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UPDATED HUMAN CHROMOSOME MAP OF LYMPHEDEMA-LYMPHANGIOGENESIS GENES: TEMPLATE FOR CURRENT AND FUTURE DISCOVERY

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ABSTRACT

We have updated the human chromosomal map of the location of known and candidate genes involved in primary lymphedema (PL) originally published in 2021. This should facilitate further discovery and provide a basis for understanding microdeletions which cause lymphedema.

Keywords: primary lymphedema, chromosomes, genes, mutations

Beginning in 2000 as an outgrowth of the Human Genome Project, multiple pathogenic mutations and their transcribed proteins have been reported to underlie human familial lymphedema, lymphatic malformations, and pleiomorphic syndromic lymphatic disorders. These "lymphedema-lymphangiogenesis" factors have consisted of growth factor receptors and their ligands, transcription factors, endothelial junctional components, among others that have been implicated in lymphatic system structure and function. In addition, many more candidate genes have been pinpointed in mouse models of these human lymphatic disorders although the human counterparts have not yet been reported. To catalog and organize these disparate discoveries, we have created in 2021 a human chromosome map from a comprehensive review of the existing literature to record, locate, and explore interactions of currently identified lymphatic disease-causing mutations and provide a template for future discovery of lymphedema-lymphangiogenesis genes and proteins.

MATERIALS AND METHODS

Since our original map reported in 2021 (1) as a complement of our extensive review on primary lymphedema (2), we have actively followed reports on novel associations between genes and PL, including complex lymphatic anomalies (CLA), using Pubmed (with the keywords "Lymphedema + Gene") and presented at international scientific meetings, such as the Gordon Lymphatics Meetings 2022 and 2024. Any paper that we identified as reporting a potential novel PL gene in patients was scrutinized and the variants reassessed for their rarity in GnomAD (https://gnomad.broadinstitute.org), their potential pathogenicity using bioinformatic tools, and their transmission in the reported pedigrees.

RESULTS

The known human or mouse genes, which are associated with primary lymphedema, including complex lymphatic anomalies, are presented in *Fig. 1.* It comprises 30 confirmed PL genes, four CLA genes, nineteen PL genes awaiting further confirmation in vitro (functional validation) or in vivo (with additional patients and/or reproduction of variants in animal models), two confirmed loci (on chromosomes *15* and *22*), five trisomies and one monosomy. Three novel genes related to human PL have been added: *MDFIC (7q31.1)* causing central conducting lymphatic anomaly (3), *ERG (21q22.2)* causing PL (4,5) and the long-awaited cause of the Aagenaes syndrome (*15q26.1*) now linked to variants in *UNC45A* (6). The lymphedema component of the Phelan McDermid syndrome (and associated renal phenotype) has now been linked to mutations in *CELSR1 (22q13.31)* (7,8), and variants in *TIE1 (1p34.2)* and *HGF (7q21.11)* now confirmed to be PL-causing by functional validations (9,10).

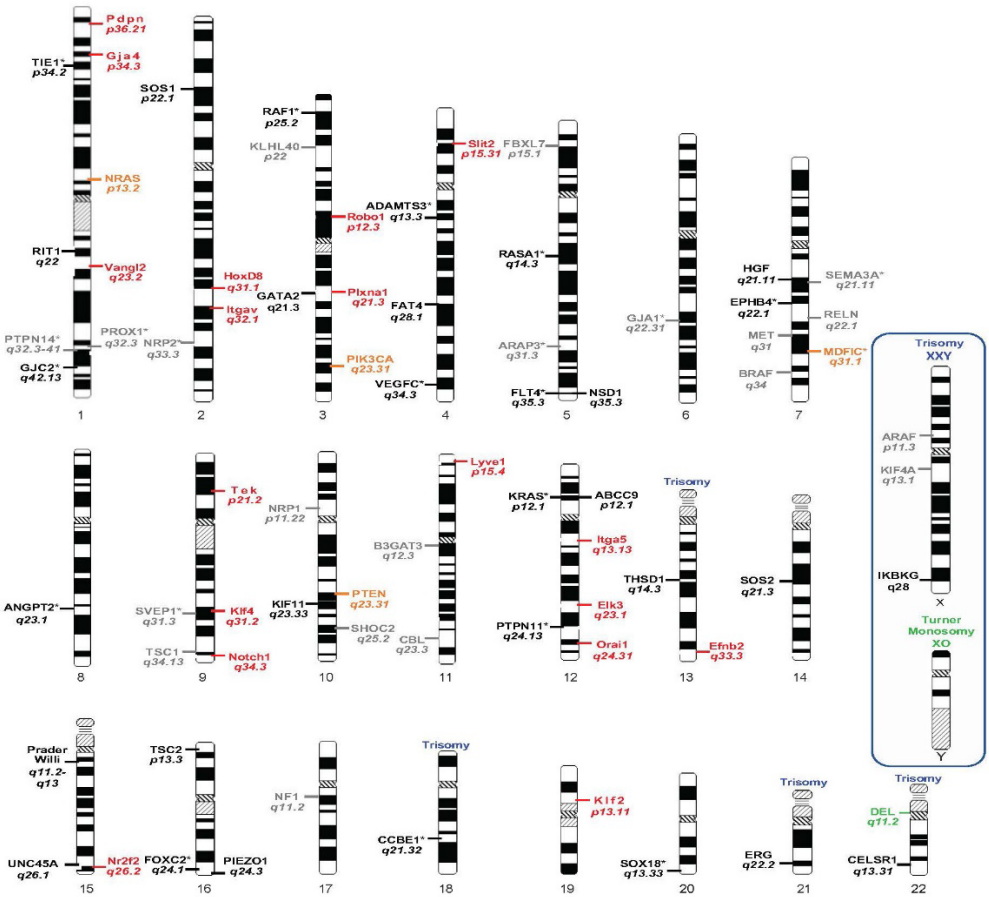

Fig. 1. *Human Chromosome Map depicting locations of gene variants and loci known to be associated with human primary lymphedema/lymphatic dysplasia syndromes, as well as candidate genes on the basis of murine studies. Black: location of confirmed human gene variants; grey: potential PL genes awaiting further confirmation; orange: genes causing complicated lymphatic anomalies (CLAs); red: corresponding human location of mouse gene variants associated with lymphatic maldevelopment (i.e., "candidate genes" not yet identified in human primary lymphedema); *: pathogenic human gene variants with corresponding mouse models; green: location of genetic deletions; blue: chromosomal duplications associated with human PL.*

DISCUSSION

Stressing on the fact that when genetic proof is weak in terms of number of clear cases and co-segregation in multiple affected family members, functional validations are required to validate the deleterious mechanisms of various candidate variants detected using bioinformatic predictions only. For example: in *ANGPT2*, most mutations generate loss of function, but one actually appeared as generating gain of function when expressed in mice (11). Another recessive *ANGPT2* variant, which at a first glance seemed to be a weak missense variant in the middle of an exon, turned out to alter splicing by the creation of a new strong splicing site and resulted in nonsense-mediated mRNA decay (NMD) causing hydrops fetalis (12). Yet, NMD is not always complete, and e.g. can result in the production of a truncated EPHB4 protein (13), or not happen at all, as seemed to be the case for the premature truncation in *CELSR1* (7). Moreover, the functional impact of missense variants should be assessed, as only a few of such variants in *HGF* significantly impacted protein function (10).

Another illustration of the importance of verifying mutations in mouse models is that of connexin 47 (encoded by the *GJC2* gene). Given that the *Gjc2* knockout mice had no lymphatic phenotype, and that the few human mutations were not fully penetrant with upper-limb instead of the more common lower-limb lymphedema, a CRISPR mouse model of one of the human mutations was made (14). Blue dye injection identified a 2-fold increase in regional lymph nodes in homozygous Cx47R259C mice, associated lymphatic channels were increased and mesenteric lymph reflux occurred. Contractility of superficial cervical lymphatics, assessed by pressure myography, was reduced in homozygous mice compared to wildtype, strongly suggesting that the human lymphedema-causing mutations were gain of function (14).

It is also important to realize that multiple mutations in one family may cause lymphedema. In a large family, exome sequencing uncovered two different mechanotransducer *PIEZO1* variants (one missense and one nonsense) and a previously described nonsense *FOXC2* transcription factor pathogenic variant in various combinations (15). Sanger sequencing confirmed the presence/absence of the three variants in affected and unaffected family members, with the *PIEZO1* nonsense change being nonpenetrant but worsening the severity of lymphedema when in combination with either of the other two pathogenic variants. The family member with all three mutations was severely affected (15).

While genome-wide association studies (GWAS) using hundreds of thousands of single nucleotide polymorphisms (SNPs) have been made for many common diseases, this seems not to have been done for post-breast surgery (secondary) lymphedema. A nomogram for risk based on phenotypic characters has been developed (16) which is of some use. Risk predictions based on a few or more genes have also been made (17,18). For common diseases, such as type 2 diabetes and Alzheimer's disease, Polygenic Risk Scores (PRS) have been developed, which predict risks that show quite wide ranges, e.g. for Alzheimer's disease, from 3-fold to plus 4-fold over the average risk (19). Given the many genes in this map, plus others, such as cytokines (18), it should be possible to develop a PRS for post-breast surgery lymphedema.

Finally, it is also becoming clear that "rare" diseases can sometimes be caused by the clustering of "common" genetic variants and this should be considered for some cases of otherwise unexplained familial lymphedema. In a GWAS study of 7000 children with rarer neurodevelopmental problems, the variance in inherited risk was attributable to common genetic variants (20). Enrichment for large-effect rare variants in putative core PRS genes for associated complex traits such as height and weight, again, especially, in children with "rare" nervous system disorders also determined the risk for the "rare" trait of delayed development (21).

CONFLICT OF INTEREST AND DISCLOSURE STATEMENT

All authors declare no financial competitive conflicts of interest.

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