Role of the genes colec11 and masp1 in embryonic development

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Mutations in the genes *COLEC11* and *MASP1* which code for lectin complement pathway proteins have been found responsible for a rare autosomal recessive disorder resulting in abnormal development. This proposes that other constituent proteins may play a role in embryonic development.

To this day, the genetic aspects of the stages involved in embryonic development that leads to certain congenital disorders are still not fully understood. As a result, researchers start off with identifying the genes that play a part in causing a congenital disorder before determining how these genes bring about the disorder. This approach was adopted by Rooryck *et al* . [1] to help prove that mutations in the genes *COLEC11* and *MASP1* , involved in the lectin complement pathway were responsible for 3MC syndrome.

The 3MC syndrome is an abbreviation of Michels, Mingarelli, Malpuech and Carnevale syndrome. These four syndromes are rare autosomal disorder that follows a recessive order of inheritance. The 3MC syndrome is characterised by blepharophimosis, ptosis of the eyelids, cleft lip and palate, craniosynostosis, hypertelorism and in some cases renal anomalies and umbilical hernia [1, 2] . Furthermore, growth retardation, mental retardation and urogenital anomalies are also common features in all four syndromes [2, 3] . After much research, it was found that the overlapping similarities in phenotype and Gestalt between these four syndromes are more significant than the differences between them [2] . Therefore, these evidence lead to the

assumption that the syndromes are allelic variations of one another and belong to the same spectrum [2] .

Rooryck and colleagues started off their study by first collecting DNA samples from individuals diagnosed with Michels, Mingarelli, Malpuech and Carnevale syndromes and all their available family members. Their genomes were then screened for single nucleotide polymorphisms which are homozygous in all affected individuals but absent in unaffected individuals. From the individuals tested, there were four consanguineous families. The genotyping results from members of these families showed a homozygous region of 2. 2 MB at 2p25. 3 in affected individuals [1] . Moreover, a region of homozygosity was found in two other families at 3q27. 3 [1, 4] . These findings suggest that the 3MC syndrome could be caused by multiple genes.

From the sequencing of the open reading frames at the 2p25. 3 region, 15 candidate genes were found, of which 9 were sequenced. Sequencing of these transcripts in affected individuals revealed three homozygous missense mutations and a single base deletion in *COLEC11* that may be linked to the inheritance of the 3MC syndrome. Furthermore, the single base deletion in *COLEC11* was found to play a role in the premature termination of the gene product CL-K1. CL-K1 is a C-type lectin that acts as a host defence by binding to carbohydrate antigens on microorganisms and activating the innate immune system [5, 6] . Due to its function, it tends to circulate in the serum. In addition, being highly conserved in various species means that a mutation would be damaging to the function of the protein. Hence, to ensure that this mutation is an underlying cause of the 3MC syndrome, a protein

blot assay was conducted to detect CL-K1 in the serum from two affected individuals. However, none was detected, indicating a connection between the syndrome and the loss of function mutation in *COLEC11* . At the same time, the expression of CL-K1 was observed in certain mouse tissues that have been known to be affected by the disorder in humans [1] .

In the same way, sequencing at the 3q27. 3 region found 16 candidate genes potentially linked to the 3MC syndrome. One of these genes is the *MASP1* gene which encodes for mannan-binding lectin serine protease 1 (MASP-1), a serine protease that is important in the lectin complement pathway [4] . Rooryck *et al* . [1] then sequenced this gene in the two affected families and discovered two homozygous single base substitutions in exon 12. As predicted, the loss of function of this gene is consistent with the inheritance of 3MC syndrome.

To study the effect of the loss-of-function of these proteins as well as to ensure that both the *COLEC11* and *MASP1* genes are accountable for the abnormalities witnessed in 3MC syndrome, knockdown studies were conducted using zebrafish. In these studies, two antisense morpholinos for *COLEC11* were injected into one-cell stage embryos of zebrafish so as to imitate the loss-of-function of the gene. The same was done for the *MASP1* gene. Based on the results from these studies, *COLEC11* and *MASP1* genes were found to cause similar phenotypes. The zebrafish morphants subjected to both these treatments displayed morphological abnormalities such as pigmentation and craniofacial cartilage defect similar to the symptoms of 3MC syndrome. Interestingly, when co-injected with a fully functional

COLEC11 mRNA, the observed abnormalities in the zebrafish are reversed. Additionally, injecting a low dosage of both *colec11* and *masp1* morpholinos into the zebrafish embryos at the same time gave rise to similar deformities observed when injected separately, suggesting an interaction between the two genes [5] .

Thanks to the pigmentation and craniofacial cartilage defects noted in zebrafish morphants, further studies were carried out to determine whether the CL-K1 and MASP-1 proteins are involved in the migration of cranial neural crest cells (NCCs) during embryonic development. Findings by Rooryck *et al* . [1] in this part of the study revealed that beads coated with CL-K1 attracted NCCs when placed in the head region of zebrafish embryo. On the other hand, control beads implanted in the same region did not display the same attraction to NCCs. This proves the possibility that CL-K1 play a role in guiding the migration of neural crest cells. Similar results were also seen in a test using CL-K1 agarose disk and HeLa cells.

At present, since the roles of both the *COLEC11* and *MASP1* genes in embryonic development have been discovered, more focus is required in investigating how the lectin complement pathway regulates embryonic development in addition to the role of the gene product, CL-K1 in early developmental processes. Likewise, the possibility of other constituent proteins and complement systems being involved in embryogenesis should also be considered. Therefore, further research on these aspects can help in the management of other congenital genetic disorders.

(996 words)

References:

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