Editorial

Gene therapy and regenerative tissue engineering in congenital heart disease

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Gene therapy in genetic heart disease is a very attractive topic within the scientific community especially as some congenital heart diseases have been treated in with genome manipulation (1). Two years after its complete affirmation, the precise editing of the genome with CRISPR-Cas9 technology (2) has allowed researchers and clinicians to enter a new era of higher-level genetic engineering and more precise gene therapy; however, the implementation of such concepts remain lagging behind. Furthermore, the availability of centres with advanced technologies in gene therapy and the inherent challenges associated with their practice have limited their widespread use. In addition, the dissemination of scientific information that often precedes solid scientific results does not contribute to clarifying this new therapeutic option for genetic diseases. Recently, the Italian press highlighted the treatment of Hunter syndrome through the manipulation of the human genome, whereas the scientific journals were yet to publish the results. This news was broadcasted immediately after the publication, in August 2017, in *Nature* (1), on the possibility of using embryos for research and therapy that has rekindled a long-standing debate regarding which precise rules should the research on the human genome be authorized.

The 44-year-old patient with Hunter syndrome was first treated with self-DNA manipulation, known as "Zinc Fingers" at Benioff Children's Hospital of Oakland. Hunter syndrome is a type of mucopolysaccharidosis where a deficiency of iduronate-2-sulphatase (I2S), the enzyme responsible for the catabolism of mucopolysaccharides or glycosaminoglycans (GAG), favors lysosomal accumulation of dermatan sulfate (DS) and heparan sulfate (HS) (3,4). Clinical evidence of Hunter syndrome is damage to organs and tissues including the respiratory tract, heart, brain, liver, spleen, bones and cartilages (5). Heart manifestations of Hunter syndrome include cardiomegaly, a pan-systolic mitral regurgitation (MR) murmur frequently associated with hepatosplenomegaly, mimicking rheumatic disease with multiple heart valve involvement. Progression to heart failure is rapid with most patients already in NYHA Class III needing single or multiple valve replacement(s) (6). Moreover, changes in tissue stroma on the face and neck are also visible with serious psychological implications to the affected person. In patients with Hunter syndrome, enzyme production may be resumed by administering a healthy gene to replace the gene that is responsible for the defective lysosomal enzyme, to the liver cell; a favorable reservoir for the required amount of protein.

The genome manipulation procedure takes utilizes a suitably modified virus, as a vector, to introduce a functioning gene into the liver cell. The right information and specific instructions are aimed at molecules called "Zinc Fingers" that insert the right gene for the production of the enzyme. This is the first time this technique of genome editing has been used in humans, and was previously only reserved for the animal model, thus representing a progressive leap in the field of genetics. The undisputed advantage is that the new healthy gene produces albumin as opposed the faulty one (7), resulting in enzyme production. In the past few years, the use of Crispr-Casg9 has greatly superseded the method of "Zinc Fingers" for genome editing in congenital heart disease allowing considerable success by means of "size and sewing". For example, in August, *Nature* published a manuscript of Shoukhrat Mitalipov that corrected a pathogenic heterozygous gene mutation

MYBPC3 related to hypertrophic cardiomyopathy (1) in human embryos with precise CRISPR-Cas9 (2). This study received extensive scientific diffusion compared to the previous one and was discussed and criticized by international experts post-publication. This discussion included the right to claim this possibility, from the opportunity to test data, methodology and queries regarding bioethics.

The enviable effort was produced by a number of suitably coordinated specialists to cure a rare disease, highlighting that scientific processes are applied not only to the most common diseases and to the large part of the population, but also to a select population with rare genetic diseases in small numbers. Scientific progresses such as these are always decisive and well accepted for the welfare of the human population. However, some caution and critical attitudes are expected, because they are not raised from competing geneticists at ground level, and supported by cardiac surgeons interested in the specific cardiac and cardiovascular complications caused by some of these genetic diseases. Therefore, important scientific events must be preceded and accompanied by publications that describe in detail the applied methodology and results to ensure clarity. The concern is linked to the speed by which some scientific innovations are presented to the public, without waiting for the trial period that is indispensable for the certainty of the success of the research. Another non-negligible aspect, concerns the involvement of the individual person who guides the sphere of social behaviour and bioethics. Certainly, the modification of the human genome for therapeutic purposes is a new frontier to be explored, but caution and reasoning are required in the presence of human embryos. Are these methods risk-free? Are the sophisticated techniques presented are reproducible with other heterozygous mutations? and are we sure there are not many other scientific considerations to be done before clinical application is possible?

Another field of scientific interest, an alternative to genetic therapy, is the development of bioabsorbable materials such as those used for the reinforcement of allogeneic tissues. Its potential application in cardiac surgery is in congenital aortic stenosis, in bicuspid aortic valve disease and in the pathologies of the left ventricular outflow tract. These cardiac diseases can be treated with the Ross operation. Newborns and children are favourable candidates to receive pulmonary autograft root insertion to replace aortic valve diseased and/or LVTO although the results are less favourable especially when compared to the adult population, as pulmonary autograft (PA) root (PAR) dilatation plays a major role in long-term outcomes. Accumulating evidence suggest that patients with bicuspid aortic valve and aortic regurgitation have a higher risk of increased pulmonary autograft root diameter thus, a higher risk of failure with shortened durability. Mechanical phenomena related to the dilatation of pulmonary autograft under systemic pressure have recently been studied highlighting the biomechanics of PA (8-20). Mookhoek *et al.* (8) evidenced that the failed PA had a nonlinear response to mechanical loading, typical of healthy human arterial tissue, as nonlinear stress-strain response was present in both failed PA and normal PAR. Evidence of a remodelling process was shown in failed explanted conduits with increased wall thickness and decreased stiffness. The authors concluded that the increased compliance may explain progressive autograft root dilatation in autograft failures. An external barrier may prevent late dilatation and failure of pulmonary autograft root. Primary experience with an inclusion cylinder technique with the native aorta and a synthetic external support, such as Dacron was performed to stabilize the autograft root and improve long-term outcomes (9,10,21-23). Concerns regarding external reinforcement of a Dacron prosthetic graft is related to the PA which results in a straight material that would encase and dramatically impaired the PA pulsatility and compliance. We previously showed the negative effect of Dacron grafts and other synthetic polyesters that severely impair aortic compliance. Their use as vascular replacement for reinforcement of PA may be determined by a strong inflammatory reaction with significant damage to vessel walls (18-20).

Two factors merit further discussion. First, the aortic root anatomy presents an increased degree of complexity and cannot be approximated to by cylindrical geometry. Second, material deformation occurs not only in axial and lateral fashions, but also via shear stress should also be applied to determine the sliding of the conduit components.

To solve these issues, we have selected a material suitable to comply with both shear stress requirements and differential dilation tendency of the root. We have chosen ePTFE that is currently used in surgery and known, from the elastomechanical standpoint, to have auxetic behaviour (9,10,16). The component of ePTFE fibers when subjected to a tensile stress "open up" structurally and expand in the direction transverse to the stress; conversely, if these materials are subjected to compression, they structurally "close" (16,17). Our results favoured the development of

a semi-resorbable composite scaffold aimed at reinforcing the pulmonary autograft during the Ross procedure (9,10,16,17). This cross-linked bioresorbable prosthesis provided increased stability and solidity to the neo-aortic root, preventing the dilation of PAR due to systemic pressure. The composite prosthesis made with ePTFE and polydioxanone (PDS) prevented pulmonary autograft dilation and promoted connective remodelling of the pulmonary autograft wall resulting in a neo-vessel formation (9,10,12,19). The final result was an increased elastin content of PA with potentially improved biomechanical properties (16,19,24).

In conclusion the future of treatment of many congenital heart diseases is with improvements of genetic practices for clinical application and tissue engineering (25). This basic path must be united by solid scientific research.

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None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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