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Functional Studies on the EPHB4 Signalling Pathway in Patients With Generalised Lymphatic Dysplasia

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Introduction: Lymphatic Endothelial Cells (LECs) have been shown to express EPHB4, a receptor tyrosine kinase (TK) which signals via the ephrinB2 ligand. Previous data revealed EPHB4 as a new causative gene for Lymphatic-Related (non-immune) Hydrops Fetalis (LRHF), a form of Generalized Lymphatic Dysplasia, a subgroup of Primary Lymphoedemas. Two missense mutations in EPHB4 were reported to be associated with this phenotype and the receptor was also identified as critical regulator of lymphangiogenesis. This study aims to further investigate the role of EPHB4 in lymphangiogenesis and the mechanisms by which the two specific mutations interfere with EPHB4 signalling and dysregulate lymphangiogenesis.

Methods: Transient transfection experiments in LECs in a liganddependent system were performed and receptor activation was analysed to functionally investigate the identified mutations. To study the effect of the two mutations on downstream signalling pathways after ligand stimulation, stable transfected cell lines expressing the wild type (wt) and the two mutant (mt) EPHB4 receptors are currently being generated. In parallel, EPHB4 expression is being silenced using siRNA with the aim to assess the effect of EPHB4 downregulation on LEC behaviour.

Results: Both mt proteins showed reduced TK activity after ephrinB2 stimulation compared to the wt receptor.

Conclusion: These results identify that the specific mutations disrupt EPHB4 signalling and suggest a possible mechanism for the lymphatic phenotype. Furthermore, they give new insights into the pathophysiological role of EPHB4 in lymphangiogenesis. Our future work will provide a better understanding of human lymphatic development and the role of EPHB4 in this process.