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THE VIRTUAL PHYSIOLOGICAL HUMAN: TEN YEARS AFTER

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ABSTRACT

Biomedical research and clinical practice are struggling to cope with the growing complexity that the progress of healthcare involves. The most challenging diseases, those with the largest socioeconomic impact (cardiovascular conditions, musculoskeletal conditions, cancer, metabolic, immunity and neurodegenerative conditions) are all characterised by a complex genotype/phenotype interaction, and in general by a "systemic" nature that the traditional reductionist approach struggle to cope with. In May 2005 a small group of researchers with different backgrounds met in Barcelona to discuss how the vision of computational physiology promoted by the Physiome Project could be translated into the clinical practice. In that meeting the term *Virtual Physiological Human* was formally proposed. We know a lot about these diseases, but our knowledge is fragmentary as it is associated with molecular and cellular processes on the one hand, and with tissue and organ phenotype changes (related to clinical symptoms of disease conditions) on the other. The problem could be solved if we could capture all these fragments of knowledge into predictive models and then compose them into *hypermodels* that help us to tame the complexity that such systemic behaviour involves. In 2005 this was simply not possible - the necessary methods and technologies were not available. Now, ten years later, it seems the right time to reflect on the original vision, the results achieved so far, and what remains to be done.

KEYWORDS

Virtual Physiological Human; Computational Physiology; *In Silico* Medicine, Physiome.

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1. FROM THE PHYSIOME TO THE VPH

Physiology has a long tradition, dating particularly from Claude Bernard in the 19th century, of quantitative research on the structure-function relationships that underpin physiological processes and the practice of medicine (1). This was a tradition that emphasized the integration of multidisciplinary knowledge using physical laws and mathematics, albeit at a simple level, to understand the complex processes of life. With the elucidation of the genetic and molecular basis of life, however, biomedical research in the latter half of the 20th century largely moved away from physiology and towards molecular biology.

One outcome of 50 years of research in molecular biology is a comprehensive 'parts list' of proteins, with their associated genetic blueprints and their interactions with other proteins, fats and carbohydrates. We have a wonderful compendium of metabolic networks, signalling and gene regulatory pathways, membrane receptors, transporters, ion channels, and many more. Biomedical scientists have easy access to vast databases of structural and biophysical information at the molecular level, greatly assisted by the development of bioinformatics web user interfaces and search tools. These reductionist discoveries, that include the human genome, metabolome, much of the proteome, and now parts of the exome and microbiome, are one of the great achievements of late 20th and early 21st century science, but they have had little impact on public health or the practice of medicine because they are not integrated into physiology which, together with anatomy, is one of the two disciplines that underpin medical science. Physiology as a discipline has lacked either the means or the inclination to deal with the increasingly complex links between molecular biology and integrative physiological processes at the tissue level.

Physiology should be providing the synthesis to match the reductionist analysis of molecular biology by integrating all the molecular information into an understanding of physiological function ('putting humpty-dumpty together again' (2)). Physiology has of course also made much progress in understanding whole body mechanisms over the past 50 years but it has had a tiny fraction of the funding in comparison with molecular biology, and the consequence is that we do not have anywhere near the same level of data and tools for synthesis. The other major issue is that the only way to quantitatively describe complex function is via mathematical models. Phenotype depends on models in the same way that genotype depends on a statement of nucleotide sequence.

Somewhat ironically, in the physical sciences and engineering the second half of the 20th century was characterized by the growth of computational science – taking advantage of the doubling of compute power per unit cost every 18 months (Moore's law (3)) and the ability of numerical techniques, such as the finite element method, to cope with complex physical and engineering challenges, such as aircraft design and space flight, by solving the well-known laws of physics that had largely been elucidated in the 19th century (e.g. mechanics, such as elasticity theory and the Navier-Stokes equations of fluid flow, thermodynamics, and Maxwell's electromagnetic equations that now underpin all of electrical engineering and communications). Again ironically, physics and engineering have been crucial to progress in the development of instrumentation that has enabled much of the progress in molecular biology (e.g. sequencing machines, PCR, mass spectrometers) and the clinical imaging that has transformed clinical medicine (e.g. MRI, CT, PET and ultrasound). The divergence between the physical and biological sciences in the use of mathematics and computational tools for scientific understanding has possibly been exacerbated by education systems that often force students to choose between the 'hard' disciplines of physics and engineering and the 'soft' disciplines of biology and medicine.

The International Union of Physiological Sciences (IUPS) recognized this problem in 1993 and established the Physiome Project to bring engineering approaches and technologies into physiology. The concept of a "Physiome Project" was presented in a report from the Commission on Bioengineering in Physiology to the International Union of Physiological Sciences (IUPS) Council at the 32nd World Congress in Glasgow in 1993. The name comes from "physio-" (life) and "-ome" (as a whole), and is intended to provide a "quantitative description of physiological dynamics and functional behaviour of the intact organism" (4-7).

2. THE VISION

During the sixth framework program (FP6, 2002-2006) the EC had funded some research and technological projects where computational physiology, biophysics, and biomechanics methods had been used to address clinically relevant problem. But in spite of the available expertise, there was a sense that Europe was missing the bus: the International Union of Physiological Societies had formally endorsed the IUPS Physiome Project in 1993, but the drive was primarily in New Zealand, in Japan, and in USA. Also, in April 2003, the Interagency Modeling and Analysis Group (IMAG) was formed, which coordinated program staff from the National Institutes of Health (NIH) and the National Science Foundation (NSF) who managed projects in this growing area. On the 1st of June 2005 a small group of researchers and officers from the European Commission (EC) met in an expert workshop in Barcelona. Out of this meeting a White Paper was published in November 2005[1], where for the first time the term "Virtual Physiological Human" or VPH was used.

Reading this document it is possible to identify three distinct themes, at that time barely integrated. The first, inherited from the Physiome Project, was the use of computational physiology in the fundamental understanding of physiology and its dysfunction (disease), where systemic interactions hard to capture with traditional reductionist approaches were relevant. In some of the early narratives (4) the Physiome Project was heavily inspired by the Human Genome Project, which started in 1990 and was completed in 2003. Similar to the Genome Project, the challenge was presented as the collaborative development of a digital representation of the human body and its relevant physiological systems (i.e. the neuromusculoskeletal system), or organs (i.e. the heart). Because of the heterogeneity of the information to be shared this also posed a grand challenge in the area of data sharing and data modelling infrastructure. The other element that permeated some early reports was the implicit idea of building a generic Human Physiome, which could eventually become a Reference Human Physiome, much like the effort of the Genome Reference Consortium[2].

The second theme was a pull towards clinical applications, in relation to the need for more individualised medical decisions, but also in the role that *in silico* methods could play in the development of new medical products. Here the vision was more toward the development of patient-specific and problem-specific models, which could be used as decision-support systems in the clinical practice, for diagnosis, prognosis, or treatment planning.

The third theme, less evident in the white paper, was a criticism of the overwhelming importance that had been given to single cell molecular biology in the past 20 years, both in terms of funding and the narrative within biomedical research. The complete mapping of the human genome had been announced only two years before, and the over-hyped public discourse of genetics as the all-solving approach to medical research was at its zenith. A

[1] http://www.vph-institute.org/upload/file517569145f61b.pdf

[2] http://www.ncbi.nlm.nih.gov/projects/genome/assembly/grc/

seminal popular science book "The Music of Life: Biology beyond genes" (8) elaborated with convincing evidence the argument for a more holistic approach. But to make real the integrative biology vision that Denis Noble advocated there was the need for a whole new "framework of methods and technologies that, once established, will allow the investigation of the human body as a whole" (9).

In 2007 the STEP Consortium published "Seeding the EuroPhysiome: A Roadmap to the Virtual Physiological Human" (9). The STEP support action, funded by the EC at the end of FP6, produced a roadmap that was the result of a large consensus process involving 318 named experts worldwide. All three themes mentioned above were still present, but now the narrative centred primarily on the second. It was acknowledged that the phenotypical variability among subjects, especially if affected by a pathology, was so large that the usefulness of a reference physiome would have been quite limited; in addition it was acknowledged that "Models are usually developed for a very specific purpose" (see (9), section 3.2.2). This is an important passage in the VPH vision: from 2007 the VPH became primarily a bioengineering effort, aimed at developing integrative patient-specific models targeting a well-defined predictive purpose of clinical relevance, which would typically be used as a decision support system. The development of repositories of data and models was seen more as a necessary tool for the VPH practitioners than an endpoint in itself; also the more fundamental reflection of the epistemological role of integrative modelling in biomedical research was framed toward the need to develop credibility for predictive medicine technologies. This focus became quite characteristic of the VPH in Europe. On the other hand, the 2007 2nd MEI International Symposium organised in Osaka by the Japanese Global Centre of Excellence on *in silico* medicine (10), and the 2009 IMAG Futures Meeting held on in Bethesda –Maryland[3], presented a use of multiscale modelling much more oriented towards the understanding of fundamental physiology and pathology mechanisms, rather than clinical applications.

In 2008 PriceWaterhouseCooper published a seminal report entitled "Pharma 2020: Virtual R&D - Which path will you take?"[4] where a second role was suggested for *in silico* technologies: the creation of Virtual Patients, computer simulations that could be used to infer the efficacy and safety of new biomedical products as they interacted with each patient's anatomy, physiology, and life style. This was a different target for the VPH, where patient-specific models of real or synthetic patients could be used to test drugs or medical devices. Along a similar line is the 2009 report "Virtual Tissues: a quantum leap in biomedical research"[5], co-authored by the US Environmental Protection agency.

In 2009 the update of the VPH Research Roadmap elaborated by the VPH Network of Excellence (11), called for the creation of a "international non-profit organisation, whose mission is to ensure that the Virtual Physiological Human is fully realised, universally adopted, and effectively used both in research and clinic". Soon after the *Virtual Physiological Human Institute for Integrative Biomedical Research* (VPH Institute for short) was established[6], as a virtual organisation to providing strategic leadership, initially in Europe but now globally, for the VPH/Physiome Project.

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[3] http://www.vph-institute.org/upload/imag-futures-report_519244d3a0aa0.pdf

[4] http://www.vph-institute.org/upload/pwc-pharma2020-virtualrd-final_519245021a53a.pdf

[5] http://www.vph-institute.org/upload/v-tissue-position-paper-2009_555460b051aaa.pdf

[6] http://www.vph-institute.org

In 2011 the vision of the VPH came to its full expression. In a position paper on the forthcoming Horizon 2020 framework program[7], the VPH Institute identified the scope of the VPH around three macroscopic targets:

- ***Digital Patient***: The VPH for the doctor; patient-specific modelling to support medical decisions.
- ***In silico Clinical Trials***: The VPH for the biomedical industry; collections of patient-specific models to augment the pre-clinical and clinical assessment of new biomedical products. *In silico* technologies for the reduction, refinement and partial replacement of animal and human experimentation.
- ***Personal Health Forecasting***: The VPH for the patient/citizen; subject-specific real-time simulations, based on data collected by wearable and environmental sensors, that provide advice to individuals affected by conditions requiring careful self-management, or to people simply at risk of developing certain diseases.

In addition to these three targets, two horizontal themes were also identified: (i) that of the infrastructure for biomedical data generation, collection, processing, modelling, and sharing, (acknowledged as a technological, socioeconomic, regulatory, and ethical challenge); and (ii) that of the methodological, epistemological, and social challenges related to the assessment and the acceptance of predictive technologies.

An in-depth analysis of the research vision for these five areas is beyond the scope of this review. The interested reader should refer to the Discipulus Roadmap for the Digital Patient area[8], and the Avicenna Roadmap for the *in silico* clinical trials area[9]. Some aspects of data sharing and of modelling based on mobile health technologies are discussed in the recent paper entitled "Big Data, Big Knowledge: Big Data for Personalized Healthcare" (12).

3. THE METHODS

The goals of the Physiome Project in 1997 were: (i) to link tissue/organ level physiological function to mechanisms at the molecular scale, (ii) to develop model and data encoding and exchange standards for multiscale modelling to ensure model reproducibility and sharing, (iii) to develop modular approaches to ensure that self-contained models could be developed and validated independently before being incorporated into a hierarchy of imported models, and (iv) to develop free web-accessible model repositories and open source software based on the Physiome modelling and data standards. The dual focus of the VPH project when it started in 2007 was to complement the physiological multiscale modelling focus of the Physiome Project with greater clinical relevance and industry opportunity.

Modelling standards

The first modelling standard developed by the Physiome Project was CellML[10] (13; 14). This standard covers models that use ordinary differential equations and algebraic expressions (both of which can be nonlinear). The 'ML' refers to 'Markup Language' and in particular the

[7] http://www.vph-institute.org/upload/vphinst-position-on-fp8-greenpaper-v3_5192443874603.pdf

[8] http://www.vph-institute.org/upload/discipulus-digital-patient-research-roadmap_5270f44c03856.pdf

[9] http://avicenna-isct.org/roadmap/

[10] CellML began as a joint public-private initiative in 1998 with funding by the US company Physiome Sciences (CEO Jeremy Levin), before being launched under IUPS as a fully open source project in 1999.

'eXtensible Markup Language' or XML, which is the Web2.0 standard[11] for exchanging information on the web. CellML treats model syntax (the mathematics, which is encoded in MathML[12]) separately from semantics (the biological and biophysical meaning of the mathematical terms, which is described via ontologies and encoded with RDF[13]). The structure of a CellML model, including the use of imports to maintain modularity, is illustrated in Figure 1.

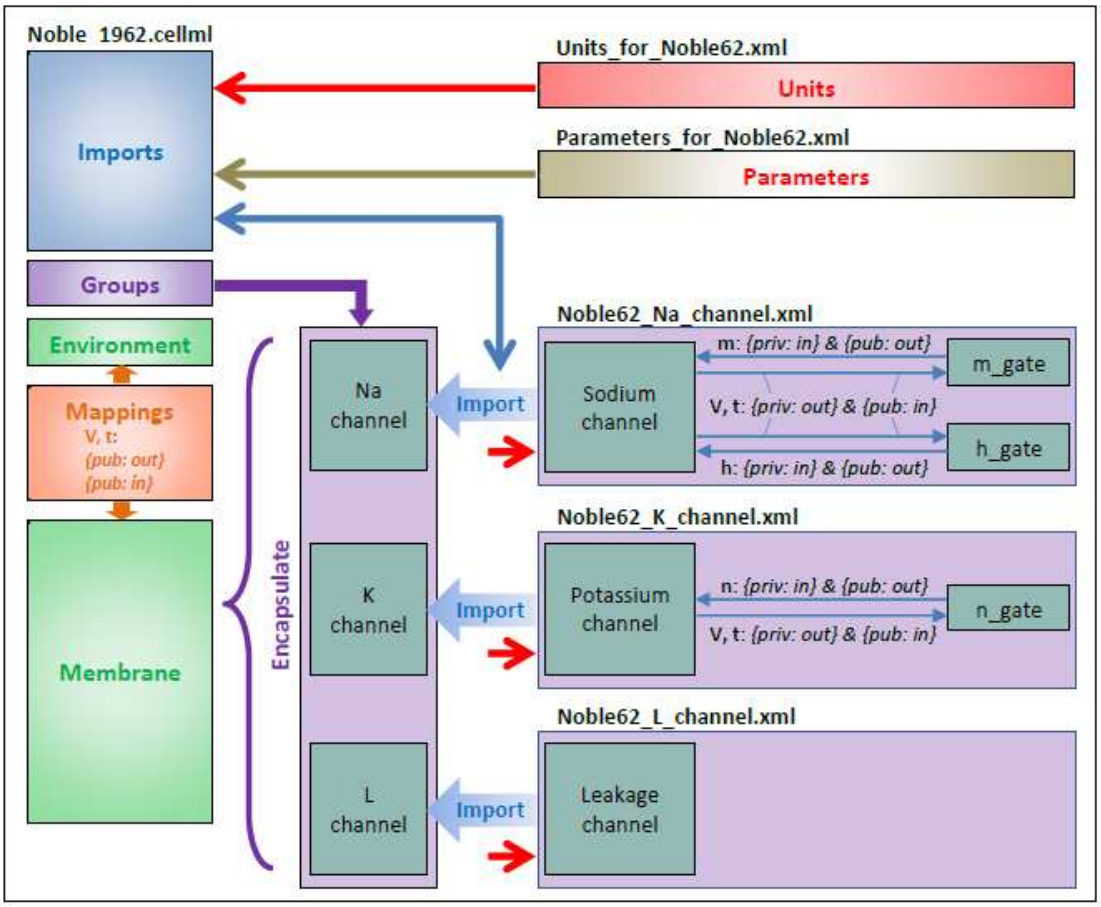

Figure 1. Overall structure of the Noble62 CellML model showing the encapsulation hierarchy (purple), the CellML model imports (blue), and the other key parts (units, components & mappings) of the top level CellML model. Note that all units are defined in a units file and imported where needed (shown by the red arrows). Also the ion concentration parameters are defined in a parameters file and imported into the top-level file but passed down to the modules that use them via the mappings.

In this example, the 1962 Noble model (15) of a cardiac action potential is built up from three separately defined sub-models (one for the sodium channel, one for the potassium channel, and one for the leakage channel). Note also that the units and the parameters for the model are also imported from separate files. Other features include *components* (for example the two types of sodium channel gate, the m-gate and the h-gate, are defined as separate components with the sodium channel model), *mappings* between components (this allows the visibility of parameters and variables to be controlled), *grouping* and *encapsulation* (to logically encapsulate a group of separate objects – say the three ion channels – into a single entity).

[11] The World Wide Web Consortium (http://w3c.org) is the governing body for Web standards and protocols. Web2.0 refers to a group of technologies, including XML, RDF and MathML, that emphasize user-generated content, usability, and interoperability.

[12] https://en.wikipedia.org/wiki/MathML

[13] https://en.wikipedia.org/wiki/Resource_Description_Framework

CellML is designed to be very general in order to handle any type of biophysical model[14] and, in order to support model imports, is a declarative language (unlike say C, Fortran or Matlab, which are procedural languages) so that the order of statements does not affect the solution. An Application Programming Interface (API) is available but more user-friendly software called OpenCOR[15], developed to create, edit and run CellML models is also available (16). A related XML standard, designed specifically for biochemical reactions and developed by the systems biology community, called SBML[16], has many alternative software platforms.

A repository of CellML models has been developed[17] that covers most areas of biology. There are about 600 model *exposures* (corresponding to 600 publications and available for public download) based on peer-reviewed publications, and another 400 *workspaces* (collaborative development environments that are password protected). Once a model has been published the workspace can generate an exposure for public access. Nearly all models are curated (checked to ensure that they have consistent units, they work and they give results consistent with the reference publication) and many are annotated (the terms in the model are linked through metadata to ontologies that provide biological and biophysical meaning). The mathematical equations for the model can be displayed directly on the website or in OpenCOR, and the model equations can be output in a variety of computer languages (C, C++, Fortran, Matlab, Python)[18]. This repository, which includes FieldML models as well as CellML models, is called the *Physiome Model Repository* (PMR)[19].

Another modelling standard developed under the VPH/Physiome Project, dealing with spatially varying structure and processes, is called FieldML. The term 'Field' refers to the concept, widely used in the physical sciences and engineering, of a spatially continuous field such as temperature or the oxygen concentration in a tissue being captured in a parametric model. The most common format for these models uses finite element basis functions that interpolate nodal parameters that are themselves the value of the field (and sometimes its spatial derivatives) at that material point. The whole field is made up of many such finite element patches, chosen with the spatial resolution needed to achieve any desired level of accuracy for either fitting measured data or representing the field solution of partial differential equations that characterize the physical processes being modelled. A model repository and open source tools based on the FieldML standard are also available (17; 18).

CellML and FieldML together provide the means to encode any biophysical model in a standardized and reproducible format. Data encoding standards are less well defined, although DICOM[20] is a well-established standard for clinical images and BioSignalML[21] is a standard developed by the VPH-Physiome community for time varying signal data. OpenCOR incorporates the BioSignalML API in order to import or export signal data in this format.

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[14] https://www.cellml.org/

[15] http://opencor.ws. This is open source and freely available.

[16] http://sbml.org

[17] https://models.physiomeproject.org/cellml

[18] http://www.cellml.org/about/publications has an extensive list of publications on CellML and OpenCOR.

[19] https://models.physiomeproject.org/welcome

[20] http://dicom.nema.org/

[21] http://www.ncbi.nlm.nih.gov/pubmed/22255626

Simulation protocols and clinical workflows

With modelling standards now in place, the focus for the VPH/Physiome community is increasingly shifting to better mechanisms, based on these standards, for comparing models with each other and with experimental data – a process called *functional curation*. In particular there is a need to define standards around the implementation of experimental protocols. One such effort, for cardiac electrophysiological models, is the Oxford 'web lab' facility[22] (19). This allows the user to compare dozens of cardiac electrophysiology models under a wide variety of conditions, such as stimulating at different pacing rates, blocking particular ion channels with specific drugs, and generating phenotypic outputs such as s1-s2 restitution curves for all models under test. To standardize the way CellML or SBML models are run and compared with experimental data, a standard called SED-ML[23] has been developed by the VPH/Physiome and Systems Biology communities. The SED-ML API has been incorporated into OpenCOR so that the simulation parameters can be read in from a file or defined in OpenCOR and exported to a SED-ML file. A tutorial on the use of CellML, OpenCOR and PMR is available on the VPH-Institute website[24].

Another VPH initiative that facilitates the development of computational models for the clinic is VPH-Share[25]. This major project, led by the University of Sheffield, has created a cloud-based IT infrastructure and universal clinical workflow development system capable of supporting the construction and operation of complete software systems to extract novel and possibly complex biomarkers from raw clinical information, and so to provide clinicians with decision support for diagnosis, stratification, therapy selection and treatment planning (20). VPH-Share provides a completely secure environment in which researchers and clinicians can quickly assemble application components – often already available within the system - into fully-operational end-to-end processing chains that can, where necessary, feature significantly complex operations including, for example, finite element models executed on remote HPC machines, programmed automatically to carry out parametric sweeps and physiological envelope explorations. Soon to be available as a supported public service for clinical researchers, where the use of anonymised data is the norm, VPH-Share includes features permitting users – if they wish - to share selected elements of their work with other named users of the system, again using fully secure mechanisms. Also available is a stand-alone version of VPH-Share for individual or collaborative use within specific communities such as industrial and healthcare organisations, where data sensitivities or local regulations require detached operation.

To bring the benefits of mechanistic models into the clinic requires that the semantic annotations mentioned above are mapped into the data storage formats used in an electronic health record (EHR). This concept is being tested with the Archetype data structure in openEHR[26] (21).

4. THE TARGETS

The term "Virtual Physiological Human" is rarely used as a scientific definition in research papers. To date in PubMed there are only 53 papers with this term, and most of them are

[22] https://chaste.cs.ox.ac.uk/FunctionalCuration

[23] http://sed-ml.org/

[24] http://www.vph-institute.org/upload/opencor-tutorial-v17_55e999c508b8a.pdf

[25] http://www.vph-share.eu/

[26] http://www.openehr.org/

policy-oriented papers, rather than original articles; also "multiscale model" returns only 260 papers, mostly in the last five years. But if we search (patient-specific[All Fields] OR subject-specific[All Fields] OR (personalised model[All Fields] OR (personalised model[All Fields])) we find 2,203 papers, with a sharp increase starting from 2005 (figure 2).

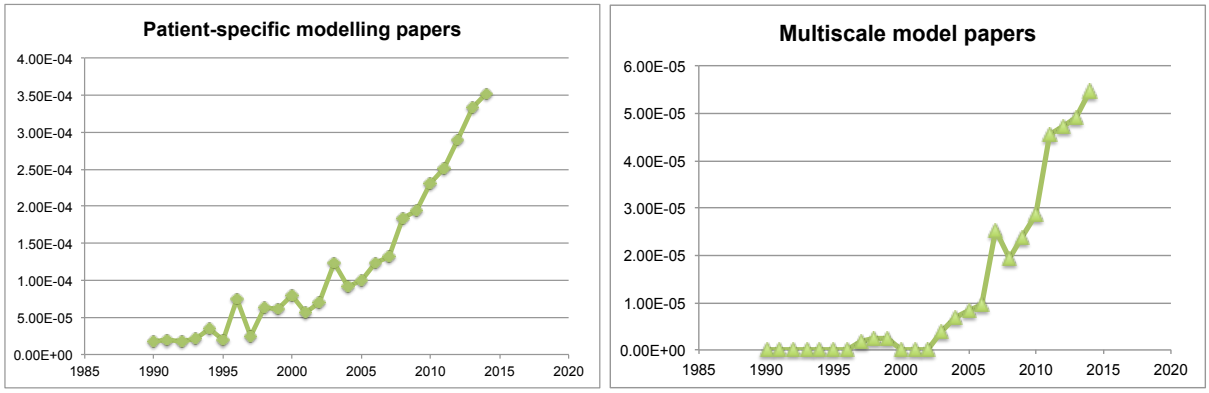

Figure 2. Number of papers in PubMed on subject-specific models (left) and multiscale models (right), divided by the total number of papers indexed by PubMed in the same year.

The VPH approach requires being able to describe pathophysiological processes in quantitative terms over space and time, and across vast space-time scales. So it should not come as a surprise that some of the most popular clinical targets for VPH models are the organ systems that have the best biophysical characterisation: the cardiovascular and respiratory system, and the neuromotor and musculoskeletal system.

The mammalian heart was one of the first targets of VPH researchers. As the VPH vision developed from the early work on the *Cardiome* Project (22-24), researchers focused more of specific clinical targets, such as the better planning of cardiac re-synchronisation therapy (25; 26), the stratification toward treatment of coronary stenosis patients (27; 28), or the patient-specific planning of the new transcatheter valve implantation procedures (29; 30).

To the authors' knowledge the first VPH technology to achieve FDA approval belongs to a special group where the quantity to be predicted can indeed be measured directly, but through a costly, invasive, and/or dangerous procedure. Fractional Flow Reserve (FFR) expresses the pressure drop across coronary stenoses; it is measured with an instrumented endovascular catheter that must be passed through the stenosis. While it has been proved that Fractional Flow Reserve has an accuracy > 90% in identifying if the stenosis is causing cardiac ischemia (31), this procedure is rarely used because of the cost, complexity, and risk that the direct measurement of the FFR involves. Using a patient-specific model generated from coronary computed tomographic angiography images, the HeartFlow service can predict the value of the FFR without any invasive procedure (32). The HeartFlow technology has received recently full FDA approval, and in now available for use worldwide. The Insigneo institute in Sheffield (UK) has completed a phase I clinical trial on a similar solution that uses rotational coronary angiography, which is far more accessible than CT angiography, especially in Europe (28). A similar application, which also just completed the phase I trial, makes it possible to perform an accurate differential diagnosis of pulmonary hypertension without the need for a delicate invasive right heart catheterisation, which is the current standard of care (33).

Complex cardiac surgery, such as transcatheter aortic valve implantation (TAVI), or paediatric percutaneous pulmonary valve implantation, require accurate planning in order to be successful. A new generation of VPH technologies facilitates patient-specific simulation of the device deployment in order to predict how it will perform if deployed in that way in that patient. See, for example, recent work on a particularly difficult paediatric case, at the

Great Ormond Street Hospital for Children in London (30). A Dutch start-up, FEOPS[27], is testing a software technology called TAVIGuide, to assist with the planning of TAVI procedures.

The accidental finding of asymptomatic aneurysms poses a significant challenge to the clinical specialist who must decide whether to treat it, which usually involves some serious risks, or leaves the aneurysm untreated, with the risk that the lesion keeps growing and eventually bursts, producing a massive haemorrhage. So predicting the risk of rupture of individual aneurysms remains one of the holy grails for VPH research. But the problem is very complex, and all technologies are in early stages of development. Some interesting results were obtained for abdominal aortic aneurysms (34), while for cerebral aneurysms a lot more research is required (35).

For the musculoskeletal system one of the first clinical problems VPH technologies confronted is the risk of bone fracture in osteoporotic patients (36) (37) (38). Today, the Insigneo predictive technology can correctly discriminate in retrospective studies fractured and non-fractured patients with an accuracy of 75%-84%, depending on the age distribution of the patients (39) (40). An interesting derivation of this research line is the stochastic modelling of neuromuscular control, which has allowed to consider sub-optimal muscle activation patterns (41; 42), enabling population modelling of the risk of hip fracture (38), but more recently showed to improve the predictive accuracy of musculoskeletal dynamics models even in relation to normal health subjects (43). Whole body musculoskeletal patient-specific models are also used in the treatment planning and functional grading of paediatric cerebral palsy patients (44); Fregly, 2009 #460; Oberhofer, 2010 #461; Ravera, 2010 #462; Scheys, 2011 #463; Scheys, 2011 #464; Bosmans, 2014 #465; Riccio, 2015 #466].

Multiscale approaches were used to investigate more fundamental problems around the role that molecular constituents and their spatial organisation plays in the macroscopic mechanical properties of bone (45) (36) (46) (47) (48), tendons (49-53), skeletal muscles (50; 54-57), and cartilage (58-61) and ligaments.

A very important feature of all connective tissues is their ability to adapt to altered environmental requirements (62) (63) (64) (65) (66), and to repair damage (67) (68); both topics have been thoroughly investigated using VPH approaches. In relation to bone remodelling, some authors focused their attention on the multiscale interaction between solid and fluid phases (69), which is suspected to play a role in bone tissue adaptation.

VPH approaches were used to investigate the complex mechanobiology of articular cartilage (60), the ethiopathogenesis of osteoarthritis (58), the mechanisms that drive the formation of focal subchondral lesions in rheumatoid arthritis (59), and the ethiopathogenesis of osteoarthritis in relation to muscle weakening (70). Rare diseases such as osteogenesis imperfecta were also targets of VPH modelling studies (71).

In dental research VPH modelling has been used to determine the mechanical stresses induced in the alveolar region during mastication (72), and in relation to the aseptic loosening of dental implants (73). Uterine biomechanics, and the role of pelvic musculature during parturition, have also been investigated (74; 75). Another interesting target is oncology, for example predicting the response for individual patients with breast cancer undergoing neo-adjuvant therapy (76).

[27] http://feops.com/clinical

5. TWO CASE MULTISCALE MODELLING STUDIES

In this section we describe two VPH/Physiome physiological modelling projects that build on the multiscale modelling methodology described in Section 3 and have reached some level of clinical relevance. There are many other clinically relevant examples involving other organ systems, but these two are chosen as exemplifying two of the first VPH/Physiome projects to address multiscale issues. In most cases clinically oriented projects deal primarily with only one or two spatial scales, but many are on a path to link clinically relevant tissue/organ level models, usually derived from clinical imaging, with molecular and cellular mechanisms. Most are also capable of being applied in a patient-specific way – using multiscale physiological measurements from the clinic to guide diagnostic analysis and therapeutic procedures.

The two examples we give here all involve international collaborations with VPH connections and funding. They are: (i) a cardiac electrophysiology modelling project for the diagnosis of cardiac arrhythmias, and (ii) a project on modelling respiratory biomechanics for the diagnosis and treatment of COPD and asthma.

(i) Cardiac electro-mechanics modelling: Diagnosis of cardiac arrhythmias

Models of cardiac ion channels by Denis Noble were among the first molecular level models developed in computational physiology (15), following the Noble-prize winning squid axon studies of Hodgkin and Huxley (77). Continuum mechanics models of the heart with anatomically realistic geometry and structure were developed later (22; 78; 79). These were soon coupled also with myocardial wave propagation to produce multiscale cardiac electro-mechanics models (80) and subsequently with ventricular flow and coronary flow models (81). This is the work that initiated the IUPS Physiome Project in 1997 and, as mentioned earlier, led to the proposal for a VPH project and subsequent investment in computational physiology by the European Commission. All four chambers of the heart are now represented in many of these models which are now used in many clinically focussed studies, including the treatment of arrhythmias (82), coronary disease (83), mitral and aortic valve failure (84) and both diastolic and systolic heart failure (85). We give one such example here, on the diagnosis of a cardiac arrhythmia, as an illustration of the need to deal with the variability of gene expression in a human population as well as the complexity of having multiple ion channels, their intracellular trafficking and their spatial distribution in tissue, contributing to the clinically observed phenotype at the whole heart level.

The cardiac action potential is generated by the flow of ions across cardiac cell membranes. The rapid upstroke is associated with a huge (rapidly self-terminated) influx of sodium ions that is responsible for the QRS complex of the EKG (Figure 1). The long (≈100ms) plateau, needed to allow time for muscle contraction before the next heart beat, is maintained by several inward ion fluxes, including the entry of the calcium ions that trigger the release of internal calcium stores to activate the contractile proteins. In most electrically active cells the resting membrane potential is maintained by an open potassium channel (with high intracellular K^+ and a Nernst potential of -85mV) that briefly conducts a positive outward current when the voltage raises towards the +35mV Nernst potential of the now open Na^+ channel. Because the cardiac cell has to maintain its depolarised (positive membrane potential) state for the period of the plateau, it cannot afford to have a prolonged outward flow of potassium ions (since these ions would have to be pumped into the cell again at high metabolic cost) and has evolved an 'inwardly rectifying' potassium channel called I_{K1}, as illustrated in Figure 1. Two other types of potassium channel, I_{Kr} (r for 'rapid') and I_{Ks} (s for 'slow'), have therefore evolved to repolarise the cell towards its resting membrane potential (where I_{K1} can take over).

Repolarisation at the cell level is reflected by the 'T-wave' at the body surface on the EKG (see Figure 3) and the time from the peak inward sodium current (the 'Q-wave' on the EKG) to the peak of the T- wave is the clinically observed 'QT interval'. Any mutation that reduces the density of I_{Kr} or I_{Ks} channels or detrimentally alters the channel kinetics leads to delayed repolarisation and a longer than normal QT interval - called LQTS ('long QT syndrome'). This is particularly dangerous since reactivation of an inward sodium or calcium current during the plateau can generate a wave of electrical depolarisation spiralling around the heart (an 'arrhythmia' referred to as 'torsade de pointes' because of the apparently random dancing pattern on the EKG) that can quickly break up into multiple spirals 'ventricular fibrillation' that render the myocardium as an uncoordinated quivering mass not able to pump blood (a 'heart attack').

Repolarising currents that produce the T-wave include I_{Ks} and I_{K1} as well as I_{Kr}. One particular inherited LQTS condition called LQTS2 (resulting from mutations of the KCNH2 gene of the I_{Kr} channel) manifests as both an LQT interval on the EKG and a splitting of the T-wave - called a 'bifid' or notched T-wave. Most (>90%) of the missense mutations of KCNH2 exert their effect through reduced trafficking of the protein from the nucleus to the cell membrane and hence reduced conductance of the I_{Kr} channel. More pronounced notching appears to be associated with more severe forms of LQTS2 and it would therefore be clinically useful to understand how the notching arises since different drugs are needed to treat different underlying causes for particular patients.

Multiscale modelling of arrhythmogenic conditions such as LQTS, to improve the precision of clinical diagnosis and therefore treatment strategies, requires anatomically based models that capture the fibrous-sheet architecture of the myocardium, the spatial distributions of ion channel densities and detailed kinetics of all the relevant ion channels. This is illustrated in Figure 3 for LQTS2.

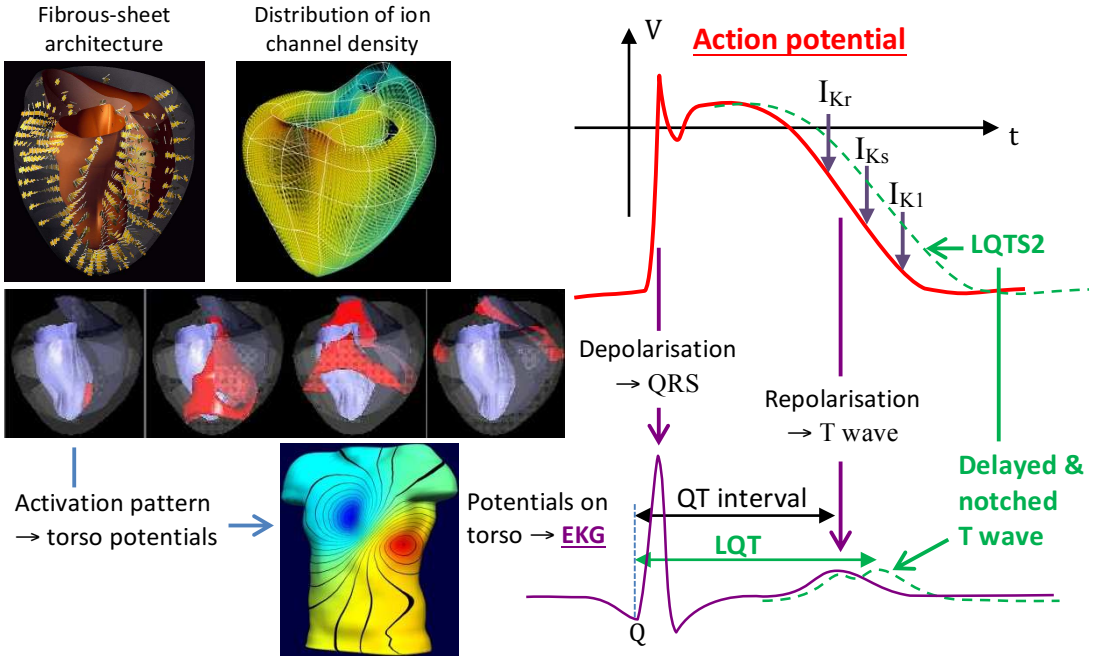

Figure 3. Anatomically based organ level models of the heart and torso include the fibrous-sheet architecture of the heart and the spatial distribution of ion channels (top left). The normal cellular action potential (top right) is repolarised by several potassium channel currents (IKr, IKs and IK1). Mutations in IKr cause LQTS2 (long QT syndrome type 2) that delays repolarisation and can be associated with a notched T wave. Modelling by Sadrieh et al (82) shows that the two peaks seen in LQTS2 (shown here in green) are associated with different myocardial cell populations, preferentially expressing IKr and IK1, respectively.

A study by Sadrieh et al (82) showed that the notched T-wave in LQTS2 is associated with two populations of cells, one with greater expression of I_{Kr}, generating the first peak of the notched T-wave, and one with greater expression of I_{K1}, generating the second peak of the notched T-wave. The dip between these two peaks (corresponding to an inward current) appears to be associated with reactivation of sodium current. The diagnosis of LQTS2 is complicated by the variability of T-wave shape.

Readers interested in the details of how this model is used clinically should consult (82). We have briefly described it here because it very nicely illustrates a clinical application of cardiac modelling that builds on the VPH foundations.

(ii) Respiratory modelling: The diagnosis and treatment of COPD and Asthma

Anatomically based finite element models of the airways and vasculature of the lungs have also been under development for some time (86). A structure-based airway model, for example when coupled with a model of airway resistance and tissue elasticity (87) can predict the distribution of ventilation (V) throughout the lungs (see Figure 4a). Similarly, a vascular model coupled with capillary recruitment and the effects of hydrostatic pressure, predicts the distribution of perfusion (Q) throughout the lungs (88) (Figure 4b). Computing the ratio of ventilation to perfusion (V/Q) for a region of tissue and coupling this to a model of the biophysics of oxygen exchange (89), yields the arterial and venous blood gases throughout the lungs (Figure 4c). These models are used to develop a treatment strategy for patients with by fitting the vessel structure to clinical CT images and using the observed 3D distribution of emboli imposed on the model.

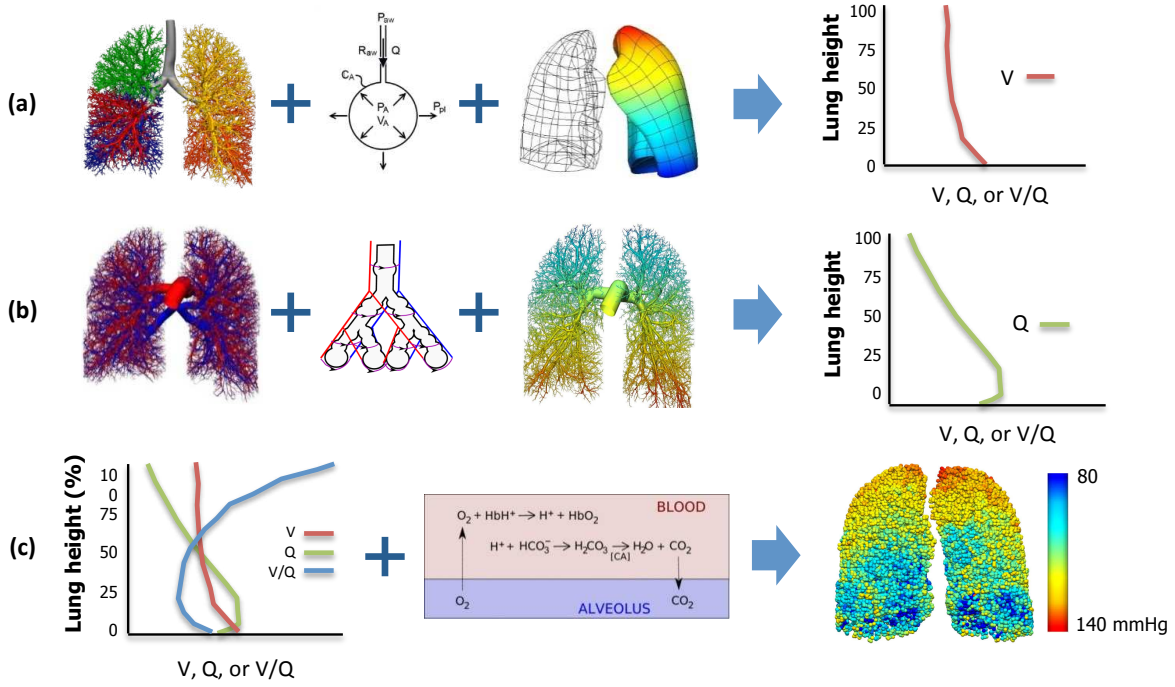

Figure 4. (a) A structure-based airway model coupled with a model of airway resistance and tissue elasticity predicts the distribution of ventilation (V), shown here as a vertical distribution; (b) a vascular model, coupled with capillary recruitment and the effects of hydrostatic pressure, predicts the distribution of perfusion (Q); (c) Left: the ratio of ventilation to perfusion (V/Q) for a region of tissue. Coupling this with a model of the biophysics of oxygen exchange yields the spatial distribution of arterial and venous blood gases throughout the lungs.

For further examples of the clinical application of these models, see (90-93).

6. THE REMAINING CHALLENGES

(i) The Physiome Project

The main task facing the nascent Physiome Project committee when it was formed in 1997 was to establish standards for encoding models and data such that physiological models could be demonstrably reproducible and reusable. The model encoding standards also needed to support modularity – since complex multiscale models must be built from independently validated modular components often developed by many different research groups. These standards ensure that the models can be curated (i.e. that they work, have consistent units and obey the laws of physics where appropriate) and are modular and annotated with bio-ontologies (to ensure reusability).

These standards and their associated modelling infrastructure have largely been completed. The challenge now is two-fold: (i) incentives are needed to persuade modellers to use the standards in order to ensure that their models are reusable by others, and (ii) a framework is needed to ensure that the multiscale models developed by independent research groups can be connected into integrative models of whole body physiology for application to healthcare – not least because the epidemic of chronic diseases that confronts the developed world can only be addressed by methods that deal with highly complex physiological processes involving multiple organs and tissue types but always linked down to molecular mechanisms.

To address these two related challenges, we propose two new developments. One is to establish a new journal, the Physiome Journal, and the other is to create a new web portal, based on models from the journal, that provides access to integrative whole body physiome models.

The journal should solicit papers that describe models and associated data encoded in physiome standards. It should complement journals that describe physiological experiments and the use of models to interpret these experiments. The prospect of citable articles that fully document a model for the benefit of others in a (hopefully) high impact journal should provide the incentive for modellers to make the effort to ensure that their models are curated, annotated and modular – since they will then be getting a second citable paper to complement the one submitted to the existing journals. Some of the essential and desirable aspects of the new journal are that it is open access (essential) and that where possible it publishes ‘smart’ papers such that readers can run the models and see results immediately, including the ability to change parameters and observe the consequences.

It is important that the Physiome Journal is not seen to be in competition with existing journals, but rather that the new journal establishes a relationship with these journals such that the two papers (one focussed on experimental data and model interpretation, the other on model sharing) can be submitted at the same time, the first to a physiology, bioengineering or biophysics journal and the second to the Physiome Journal. Ideally the curated and annotated model(s) associated with a pair of papers would be available for download from the Physiome Journal website at the same time as the experimental paper is published. The Physiome Journal should also publish papers that provide a comparative review of models in an area of physiology.

The second proposal for a web portal is being developed by the VPH-Institute such that models published by the Physiome Journal would be automatically linked into the evolving whole body physiological models, including whole body circulation models, physiologically

based pharmacokinetic (PBPK) and pharmacokinetic-pharmacodynamic (PKPD) models, available on the VPH portal. One role for the portal will be to identify areas where there are no models and to encourage new experimental and modelling efforts to fill those gaps.

(ii) VPH Project

The types of challenges that still remain open for the VPH vary considerably depending on the specific application and on the Technology Readiness Level (TRL) of the technology at hand. A possible definition of the TRLs in this context is the following:

Technology Readiness Level	**Description of RTD activities**
TRL1: Technical Watch	Monitor fundamental technological innovations; monitor discovery of fundamental biological mechanisms.
TRL2: Basic Technology Research	Apply fundamental technological innovation to the quantification, prediction, and modification of fundamental biological mechanisms.
TRL3: Research to Prove Feasibility	Hypothesis testing and initial proof of concept (PoC) is demonstrated in a limited number of in silico & in vitro models.
TRL4: Technology Demonstration	Proof of concept and safety are demonstrated in a defined laboratory or animal model.
TRL5: Technology Demonstration	Pre-clinical studies, including GLP animal safety & toxicity, sufficient to support industrial application.
TRL6: Technology Demonstration	Phase 1 clinical trials: support to proceed to phase 2 clinical trials; safety, usability, impact, accuracy on small cohort.
TRL7: System/Subsystem Development	Phase 2 clinical trials: accuracy and efficacy on medium cohort; scalability and impact
TRL8: System Test, Launch, & Operations	Phase 3 clinical trials: efficacy on large cohort. Cost-benefit analysis, primary research for Health Technology Assessment.
TRL9: Post marketing studies and surveillance	Post marketing studies and surveillance; registers, failure analysis, post-mortem examinations.

For those technologies that have completed TRL7 or higher (vFFR, osteoporosis, abdominal aneurysms) most of the challenges are in the re-engineering of the process to handle large volume at low costs, i.e. a need for higher degree of automation. A lot of patient-specific models require massive manual pre- and post-processing done by a highly qualified operator; while this is acceptable during the development phase, large scale deployment, especially under tight cost-benefit targets, require a massive re-engineering of the technologies.

A second problem for mature applications is the turn-around time. Today most VPH applications work like a laboratory test, the patient comes in today, and the doctor will receive the results in 1-4 days; however, in many clinical applications, the ideal time-frame is that of a clinical consultation, typically 15-45 minutes: the patient comes in, the nurse collects all the data and requests the VPH simulation, so that when the specialist visits the patient a few minutes later, the prediction is already available to support the clinical decision. The extreme case is when the VPH model is integrated in a real-time application, such as pre-operative planning (94). In many cases the turn-around time is dominated by the pre- and post-processing, so the issues falls back to the need for re-engineering the processing workflow; but in many others the computational complexity is the limiting issue. Here research goes in many different directions from pre-computed solutions, parallelisation or other hardware related optimisations (such as for example GPU porting), but also the development of reduced-order models that provide the results at a fraction of the computational cost in

exchange for a small reduction of the predictive accuracy, small enough to remain clinically useful.

For technologies that have achieved a TRL5, the biggest challenge is clinical credibility. Here what is necessary is clinical research, where the new VPH-based clinical pathway is compared in term of accuracy and/or efficacy to the current standard of care, in order to demonstrate that the increased complexity that a VPH-based pathway usually involves is fully justified by the massive benefits it yields.

For applications with lower RTL, the main challenge is to bridge the large volume of work that is being done around the biochemical modelling of molecular pathways in a single cell (what is normally referred as molecular system biology) to the biophysics modelling at tissue, organ and organism scales. There are operational challenges (for example bridging the cell-tissue scale is particularly difficult), but also cultural issues, as this scale transition marks the passage from physiologists, biophysicists and bioengineers to pharmacologists, biologists, and geneticists.

7. CONCLUSIONS

Charles Taylor, the principal investigator behind the HeartFlow CT-vFFR technology, published his first paper on this topic in 1999 (95). The development of the sugar metabolism model now used in the FDA-approved Cobelli-Kovatchev pre-clinical simulator (96) started in 1979 (97). The Oxford Virtual Assay for in silico cardio-toxicity is based on the work of Denis Noble that started in the '60s (15). While developing and validating VPH models today is probably easier than it was for these pioneers, and it will get easier in the future, it is clear that the development of a subject-specific multiscale predictive model fully validated for clinical use requires a considerable investment of time and money. But the potential payback is huge: an FFR procedure cost $3000-$5000 USD, and can be performed only in specialised centres; a vFFR cost considerably less, and almost every hospital with a CT system and access to the HeartFlow service can perform it. But much more important, with a vFFR model that support virtual stenting, the cardiologist can play "what-if", and deal much more effectively with patients with multiple stenoses, traditionally difficult to plan.

But these are the low-hanging fruit. In the long-term VPH models enable *Predictive Medicine*, where the clinical specialist can make critical decisions based on reliable, patient-specific predictions, with significant improvements in the efficacy of care. Reliable predictions of clinically relevant disease manifestations make finally possible a *Personalised Medicine*, currently burdened by unrealistic expectations of molecular biology technologies. Personalised predictions are the precondition for the expansion of *Preventive Medicine*; only accurate predictions ahead of time can truly enable a personalised prevention. Last, but not least, we believe predictive technologies can play a role also in enabling a more *Participative Medicine*. A typical example is geriatric oncology, a clinical domain frequently at risk of therapeutic obstinacy; a much more reliable ability to predict the prognosis as a function of the treatment options that are available, would make recommendations of palliative therapy much easier than the current prognostic uncertainty allows.

The first ten years of VPH research have shown the enormous potential of this approach; now it is time for massive research investment in this area, to deliver in full the promise of predictive medicine.

CONFLICT OF INTEREST

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