GJC2 Mutations Cause Primary Lymphedema

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Abstract

Lymphedema results from abnormalities in either the structure or function of the lymphatic system. Mutations (changes) identified in genes controlling development of the lymphatic system's structure result in primary (inherited) lymphedema. Until now, no mutations causing primary lymphedema had been identified in genes affecting the function of the lymphatic system. Other researchers have implicated a particular gene family, the gap junction/connexin genes in maintaining lymphatic flow. Studies of cells from the lymphatic system identified expression (activity) of several of these gap junction genes within the lymphatic system. By sequencing (reading) three gap junction genes in a group of families with dominantly inherited lymphedema, we identified six families with unique mutations in GJC2 (the gap junction gene which encodes for the connexin [Cx] 47 protein). We hypothesize that mutations in GJC2 alter gap junction function and disrupt lymphatic flow. The identification of GJC2 mutations as a cause of primary lymphedema raises the possibility of developing gap-junction-modifying drugs as potential therapy for some forms of lymphedema.

Main Text

Lymphedema is the abnormal accumulation of lymphatic fluid in the body tissues, which can result from a damaged or poorly functioning lymphatic system, as well as a lymphatic system with an abnormal structure. Some examples of possible inherited lymphatic structural abnormalities are a deficiency of lymph vessels or valves that do not function properly, allowing back-flow of lymph fluid. Patients with lymphedema suffer from recurrent infections, physical and cosmetic impairment, and sometimes even psychosocial stigmatization. They may also be at increased risk for developing a type of cancer called lymphangiosarcoma. The population prevalence of lymphedema is estimated in the range of 1.3–1.4 per 1000 individuals. Primary (inherited) lymphedema is less common than secondary lymphedema, which is associated with conditions such as cancer therapy, injury, and filariasis (a parasitic infection). Recent studies in families with inherited forms of lymphedema have identified six genes, FLT4,1,4 (encoding VEGFR3), FOXC2,5,6, SOX18,1, HGF8, MET8, and CCBE19,10, that cause primary lymphedema.

Primary lymphedema is usually dominantly inherited with incomplete penetrance and variable expression. “Dominant” means that all children of individuals with this type of lymphedema have a 50% (1 in 2) chance of inheriting the lymphedema-causing gene mutation from a parent with primary lymphedema. However, there is “incomplete penetrance” in primary lymphedema, meaning that some family members might inherit the mutation and have no signs of swelling at all, which tends to occur more often in males than females. There is also “variable expression,” meaning that the severity and amount of swelling can vary significantly within a family. Incomplete penetrance and variable expression are not specific to primary lymphedema, as they also occur in many other genetic disorders.

Although some genes are active in cells throughout the body, most genes are expressed (active) only in certain cell types. For example, some genes function only in the brain, while others are only expressed in the heart. To identify other causal genes for lymphedema, we reviewed the gene activity in lymphatic endothelial cells (LECs, which form the lining of lymphatic vessels) versus blood endothelial cells (BECs, which form the lining of blood vessels). It was noted that GJA1 is expressed in both BECs and LECs, whereas GJC2 is expressed only in LECs. That observation led to the hypothesis that GJC2 could be a significant gene in the lymphatic system. Gap junction genes, such as GJC2, are important for sending signals in the body, particularly in tissues such as muscles and nerves. Six con-
nexin proteins join together in the plasma membrane (cell wall) to form a connexon, which then connects to another connexon in an adjacent cell membrane (Figure 1). This connection, called a gap junction, is a channel (hole) connecting two cells; the channel can be opened to transmit messages from one cell to another. Rhodin first suggested a role for gap junctions in lymphatic vessels, but there has been limited research on gap junction intercellular communication (GJC) in lymphatic vessels or LECs.

We investigated the connexins as potential genes for causal lymphedema mutations in the families ascertained through the University of Pittsburgh Lymphedema Family Study (UPLFS). This study was approved by the Institutional Review Board of the University of Pittsburgh, and informed consent was obtained from all subjects. Initially, families were enrolled in the study if there was a physician’s diagnosis of lymphedema in one individual and lymphedema in a first-degree relative (parent, child, or sibling). Most families were self-referred through the Lymphedema Family Study Website: www.hgen.pitt.edu/projects/lymph/. We screened 150 families from the UPLFS for mutations in GJA1, GJA4, and GJC2. Mutations in FLT4, FOXC2, and SOX18, known lymphedema genes, were previously excluded in these families. Six lymphedema families of mixed European ancestry were identified with GJC2 mutations as shown in Table 1.

Two families, in which we identified GJC2 mutations, were large enough for linkage analysis. Linkage analysis is a method used to determine the probability that a specific mutation is responsible for a specific trait or disease, by examining how they co-segregate in a family (i.e. do the mutation and disease occur together in the same family members?). The statistic obtained from linkage analysis, called a LOD score, is used to assess the support for co-segregation of the mutation and disease. Values above 3 indicate co-segregation of the disease with the mutation in question. Linkage analysis in these two families yielded a LOD score of 6.5, which is highly supportive of linkage of their GJC2 mutations to lymphedema (a LOD score of 6.5 indicates that the odds are 106.5 to 1 or 31,622,777 to 1 in favor of the mutations causing the lymphedema). Four additional unique GJC2 mutations were observed in other smaller families demonstrating inheritance from an affected parent to an affected child (Figure 2 [see page 31]; Table 1).

The GJC2 mutations identified (1) occurred only in affected or at-risk individuals, (2) were not present in 250 matched control subjects who were unaffected with lymphedema, and (3) caused a change in a conserved amino acid of Cx47. Conserved amino acids are portions of the protein that are highly similar in other mammals, indicating their importance in biological function. In particular, the mutation in one of the linked families (i.e. a family demonstrating “linkage”) is located within a highly conserved region of the gene that is important for attachment of other connexins to form the gap junction.

In the two linked families, uncomplicated lymphedema of the leg or hand was the only constant feature reported in the affected individuals. Many affected individuals had onset of lymphedema in childhood or adolescence. However, four males in the 2 linked families carried the GJC2 mutation, but were not diagnosed with lymphedema, demonstrating incomplete penetrance of GJC2 mutations in these families, particularly among males. Generally, males also showed a later age at onset of swelling than females. Other features reported in some lymphedema families (ptosis, cellulitis, venous insufficiency, etc.) appeared sporadically. Four individuals within family 135 reported recurrent skin infections. In the four smaller families with mutations, the clinical picture was similar to the families demonstrating linkage, including a later age at onset, typically around puberty.

We show here that mutations in GJC2 cause primary lymphedema, through linkage in two families and significant genetic evidence from four additional independent families. We hypothesize that coordinated gap junction function is needed to optimize the movement of lymph fluid through the body, and is compromised in individuals with GJC2 mutations. The GJC2 mutations are notable because they cause an abnormality in lymphatic function rather than the previously identified mutations in genes causing abnormal lymphatic development. Such functional abnormalities could potentially benefit from the creation of gap-junction-modifying drugs, offering a novel medical treatment for lymphedema.

The role of GJC2/Cx47 in lymphatic function is unexpected, because this gene has a previously demonstrated role only in a particular cell type in the brain and spinal cord. The GJC2 lymphedema mutations are distributed throughout the gene, with no clustering within a specific section of the gene. However, the two mutations located in the extracellular (outside the cell) loop domains (the S45L and R257C mutations from Table 1) are predicted to interfere with connexon assembly into functional channels. Specifically, the R257C mutation is located in a conserved area of the gene that is important for connexon attachment to the cell. We expect these two extracellular mutations to result in impaired gap junction activity and propose that this might result in poor coordination of lymphatic flow. The mechanism through which the identified intracellular (within the cell) mutations affect the gap junctions, however, is not clear. Further characterization of the mutations reported here will contribute to our understanding of the role of connexins in lymphatic function.

Although we are approaching the 400th anniversary of the first description of the lymphatic system in 1627, lymphatics remain a poorly understood and largely neglected biological system. Despite an essential and active role in the maintenance of fluid homeostasis, transport of immune cells, and pathological processes like chronic inflammation and cancer, the lymphatic vascular system is often viewed as a passive conduit rather than an interactive participant in these processes. The identification of mutations in the Connexin gene family in primary lymphedema justifies further investigation into the manipulation of gap-junction-coordinated lymphatic flow and function as a possible therapeutic option for primary lymphedema.
References: