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Genetic Mapping in Mice Reveals the Involvement of *Pcdh9* in Long-Term Social and Object Recognition and Sensorimotor Development

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ABSTRACT

BACKGROUND: Quantitative genetic analysis of basic mouse behaviors is a powerful tool to identify novel genetic phenotypes contributing to neurobehavioral disorders. Here, we analyzed genetic contributions to single-trial, long-term social and nonsocial recognition and subsequently studied the functional impact of an identified candidate gene on behavioral development.

METHODS: Genetic mapping of single-trial social recognition was performed in chromosome substitution strains, a sophisticated tool for detecting quantitative trait loci (QTL) of complex traits. Follow-up occurred by generating and testing knockout (KO) mice of a selected QTL candidate gene. Functional characterization of these mice was performed through behavioral and neurological assessments across developmental stages and analyses of gene expression and brain morphology.

RESULTS: Chromosome substitution strain 14 mapping studies revealed an overlapping QTL related to long-term social and object recognition harboring *Pcdh9,* a cell-adhesion gene previously associated with autism spectrum disorder. Specific long-term social and object recognition deficits were confirmed in homozygous (KO) *Pcdh9*-deficient mice, while heterozygous mice only showed long-term social recognition impairment. The recognition deficits in KO mice were not associated with alterations in perception, multi-trial discrimination learning, sociability, behavioral flexibility, or fear memory. Rather, KO mice showed additional impairments in sensorimotor development reflected by early touch-evoked biting, rotarod performance, and sensory gating deficits. This profile emerged with structural changes in deep layers of sensory cortices, where *Pcdh9* is selectively expressed.

CONCLUSIONS: This behavior-to-gene study implicates *Pcdh9* in cognitive functions required for long-term social and nonsocial recognition. This role is supported by the involvement of *Pcdh9* in sensory cortex development and sensorimotor phenotypes.

Keywords: Associative learning, Autism spectrum disorder, Genetic mapping, Information processing, *Pcdh9*, QTL, Quantitative trait locus, Recognition, Sensory cortex, Social cognition

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The genetic architecture of psychiatric disorders is complex and composed of multiple interacting factors. Although many candidate genes have been identified, not much is known on how these genes impact specific components of behavior and social functioning (1,2). One of the reasons is the difficulty of linking gene products or molecular pathways to the expression of social behavior; another, the challenge to find meaningful genetic phenotypes that directly relate to cognitive deficits in human psychopathology. A commonly proposed solution to enhance the relevance and specificity of genetic phenotypes is to focus on underlying quantitative traits that more directly index neurobiological disruptions (3–5). This is an attractive approach, though highly demanding, requiring large human samples characterized for relevant phenotypes. To overcome this limitation, we have proposed a complementary strategy to perform quantitative genetic analysis of basic social behaviors in mice that are regulated by processes likely to be conserved across species (6). The advantage of this approach is that genetic variation underlying differences in specific traits can be detected using sophisticated mapping panels to gain understanding of brain function.

In this study, we followed this strategy and performed the first forward genetic screen of social recognition (SRE) in mice followed by a functional characterization of a candidate gene

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in a newly generated knockout (KO) model. We chose SRE, as it represents a basic behavior to form social relationships and to establish social hierarchies essential for survival (7,8). Taking advantage of this, SRE tests provide a unique approach to test essential brain functions required for social behavioral adaptation. Social recognition can be analyzed in a social discrimination task that takes advantage of the innate drive of animals to investigate unfamiliar over familiar social stimuli and allows direct stimulus presentation for the acquisition of the full olfactory signature (9,10). This test is inexpensive and fast with easy application and therefore highly suitable for quantitative trait locus (QTL) studies involving large numbers of mice. The SRE capacity can be tested after one-trial learning with different time intervals, offering the unique opportunity to separately investigate different processes underlying recognition during social interaction (9). For instance, impairments in both short-term and long-term SRE may indicate inadequate sensory (e.g., smell) perception or basic encoding problems, while an impairment restricted to long-term but not short-term SRE indicates proper encoding but reduced consolidation or cognitive processing of social information. Impairments in such higher order processes will have consequences for long-term social interactions and may affect behavioral development. Furthermore, cue specificity of recognition can be investigated by comparing the performance in social versus nonsocial (object) discrimination (11).

Here, we describe the QTL mapping of social recognition in a panel of consomic strains, leading to the implication of protocadherin 9 (*Pcdh9*) in long-term social and object recognition and other phenotypes related to higher order information processing.

METHODS AND MATERIALS

Chromosome Substitution Strains

Breeding pairs for C57BL/6J, A/J, and all 21 C57BL/6J-Chr #^{A}/NaJ (chromosome substitution strain [CSS]) strains (# = 1–19, X or Y) were obtained from the Jackson Laboratory (Bar Harbor, Maine). Strain colonies were subsequently bred in-house. All experiments were approved by the ethical committee for animal experimentation of the University Medical Center Utrecht and performed according to the University Medical Center institutional guidelines that are in full compliance with the European Council Directive (86/609/EEC).

Social and Object Discrimination Tests

Two-day versions of social and object discrimination paradigms were used to measure social and object recognition capacity, as we performed previously (12) (Supplement 1). In brief, for social discrimination, test animals were habituated in the test cage for 5 minutes and initially exposed to an age- and gender-matched A/J conspecific for 2 minutes and then, after intertrial intervals (ITI) of 5 minutes, exposed to the familiar conspecific and a first novel A/J conspecific for 2 minutes. On day 2, after the 24-hour ITI, the test animal was habituated for 5 minutes and re-exposed to the same familiar intruder of day 1 and to a different novel intruder animal from a different cage and housing room than the intruder of day 1 for 2 minutes. For object discrimination, a similar test was used involving novel and familiar objects instead of intruder mice (Supplement 1). Behavioral assessments were conducted at 3 to 5 months of age and only male mice were used in the current study.

QTL Mapping

A CSS14-F_1 generation was derived by reciprocal mating of C57BL/6J and C57BL/6J-Chr 14^{A}/NaJ (CCS14) animals. The F_1 hybrids were intercrossed, producing CSS14-F_2 animals. The F_2 and control C57BL/6J animals were tested at the same age range as the CSS mice with social and object discrimination tests consecutively in fixed order, with 1 to 2 weeks between the tests. DNA samples of the CSS14-F_2 mice were obtained according to a standard procedure (13). For generating a genetic map of chromosome 14, 19 microsatellites were chosen from the mouse genome database (Mouse Genome Informatics, Jackson Laboratory, Bar Harbor, Maine; http://www.informatics.jax.org/) (Supplement 1). Segregation ratio of the genotypes of individual microsatellite markers was checked with the chi-squared goodness-of-fit test. None of the markers showed significant segregation distortion ($p > .05$). Cox *et al.* (14) have constructed a revised genetic map of the mouse genome and demonstrated that utilization of the revised map improves QTL mapping. Therefore, marker positions were taken from this map by using the mouse map converter (Jackson Laboratory; http://cgd.jax.org/mousemapconverter/). The location of the QTLs affecting the measured quantitative traits was determined by using the scan one function in the R/qtl package (http://www.R-project.org) and using cross as an additive and interactive covariate (15). Because the traits were normally distributed, the interval-mapping module was used. Results were expressed as logarithm of the odds (LOD) scores. The LOD score threshold level was determined through permutation tests (random shuffling of genotypes with phenotype based on 10,000 permutations). The LOD score threshold level, set at a confidence level of .05 (this level is generally accepted for statistical significance) was 3.31, whereas at tenfold, increased confidence level (.005) corresponds to a LOD score of 4.52. The power to detect a main effect locus was calculated using R/qtlDesign (http://www.R-project.org)(16). Based on an average marker density of $\sim$ 3.0 cM, 192 CSS14-F_2 mice are sufficient to detect a QTL that has a LOD score of 3.31 and accounting for 10% of the variance in the phenotypes with a power of 80%.

Generation and Breeding of *Pcdh9* Knockout Mice

Pcdh9-deficient mice were generated using a standard procedure (17) (Supplement 1). A targeting vector was designated to delete the second exon of the mouse *Pcdh9* gene, which encodes extracellular, transmembrane, and part of cytoplasmic domains (Supplement 1).

Developmental Behavioral and Neurological Screening

The extended SmithKline Beecham, Harwell, Imperial College, Royal London Hospital, phenotype assessment was used to assess basic sensorimotoric functions, locomotor activity, and various reflexes in mice (18,19) (Supplement 1). Sociability was measured using the three-chambered apparatus (20) and a variant of the reversal/set-shifting task was used to assess multi-trial discrimination learning and behavioral flexibility (12) (Supplement 1). Anxiety-like behavior in a novel environment