Normalizing levels of MeCP2 in mouse model of MECP2 duplication syndrome restores neurological dysfunction

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Gene duplications are a common cause of intellectual disabilities and autism as well as various other neurological disorders. In a new study that appears online in the journal *Nature*, Dr. Huda Zoghbi, professor of molecular and human genetics at Baylor College of Medicine, and director of the Jan and Dan Duncan Neurological Research Institute at Texas Children's Hospital, and her team showed that there is a new potential way to treat such disorders.

MeCP2 (methyl-CpG-binding protein) is a cellular maestro, that modulates the expression of thousands of other genes in the brain, but its levels must be carefully controlled. Too little of the protein results in Rett syndrome, a childhood neurological disorder characterized by decreased cognition, inability to perform motor functions, particularly with hands, and autism-like behavior.

More than 10 years ago, Zoghbi who is also a Howard Hughes Medical Institute investigator, discovered that MeCP2 is a "Goldilocks" protein - too little causes Rett syndrome, but too much can cause a different neurological problem. The mouse models Zoghbi's lab generated with an extra copy of the MeCP2 gene developed a progressive neurological disorder. She suspected there must be children or adults with analogous neurological problems due to duplication of the MECP2 gene and this proved to be the case. Boys with the MECP2 duplication syndrome suffer from poor muscle tone and motor function, cognitive disability, epilepsy, autistic behaviors, respiratory infections, and premature death.

Once considered rare, it appears that the disorder is more common than previously thought, said Dr. Yehezkel Sztainberg, a post- doctoral fellow in Zoghbi's laboratory and first author of the report.

Now, using both state-of-the-art genetic techniques and a small molecule that can target specific genetic material (in this case the extra MECP2 gene), Zoghbi, Sztainberg and their colleagues have shown that it is possible to reverse the terrible effects of the duplicated gene.

In a series of experiments, they showed that mice engineered to have an extra copy of MECP2 develop all the symptoms of the human disorder. When such mice became fully-symptomatic, deleting the extra MECP2 gene normalized the levels of MeCP2 and reversed many of the phenotypes" providing the proof of principle that the disorder is reversible in adult animals," Sztainberg explained.

However, while deleting the gene proved the concept that the neurological dysfunction in the mice can be reversed, it is not a feasible treatment for human patients. To develop a translational tool, Zoghbi and her colleagues turned to antisense oligonucleotides (or ASOs for short). They found that normalizing the levels of MeCP2 with ASOs largely reversed the behavioral, molecular, and other deficits that plagued the mice. After they treated young adult mice, they used the ASOs in older mice who, by that time, were having many seizures. With treatment, the seizures stopped, said Zoghbi. "That was very encouraging," she said. It meant that the brains of the mice were not permanently damaged and could recover.

ASOs are being tested in individuals with spinal muscular atrophy, a devastating genetic disorder, providing hope that this approach can be used in human MECP2 duplications in the future.

ASOs take advantage of the most basic biology of genetics - the base adenine (A) always seeks out and pairs with the base thymine (T) in DNA or uracil

(U) in RNA and cytosine (C) pairs with guanine (G). When these antisense oligonucleotides (or small chemically modified nucleic acids) pair up with messenger RNA that has been transcribed from a gene, they silence the message, correcting the deficit caused by excess MeCP2 protein. In other words, the antisense base pairs seek out their natural partners and silence the extra gene.

"Now it's translational," said Sztainberg. The antisense treatment was developed in collaboration with Isis Pharmaceuticals and targets the human copy of the gene. Zoghbi hastened to add: "More work must be done in the animal model before we consider clinical trials. The additional experiments are to determine how to titrate and monitor the dose of ASOs to safely normalize MeCP2 levels without lowering them below the normal range to avoid causing Rett-like symptoms."

The antisense approach has potential for treating other disorders in which there is duplication of genetic material by targeting genes in the critical regions, said Sztainberg. Among these are autism and intellectual disabilities, Charcot-Marie-Tooth disease, Potocki-Lupski syndrome, as well as Down syndrome, the most common of these disorders, Sztainbergsaid. Treating Down syndrome, in which an entire chromosome is duplicated, would require determining the critical gene(s).

"Down syndrome is the prototypical gene dosage disorder," said Zoghbi. While the disorder results from a duplication of an entire chromosome, using antisense oligonucleotides to knock down some of the critical genes that affect learning and memory as well as dementia could prove important.

More information: Reversal of phenotypes in MECP2 duplication mice using genetic rescue or antisense oligonucleotides, *Nature*, DOI: 10.1038/nature16159