Pathogenic *ELN* gene mutations (*ELN*, MIM#130160) cause AD Supravalvular Aortic Stenosis (SVAS) a congenital narrowing of the ascending aorta, and Cutis Laxa (CL) characterised by inelastic, loose-hanging skin. Variable phenotype and penetrance is apparent. Pathogenic *ELN* variants result in loss of function and include frameshift (most common), nonsense, splice site and missense variants. The well characterised contiguous gene deletion syndrome, Williams-Beuren syndrome includes SVAS and encompasses at least 114kb on 7q11.23 including the *ELN* gene; however, there are only 5 case reports of CNVs within *ELN* (single or multiple exons).

Bristol Genetics Laboratory provides a UKGTN approved service for *ELN* gene sequencing (33 coding exons). In three years, 52 UK and foreign patients with SVAS, CL or features such as pulmonary artery stenosis and aortic dilation have been tested. 18/52 (34%) patients were heterozygous for a likely pathogenic variant including frameshift (6), nonsense (4), splice (4), and missense (4). 12 of these cases were novel variants, 5 are supported by segregation analysis and 1 is sporadic. The remaining novel variants are classed as possibly pathogenic as they are phenotypically compatible.

12/35 patients negative on sequencing have so far been screened for CNVs by MLPA (MRC Holland) covering the Williams-Beuren syndrome region, including 10 exons of the *ELN* gene (1, 3, 4, 6, 9, 16, 20, 26, 27 and 33) and in addition a bespoke MLPA assay including probes for exons 28 to 30, 32 and 3'UTR.

4/12 (33%) patients have a heterozygous deletion within the *ELN*gene. A mother and daughter with pulmonary stenosis and an extended family history have a deletion spanning exons 30 to 33. This deletion was also identified in another patient with SVAS and arteriopathy. A deletion of the 5' end of the gene, involving at least exon 1 (but not exon 3) was identified in an infant with SVAS and pulmonary branch stenosis, and a deletion involving the entire coding region of the *ELN* gene and at least the first two exons of the adjacent 3' gene *LIMK1* was detected in a neonate who died at 2 months with SVAS, pulmonary stenosis and mild hypoplasia with PDA. The deletion was detected in this patient's father who consequentially was found to have an aortic regurgitation and in a subsequent pregnancy of this family which was lost at 31 weeks with pulmonary stenosis and significant aortic stenosis

MLPA analysis has enhanced the clinical utility of this service giving an increased diagnostic yield in patients with SVAS and CL and related presentations.

204 GLYCOMIMETICS; A NOVEL CLASS OF DRUGS TO PROTECT AGAINST FREE FATTY ACID-INDUCED ENDOTHELIAL DYSFUNCTION

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10.1136/heartjnl-2016-309890.204

Background Endothelial dysfunction is a key player in cardiovascular disease (CVD) complications and novel drugs are required to treat this pathological process. Glycosaminoglycans (GAGs) are key molecules that regulate signalling in many biological processes and drugs that mimic their structure could be a novel source of therapeutics to target specific CVD pathways.

Purpose We have synthesised a set of four glycomimetic compounds and our objective was to determine whether they could activate protective pathways in endothelial cells subjected to fatty acid-induced endothelial dysfunction.

Methods Glycomimetics, C1-C4, were synthesised by the stepwise transformation of 2,5-dihydroxybenzoic acid to a range of 2,5-substituted benzoic acid derivatives, incorporating the key sulphate groups to mimic heparan sulphate. Human Umbilical Vein Endothelial Cells (HUVECs) were treated with glycomimetics (1ÂμM) in the presence or absence of the free fatty acid, palmitate. DAF-2 and H_2DCF-DA assays were used to determine NO and reactive oxygen species (ROS) production, respectively. Lipid peroxidation colorimetric and antioxidant enzyme activity ssays were also carried out. RT-PCR and western blotting were utilised to measure Akt, eNOS, Nrf-2, NQO-1 and HO-1 expression. Endothelial function was determined *ex vivo* using acetylcholine-induced endothelium-dependent relaxation in mouse thoracic aortic rings by wire myography.

Results All four glycomimetics protected against palmitate-induced oxidative stress and enhanced NO production *in vitro* via upregulation of Akt/eNOS signalling, activation of the Nrf2/ARE pathway and down-regulation of ROS-induced lipid peroxidation. Under palmitate-induced oxidative stress, *ex vivo* endothelium-dependent relaxation was significantly enhanced by all four glycomimetics. Furthermore, the glycomimetics did not induce HUVEC activation, as determined by lack of ICAM-1 protein.

Conclusion We have developed a new set of small molecule glycomimetics that do not activate ECs and protect against free fatty acid-induced endothelial dysfunction both *in vitro* and *ex vivo*. Future work will focus on developing the glycomimetics into drug-like therapies that target endothelial damage.

205 INFLUENCE OF NOX NADPH OXIDASES ON HUMAN PARTIAL INDUCED PLURIPOTENT STEM CELL-DERIVED ENDOTHELIAL CELLS

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10.1136/heartjnl-2016-309890.205

Background Human induced pluripotent stem (iPS) cell-derived endothelial cells (ECs) hold clear potential for therapeutic angiogenesis as a novel strategy for ischaemic disease. Recently, our group has developed a novel method for direct reprogramming of partial iPS (PiPS) cells, which unlike iPS cells, are generated before pluripotency so do not form tumours. Importantly, PiPS cells may be differentiated into ECs with characteristic morphology and pro-angiogenic actions, which *in vitro* and *in vivo* studies have demonstrated are comparable to mature ECs with regard to their capability of forming vascular-like tubes and re-endothelialisation of ischaemic tissue. It is well established that oxidative stress and reactive oxygen species (ROS), which are characteristic features of ischaemic disease, are important regulators of both endothelial and stem cell biology, with recent evidence suggesting a key role for NADPH oxidases. Notably, we have previously identified a key role for the Nox4 isoform in regulating