Every autistic individual is unique and has different strengths and co-occurring medical conditions. Research has shown that one medical condition that occurs in many autistic individuals is macrocephaly, which is characterized by a larger-than-normal head size. Since macrocephaly occurs in early brain development, it has been difficult to analyze the condition in autistic individuals since the average age of an autism diagnosis in the United States is five years old.

In recent years, scientists discovered a way to retrospectively investigate macrocephaly and early brain development in autistic children through the use of organoids. Organoids are simplified versions of organs or tissues that researchers grow in a laboratory. They are typically created from tissue samples, and can mimic the structure and function of real organs to some extent. Organoids provide a valuable tool for studying organ development, modelling various diseases, and testing the effects of drugs or treatments in a controlled environment without the need for animal or human subjects. They have been particularly useful in advancing research in fields like regenerative medicine, cancer studies and developmental biology, and have provided insights into the complexities of human biology.

One specific organoid — the cortical organoid — resembles the developing human cerebral cortex, which is the outermost layer of the brain. A recent study led by Dr. Flora Vaccarino explored the different cell types in cortical organoids to gain a better understanding of the development of macrocephaly in autistic individuals.

**Study**
In this study, brain organoids were generated using stem cells from male autistic individuals and their non-autistic fathers. A total of 13 pairs of fathers and sons participated in the study. Eight were autistic males with macrocephaly, and five autistic males had typical-sized heads. Participants were considered macrocephalic if their head circumference was at or above the 90th percentile.

Each participant underwent whole genome sequencing, and the results for fathers and sons were compared to each other. The researchers also identified excitatory and inhibitory neurons from the early forebrain organoids studied.
Results

• The autistic participants with macrocephaly had an unusually high number of excitatory neurons in the cortical plate, the structure that eventually develops to become the cerebral cortex.

• Autistic participants without macrocephaly had a decreased number of excitatory neurons in the cortical plate.

• Some genes associated with excitatory neurons showed an increase in expression in the macrocephalic patients. The same genes had a decreased expression in the participants with typical-sized heads.

• Of the 324 known autism risk genes previously identified by researchers, 111 are associated with macrocephaly and 47 are associated with normocephaly, or a normal-sized head.

Conclusions
This study points to the intriguing possibility that there might be two distinct subtypes of autism emerging from different paths in fetal brain development. The researchers showed this by identifying an imbalance between excitatory and inhibitory neurons in the early development of the brain. Such differences potentially led to some autistic children having enlarged heads. This suggests that autistic individuals with macrocephaly might be fundamentally different from normocephalic autistic individuals. The findings could lead to unique therapeutic avenues for each subtype of autism and emphasize the importance of personalized treatment strategies.

The researchers recognize that a larger sample size is needed in order to validate these findings and unravel the underlying molecular mechanisms responsible for these imbalances. As mentioned, this exploratory study only involved 13 pairs of fathers and sons. It is also important for future studies to investigate molecular pathways that might be responsible for the differences and imbalances seen in these autistic individuals.

Since they are capable of reproducing the cellular diversity of the human forebrain, organoid models of early brain development offer a promising way to further investigate and understand these possible subtypes of autism.

References