Review

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The role of Down syndrome cell adhesion molecule in Down syndrome

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Abstract: Down syndrome (DS) is caused by the presence of an extra copy of the entire or a portion of human chromosome 21 (HSA21). This genomic alteration leads to elevated expression of numerous HSA21 genes, resulting in a variety of health issues in individuals with DS. Among the genes located in the DS "critical region" of HSA21, Down syndrome cell adhesion molecule (DSCAM) plays an important role in neuronal development. There is a growing body of evidence underscoring DSCAM's involvement in various DS-related disorders. This review aims to provide a concise overview of the established functions of DSCAM, with a particular focus on its implications in DS. We delve into the roles that DSCAM plays in DS-associated diseases. In the concluding section of this review, we explore prospective avenues for future research to further unravel DSCAM's role in DS and opportunities for therapeutic treatments.

Keywords: Down syndrome; DSCAM; neuron; axon; GABAergic synapse

Challenges of discovering the molecular mechanisms underlying Down syndrome

Down syndrome (DS) is a chromosomal disorder that is caused by the presence of a complete or partial extra copy of human chromosome 21 (HSA21), a genetic condition called trisomy 21 [1, 2]. It occurs in about one in 800 live births worldwide [3]. Advanced maternal age at conception is a key risk factor for trisomy 21 [4, 5], as is true for all human autosomal trisomy. DS affects multiple systems and causes both structural and functional defects that lead to many health issues [6]. In neonatal DS patients, about 40 % have congenital heart diseases [7], 12 % have congenital gastrointestinal malformation [8, 9], and 3 % have imperforate anuses that require immediate operation [10]. In childhood and early adolescence, most DS patients have eye disorders and middle ear deficits [11, 12]. About 3 % of patients have dense congenital cataracts that must be extracted after birth to allow light to pass into the retina [13]. Some patients develop sleep apnea or suffer from seizures [14, 15]. During the stages of childhood and early adolescence, most patients show moderate intellectual disability. In rare cases that the patients have little to no cognitive deficits, they often do not possess the intellectual and behavioral capabilities to achieve complete independence [16]. DS is the most commonly identified genetic cause of intellectual disabilities [17], and the patients have developmental abnormalities causing smaller brains, cortical dysplasia, and hippocampal dysplasia [18, 19]. These abnormalities might be related to the cognitive impairments and sensory problems [20]. As DS individuals age, almost all patients aged 40 years and above develop indications of Alzheimer's disease [21]. About 30 % of DS individuals have early onset Alzheimer's disease at the age of 50, which rises to 50 % at the age of 60. The life expectancy for individuals with DS is approximately 60 years old, much lower than the average life expectancy, with congenital heart disease being the leading cause of death [22, 23].

Because DS is a chromosomal disorder, a major challenge in DS research is to identify genes that contribute to specific medical conditions. Although HSA21 is the smallest human autosome, GENCODE/ENSEMBL lists 233 protein-coding genes, 423 non-protein-coding genes, and 188 pseudogenes on HSA21 [24]. Many genes are yet to be annotated on this chromosome [25]. Full trisomy 21 increases one copy of every single HSA21 gene, and the expression of most HSA21 genes thus increases [26]. Approaches for studying single-gene

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disorders are often not applicable or ideal for identifying the disease-causing gene from the trisomic background. Moreover, the symptoms of DS are influenced by overexpression of multiple genes on HSA21, which may work together to cause polygenic symptoms. For example, overexpression of both Down syndrome cell adhesion molecule (DSCAM) and α2 chain of type VI collagen (Col6A2) triggers congenital heart disease, while DSCAM or Col6A2 overexpression individually does not cause heart disease in mice [27]. It is usually difficult to test each individual gene, not to mention the interactions among HSA21 genes, for their contributions to DS-related symptoms. Comprehensively testing each gene or a group of genes and associating them with specific symptoms is a monumental task. As a result, our understanding of the molecular mechanisms that underlie specific diseases in DS is very limited. Yet, the identification of the genes and their interactions that underlie the pathogenesis of individual symptoms of DS is a key step for the development of therapy for the treatment of these symptoms.

One approach for identifying HSA21 genes that contribute to DS-related medical conditions is genetic mapping using partial trisomies, with the idea that the missing part of HSA21 correlates with the missing symptom. Reporting negative or positive clinical diagnoses in the presence or absence of various trisomy 21 regions has allowed researchers to correlate various regions of HSA21 to these health complications [28]. For example, overexpression of β-amyloid precursor protein (APP) has been linked to increased brain β-amyloid and early on-set Alzheimer's disease in DS patients. An individual with partial trisomy 21 missing the extra genetic copy of APP did not develop plaques indicative of the early onset of Alzheimer's disease and did not present with the early onset of the disease at any point of this individual's life [29]. From these types of studies, the distal half of the long arm, 21q22, was found to harbor most of the gene transcribing sites for the chromosome and has been termed the "Down syndrome critical region" [28].

The DSCAM gene, which is the focus of this review, is on 21q22. The discovery of DSCAM's role in DS-related pathology benefited from studies in *Drosophila* [30]. The simplicity, high efficiency, and well-established genetic tools available in *Drosophila* make it an excellent model for screening transgenes to evaluate their effects on cells. Studies of the fly homologous gene provided the guidance for investigations in mouse models of DS and led to the discovery of DSCAM's contribution to excessive GABAergic synapses in mouse models [31].

The roles of DSCAM in neuronal development

DSCAM was initially discovered in an effort to characterize genes located in the DS critical region [32]. The name came from the gene's location within the DS critical region and the homology of the protein that it encodes to cell adhesion molecules. The relevance of DSCAM to the pathogenesis of DS was only established later, after its functions in normal development was discovered through studies in various species, including those in *Drosophila*.

The *Drosophila melanogaster* homolog of DSCAM, termed *Dscam*, is subject to extensive alternative splicing to create up to 38,016 variants [33]. These variants allow for recognition of neurites of the same neuron (termed 'self-recognition'), through which neurites from the same neuron avoid each other for appropriate arbor formation [34–37]. Loss of *Dscam* function in *Drosophila* leads to abnormal dendritic crossing [35, 38, 39]. Additionally, diversity of the *Dscam* extracellular domain via alternative splicing is required for the specificity of synapse formation and axonal guidance [37, 40–44].

Mammalian DSCAM genes lack the splicing diversity, but are also involved in neurite self-avoidance. In DSCAM knockout mice, dopaminergic amacrine cells in the retina exhibit an increase in soma clusters and neurite fasciculation in comparison to wild type controls [45–47]. This effect is absent in retinal cells that do not normally express DSCAM [48]. These results suggest that DSCAM modulates the distribution of the soma and neurites to form proper arbors. This discovery leads to the hypothesis that DSCAM reduces cell type-specific adhesion, such as that mediated by cadherins, and consequently allows the somas to be separated from each other. This hypothesis has been supported by a partial reduction in soma clustering and neurite fasciculation in DSCAM knockout cells by reducing the amount of cadherin-3, cadherin-6, and α-catenin [49]. Inversely, increasing the expression of cadherin-3 in the absence of DSCAM leads to an even greater amount of soma clustering and neurite fasciculation in comparison to cells that only missing DSCAM.

Later studies support the notion that DSCAM weakens cadherin-mediated cell adhesion. DSCAM regulates neuronal delamination at the apical endfeet of neuronal stem cells in the dorsal midbrain by suppressing the RAPGEF2-Rap1-N-cadherin signaling cascade. DSCAM associates with RapGEF2 to inactivate Rap1, which is required for the localization of N-cadherin on membranes. DSCAM

knockdown increases N-cadherin localization and ventricular attachment area in the apical endfeet of neuronal stem cells during development. This increased attachment impairs neuronal detachment from the ventricles and restricts neuronal delamination [50]. Additionally, DSCAM is required for migrating pyramidal neurons of the cortex to bypass their post-migratory predecessors during upper cortical layer development [51]. Loss of DSCAM reduces the thickness of the neocortex in early postnatal days [52]. Particularly, the upper cortical layers (II–IV) are thinner in DSCAM−/− cortices compared with wild-type or heterozygous cortices, while the deeper cortical layers (V–VI) are unaffected. This phenotype is not caused by changes in neurogenesis or cell death [51, 52]. Rather, in DSCAM-deficient cortices, migrating neurons stop prematurely at the front of the cortical plate, leading to thinner upper cortical layers with higher density of neurons [51]. This function of DSCAM is achieved by weakening N-cadherin-mediated cell adhesion in the upper cortical plate [51].

Studies in different species have demonstrated that DSCAM regulates the growth of presynaptic terminals or axons, a function that is independent of its molecular diversity in *Drosophila*. *Drosophila* neurons homozygous for *Dscam* null mutations display significantly decreased presynaptic terminals [30] (Figure 1A). Conversely, overexpression of *Dscam* results in overgrowth of these terminals. The extent of the overgrowth is proportional to the level of *Dscam* overexpression. This result raised the possibility that *Dscam* is involved in the pathogenesis of neuronal defects in DS. Later studies found that this function of DSCAM is conserved in mammals. In mice, axon growth of retinal ganglion cells (RGCs) is impaired in DSCAM loss-of-function mutants [53]. Conversely, RGC axons overgrow in DSCAM gain-of-function mutants. Moreover, studies on GABAergic inhibitory neurons in the mouse neocortex showed decreased presynaptic terminals, bouton number, and bouton size in mice that are homozygous for DSCAM loss-of-function mutations [31]. The effect of DSCAM on GABAergic presynaptic terminals is proportional to the level of DSCAM in the neocortex.

DSCAM has also been reported to slow down the maturation of dendritic spines in excitatory neurons. DSCAM deficiency leads to more dendritic spines at earlier postnatal days (e.g., P12 and P21), but not later postnatal days (e.g., P42), in the neocortex [52, 54]. These mice also exhibit increased glutamatergic transmission – as measured by miniature excitatory postsynaptic currents – in layer 2 and 3 of sensory cortices [54]. The extracellular domain of DSCAM interacts with neuroligin1 (NLGN1) to inhibit the NLGN1-neurexin1β interaction [54]. DSCAM deficiency results in an enrichment of NLGN1 in synaptic membranes, which may cause precocious maturation of dendritic spines. Ablation of DSCAM in neurons and astrocytes leads to deficits in social novelty tests and enhances repetitive behaviors (e.g., circling and grooming) [54].

The contribution of DSCAM to the pathogenesis of DS and associated disorders

The protein levels of DSCAM are elevated in brain tissues of several brain disorders, including DS, intractable epilepsy, and bipolar disorder [55–57]. In DS, DSCAM is believed to play a role in intellectual disability [30, 31, 58–62], congenital heart disease [22, 23, 27, 63], and congenital intestinal obstruction (Hirschsprung's disease) [22, 64, 65].

DSCAM's role in synaptic development in DS

The importance of *Dscam* levels in neuron development was established by studies conducted in the class IV dendritic arborization (C4da) neurons of the *Drosophila* larva. These neurons are a classic model for studying dendrite and axon development [66] and were used to establish *Dscam*'s role in dendrite self-avoidance [35, 38, 39]. Overexpression of *Dscam* in C4da neurons increases their presynaptic terminals without increasing dendritic growth [30] (Figure 1A). Importantly, this increase is due to *Dscam* expression levels and independent of ectodomain diversity. Additional studies show that triplicating the *Dscam* gene induces abnormal branching of sensory axons in adult flies [67] and alters synaptic transmission at the neuromuscular junction of *Drosophila* larvae [68].

These discoveries in *Drosophila* set the stage for investigations of DSCAM's contribution to presynaptic development in DS. The size of presynaptic terminals is typically much larger in mammals than in *Drosophila*. Moreover, axons are usually projected longer distances in mammals than in *Drosophila*. These make the evaluation of presynaptic terminals more difficult in mammals. GABAergic interneurons usually elaborate their axons around the cell body, making it easier to identify their presynaptic terminals. Importantly, it is well established that DS brains have an excessive amount of GABAergic synapses [58–62]. For these reasons, the first study testing DSCAM's contribution to presynaptic development was conducted on GABAergic neurons.

Liu et al. carried out this study in Ts65Dn mice, an animal model containing an extra copy for two-thirds of the homologous chromosomes of HSA21 [69]. Studies have

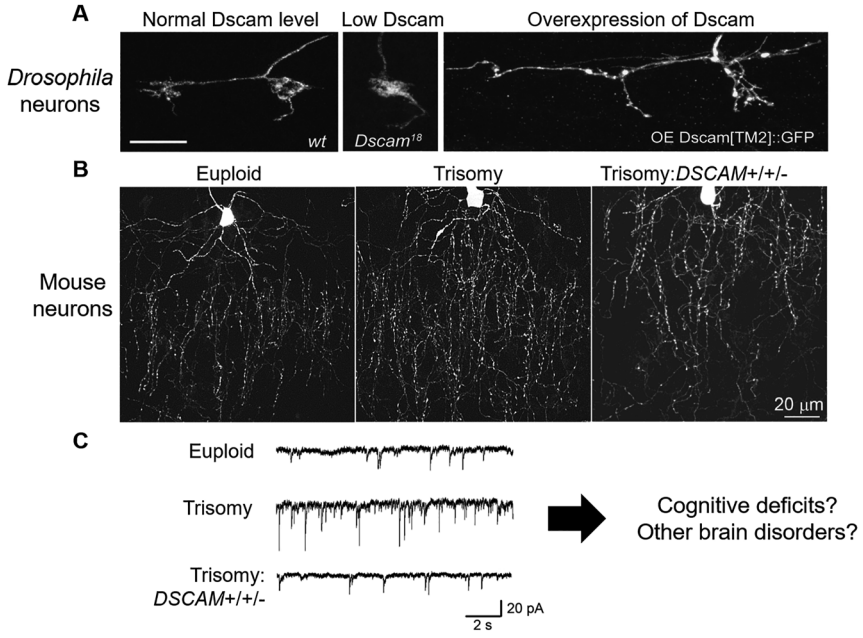

Figure 1: DSCAM expression levels determine the sizes of presynaptic terminals in *Drosophila* and mouse neurons. (A) Presynaptic terminal length of *Drosophila* C4da neurons are determined by *Dscam* levels. Low *Dscam* levels (*Dscam*^{18}) lead to a decrease in presynaptic terminal length in comparison to wild-type (wt) neurons, while overexpression of *Dscam* leads to an increase. Scale bar: 10 µm. (B) The Ts65Dn ("Trisomy") mouse model of DS displays an increase in the lengths of presynaptic terminals of the GABAergic chandelier cells in comparison to euploid mice. Normalizing DSCAM gene dosage to two copies in the trisomy mice (trisomy:*DSCAM*+/+/–) eliminates the overgrowth of chandelier presynaptic terminals. (C) The excessive GABAergic synapses in trisomy mice lead to an increase in the frequency of mIPSCs, which is eliminated in trisomy mice that carry only two copies of the DSCAM gene (trisomy:*DSCAM*+/+/–). Whether the excessive GABAergic synapses that are caused by DSCAM overexpression in the trisomic neocortex lead to cognitive deficits or other brain disorders remains to be determined. Panel A is adapted from Kim et al. [30] with permission from the publisher. Panel B and C are adapted from Liu et al. [31].

shown increased GABAergic inhibition that impairs cognitive functions in these mice, which are mitigated by GABAergic receptor antagonists [60, 70–73]. The number and size of GABAergic synapses are increased in the Ts65Dn neocortex [74–76]. By crossing Ts65Dn mice with heterozygous DSCAM^{2j} mice, a DSCAM loss-of-function mutant [77–79], it is possible to generate Ts65Dn mice with two functional DSCAM alleles and one nonfunctional DSCAM allele (Ts65Dn:DSCAM+/+/–) for comparisons with euploid and Ts65Dn mice [31]. The number of GABAergic synapses formed around the cell bodies of excitatory pyramidal neurons in the neocortex was increased in Ts65Dn, but not in Ts65Dn:DSCAM+/+/–. Moreover, the presynaptic terminals of chandelier cells, which are GABAergic inhibitory neurons that synapse onto the axon initial segment of pyramidal neurons [80–82], exhibited increased axonal cartridge length, bouton number, and bouton size in Ts65Dn but not in Ts65Dn:DSCAM+/+/– (Figure 1B). Consistent with the morphological phenotypes in GABAergic presynapses, Ts65Dn mice showed an increase in the frequency of miniature inhibitory synaptic current (mIPSC) frequency, in Ts65Dn but not in Ts65Dn:DSCAM+/+/– (Figure 1C). By specifically correcting DSCAM gene dosage in GABAergic neurons with the Cre-Lox approach, it was determined that the synaptic phenotypes are caused by the triplication of the DSCAM gene within GABAergic neurons [31] (Figure 2).

A study in *Drosophila* proposed a therapeutic approach for treating the neuronal deficits that are caused by DSCAM overexpression. Similar to overexpression of *Dscam*, overexpression of Abelson tyrosine kinase (Abl) increases presynaptic terminal growth in *Drosophila* neurons [30, 83, 84]. While loss of Abl leading to only a minor reduction in presyanptic terminals, it completely blocked the overgrowth caused by *Dscam* overexpression, demonstrating that overexpressed *Dscam* requires Abl to promote the overgrowth of presynaptic terminals [84]. Additionally, *Dscam* was shown to bind to and activate Abl. These findings led to the idea that neurological disorders caused by DSCAM overexpression could be treated by inhibiting Abl activity. In a proof-of-principle test, treating *Dscam* overexpressed animals with nilotinib or bafetinib, two FDA-approved Abl inhibitors [85–87], mitigated the overgrowth of presynaptic terminals [84].

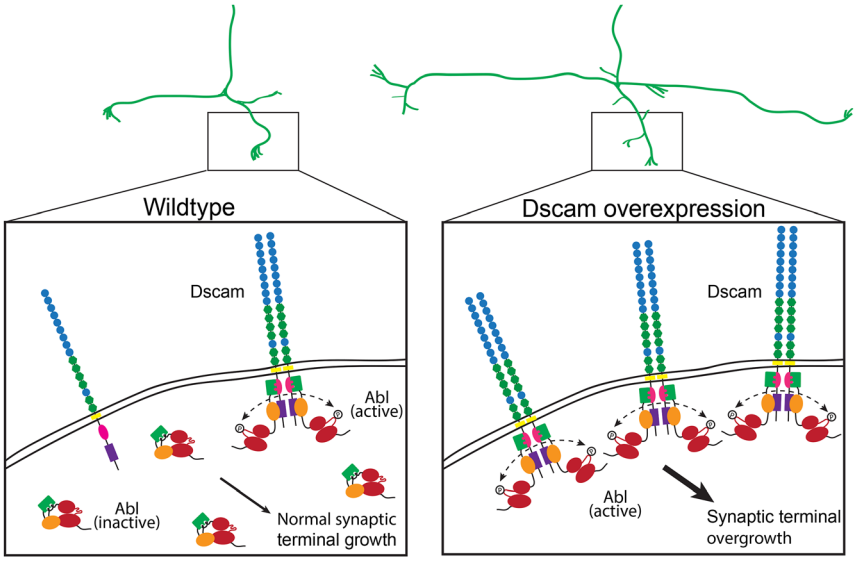

Figure 2: Increasing *Dscam* levels activates Abl through its cytoplasmic domain, leading to the overgrowth of presynaptic terminals in *Drosophila* neurons. Image credit: Gabriella Sterne.

DSCAM's role in altering retinogeniculate refinement in DS

The lateral geniculate nucleus (LGN) is a crucial relay station in the visual pathway, receiving axon input from RGCs and transmitting this visual information to the primary visual cortex. The arrangement of axons from the two eyes in the LGN is essential for the formation of binocular vision and depth perception. During development, the axons from the two eyes initially intermingle in the LGN, but later segregate into distinct layers before eye opening. It is crucial for the brain to process visual information from each eye separately. Instead of a typical pattern with some overlap between ipsilateral and contralateral axons, DS mouse models exhibit a supranormal separation without overlap [88]. Although the dosage of the DSCAM gene is positively correlated with the refinement of the LGN, normalizing DSCAM gene dosage to two copies did not eliminate the over-segregation of the axons from the two eyes in the Ts65Dn trisomy mice [88]. DSCAM might still play a role in the over-segregation phenotype in LGN, but additional HSA21 genes are likely involved (Figure 3).

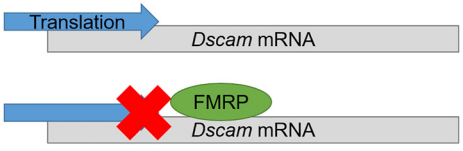

Figure 3: FMRP binds to *Dscam* mRNA to block translation. Loss of FMRP increases the level of *Dscam* proteins, likely by blocking the translation of *Dscam* mRNAs.

A molecular link between DS and fragile X syndrome (FXS) through DSCAM expression

FXS, which is caused by loss of the gene *fragile X mental retardation* (*FMR*), is another common cause of intellectual disability [89]. FMR encodes the Fragile X mental retardation protein (FMRP), which binds to its target mRNAs to repress their translation [90, 91]. FMRP targets include the mRNAs of several HSA21 genes, including DSCAM, DYRK1A, NCAM2, and APP [92]. Like DS patients, FXS patients exhibit increased expression of DSCAM, DYRK1A, NCAM2, and APP. In DS patient-derived pluripotent stem cell models, an acute increase in FMRP reduces the expression levels of DS-related proteins and reverses 40 % of global transcriptional changes [92].

Two separate studies in rodents suggested that FMRP binds directly to mRNAs of DSCAM in brain tissues [93, 94], which was corroborated by two independent studies in *Drosophila* [30, 67]. The *Drosophila* studies further demonstrated that FMRP represses *Dscam* expression. Both the loss of FMRP and the overexpression of *Dscam* lead to overgrowth of presynaptic terminals in fly neurons. Epistasis tests showed that FMRP requires *Dscam* to promote presynaptic growth. In C4da neurons, both *Dscam* overexpression and loss of FMRP function require Abl to cause presynaptic overgrowth [84]. Moreover, Abl inhibitors rescue the overgrown presynaptic terminals in C4da neurons that overexpress *Dscam* or carry FMRP loss-of-function mutations. Therefore, Abl might be a common target for treating aspects of DS and FXS (Figure 4).

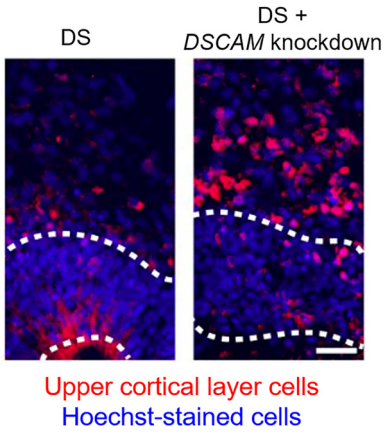

Figure 4: Knockdown of DSCAM rescues the neurogenesis defects in organoids derived from DS cells. Neurons in the upper cortical layer express SATB2 (red). Hoechst dye stains all cells (blue). Adapted from Tang et al. [99] with permission from the publisher.

Possible roles of DSCAM in neurogenesis

Postmortem DS patient tissues exhibit reduced neuronal densities and abnormal neuronal distribution, especially in cortical layers II and IV [95, 96]. Additionally, there seems to be a substantial increase in glial cells [97]. Induced pluripotent stem cell (iPSC) lines from DS patients have opened new avenues for understanding the mechanisms underlying DS pathogenesis [98]. Cerebral organoids derived from DS iPSCs provide a more complex and three-dimensional model to study the effects of extra HSA21 genes on brain development. The DS organoids showed decreased proliferation of neural stem cells. The growth of the DS cerebral organoids was delayed from the beginning of the culture and exhibited dramatically decreased expansion rates. Knockdown of DSCAM and its down-stream molecule PAK in the DS organoids rescued the proliferation defects [99], suggesting that DSCAM overexpression causes the defective neurogenesis in DS brains (Figure 5).

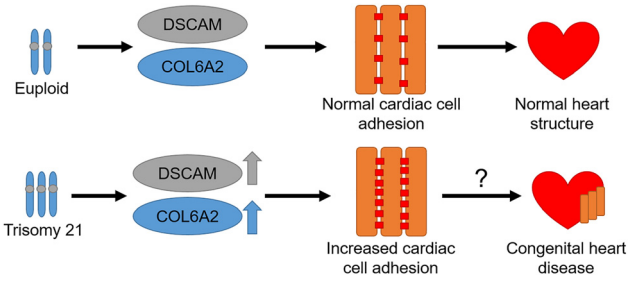

Figure 5: Co-overexpression of DSCAM and COL6A2 caused cardiac defects. Overexpression of DSCAM or COL6A2 individually does not cause severe heart diseases, but overexpressing both genes leads to increased cell adhesion and severe cardiac defects. Whether the increased cell adhesion is responsible for the congenital heart disease remains to be determined.

Overexpression of DSCAM and COL6A2 cooperatively causes congenital heart defects

DS is the most common cause of congenital heart diseases [100], which are the leading causes of death in the DS population. About 50 % of DS patients suffer from congenital heart diseases, many of which require surgical corrections [100–102].

With molecular mapping of DS patients with truncated or translocated trisomy 21, in which only a portion of chromosome 21 is triplicated, a particular region on the distal end (in 22.3) of HSA21 is associated with the occurrence of congenital heart diseases [23]. DSCAM is located in this region and highly expressed in the developing heart, raising the possibility of a potential link between DSCAM and the congenital heart diseases in DS. Congenital heart defects in DS are characterized by increased intercellular adhesiveness of cardiac fibroblasts [103], which could be caused by elevated DSCAM expression that has been shown to mediate cell adhesion [63].

Based on the findings of Barlow et al.[23], Grossman et al. tested the effects of overexpression of either a single or a pair of candidate genes on the heart of *Drosophila* [27]. Cardiac functions were assessed by measuring three parameters, which included the basal heartbeat rate, the response to stress-induced heart failure, and the recovery of heart function after heart failure. Whereas several genes, including DSCAM, COL6A1, COL6A2, COL18A1, and SH3BGR, led to an increase in stress-induced cardiac dysfunction in one or two parameters measured, with none of them increasing all three physiological parameters. This raised the question whether these genes worked cooperatively to cause severe heart diseases. To understand potential interactions between these genes, different combinations of gene pairs were co-expressed in the fly hearts. Gene pairs DSCAM/COL6A2 and DSCAM/SH3BGR were particularly significant, causing a substantial increase in heart failure rates across all three assessed parameters in over 60 % of the flies [27].

The effects of the DSCAM/COL6A2 gene pairs were further tested in mice with one mouse line for DSCAM overexpression and another for COL6A2 overexpression [27]. The mouse line with DSCAM or COL6A2 overexpression did not affect the viability or cause defective cardiac physiology and morphology. However, co-overexpression of DSCAM/COL6A2

caused lethality in over 50 % of mice and severe cardiac morphological and physiological defects in the surviving population. DSCAM and COL6A2 co-overexpression dramatically increased cell adhesion, while DSCAM or COL6A2 individual overexpression did not. Interestingly, a high-resolution genomic mapping suggested that COL6A2 and several other genes are not the primary genes that cause DS-CHD [22], due to a few individuals with DS-CHD not having an extra copy of these genes. However, this logic is based on the assumption that DS-CHD is caused by a single gene. It is possible that interactions between different HSA21 genes cause DS-CHD, as Grossman et al. found [27].

It is unclear how the change in cell adhesion affects the heart structures. Nevertheless, these findings show that overexpression of both DSCAM and COL6A2 amplifies their individual effects and causes congenital heart diseases. They also highlight the importance of studying the cooperative effects of HSA21 genes in causing DS-related diseases. Furthermore, the successful discovery of the DSCAM/COL6A2 gene pair in congenital heart diseases exemplifies the power of combining different model systems in parsing out the mechanisms that underlie DS.

Possible involvement of DSCAM in Hirschsprung's disease

Hirschsprung's disease is an enteric nervous system disorder that decreases colon mobility and is a leading cause of intestinal obstruction [104, 105]. Hirschsprung's disease is characterized by a decreased number of enteric ganglia in the myenteric and submucosal plexus along the hindgut, which is due to a failure of neural crest cells to migrate fully during embryonic development [106, 107]. DS is the most frequent chromosome abnormality associated with Hirschsprung's disease [106], contributing to more than 90 % of Hirschsprung's disease cases – which affect approximately 2 % of the DS population [108–110]. Gene mapping of DS phenotypes based on partial trisomy identified a Hirschsprung-associated region spanning 33.50–46.25 Mb on HSA21, which includes DSCAM [22]. Further dose-dependent associations of HSA21 genes in DS patients with Hirschsprung's disease identified two single-nucleotide polymorphisms (SNPs) associated with this disease in DS [64]. Both polymorphisms are located in the intron 3 of DSCAM and are consistent with DSCAM expression during the development of the enteric nervous system. Additionally, one of these polymorphisms was shown to be associated to Hirschsprung's disease independent of DS in the Chinese population [111], supporting the involvement of DSCAM in Hirschsprung's disease.

Although these studies associate DSCAM with Hirschsprung's disease, it is unclear whether the extra copy of DSCAM gene causes the disease in DS. Two mouse models of DS, Ts65Dn and Tcl, have less neurons in the submucosal plexus of the enteric nervous system, but deletion of the extra copy of DSCAM does not rescue this defect [112]. Nevertheless, DSCAM cannot be ruled out as a contributing factor to Hirschsprung's disease by cooperating with other risk genes, such as the HSA21 gene *Bace2*. In a zebrafish model, morpholino-mediated knockdowns of DSCAM homologs (*dscama* and *dscamb*) and *bace2* decreased the number of enteric neurons [65], indicating a role for these genes in the development of the enteric nervous system.

Summary and future directions

There remain much to be done to elucidate DSCAM's contribution to DS-associated diseases. In the nervous system, while we know that DSCAM triplication leads to morphological and synaptic changes in inhibitory synapses in the neocortex, we do not know if this leads to cognitive deficits. Neither do we know if the changes in ocular segregation in the LGN are indeed contributed by DSCAM triplication, and if so, what other genes cooperate with DSCAM in this process. Moreover, additional evidence in animal models is needed to corroborate the possible roles of DSCAM in neurogenesis and to demonstrate the consequences on nervous system functions. Furthermore, we do not know whether DSCAM is involved in changes to other parts of the nervous system, such as the hippocampus, glial cells, and other systems.

The cooperative effects between DSCAM and other genes have just begun to be discovered. In this regard, efficient experimental systems such as *Drosophila* and cultured neurons, especially the stem-cell-derived neurons from DS patients, will be very useful, as they can be efficiently applied to test the cooperative functions of multiple genes. The interactions between DSCAM and other genes might also be relevant to the question of whether DSCAM's contribution to DS-associated disorders varies in different human populations, as DSCAM's roles might change in different genetic backgrounds.

The studies of DSCAM signaling have led to ideas about targeting these signaling mechanisms to treat diseases in DS. In addition to Abl [84], DSCAM has been shown to bind to Dock in *Drosophila* [33], and FAK, Fyn kinase, as well as PAK1 in mammals [113]. Targeting these signaling pathways downstream of DSCAM could block the overexpression effects caused by DSCAM triplication in DS. Whether the signaling molecules downstream of DSCAM can be used as

therapeutic targets for DS needs to be tested in the future. For diseases in which the affected tissues/organs are amenable to therapeutic viral infections, gene therapies might be developed by reducing the expression levels of DSCAM or its downstream effector genes.

The understanding of the molecular mechanisms that underlie DS impacts not only the DS population, but also many other developmental and degenerative disorders. For example, DSCAM variants have been found to be strongly associated with intellectual disabilities and autism spectrum disorder [54, 114]. The knowledge about DSCAM functions in the brain, its interacting genes, and its downstream signaling pathways in DS brains will provide insights into the understanding of other human conditions and the development of therapeutic approaches.

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