

Scientific progress and clinical uncertainty

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In the path underway towards Precision Medicine, two areas are in rapid development: genetics and artificial intelligence. In the genetic area, there are two current problems, both of the highest social importance. The first concerns the project, emerging in some countries, of systematic sequencing of the genome in the whole population. The problem is that reading the genome is very complex, requires specific knowledge, and the medical class is now unprepared. The second problem concerns the now achieved ability to modify the genome, which might be applied in the treatment of genetic diseases previously considered incurable. The techniques that can be used today are extremely delicate and expose to high risks. Artificial intelligence (AI) is a branch of neuroscience ('computational neuroscience') and advanced computer science which aims to apply the operational models of the human mind with the mnemonic and calculating power of advanced cybernetics. It is applied by conventional smartphone 'apps' to the most advanced computers used in various areas of diagnostic and prognostic medicine, image reading, big data management, setting of new pharmacological molecules, up to completely different applications, such as spoken language, automatic driving of vehicles, insurance plans, financial strategies, etc. Of course, with enormously different degrees of complexity. Will the doctors' role survive?

Preamble

The following paper was written before the COVID outbreak. After this tragedy and having experienced the ocean of clinical uncertainty in which all physicians have been immersed and too many died, the matter of this article would have been completely different.

Introduction

Today medicine is an ex-art that reels in a universe that has become digital. In practice, it tries to float, orienting itself in a still disordered sea of exponentially increasing numbers, sometimes generating interpretative complexity and uncertainty. In reality, the doctor lives in uncertainty, today as yesterday, even if the methodological, operational, and social context is rapidly changing. Overall, clinical research is moving from evidence-based philosophy, mainly

deriving from large pragmatic population trials, to the 'individual patient' in some cases (patients with specific, rare, or simple genetic pathologies), or to groups of patients identified on the basis of defined clinical features and pathophysiological mechanisms that unite them (phenotypes). In other words, we are moving towards the precision medicine.

Medical genetics

Obviously, the sequencing of the human genome, completed in 2003—together with that of a multitude of plant and animal species—represents a fundamental scientific achievement. The history of humanity will change because of that. Outside of the research labs, knowledge of the functional aspects of individual genes and their interactions is still relatively poor. Except for a few specialist niches, the clinical effects of genome studies are even more scarce, taking into account the fact that identifying a causal genetic mutation of a disease does not mean having solved the clinical problem. A typical example is sickle cell

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anaemia, endemic in Sardinia, with a causal gene identified 70 years ago, that, although, remains without therapy. Promising exception is the oncological pathology of solid tumours, where the introduction of the genome sequencing of the neoplastic tissue offers large prospective spaces for more individualized and active therapies against cancer. The first map of cancer vulnerabilities on a genomic scale with the identification of 600 key genes for the viability of cancer cells and hundreds of new possible drug targets¹ has only recently been made completed.

In the cardiovascular area, clinical genetics has been determinant for the diagnosis of some pathologies with specific non-complex genetic aetiology, and research is being developed on risk factors and conditions with complex pathogenesis.

Regardless of clinical practice, there are two main problems—scientific and social—which arise today in medical genetics. The first concerns the possible systematic application of genome sequencing in all people in whom it is possible to do so. The second concerns the therapeutic modifications of the genome.

Genome sequencing for all?

The sequenced genome is the detailed description of each of us, that identifies and characterizes us. The cost is a few hundred euros/dollars. In fact, the concept, and to a much lesser extent the realization, of mass genotyping is spreading. In the USA, three independent programmes are underway (All of Us, the Cancer Moonshot, and the Million Veterans Program), each calibrated to enrol a million volunteer subjects available to participate in scientific research programmes and the sequencing of their genome. Recently, both the American College of Cardiology and the British Royal Society of Medicine have publicly invited all citizens to genotyping using the ‘direct to consumer’ method, suggesting then to turn to their doctors for interpretations and clarifications.² In both the UK and the USA, educational support initiatives have been launched for health professionals by major Journals, such as *Lancet* and *JAMA*, which had set up a programme of short articles to improve the medical culture on clinical genetics.^{3,4} Personally, the author believe that considering today’s volatile publications, the uncontrollability of social media, the rampant digital delinquency, and the current fragmentation of medicine, the popularization of genomics will be very difficult to manage. It will likely become a powerful sower of uncertainty and at least temporarily, it will be able to lead to trouble with interpretative ambiguities and extension of interpretative and operational inappropriateness. The sequenced genome is an impractical maze for the inexperienced, and well practicable by malicious inexperienced. However, the use of the genome to identify individual prospective risk profiles, if managed competently, will be useful for prevention as long as some basic concepts are taken into account.⁵

Polygenic risk scores are mathematical aggregates of many DNA variants found more or less weakly associated with a disease, that overall can be used to estimate the probability of incurring that disease. So, the genetic risk profile is not a diagnosis, and it has nothing predictively

inevitable. The global individual risk profile adds the genetic risk profile to known non-genetic risk factors. The relative weight of the genetic profile can vary substantially based on the individual weight of the other risk factors and is a function of age, environmental factors, habits, and comorbidities as well as any preventive measures put in place. The polygenic risk score for the most common cardiovascular diseases can include thousands of modest impact genetic variables, which in the long run can confer a significant, more or less marked, propensity to cardiovascular disease. It is also important to consider that the existing polygenic scores are not internationally standardized.⁶

Genome transduction: how it can be done

There are two ways of inserting/changing/deleting DNA, i.e. DNA transduction.

An *ex vivo* procedure, according to which circulating and/or medullary progenitor cells are taken, transduced with the modified gene tract, tied to a non-pathogenic virus or a transporter that knows where to go and then reinfused. A second modality is *in vivo*, the gene fragment is joined to a transporter and infused as an intravenous drug.⁷ The procedural modality is not irrelevant, because it implies different formal classification/and different regulatory paths. Upon arrival in the cell nucleus in some cases, the transduced DNA is incorporated into the patient’s genome and therefore the ‘normal’ character will be transmitted to the offspring. In other cases, the transduced element is not incorporated into the genome but is fixed inside the cells as an episome. This will lead, as a consequence, which the new ‘fixed’ gene will not be passed down to the offspring, which therefore will receive the causal altered gene of the disease.

Some relevant, potential risks for the recipient should be considered. The major risk associated with the use of viruses as genes carriers is their integration into the chromosome, making an insertional mutagenesis that may generate a cancer. In case of *in vivo* transduction via the venous route, the risk is linked to the activation of the immune defence, which can be controlled with appropriate therapy (as for transplants).⁸ Having said that, however, the weapon, the smoking gun: the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) Cas9, is missing: The CRISPR Cas9 system was originally made up of programmable nuclease enzymes. Driven by an RNA molecule to the target chromosome site, CRISPR Cas9 enzymatically cut the gene tract to be eliminated by replacing it with a normal segment. The operation was rather violent (on a chromosomal level) with the risk of causing damage to neighbouring chromosomal tracts. Since 2016 Cas9 has been modified in order to ‘fix’ the altered gene by reassembling the correct sequence of letters (amino-acids) of the gene without cutting anything, therefore more softly and accurately. Today the weapon has been further improved by accentuating the central role of RNA in the process. In an article published in *Nature*, December 2019,⁸ the authors state that the technique could allow to correct 89% of gene variants associated with diseases, which are around 75 000.

What has been done in practice and what could be done

Since 2016, the European Medicines Agency (EMA) and/or the US Food and Drug Administration (FDA) have approved six gene therapy products currently available on the market. They concern serious monogenic diseases, including β -thalassaemia, some forms of blindness, spinal muscular atrophy, and forms of primary immunodeficiency.⁹ There are >800 cell and gene therapy programmes in clinical development for incurable diseases, such as Duchenne, Huntington, and other muscular dystrophies. In the USA, the regulatory assessment carried out in the past by both NIH and the FDA, is now performed by the FDA alone,^{10,11} which should shorten the time of the authorization assessments to carry out the studies. The CRISPR Cos9 genomic editing technique has already been used clinically in the USA,¹² in a patient with beta thalassaemia and in one with sickle cell anaemia about a year ago with beneficial results that were maintained over time. More recently, in three patients with advanced cancer, the immune competent T lymphocyte genome was modified *ex vivo* (removed three genes, added one) to make T lymphocytes aggressive towards neoplastic cells and then re-injected into the patient, where they multiplied as expected. After about a year, there were no significant side effects. The matter is so delicate that the researchers waited 2 years for the consent of the US regulatory bodies to carry out the study.

Already largely used in fruits and plants it is not emphatic to say that the era of the modification, cautiously guided, of the identity structure of the human being, the genome, is beginning. The increasingly sophisticated technology will develop more refined, flexible, and efficient methods of genetic therapy (as long as it remains 'therapy'), that will be safer as well. However, the question will be whether to put limits to the interventions: i.e. formal (legal?) constraints, and also who will put them (national, supranational bodies?), on behalf of whom (citizen involvement, potential users?), how informed and involved (in referendum?), etc.^{14,15} The Second International Human Genome Editing Summit held in Hong Kong in November 2018 released a statement reading 'the experimental approach consisting in the modification of the human genome is not sufficiently tested and is too dangerous to be allowed'.¹⁸ The Multidisciplinary Association for Responsible Research and Innovation in Genome Editing¹³ stated that 'the use of technology should not be allowed or authorized until it is deemed safe and effective for humans for precise therapeutic applications justified by a broad debate'. An article on *Nature* written by prestigious scientists is in line with these positions and explicitly calls for an international moratorium on clinical trials.¹⁴

However, the trials will continue, unstoppable, even if with positions sometimes disruptive and serious risks of abuse. For example, a Chinese biophysicist just over a year ago practiced genetic editing in embryos of twins 'to reduce susceptibility to the HIV virus' (sic). The scientific press was mostly critical and the biophysicist was tried in a Chinese court and sentenced to 3 years in prison convicted of 'illegal medical practice' and 3 million yuan (US\$430 000) of sanction.

However, while taking into account the approximation of information that is detectable in more than half of the world, the prevalence of genetic diseases in children of non-parental couples is high and is estimated at around 1-4%. A strategy will have to be outlined. The demand for 'total' reassurance about children's health before engaging a pregnancy is increasing. A 'proxy' answer could only come from the ascertainment of potential parents' pedigree and their sequenced genomes. The individually agreed systematic therapeutic genetic engineering of very severe genetic diseases over time could become the virtuous path towards eradicating them.

Given the rapid development of knowledge and interventional techniques of genomic editing, the debate may move from a categorical opinion (yes/no) towards a definition of the ethically and socially permissible limits to the procedure. For now, the parties that have a voice—mostly from the ethical and scientific fronts—are firmly opposed to gene transduction not aimed at 'avoiding diseases or preserving health'. Although in this matter each word requires a precise definition—for example, 'health preservation'—it is still explicit that interventions to optimize traits that can be classified as 'positive subjective qualities', including intelligence, memory, physical strength, creativity, courage, are far from being included in such category. But it is already significant (and alarming) that we are talking about it!

Lifetime treatment of some genetic diseases can cost a lot. For example, the drug treatment of haemophilia in the US costs up to about \$400 000,⁸ therefore being economically unsustainable for 99% of the world's population. The genomic editing intervention can be resolving with a single intervention. In perspective this could be a significant point in favour of the procedure. In the meantime, there are already companies in the USA that sell 'genomic editing procedures' by offering it in medical conferences and obviously on the internet.

The environment of precision medicine: artificial intelligence (perceived by a cardiologist)

From a technical-methodological point of view, great opportunities are emerging for medical science today: (i) rampant and penetrating technology; (ii) universal and often free or inexpensive digitalization (democratic, we could say, at least for now); (iii) huge modular and decomposable networks of people, even equipped with 'medicalized' smartphones, which can facilitate the performance of otherwise complex and expensive trials; (iv) a massive increase in the ability to systematically store numeric data obtainable from many different sources; (v) a revolutionary ability to order, manage, and analyse them with increasing speed and efficiency, using artificial intelligence techniques.

General information on the artificial intelligence

In the current perception, the intriguing denomination associated with a real technical complexity contribute to placing the artificial intelligence (AI) in a corner between

the arcane and the magical. Like genetics, AI associated with robotics will also change man's life, if not history. Artificial intelligence is exactly what the two words that define it express: a science designed and developed to imitate the processes of the human brain in the most technically suitable way, to solve the most diverse problems. There is a specific area of neuroscience, 'computational neuroscience', which aims at the development of models that follow the functioning of biological neurons and their networks. Although little is known about how neurons function in learning and decision-making logic, AI has now quietly entered the daily data analysis, even for usual operations, such as searching for keywords on an engine search, or a shorter road route with a smartphone.

The experimental AI that enters directly into today's reasoning on the topic is young; it is about 14 years old. In 2006, Geoffrey Hinton introduced simple computing units (now called neurons) arranged on several interconnected levels (using a technique called back-propagation and others developed later), and with a series of mathematical artifices he 'trained' them (this is the technical term in use) to develop expressive algorithms of information contained in datasets. Performance and number of computing units were rapidly multiplied, building the so-called neural networks, and machines were developed which could be 'trained' to 'learn' and 'develop new analytical procedures' based on the experience acquired (Machine Learning). In practice, by accumulating a lot of data concerning a specific area of interest, the computer (in a broad sense, or a computer network if they run big data) appropriately arranges them in its neural network, becoming an expert in the area. Subsequently, an analytical modality was developed—*Deep Learning*—where the concept of depth is technically linked to a high amount of neurons that can be involved and provide the ability to learn and apply also in an innovative way. Each neuron captures a predefined part of the whole (in an image: curves, segments, edges, colours, etc.) which will eventually emerge as a whole. As such, neurons can be considered as an assembly line of complementary aspects. According to the subject under examination, an image, a spoken phrase, an administrative plan ... or a phenotype may emerge. Currently, deep learning holds 40% of the entire data analysis market, with an estimated annual economic potential of between \$3.5 and \$5.8 billion (data from the McKinsey Global Institute, April 2018).

In summary, interesting prospects are emerging today for a Healthcare System equipped with a widespread digital system with AI applications and adequate technical expertise, which include: (i) analytical skills that can quickly solve very intricate situations; (ii) rapidity, high resolution, and reliability in the recognition and management of fields rich in images; (iii) risk stratifications and accurate individual and population predictive models; (iv) high performance in organizational health care plans (diagnostic procedures, follow-up, administrative and insurance plans); (v) electronic secretariats trained in 'spoken language' (including colloquial responders and automatic translators); various services: surgical robots;

computerized clinical radiology (from patient's reception to the report), analysis of biological material, etc.¹⁴

Did Sesame open? Uncertainties and limits of artificial intelligence

If Sesame has not opened wide, certainly a crack has opened. The most interesting aspect of the ongoing process is that physiologists of the human mind, scientists of the most diverse technical disciplines and now, at least as end-users, clinicians, also find themselves on intertwined paths. Although, there are problems.

At the moment, the most successful deep learning applications in medicine have been images, particularly when used in diagnostic processes.¹⁵ The training, often heavy, consists in submitting images of the subject of interest to train the machine to learn until it is almost never wrong, according to the judgement of the (human) referent. In total, 128 175 retinal images were required to develop a pattern of recognition of diabetic retinopathy (an FDA approved system in 2018). The application of deep learning techniques to more complex and heterogeneous pathologies, such as chronic heart failure would require tens of millions of examples to generate a reliable diagnostic model.¹⁴ However, AI will not be used to diagnose heart failure, but to identify phenotypes (or geno-phenotypes) from the aggregate complex of heart failure syndrome, on which the forces of research are converging. It will also be used as a dynamic 'fishing' path moving on in a sea of data associations, finding out some that are not compatible with randomness, configuring clinical profiles that we would have missed otherwise.

Unfortunately, in the world of images, even minimal but precise changes, such as rotating the image on definite positions, can affect the results. This potential source of uncertainty binds another serious problem of AI in medicine, that is the complex logical-mathematical path of the neural network leading to clinical problems' solution cannot be reconstructed. So, the machine works as a black box. However, every medical decision should be justifiable, even *a posteriori*, through open logical processes and available findings. The new European Data Protection Regulation (GDPR) also restricts the use of 'opaque' data processing. Accordingly, the concept of 'Explainable AI (XAI)' is thus emerging, being actually the major present limitation to a rapid extension of AI techniques in clinical practice.

The 'post-physician' era? What will the doctors do?

In 1976, Maxmen, dreaming of AI, predicted that in the 21st century AI would lead a post-medical era managed by computers and non-medical health workers. The mass extinction of doctors for now has not occurred and seems unlikely in the near future, but undoubtedly something will change. In the silence of the medical-scientific societies, some editorialists addressed the issue, with substantially benevolent and reasonable conclusions. Computerized medicine will advance; smartphones will be medicalized, inflated with apps, patients, and healthy subjects will be

(tele) monitored with handy wearable sensors. The clinical trials will be global, largely funded by data managers, sensor manufacturers, and companies active in the AI technical evolution. For example, an ‘all-digital clinical trial’ funded by Google (the ‘CHIEF-HF virtual trial’) tests a new heart failure drug. Google recently acquired ‘Wearable FitBit’, a wearable sensor company, for \$2.3 billion. Facebook has launched a large ‘Preventive Health’ programme and has scheduled trials. Apple is funding three trials testing prevention programmes, virtual of course (very few people needed to work on it), involving other communication companies (iPhones, Watches), distributing apps, and entrusting the coordination of the study to very experienced clinical researchers of prestigious universities. Tech futurists believe that these large digital companies will be able to obtain and manage individual health data and release clinical recommendations by lowering the costs of health care (which has reached 18% of GDP in the USA) and without taking on the associated risks.

In daily clinical activities, doctors and machines will collaborate. The slogan is ready: ‘shared decision-making in navigating uncertainty’. In this scheme, the machine will have the role of an expert consultant, probably unbeatable in the solution of specific problems, even rare, solved in the literature with standardized and well-presented recommendations. Once recognized, solving these problems having memorized the updated worldwide literature, will take few milliseconds. Perhaps less prompt or reliable in holistic evaluations, with significant but not prominent multi-morbidity, in short, complex cases. In any case, the doctor should remain the final decision maker, referent for the patient to whom he will give explanations and instructions and offer humanity, which is not among the characteristics of the thinking machine, and will remain a doctor’s characteristic. Will it be like this, thus better than today? Probably yes, at least for the patient, considering the data published by the *British Medical Journal* relating to a large-scale survey carried out in the USA that reports medical errors as the third cause of disability or death, after cardiovascular causes and cancer.¹⁹

Conclusions

In conclusion, will precision medicine and artificial intelligence introduce us into an era of certainties in medicine? I tend to believe otherwise. The new technical and conceptual means will offer a multitude of more or less clinically relevant information and will in any case generate further questions. Usually, the desire to know increases with the increase of knowledge and in parallel with the uncertainty. The statisticians write ‘We must learn to embrace uncertainty’.²⁰ A thought was taken from a recent editorial by Marco Cattaneo titled ‘The unsustainable uncertainty of reality’. (*Le Scienze* 2019; 615:7) says, in an interrogative form, ‘Why does science remain the best tool we have to interpret the world?’. And replies: ‘Because it measures

the uncertainty’. Future doctors will also have to live with it.

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