene *CHD2* and on the same strand of DNA; *CHD2* is overexpressed in the three affected children.

WHAT ARE LNCRNAS?

A lncRNA is an RNA transcript longer than 200 nucleotides that lacks protein-coding potential.[2] Most lncRNAs are expressed at a low level and in a manner specific to the cell and tissue type. A diverse catalog of lncRNAs has been described. Many share features with messenger RNAs (mRNAs) (see Key Concepts), such as an N7-methylguanosine cap at the 5′ end and a polyadenosine tail at the 3′ end, which suggests that they are processed by the same (or similar) machineries as those that process mRNAs. Unlike mRNAs, however, many lncRNAs are retained in the nucleus. Some remain tethered to the DNA from which they are transcribed.[2] Indeed, *CHASERR* lncRNA localizes to a topologically associated domain containing *CHASERR* and *CHD2*, in addition to other genes.[3] (Topologically associated domains occur across each chromosome, like beads on a string. The domains are physically arranged such that elements within each domain are close to one another — more so than elements outside that domain. This physical apposition is thought to influence the expression of genes within the domain.)

HOW DOES *CHASERR* WORK?

Research shows that lncRNAs regulate gene expression,[4] but understanding their modes of action has been challenging, owing to their large size, flexible conformation, and various interacting proteins. However, their subcellular localization patterns often provide clues. For example, the location of the *CHASERR* lncRNA (at the site of its own transcription) suggests that it may regulate *CHD2* (Fig. 1A).[5]

A heterozygous de novo pathogenic variant in *CHD2* has been linked to neurodevelopmental disorders (Fig. 1B).[6] Indeed, altered transcriptional activity at the *CHASERR* locus may be the pathogenic event in persons with haploinsufficiency for *CHASERR*. Rom et al.[5] observed that in mice, the promoters of *Chaserr* and *Chd2* compete to bind the same enhancer element, located approximately 200 kb upstream of *Chaserr*. (Enhancer elements are regulatory DNA sequences that activate transcription and thereby enhance gene expression.) They found that deletion of the *Chaserr* promoter resulted in increased contact between the *Chd2* promoter and this upstream enhancer.

There are additional mechanisms through which disruption of a lncRNA may disrupt the expression of other genes. For example, the lncRNA may recruit transcriptional regulators to a neighboring gene or form a three-stranded RNA–DNA hybrid known as an R-loop, affecting local gene expression.[2] Whether such modes of action occur at the *CHASERR–CHD2* locus has yet to be determined. The lncRNAs can also act in *trans* by decoying, chaperoning, and scaffolding microRNAs and proteins.[2] Although Ganesh et al.

propose that in-*cis* effects of *CHASERR* cause disease, in-*trans* effects cannot be ruled out at this stage.

WHAT ABOUT LNCRNAS IN NEURONAL DISEASES MORE GENERALLY?

Approximately 40% of the identified lncRNAs are expressed in the brain,[7] and there is some experimental support that at least a few of them contribute to neuronal disease. For example, *BACE1-AS* was shown to facilitate amyloid-beta plaque formation, and its expression is elevated in persons with Alzheimer's disease.[8] *KCNA2-AS* was suggested to tamp down the expression of the potassium channel subunit KCNA2, and *KCNA2-AS* overexpression induced neuropathic pain in rats.[9] Others have reported dysregulated lncRNAs in persons with schizophrenia, autism spectrum disorder, and Parkinson's disease.[4] However, because of the poor conservation of lncRNAs across species — only approximately 20% of human lncRNAs have homologues in mice[10] — evaluating their pathophysiological roles in vivo has been challenging.

AND THE STUDY BY GANESH ET AL.?

Inspired by observations in mice that *Chaserr*$^{+/-}$ led to substantially increased *Chd2* expression and severe growth retardation,[5] Ganesh et al. hypothesized that *CHASERR* haploinsufficiency in persons with neurodevelopmental disease increases CHD2 protein expression. CHD2 is a chromatin-remodeling enzyme that facilitates disassembly, eviction, sliding, and spacing of nucleosomes.[11] *CHD2* haploinsufficiency causes neurodevelopmental disorders,[6] and Ganesh et al. found increased expression of CHD2 accompanying *CHASERR* haploinsufficiency (Fig. 1C). These studies suggest that proper neurologic function is dependent on calibrated levels of expression of CHD2, raising the bar for a potential therapeutic intervention at the level of CHD2.

WHAT'S NEXT?

As noted by Ganesh et al., it would be prudent to analyze *CHASERR* in existing genome-sequencing data from patients with neurodevelopmental disorders. More broadly, the depletion of lncRNAs (at least those with evident neuronal functions in animal models) should be evaluated as the potential risk factors.

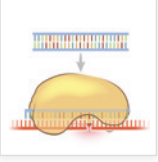
Key Concepts

Messenger RNA (mRNA)

RNA that is a template for protein synthesis. It is derived from pre-mRNA, which is directly transcribed from DNA. Pre-mRNA is processed in the nucleus: the exons (segments of coding sequence) are spliced together, introns (segments of noncoding sequence) are excluded, and a N7-methylguanosine "cap" is added to one end and a poly-A tail to the other. Pre-mRNA may be alternatively spliced, yielding two or more mRNA and protein isoforms. Mature mRNA is transported from the nucleus to the cytoplasm, where it may be translated into protein by the ribosome.

Transcription

The synthesis of an RNA molecule using a sequence of DNA as a template. The sequence of the RNA is therefore complementary to the strand of DNA from which it was transcribed. Transcription of chromosomal DNA, which takes place in the nucleus of eukaryotic cells, can generate functional RNAs (such as microRNAs and long noncoding RNAs) that are not translated into protein or can generate pre-mRNA that is processed into mRNA; the mRNA is transported to the cytoplasm, where it is translated into protein.



Small (or short) interfering RNA (siRNA)

A short, double-stranded regulatory RNA molecule (20 to 25 nucleotides) that interferes with the expression of a specific gene. It does so by binding to and targeting for degradation the mRNA of that gene. The siRNA is processed from a longer double-stranded RNA molecule by the enzyme Dicer. One strand of the siRNA (the guide RNA) creates a complex with other proteins to form the RNA-induced silencing complex (RISC). The other strand (the passenger RNA) is degraded during or after the formation of the RISC. The residual guide RNA, ensconced in RISC, snares mRNA with complementary sequence, bringing it into close contact with the RISC, which then degrades the mRNA. Artificial siRNAs, synthesized and chemically modified ex vivo, are the basis of some approved drugs and drugs in development.

Antisense oligonucleotide

A short (typically 12 to 30 nucleotides) single strand of chemically modified nucleotides that target mRNA to prevent translation into protein. Antisense oligonucleotides can bind directly to mRNA, leading to mRNA degradation; can inhibit generation of mature mRNA by blocking splicing of precursor forms of mRNA; or can block ribosome recruitment to inhibit protein translation. Antisense oligonucleotides can also be designed to target other RNAs, such as microRNAs and long noncoding RNAs.

An expanded illustrated glossary is available at NEJM.org

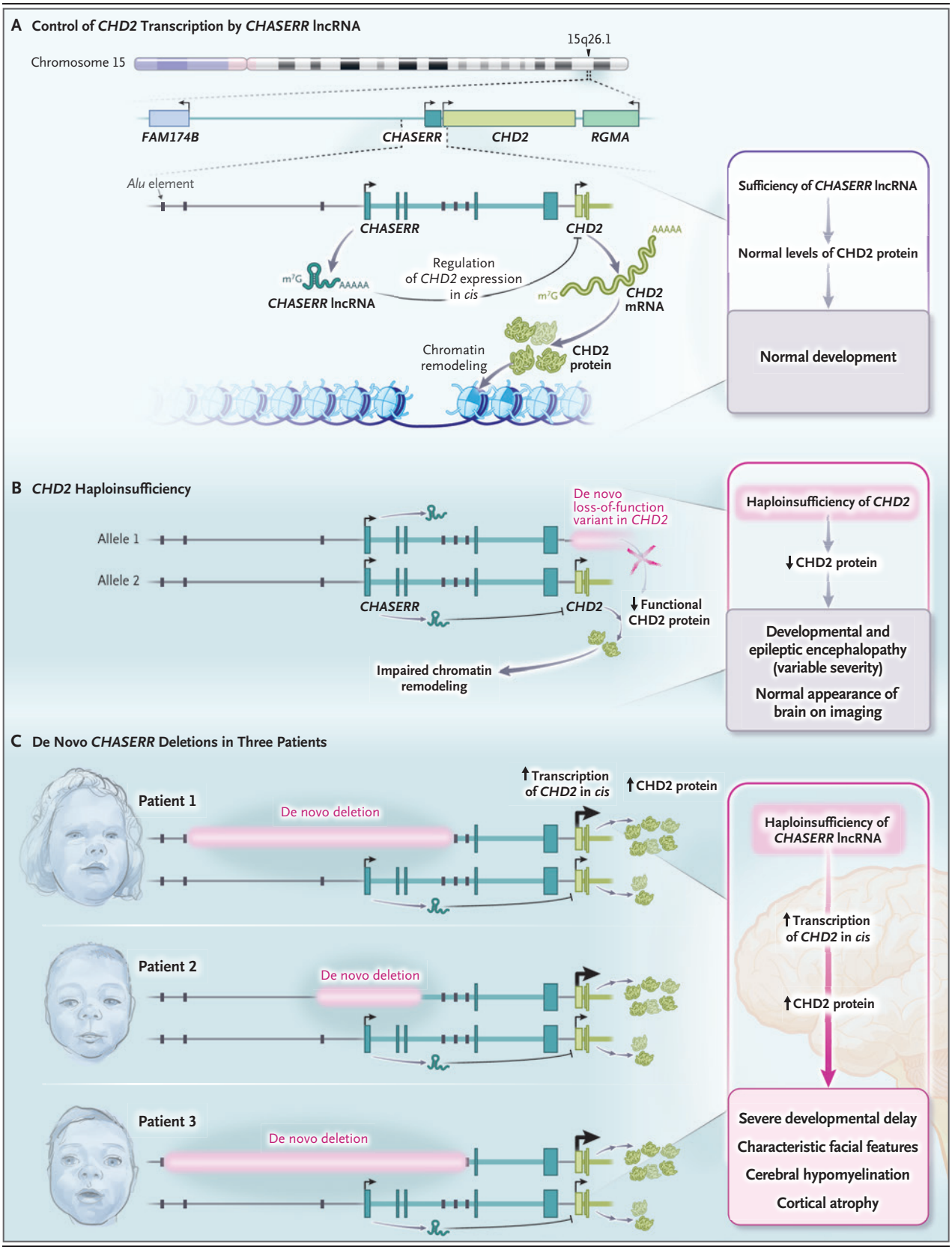


Figure 1 (facing page). *CHASERR* Haploinsufficiency in a Neurodevelopmental Disorder.

CHASERR is a long noncoding RNA (lncRNA) transcribed from human chromosome 15 (Panel A). Data from mouse and cellular models suggest that *CHASERR* suppresses *cis* expression of its adjacent gene, *CHD2*, which encodes a chromatin-remodeling protein essential for proper gene expression. In healthy persons, the transcription of an intact *CHASERR* gene ensures an appropriate level of *CHD2* transcription and thus CHD2-related gene expression. Haploinsufficiency of *CHD2* resulting in a substantially reduced level of functional CHD2 causes developmental and epileptic encephalopathy with variable severity and normal appearance of the brain on magnetic resonance imaging. Whether the expression and function of *CHASERR* are altered in the absence of CHD2 remains unclear (Panel B). Ganesh et al.[1] report that three unrelated children with de novo deletions of *CHASERR* have a severe neurodevelopmental disorder with characteristic facial features, cortical atrophy, and cerebral hypomyelination. These clinical features are attributed to the increased transcription of *CHD2* (in *cis*) and abundance of CHD2 protein (Panel C). The term mRNA denotes messenger RNA.

As for therapeutic strategies for patients with *CHASERR*-related neurodevelopmental disorders, small-interference RNA–based or antisense oligonucleotide–based therapies targeting *CHD2* expression could be considered. However, dose determination could prove challenging: too little may be just as bad as not enough.

Disclosure forms provided by the author is available with the full text of this editorial at NEJM.org.

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