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LETTER TO THE EDITOR

Involvement of Clustered Genes in Mammalian Functions: Their Relation in a Rat Mutant Strain

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Gene duplication and genome duplication are considered to play significant roles in vertebrate evolution. Although DNA sequences are highly homologous in mammalian species, differences are present in the number of genes in gene families. With the exception of a few gene families, the involvement of particular gene families in mammalian functions is yet to be clarified. Our recent studies have revealed that a rat mutant strain, Hirosaki hairless rat (HHR), shows defects in certain mammalian functions, resulting from deletions of duplicated genes belonging to particular gene families. Mammals are characterized by a number of common traits, including lactation and hair growth (McClellan et al. 2008). Mammary glands are essential for mammals as organs to produce milk for their young. In eutherian placental mammals, embryonic and fetal development occur in the maternal uterus, and immuno-suppressive regulatory T cells (Tregs) are required to maintain successful pregnancy by inducing tolerance to the semiallogeneic fetus (Rowe et al. 2012; Jiang et al. 2014). Thus, Tregs, as well as mammary glands, are essential for mammalian development.

HHR is a mutant strain spontaneously derived from Sprague–Dawley rats (SDRs), and its inheritance is autosomal recessive (Fig. 1, Hanada et al. 1988; Akita et al. 2009). The hairless phenotype of HHRs is due to the deletion of an 80-kb genomic DNA, ranging from exon 9 of *Kb25* (*Krt85*) to exon 9 of *Krt2-25* (Fig. 2a). The breakpoints of *Krt85* and *Krt2-25* are within a 95-bp portion shared by the two genes, indicating that the deletion is due to non-allelic homologous recombination (Nanashima et al. 2008). Hard keratin-based hair, lactation, and mammary glands are all characteristics common in mammals (McClellan et al. 2008). Female HHRs show involution of the mammary glands at an early stage of lactation (Nanashima et al. 2005). Array comparative genomic hybridization revealed the deletion of 50 kb of genomic DNA from 1q21 in HHRs, including the *Pla2g4c* gene and neighboring *LOC691813* gene (Fig. 2b, Nanashima et al. 2015). Both genes are highly homologous and their breakpoints are within a shared 149-bp region. Inhibition of *Pla2g4c* expression in rat mammary cells has revealed that it is involved in the prevention of apoptosis induction.

In the HHR thymus, the number of naturally occurring Treg (nTreg) was decreased. The *Ly49* genes: *Ly49s3, s4, i3*, and *i4*, in the chromosome 4q42 region, are deleted in HHRs (Fig. 2c). Among these genes, the *Ly49s3* gene is expressed in thymic dendritic cells in wild-type SDRs, but not in HHRs. CD4-positive SDR thymocytes can be differentiated to nTreg by co-culture with SDR dendritic cells, whereas HHR dendritic cells show deficient induction of nTreg differentiation (Yamada et al. 2013). The direct involvement of *Ly49s3* in the induction of nTreg differentiation is demonstrated by forced expression of *Ly49s3* in HHR dendritic cells.

Thus, basic hair keratin genes, *Pla2g4c* and *Ly49*, are deleted in HHRs, and these deletions result in alterations to mammalian functions. Interestingly, these genes are members of gene families that form gene clusters, suggesting that such clustered genes may be important for the

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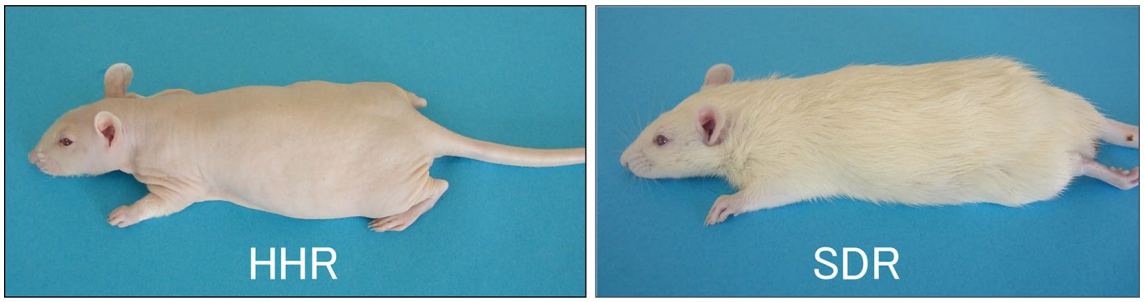

Fig. 1 Gross appearance of HHR. SDR (*right*) has a normal pelage, but HHR (*left*) shows short and sparse pelage

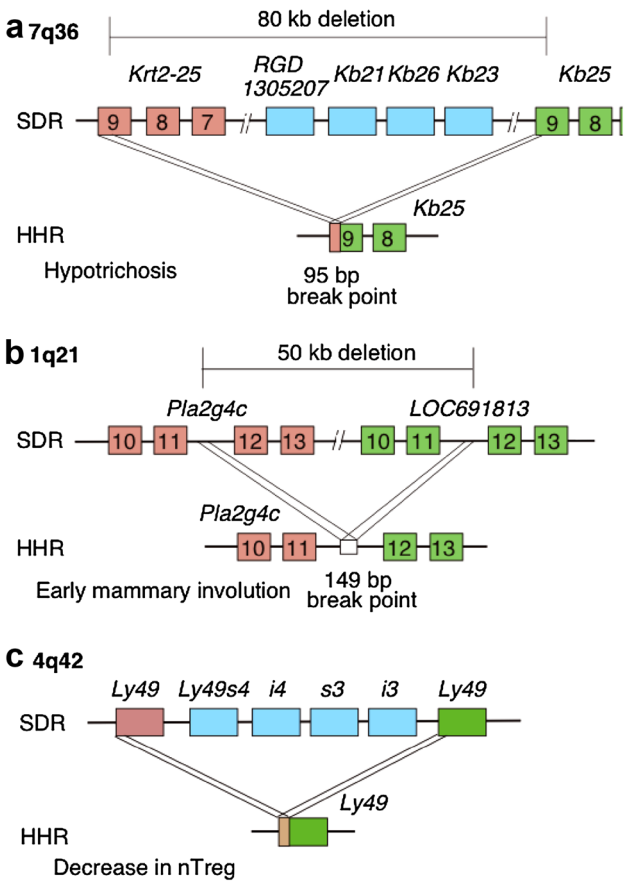

Fig. 2 Gene deletions and loss of mammalian functions in HHRs. *Upper* gene loci in SDR genome at 7q36 (**a**), 1q21 (**b**), and 4q42 (**c**). *Lower* gene loci in HHRs, with gene deletions and fusion between highly related genes. **a** *Brown boxes* exons 7–9 of *Krt2-25* gene; *blue boxes RGD1305207, Kb21, Kb26*, and *Kb23* genes, deleted in HHR; *green boxes* exons 7–9 of *Kb25* gene. Breakpoints are within a 95-bp portion shared by both exon 9 of *Kb25* and exon 9 of *Krt2-25*. **b** *Brown boxes* exons 10–13 of *Pla2g4c* gene; *green boxes* exons 10–13 of *LOC691813*. Breakpoints are within a 149-bp portion shared by both intron 11 of *Pla2g4c* and intron 11 of *LOC691813*. **c** *Brown* and *green boxes* members of the *Ly49* gene family, but not identified; *blue boxes Ly49s4, i4, s3*, and *i3* genes, deleted in HHR. The rat *Ly49* gene superfamily includes more than 30 members and breakpoints are not identified in HHR

development in mammals. Tandem gene duplication plays a major role in the formation of clustered genes (Liao and Chang 2014), and comparative sequence analyses of clustered gene families, such as the β-*globin* gene, in different mammals revealed species-specific differences in gene loss, fusion, and conversion (Strachan and Read 2004). These findings suggest that gene duplication may play an important role in vertebrate evolution and development in mammals.

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