Autism-like behaviors in male mice with a Pcdh19 deletion

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Abstract

Mutations in protocadherin 19 (PCDH19), which is on the X-chromosome, cause the brain disease Epilepsy in Females with Mental Retardation (EFMR). EFMR is also often associated with autism-like symptoms. In mice and humans, epilepsy occurs only in heterozygous females who have a mixture of PCDH19 wild-type (WT) and mutant cells caused by random X-inactivation; it does not occur in hemizygous PCDH19 mutant males. This unique inheritance pattern strongly suggests the underlying disease mechanism operates via interference between WT and mutant cells rather than being a result of complete loss of PCDH19 functions. Although it remains unclear whether the other symptoms of EFMR also conform to this unique genotype-phenotype relationship, PCDH19 mutant males were recently reported to demonstrate autism-like symptoms. We, therefore, used a Pcdh19 knockout (KO) mouse model to ask whether a complete lack of PCDH19 causes autism-like behaviors. Consistent with the autism observed in EFMR females, we found Pcdh19 heterozygous KO female mice (with mosaic expression of PCDH19) show defects in sociability in the 3-chamber test. Surprisingly, hemizygous Pcdh19 KO male mice (without any PCDH19 expression) exhibit impaired sociability in the 3-chamber test and reduced social interactions in the reciprocal social interaction test. We also observed that, compared to WT mice, mutant mice display more repetitive behaviors, including self-grooming and rearing. These findings indicate that hemizygous Pcdh19 KO male mice show autism-like phenotypes.

Epilepsy in Females with Mental Retardation (EFMR) is reportedly caused by mutations (i.e., missense, nonsense, and deletion, etc.) in the X-linked gene protocadherin 19 (PCDH19) [1]. EFMR patients have early-onset seizures frequently associated with varying degrees of intellectual disability (ID) and autism-like symptoms [2–4]. As the name of the disease implies, it is highly sex-limited. Epileptic symptoms appear only in females heterozygous for PCDH19 mutations, whereas males hemizygous for PCDH19 mutations are unaffected carriers [5]. Because PCDH19 is X-linked, it is subject to random X-inactivation, producing mosaic expression in heterozygous mutant females [1]. The identification of male patients affected by postzygotic somatic PCDH19 mutations supports the idea that the disease mechanism is related to mosaic expression of PCDH19 [6, 7]. This idea is further supported by the case of an epileptic patient with Klinefelter Syndrome (47, XXY) heterozygous for a PCDH19 mutation [8]. This unique genotype-phenotype relationship suggests the symptoms of EFMR emerge from the abnormal interaction of two different populations of brain cells, some with and some without PCDH19 expression. This cellular mechanism is referred to as “cellular interference” [1, 6].

Recently, Pederick et al. [9] demonstrated a dramatic and abnormal segregation of PCDH19(+) and PCDH19(−) cells in the developing brains of Pcdh19 heterozygous KO female mice that was well-correlated with seizure-like activities as recorded by electrocorticogram. This result provided the first experimental evidence of cellular interference as a key pathogenic mechanism in EFMR. It is unclear, however, whether autism spectrum disorder (ASD) in EFMR also conforms to both this unusual inheritance pattern and cellular interference mechanism. It is notable that recent human studies identified some males with ASD who have mutations in PCDH19 [3, 10, 11], suggesting PCDH19 mutations may also play a role in producing the symptoms of males with ASD via mechanisms other than cellular interference. In this study, we investigate this possibility using a Pcdh19 KO mouse model.

Consistent with previous studies [9, 12], heterozygous Pcdh19 KO female mice show an abnormal “tiger-striped” pattern of segregation between PCDH19(+) and...
Fig. 1 Pcdh19 hemizygous KO male mice exhibit autism-like behaviors. a Representative images of tdT-expressing cells (PCDH19-negative cells) in coronal sections of HET-tdT female and KO-tdT male brains at P56 (left). Scale bar, 200 μm. Schematic diagrams of the X and Y chromosomes of heterozygous KO female and hemizygous KO male mice, showing tdT and Pcdh19-null alleles (in which the PCDH19 coding sequence is replaced with a LacZ cassette) are located on the same X-chromosome; X\textsuperscript{LacZ-tdT} (right). b Group-averaged heat map images for the movement of WT (X/Y) and Pcdh19 hemizygous KO (X\textsuperscript{LacZ-tdT}/Y) male mice during the 3-chamber sociability test (S1 vs O). c Quantification of the results shown as sniffing time, based on the time spent sniffing S1 vs O in the sociability test and S1 vs S2 in the social novelty test (*p < 0.05, **p < 0.01, paired Student’s t test). d The time of social interaction of Pcdh19 KO male mice during the reciprocal social interaction test. e-f Repetitive behavior tests: the number of rearing incident (e) and the time spent self-grooming (f) in Pcdh19 KO male mice (*p < 0.05, **p < 0.01, ***p < 0.001, unpaired Student’s t test). n = 10–12 male mice per genotype. All data are presented as means ± SEM.
PCDH19(−) cells in the brain (Fig. 1a). In contrast to heterozygous Pcdh19 KO female mice, we did not observe a similar segregation in hemizygous Pcdh19 KO male mice, despite both having a tdTomato (tdT) reporter gene on the X-chromosome where the Pcdh19 gene was deleted (Fig. 1a). This finding suggests any phenotypes in hemizygous Pcdh19 KO male mice are independent of the abnormal sorting mechanism observed in heterozygous Pcdh19 KO female mice.

First, we asked whether heterozygous Pcdh19 KO female mice show autism-like behaviors in our experimental setting (see Additional file 1 for the detailed methods). We found that heterozygous Pcdh19 KO female mice (X<sup>lacZ</sup>/Y) do not show any preference toward exploring a novel mouse (S1) versus a non-social novel object (O) in the 3-chamber test (the sociability test). This suggests heterozygous Pcdh19 KO female mice recapitulate the autism-like symptoms of female EFMR patients. In the social novelty test, in which we measured preference toward a familiar mouse (S1) and a novel mouse (S2), we found the heterozygous Pcdh19 KO mice (X<sup>lacZ</sup>/Y) spend more time exploring the novel mouse (S2) (see Additional file 2).

We next examined the male mice to determine whether they also show any social impairment. In the 3-chamber sociability test, we found male Pcdh19 KO (X<sup>lacZ</sup>/Y) mice spend significantly more time sniffing a non-social novel object (O) rather than a novel mouse (S1), suggesting abnormal sociality. Both hemizygous males and controls, however, show similar preference towards a novel mouse (S2) in the social novelty test (Fig. 1b–c). We then measured reciprocal social interactions to confirm the social abnormalities of male Pcdh19 KO (X<sup>lacZ</sup>/Y) mice. We found they spend significantly less time interacting with a stranger mouse than WT (X/Y) mice do (Fig. 1d). To determine whether male Pcdh19 KO (X<sup>lacZ</sup>/Y) mice show increased repetitive behavior—another autism-like phenotype—we monitored their rearing and stereotyped grooming behaviors. We found Pcdh19 X<sup>lacZ</sup>/Y male mice spend more time rearing and self-grooming (Fig. 1e–f) than WT (X/Y) male mice. Thus, their abnormal social interaction results and increased repetitive behaviors suggest hemizygous Pcdh19 KO (X<sup>lacZ</sup>/Y) mice show autism-like behaviors.

We do not yet know how the complete loss of Pcdh19 causes autism-like behaviors in male mice, but considering the fact that the abnormal segregation pattern occurs only in the brain of female heterozygous Pcdh19 KO mice [9, 13], but not in male KO mice, our present findings suggest the mechanism will be distinct from the cellular interference mechanism that underlies the epileptic symptoms of EFMR. It is possible a loss of PCDH19-mediated cell-to-cell adhesion may contribute to autism-like behaviors in hemizygous Pcdh19 male KO mice. PCDH19 regulates intracellular binding proteins like NONO and the GABA<sub>A</sub> receptor alpha subunits [14, 15]. Hence, it is also possible the absence of PCDH19 disrupts the function of unidentified autism-related binding proteins. Our social interaction results were inconsistent with a previous study in which heterozygous KO female and hemizygous KO male mice showed no abnormalities in the social interaction tests [12]. In fact, we are still unclear why the mice showed this inconsistency, but we found that the background of the mouse, the targeted exons, and the size of the behavioral apparatus used for the experiment were all different.

This is the first report showing, in genetically modified mice, that autism-like behaviors induced by Pcdh19 mutations are not subject to the same genotype-phenotype relationship observed in epileptic symptoms of EFMR. From this finding, we postulate that both mosaic expression of PCDH19 and PCDH19 insufficiency contribute to the pathogenesis of EFMR. Considering the fact that male patients affected by mosaic PCDH19 mutations also show autism [6], the induction of autism in a male patient may not require complete loss of PCDH19 in every cell. In the future, the generation of male mice with mosaic Pcdh19 deletions will help address the question of whether both mosaic loss and complete loss of PCDH19 result in autism-like behaviors.

**Supplementary information**

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**Abbreviations**

ASD: Autism spectrum disorder; EFMR: Epilepsy in females with mental retardation; ID: Intellectual disability; KO: Knockout; PCDH19: Protocadherin 19; SEM: Standard error of the mean; tdT: tdTomato; WT: Wild-type

**Authors’ contributions**

CHK conceptualized and designed the research. JL, JR, SK, HJN conducted the behavioral experiments. CHK, JL analyzed, interpreted the data. CHK, JL prepared the manuscript. All authors read and approved the final manuscript.

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**Availability of data and materials**

All data and materials are available upon requests.
Ethics approval
All animal experiments were performed in compliance with guidelines approved by the Institutional Animal Care and Use Committee (IACUC) of Yonsei University Health System (reference number: 2018–0285).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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