Does an ancient virus genome drive autism?
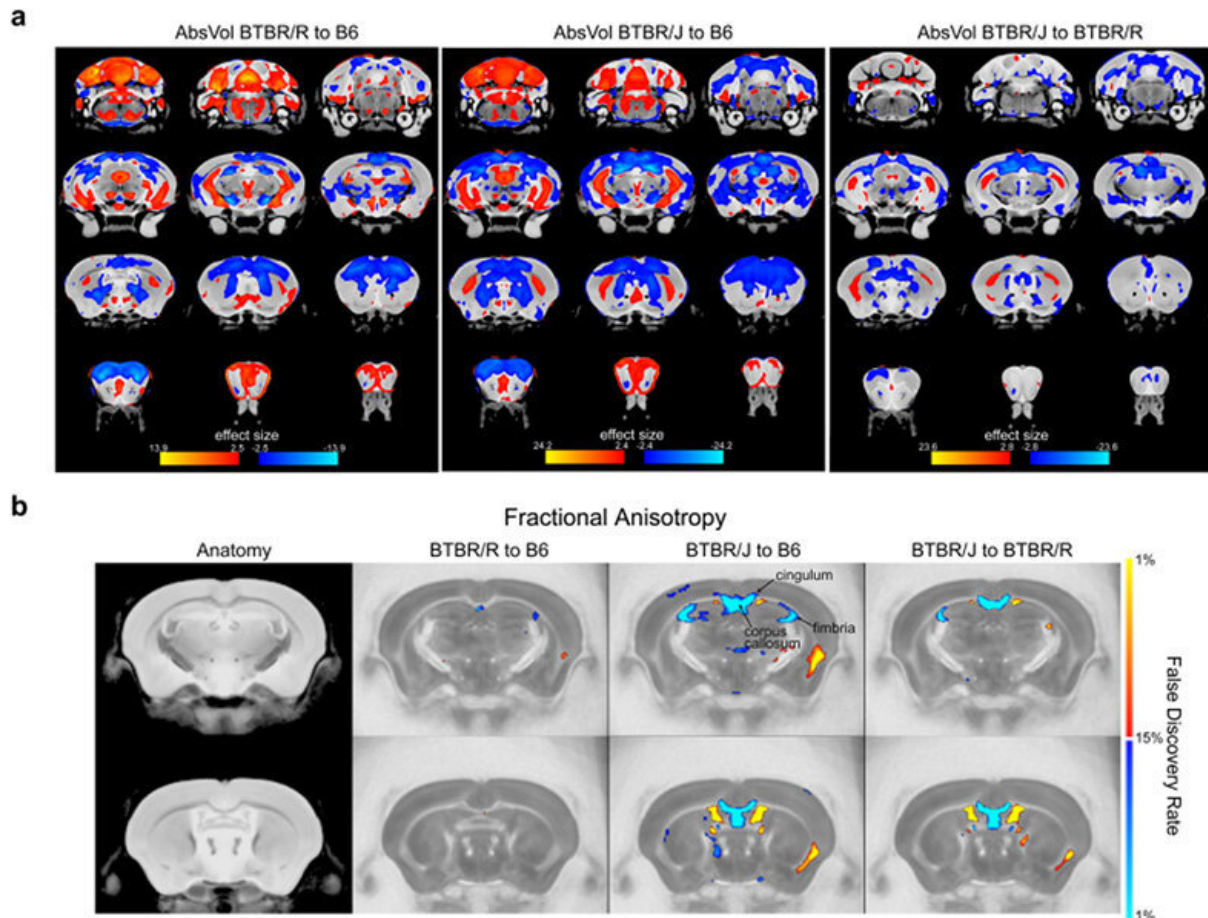
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Fig. 1. a. Brain structure comparisons of the following mice: Left: BTBR/R and B6 (normal mouse), Center: Comparison of BTBR/J and B6, Right: BTBR/J and BTBR/R. b. Diffusion tensor imaging to compare differences in nerve fibers. Red indicates the brain regions that were either bigger or had increased numbers of nerve fibers in BTBR/J mice in comparison to either B6 (left and center images) or BTBR/R (right image). Conversely, blue indicates brain regions in BTBR/J mice that were comparatively smaller or had decreased numbers of

nerve fibers.These scans revealed particularly significant differences between BTBR/J and BTBR/R mice's corpus callosum. Credit: *Molecular Psychiatry* (2023). DOI: 10.1038/s41380-023-01999-z

Although autism is a common neurodevelopmental disorder, the multiple factors behind its onset are still not fully understood. Animal models of idiopathic autism, especially mice, are often used to help researchers understand the complicated mechanisms behind the disorder, with BTBR/J being the most commonly used mouse model in the world.

Now, an international research collaboration including Kobe University's Professor Takumi Toru and Researcher Chia-wen Lin and colleagues have made new discoveries regarding autism onset in mouse models.

In their detailed series of experiments and analyses of BTBR/J mice and the other subspecies BTBR/R, they revealed that endogenous retrovirus activation increases a fetus's susceptibility to autism. They also discovered that BTBR/R exhibits autistic-like behaviors without reduced learning ability, making it a more accurate model of autism than the widely-used BTBR/J model. These research results were published in *Molecular Psychiatry* on March 7, 2023.

It is hoped that further research will contribute towards better classification of autism types, as well as the creation of new treatment strategies for neurodevelopmental disorders.
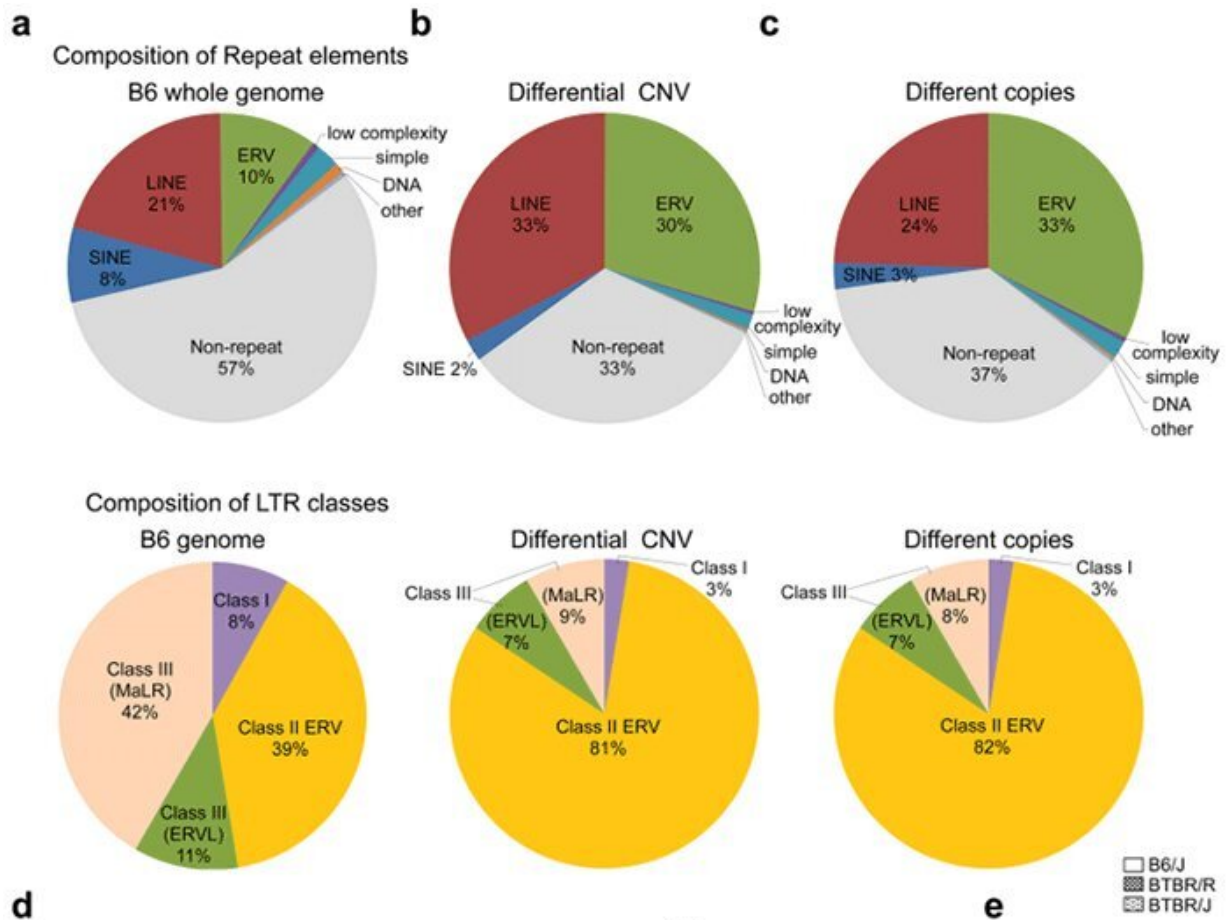
Fig. 2. a–c. Comparison of the repeated sequence of copy number variations, including retrovirus genes on the genome. d and e. Gene expression level analysis of repeated sequences: BTBR mice had more copy numbers of the endogenous retrovirus gene ERV than normal (B6) mice, and a portion of these genes were activated in BTBR mice. Credit: *Molecular Psychiatry* (2023). DOI: 10.1038/s41380-023-01999-z

Autism (autism spectrum disorder) is a neurodevelopmental disorder that remains largely unexplored despite the rapidly increasing number of patients. Reasons for this continuing increase in people diagnosed with autism include changes to diagnostic criteria and older fathers becoming more common.

Autism is strongly related to genetic factors and can be caused by abnormalities in DNA structure, such as copy number variations. Animal models, especially mice, are often used in research to illuminate the pathology of autism. Among these models, BTBR/J is a mouse model of the natural onset of autism that is commonly used. Studies have reported various abnormalities in BTBR/J mice including impairment of the corpus callosum (which connects the left and right hemispheres of the brain) and excessive immune system signaling. However, it is not fully understood why this particular lineage displays autistic-like behavioral abnormalities.

The aim of the current study was to shed light on the onset mechanism of these autistic-like behavioral abnormalities by conducting comparative analysis on BTBR/J and its subspecies BTBR/R.
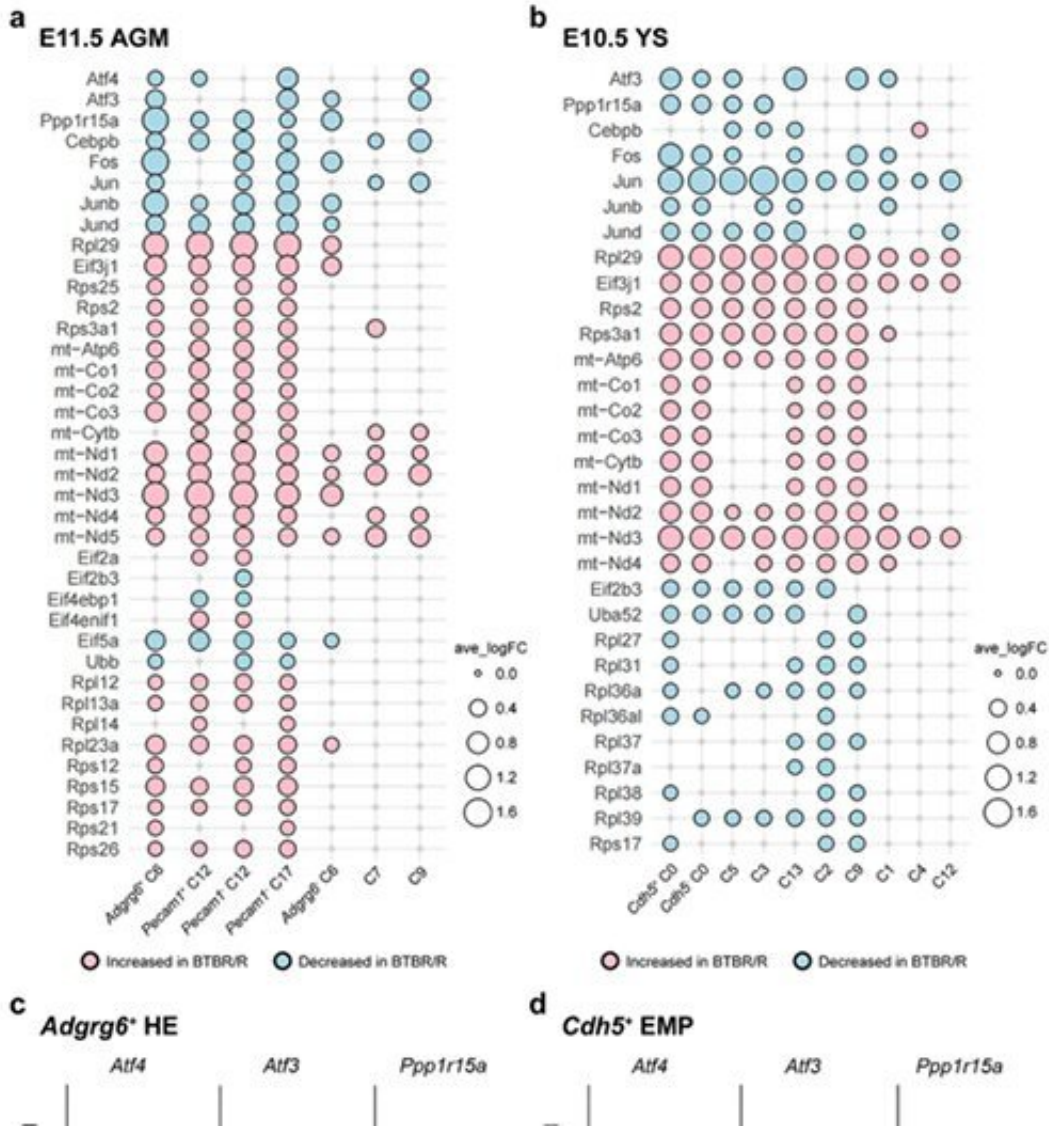
Fig. 3. a. AGM. B. Yolk sac: For BTBR/R autism model mice, genes with increased expression are indicated in pink and genes with decreased expression are in light blue. c–f. Comparison of gene expression levels in each cluster. In BTBR/R mice, there were changes in the expression of various genes (including stress response genes) that are indicative of endogenous retrovirus (ERV) activation. Credit: *Molecular Psychiatry* (2023). DOI: 10.1038/s41380-023-01999-z

First of all, the researchers conducted MRI scans on BTBR/J and

BTBR/R mice to investigate structural differences in each region of the brain. The results revealed that there were differences between BTBR/J and BTBR/R mice in 33 regions including the amygdala. A particularly prominent difference discovered was that even though BTBR/J's corpus callosum is impaired, BTBR/R's is normal (Fig. 1).

Next, the research group used the array CGH method to compare BTBR/R's copy number variations with that of a normal mouse model (B6). They revealed that BTBR/R mice had significantly increased levels of endogenous retroviruses (ERV) in comparison to B6 mice (Fig. 2 a–c). Furthermore, qRT-PCR tests revealed that these retroviruses were activated in BTBR/R mice (as shown in Fig. 2d). On the other hand, in B6 mice there was no change in the expression of LINE ERV (which is classified in the same repetitive sequence), indicating that this retroviral activation is specific to BTBR (Fig. 2e).

Subsequently, the researchers carried out single-cell RNA analysis on the tissue of embryonic BTBR mice (on the AGM and yolk sac). The results provide evidence of ERV activation in BTBR mice, as expression changes were observed in a group of genes downstream of ERV (Fig. 3).
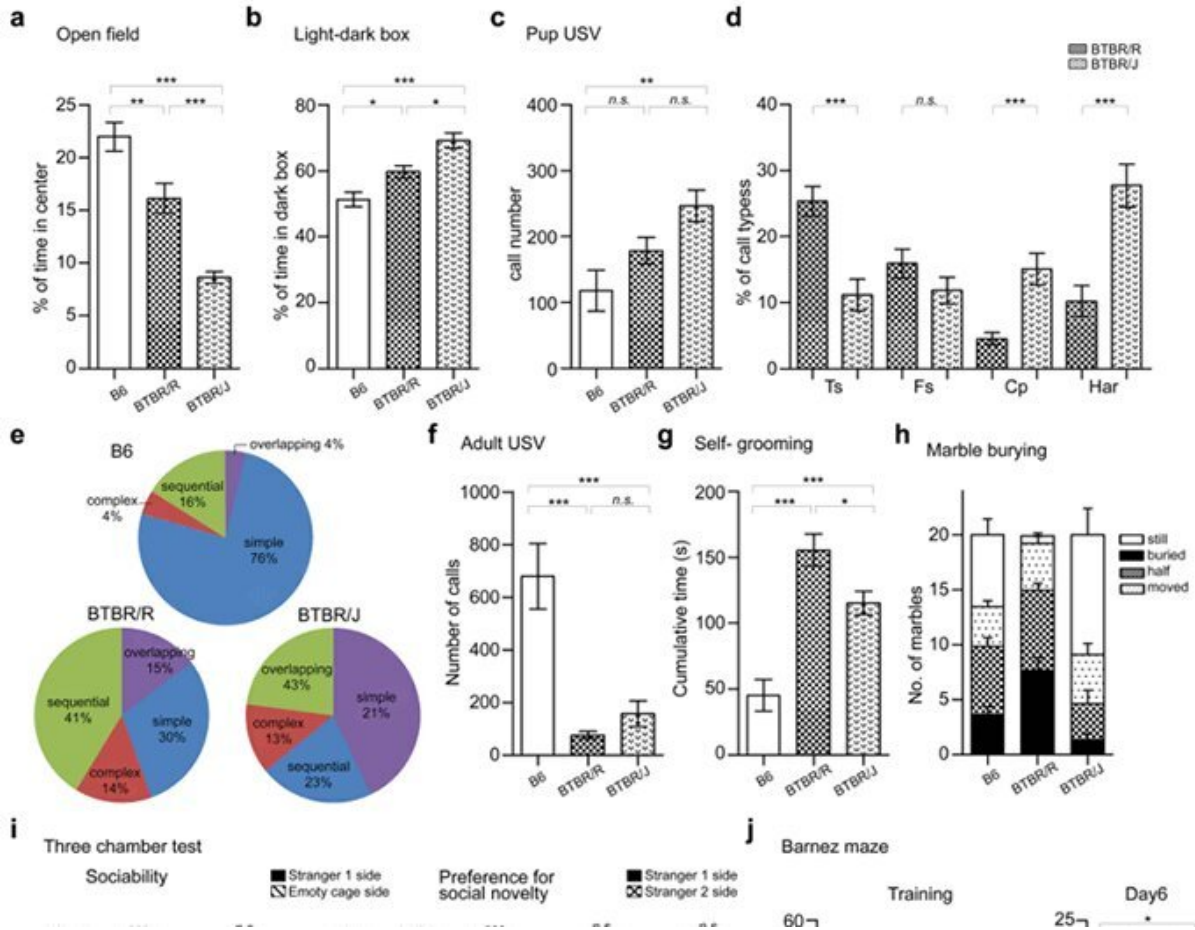
Fig. 4. a: Open field experiment b. Light-dark box experiment c-e. ultrasonic vocalizations made by mouse pups when separated from the mother. f. ultrasonic vocalizations of adult mice (in the presence of a mouse of the opposite sex). g. Self-grooming behavior. h. Marble burying test. i. 3-chamber social interaction test. j. Barnes maze spatial learning test. BTBR/R and BTBR/J mice share key autistic-like behavioral abnormalities demonstrated in the results for experiments c through i. However, there are differences in anxious behavior (a, b) and spatial learning (j). BTBR/R mice did not demonstrate spatial learning difficulties. Therefore, BTBR/R is a more suitable model of autism than the existing BTBR/J model because it exhibited autistic-like behavior without compromised spatial learning ability. Credit: *Molecular Psychiatry* (2023). DOI: 10.1038/s41380-023-01999-z

Lastly, the researchers comprehensively investigated the differences between BTBR/J and BTBR/R on a behavioral level. BTBR/R mice were less anxious than BTBR/J and showed qualitative changes in ultrasound vocalizations, which are measured as a way to assess communicative ability in mice (Fig. 4a–f). BTBR/R mice also exhibited more self-grooming behaviors and buried more marbles in the marble burying test (Fig. 4g, h). These two tests were designed to detect repetitive behavioral abnormalities in autistic individuals.

From the results, it was clear that BTBR/R exhibits more repetitive behaviors (i.e., it is more symptomatic) than BTBR/J. The 3-chamber social interaction test, which measures how closely a mouse will approach another mouse, also revealed more pronounced social deficits in BTBR/R than BTBR/J mice (Fig. 4i). In addition, a Barnes maze was used to conduct a spatial learning test, in which BTBR/J mice exhibited reduced learning ability compared to B6 (normal mice). BTBR/R mice, on the other hand, exhibited similar ability to B6 (Fig. 4j).

Overall, the study revealed that retrovirus activation causes the copy number variants in BTBR mice to increase, which leads to the differences in behavior and brain structure seen in BTBR/J and BTBR/R mice (Fig. 5).
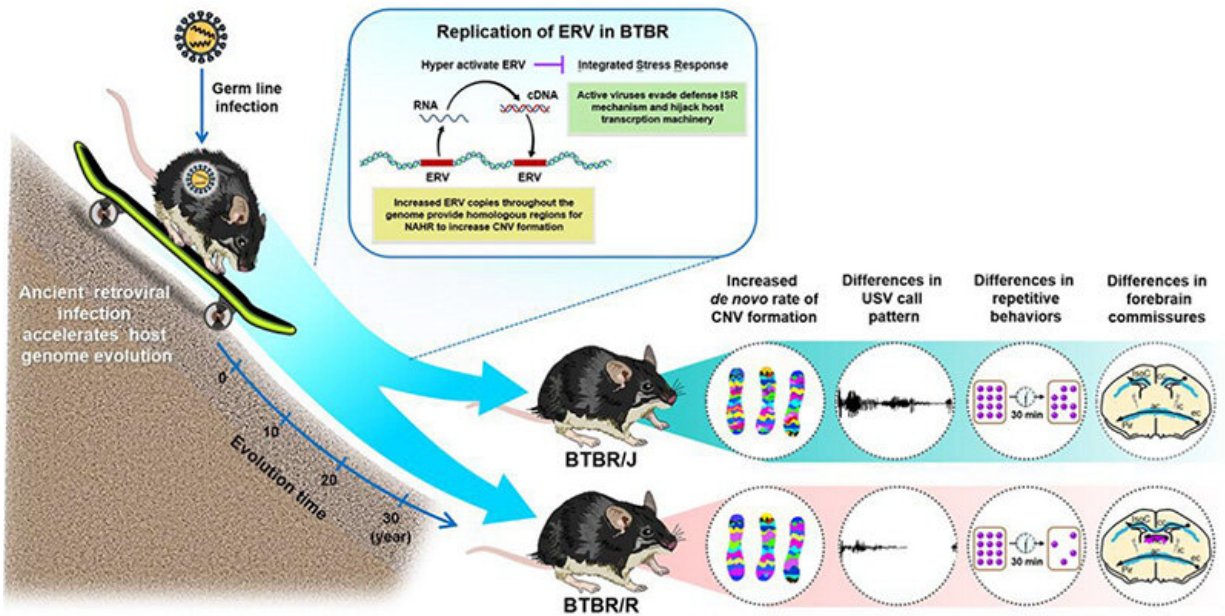
Fig. 5. In BTBR autism model mice (BTBR/J and BTBR/R variants), retrovirus activation makes copy number variations occur easily. In other words, they could be said to evolve at a faster speed than normal mice. The supporting evidence for this is that although BTBR/J and BTBR/R mice share a common ancestry, a mere 30 years of being raised in different environments has led not only to behavioral differences between the two species but also significant differences in brain structure (i.e. BTBR/R mice have a functioning corpus callosum, while BTBR/J mice do not). Credit: *Molecular Psychiatry* (2023). DOI: 10.1038/s41380-023-01999-z

BTBR/J mice are widely used by researchers as a mouse model of autism. However, the results of this study highlight the usefulness of the other lineage of BTBR/R mice because they exhibit autistic-like behavior without compromised spatial learning ability. The results also suggest that it may be possible to develop new treatments for autism that suppress ERV activation. Furthermore, it is necessary to classify autism subtypes according to their onset mechanism, which is a vital first step towards opening up new avenues of treatment for autism.