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FROM CHEMICAL TO GENETIC INDIVIDUALITY EVOLVING CONCEPTS AND THERAPEUTIC APPROACHES

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SUMMARY

At the beginning of XX century the British physician Archibald Garrod put forward seminal aspects of medical genetics and Mendelian transmission of a few human metabolic diseases, highlighting the close relation between heredity and abnormal conditions for the first time. After the mid-1950s, advances in biochemistry, genetics and, then, functional genomics have eventually shown that Garrod's pioneering concept of "chemical individuality", which is expressed through protein variants, is still topical. As from 2002, the mapping of single nucleotide of polymorphisms (SNPs) – changes of single "letters" in DNA sequence common in human population – provided one of the main sources of genetic variations, relevant for complex traits. The paper will track the evolution of the concept of individuality, from the Garrodian to the current post genomic perspective, exemplified by specific definitions. Further, it will be shown that such evolution has been paralleled by a shift of focus in medical thought: from a reactive to an always more preventive and personalized oriented therapeutic approaches.

Introduction

The fact that no two individuals are genetically the same due to a huge number of genetic variations responsible of differences in susceptibility to, or protection from, certain diseases is a well-known notion in the post genomic era. But that a British physician would have foreseen it at the very beginning of the XX century is remarkable.

Key words: Archibald E. Garrod - Individuality - Single nucleotide polymorphisms (SNPs) - Genome wide association studies (GWAS) – Personalized medicine

Chemical individuality was the pivotal concept put forward by Archibald Garrod[1]. All human differences have a chemical basis that is expressed through the variations of the amino acidic composition of proteins[2]. It reflects a qualitative modification of the *molecules of chromosomes* and explains evolutionary changes resulting into inter-individual characteristics[3]. Hence, Archibald Garrod's genius can be deeply appreciated only if his pioneering observations are adequately put in a specific temporal and cultural frame.

His first studies about "inborn errors of metabolism", recessively hereditary disorders due to deficiency of enzymes involved in metabolic pathways, can be traced back to the end of the XIX and the beginning of the XX century when Mendel's laws of inheritance were barely rediscovered. Garrod foresaw genetic roots of biochemical abnormalities and anticipated key aspects of molecular medicine, thus linking biochemistry, genetics and medicine. Mostly, one of the latest and crucial Garrod's contributions concerned the evolutionary significance of chemical individuality. He put forward the concept of "inborn factors in disease" related to the inherited susceptibility to specific pathological conditions reflecting chemical differences[4].

Garrod's salience remained underestimated for decades due to several reasons: despite of attributing a relevant role to heredity, Garrod had only a vague idea about the nature of the genes; during his own time there was no way to detect them and, consequently, to test his ideas until the late 1940s and early 1950s, when analytical tools to identify chemical variations by separating and isolating proteins were introduced; communication between biochemistry and genetics was scant and physicians were more interested in full-blown phenotypes determined by evident causes[5]. Hence, the breadth of his vision was appreciated only after the second half of the XX century when eminent scientists, as Harry Harris, and distinguished pediatricians, as Charles Scriver, Barton Childs and David Valle, shed light on his invaluable work. Harris promoted the reprint of "Garrod's Inborn

Errors in Metabolism" in 1963, while Scriver, Childs and Valle contributed to write the landmark handbook, "The Metabolic Basis of Inherited Disease" (MBID)[6]. First published in 1960, it went to further editions until an online version (OMMBID), including also the *molecular* basis of inherited disease.

The renowned George W. Beadle and Edward L. Tatum's "one gene-one enzyme" principle (1945) and the increasing identification of polymorphisms in humans (1950s) mainly contributed to rediscover Garrod's genius. Indeed, he was the first to hypothesize that the function of each enzyme is controlled at a genetic level[7]. Polymorphisms, which are variants of a particular DNA sequence, provide a *genetic counterpart* of chemical individuality[8].

In this paper the evolution of "chemical" individuality prime concept in "genetic" and, finally, "metabolic" will be described. Further, how such evolution has been influenced by advances in post genomic studies will be shown. Finally, a resulting shift of focus of biomedical thought from an "interventionist" to an always more genetic-based individualized approach will be highlighted. To this aim, Garrod's insights will be reported and followed by the description of some fundamental studies about polymorphisms. These will be analyzed in order to describe how they defined genetic individuality. Finally, recent integrated approaches, combining whole-scale genome screening and "omics" (genomics, transcriptomics, proteomics, epigenomics and metabolomics) aimed at identifying potentially therapeutic targets, will be explored.

Chemical individuality and Garrod's insight about human genetic variations

Garrod's first intuition about the nature, the causation and the familiar transmission of the metabolic disorder already emerged in 1901 in the paper "About alkaptonuria"[9] originally published in *The Lancet*, as clearly exemplified by the paragraph "The rela-

tion of alkaptonuria with consanguinity of parents"[10]. This work caught the attention of William Bateson, one of the main promoters of Mendel's laws of inheritance, with whom he kept up a prolific correspondence about his clinical observations. However, "The Incidence of Alkaptonuria: A Study in Chemical Individuality" published in 1902 represents Garrod's milestone paper in which the author described three different *chemical abnormalities* showing chemical basis: alkaptonuria, albinism and cystinuria[11]. All of them were reported as extremely rare conditions with a similar incidence, which was higher in the offsprings from consanguineous marriages. However, the pivotal point expressed by Garrod was that the mere consanguinity could not explain such phenomena. He referred to a *[…] 'peculiarity' of the parents, which may remain latent for generations […]* that has more chances to express in members of the same family and that should have been investigated[12]. According to this perspective, alkaptonuria, albinism and cystinuria represent alternative mode of metabolism or '*Individualities of metabolism' […]* that are *merely 'extreme examples of variations of chemical behavior' which are probably everywhere present in minor degree […]*[13]. Further, Garrod pointed out, in evolutionary terms, that two individuals of the same species show differences in body structure as well as in chemical processes[14].

Such pivotal concept emerged in his four "Croonian Lectures" delivered at the Royal College of Physicians in 1908, then assembled in "Inborn errors of metabolisms", first published in 1909, followed by a reprint in 1923, in which Garrod deepened his initial studies.

Here chemical individuality is described as *[…] 'diversity' which is no less real than that of structure, although far less obvious* and it is related to a chemical specificity due to differences in each individuals of a species made up of distinct proteins[15]. Garrod foresaw that factors determining human evolution worked just on such chemical and structural variations. Further, he introduced a dynamic inter-

pretation of metabolic pathways, in which an intermediate product can turn into another in a building up and breaking down fashion. Thus, a possible block due to an inborn event caused its accumulation. Moreover, inborn errors of metabolism, much considered as *metabolic sports*, *i.e.* conditions following a benign trend, are distinguished from *disease of metabolism*, such as diabetes, gout or obesity in which the metabolic alteration is the most evident aspect[16].
The salience of Garrodian thought in the modern medicine emerges in an undeniable manner from his last manuscript to *Inborn Factors in Disease* (1931). He proposed that the inherited predisposition, the tendency to be more or less liable or immune to certain diseases, expressed by the long lasting term *diathesis*, is *inherent* in our chemical individuality (essentially a proteic individuality)[17]. Now a more suitable conveying such concept would be the topical "risk factor"[18]. Already few years earlier, in the "Huxley Lecture on Diathesis" (1927), Garrod put forward this concept together with other cutting-edge ideas for his own time concerning evolution, individual deviations and differences:

> *Unfavorable modifications tend to be eliminated, because they diminish the capacity of the organism to conform to its environment; and among the factors at work in the elimination of the unfit none is more potent than disease. A new departure may be both good and bad, and if the advantage outweigh the disadvantage it may persist*[19].

He underlined that various factors influence predisposition – relying on chemical characteristics – to morbid conditions. In view of the variations among individuals within the same genera and species, it should be taken into account that metabolic processes could show differences *of like kind but of less degree*[20]. Hence, Garrod anticipated that *A systematic search for such mutations would involve elaborate metabolic studies of large numbers of individuals, and much of the labour expended would probably be in vain*[21].

From chemical to genetic individuality

The identification of polymorphisms, which make unique each individual, has been crucial to understand genetic variations underlying humans' individuality. The British biochemist Harry Harris' papers "Enzyme polymorphisms in man" (1966) and "Enzyme variants in human populations" (1976) are seminal in this context. He observed that several enzymes synthesized in humans showed one or more structurally variants. Moreover, a few of the genetically determined enzymatic deficiencies found in the so called "inborn errors of metabolism" were quite common in certain human populations. Hence, the enzymatic diversity could result from the genetic one. In order to investigate in which way *such genetically determined enzyme diversity* could concern *normal individuals*, he screened several categories of enzymes by electrophoretic techniques[22]. Later, Harris reported that enzymatic variants could be ascribed to allelic differences at a genetic locus[23] encoding an amino acidic stretch in a certain protein or enzyme and likely rooted in gene mutations in earlier generations. Many of these mutations were really rare while others occurred more frequently in human population, giving rise to polymorphisms, defined as *[…] those loci in which the commonest identifiable allele has a frequency no greater than 0.99*[24]. In addition, uncovering the distribution and the frequency of polymorphic loci as well as whether they generated functional differences in enzyme phenotypes were the forerunning issues reported by Harris, even now still investigated[25].

In a sense, Garrod already broached the question. As underlined by the Italian pediatrician Roberto Burgio in 1986, the inborn factors are etiological, thus establishing a connection between diseases and individuality of each members of a certain species. Further, they also define the latter in relation to the Garrodian "metabolic sports". If we think the inborn errors of metabolism as such, thus affecting metabolic pathways in various degrees depending on chemical individuality, the finding of polymorphisms broaden this perspective from a genetic point of view[26].

Some years later, polymorphisms as risk factors in disease were a central topic debated by Arno G. Motulsky. *What do we know about the genetic contribution to human individuality?* is the question posed by the father of pharmacogenetics when he speculated on *human variability due to intraspecies genetic variation* as elements to look at for risk assessment[27]. Just because of their high frequency in the population, polymorphisms are by far more relevant than rare mutations and make presuming that they differently influence responses to drugs or environmental factors. Relevant genetic differences are established: *e.g.* those concerning HLA locus in blood cells, which encodes proteins presenting the antigens to the immune system or others affecting the breakdown of substances and the binding between ligands. However, Motulsky argued that, although most of the variants at DNA level are phenotypically silent, too little is known about them and should further investigated[28]. Actually, now we know that in healthy patients one of the main sources of genetic variation is represented by single nucleotide polymorphisms (SNPs), changes of a single "letter" of DNA that occurs with high frequency in the human genome. Typically these changes have either no effect or can slightly affect the gene product and its function, thus influencing individuals' response to drugs or predisposition to diseases[29].

Also the American pediatrician David Valle deeply discussed the importance of individuality for the future of medicine through a genetic perspective. In an article presented in 2003 at the annual meeting of The American Society of Human Genetics he speculated on the recent advances in genomics, firstly the outcomes of the completion of the Human Genome Project. The information collected would have opened novel perspectives in medicine by understanding the genetic and molecular bases of diseases. Further, it would have put out new challenges emerging from the intrinsic complexity of human genome relying on the still unknown "individuality". It was defined as

> *[…] the biological qualities that distinguish one person from another. These include variations in bodily or cellular structure or function and in homeostasis and adaptation. These are all properties mediated by proteins, which themselves express the individuality of the genes that specify the. Thus, the root of individuality expressed in these terms is 'genetic'*[30].

According to Valle, understanding the incredibly high degree of individual variability would means to fully grasp individuality through biological, genetic, medical and clinical points of view. Indeed, finding out the origins and the effects of individuality contributes to deepen evolutionary aspects shaping human beings. Moreover, genetic differences could result in distinct phenotypes, which are peculiar from a patient to another, even resulting in the outlier ones. Mostly, the efforts of genomic studies should be directed towards appraising and integrating individuality into an effective preventing medical approach[31].

Later, the American scientist Maynard V. Olson used the expression *human genetic individuality […] to refer to the assemblage of heritable traits that are unique to each individual in a population*[32]. He focused on the plenty of variable traits constituting the "normal" phenotype and on which are directed many actual genomic efforts. Olson argued that the real endeavor of genomic science would have been spent in discerning the very small fraction of significant genetic variations with an appreciable phenotypic effects in an *ocean of noise*[33].

Metabolome, metabotypes, metabolic individuality and potential therapeutic strategies

Therapeutic approaches for the so-called inborn errors of metabolism (IEMs) fall into few treatments, mainly dietary interventions, both restriction and supplementation diets, and the replacement of the deficient enzyme. Nevertheless, they are not always suitable and resolving solutions for all conditions since polymorphisms, pleiotropy[34] and allelic heterogeneity[35] make inborn errors of metabolisms (IEMs)

extremely variable from both a genotypic and phenotypic point of view. Indeed, even though they are usually classified as Mendelian disorders, thus caused by a single gene mutation, actually they are the best example of complex diseases. This is particularly evident for phenylketonuria (PKU)[36], which shows in an exemplary way that *Monogenic traits are not simple*[37]. As matter of facts, a mutation at the single locus encoding the enzyme phenylalanine hydroxylase could result in disparate phenotypes, respectively enzymatic, metabolic and cognitive[38]. At the moment more than 500 mutations involving the enzyme responsible for the metabolic pathway of PKU have been identified. Many of them are SNPs, of which several result in changing amino acidic sequence ("nonsynonymous" SPSs), thus influencing protein folding, levels and activity; many others, have no effects on protein sequence ("synonymous" SPSs) (http://ghr.nlm.nih.gov/gene/PAH; http://www.nature.com/subjects/functional-genomics). How these last could affect gene transcription and expression is dissected by "functional genomics", which studies the relation between genotype and phenotype by high-throughput technologies[39].

Indeed, in the last decade great expectation has been posed on the *close correlation between the mutant genotype and the variant phenotype*[40]. This has been addressed through whole genome analysis, the so-called "Genome Wide Association Studies" (GWAS), which have evolved over the last ten years. They aim to identify common genetic variants in human populations associated with complex traits (phenotypes). They focus on those genetic loci including SNPs that may contribute to the susceptibility of common disorders (diabetes, auto-immune diseases, hearth diseases etc.), thus representing risk factors of diseases[41]. Hence, they could be crucial for designing diagnosis and therapeutic strategies. Nevertheless, GWAS alone have been largely disappointing from a statistical point of view and little enlightening about the biological processes underlying diseases, even more so for complex ones[42].

As a matter of facts, just because including both characteristics of monogenic and complex diseases, IEMs represent an appealing and challenging target to investigate because difficult to classify, diagnose, manage and treat. In this context the two concepts of *metabolome* and the *metabolic flux* are pivotal. The first refers to the complete set of chemical compounds (metabolites) involved in an organism's metabolism; the second, embracing and broadening the dynamic view already put forward by Garrod, can be imagined as an entangled network of biochemical pathways. Thus, the enzymatic defect affecting a single metabolic process could have "side effects" in others, determining different phenotypic outcomes. This has been shown, for example, in urea-cycle disorders[43], in which the association between a certain SNP of one of the gene involved (carbamyl phosphate synthetase-1, *CPS1*) and the plasma levels of arginine/citrulline (aminoacids metabolized in the cycle) could affect newborns with persistent pulmonary hypertension. Thus, the extreme genotypic and phenotypic variability of this class of disorders would require gene-based personalized therapies. These would profit of advanced analytical techniques as well as of integrated approaches, including multiple "omics". If applied to large population based cohorts, they could contribute to understand the correlation between several factors and the predisposition to diseases. Mostly, such integrated strategies could help to define *metabolic individuality* that can be considered as the result of "differentiated metabolic phenotypes", thus influenced by functional genetic variants present in loci involved in metabolism[44].

Actually, one of the current strategies in biomedical and pharmacological researches aim to find out prognostic and diagnostic data contributing to design individualized therapies relying on the combination of GWAS with "omics". This novel strategy is exemplified by emblematic titles of a few studies, such as "Epigenome-wide association studies for common human diseases"[45], "Global genomic

and transcriptomic analysis of human pancreatic islets reveals novel genes influencing glucose metabolism"[46] etc.

An example is also the integration of GWAS and metabolomics, that is the study of the chemical processes involving a broad range of metabolites in biological fluids (blood and urine), which often reflects biological processes associated to complex disorders, as diabetes and cardiovascular disease[47]. The actual GWAS approach is being shifting always more from analyzing the direct correlation with disease endpoints towards the association with intermediate traits (phenotypes), representing risk factors. For example, levels of blood triglycerides and cholesterol, which are risk factors of cardiovascular disease[48].

Nevertheless, while several disease-associated changes result in specific metabolic phenotypes and, therefore, represent a potential target of therapeutic interventions, other conditions are characterized by changes in levels of metabolites as a consequence of a disease, thus representing prognostic and diagnostic markers. Noteworthy, many metabotypes (metabolic phenotype) do not translate in a classical IEM, characterized by a loss of function of genes with the accumulation of toxic levels of metabolites in homozygous, but are determined by genetic variants in gene coding enzymes, cofactors, transporters etc. To this purpose it is necessary to understand the clinical significance of the association of certain SNPs and the concentration of metabolites: a large GWAS plus metabolomics study showed the association of a specific polymorphism of *NAT8* locus (linked to a detoxifying biochemical process, N-acetylation, essential for kidney function) with high levels of serum N-acetylornithine. Thus, *NAT8* variant represents a risk allele for chronic kidney disease. However, since a clear causal relation cannot be derived, further investigation is suggested. Further, many metabotypes are associated to specific responses to drugs. For example, a locus coding for a family of transporters (*SLO1B1*) associates with the risk of statin-induced my-

opathy. Then, an integrated research showed that it is also associated with fatty acids, thus suggesting to redesign several drugs and using levels of such fatty acids as indicator of drug effects[49].

Conclusions

The prime Garrodian thought finds today genetic and molecular explanations. "Individuality" means "heterogeneity". It could represent the cause of certain pathological conditions as well as the target of diagnostic and therapeutic approaches. Hence, a great emphasis has been always more posed on it: the more a detailed genetic analysis is, greater the number of genetic loci and variants can be identified, thus contributing to unravel the uniqueness of each individual[50]. Such idea already emerged around the mid-1950s from the first biochemical screenings of polymorphisms and from the early attempts by the "International Commission of Enzymes"[51] in classifying these last in specific categories[52]. Further, more recent efforts were performed starting just after the completion of the first draft of the Human Genome Project (2001), which showed that two individuals are identical for the 99.5% of their genome. This data sparked intense investigation to pinpoint large genomic regions to find out sources of human variations. As already Garrod anticipated, they are fundamental indicators of biological history resulting from evolution. Since they are *makers of genes and genome*, they are so crucial that *The field of human and medical genetics simply cannot exist without understanding this variation*[53]. Researches showed that, among them, SNPs occurred in the human genome as the most common type of DNA sequence variation. Hence, an International Consortium in 2002 launched the HapMap Project[54] in order to identify and localize "haplotype" or groups of closely spaced and inherited polymorphisms in hundreds of human genomes (259)[55].

The resulting maps and the commercially available SNPs array (*SNPs chips*) showed the way forward to a new type of research

efforts, the genome wide association studies (GWAS), aimed to investigate those SNPs associated to complex *medically (and commercially) important traits*[56].

In 2007 the Wellcome Trust Case Control Consortium performed a landmark study that led to identify new genetic markers correlated to an increased risk for complex disorders (heart diseases, diabetes etc.). However, other results were already reported in 2005 and 2006[57].

Although GWAS aided in advancing genomic medicine, they have been largely disappointing. The correlation between a genetic variant and the risk of developing a certain disease rely on statistical estimation, occasionally resulting straightforward. Consequently, they may not provide an indisputable prediction. Interacting factors underlying disorders, including environmental ones, should be considered.

However, information deriving from high throughput genome-scale analyses have turned out to be appealing for detecting novel biological targets and providing tools on which design prevention and treatments. In a sense, they prepared the ground for *personalized medicine*, a medical model aiming at tailoring healthcare on each patient' *genetic constitution* and *other biological features*[58].

The development of "omics" and their integration with GWAS, supported by powerful computational tools and comprehensive database have laid the foundations of a shift of focus in medical thought: from *reactive* to *proactive* approaches. A *4P medicine (predictive, personalized, preventive, participatory)* medicine has been proposed, although actually still not feasible[59]. Further, at the beginning of 2015 the US President Barack Obama announced a novel medical effort, the *precision medicine*. It refers to strategies of prevention and treatment of diseases focusing on individual variations in genes, environmental factors and life styles. It would require integrated research approaches, large cohorts of people (>1 million American volunteers), numerous trials, extensive characterization of various

biological specimens. The project promise to deepen mechanisms underlying cancers (for the moment), to identify factors influencing risk assessment, to provide diagnosis and treatments, even *the right drug at the right dose to the right patient*[60].
However, although the huge bulk of novel information will lead to crucial advances in genomic medicine, all cannot be ascribed merely to a simple *switch from gene to function*. They should be put into a wider frame taking into account that genetic expression is the result of complex integrated signals, elements and systems, which are comprehensive only if analyzed through an evolutionary lens[61].

BIBLIOGRAPHY AND NOTES

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1. GARROD A. E., *The Incidence of Alkaptonuria: A Study in Chemical Individuality. 1902 [Classical Article]*. Yale J Biol Med 2002; 75(4): 221-31.
2. Actually, Garrod used the expression "molecular groupings": SCRIVER C. R., *The Salience of Garrod's 'Molecular Groupings' and 'Inborn Factors in Disease'*. J Inherit Metab Dis 1989; 12(1): 9-24. CHILDS B., *Medicina Genetica. Una Logica Della Malattia*. Translated by Gabaglio Letizia, edited by Canali Stefano. Roma, Giovanni Fioriti Editore, 1999, p.15.
3. CHILDS B., *Sir Archibald Garrod's Conception of Chemical Individuality: A Modern Appreciation*. N Engl J Med 1970; 282(2): 71-7, p. 71.

4. SCRIVER C. R., *Garrod's Croonian Lectures (1908) and the Charter 'Inborn Errors of Metabolism': Albinism, Alkaptonuria, Cystinuria, and Pentosuria at Age 100 in 2008*. J Inherit Metab Dis 2008; 31(5): 580-98. BEARN A. G., *Archibald Garrod and the Individuality of Man*. New York, Oxford University Press Inc., 1993.
5. SCRIVER C. R., ref. 2. HARPER P. S., *A Short History of Medical Genetics*. Oxford University Press, 2008. CHILDS B., ref. 3.
6. CORBELLINI G., *Individuality and Disease in Garrod's Theory of Medicine*. Nuncius 1994; 9(2): 809-17.
7. BURGIO G. R., *"Inborn Errors of Metabolism" and "Chemical Individuality", Two Ideas of Sir Archibald Garrod Briefly Revisited 50 Years after His Death*. Eur J Pediatr 1986; 145(1-2): 2-5.
8. *Ibid*., p. 3.
9. In alkaptonuria (classified as E70.2, *Disorders of of tyrosine metabolism*, in ICD-10) the mutation of the gene encoding the enzyme *homogentisate 1,2 dyoxigenase* involved in the metabolic pathway, causes the accumulation of an intermediate product in blood and urine, the homogentisic acid, commonly called "alkapton". It turns the urine black when they are in contact with the air and produces cartilage and heart alterations.
10. GARROD A. E., *About Alkaptonuria*. Med Chir Trans. 1902; 85: 69-78, 69.
11. GARROD A. E., ref. 1, p. 229.
12. *Ibid*., p. 226, inverted commas are mine.
13. *Ibid*., p. 227, p. 230, inverted commas are mine.
14. GARROD A. E., ref. 1.
15. GARROD A. E., *Inborn Errors of Metabolism*. UK, Oxford University Press, 1923, Second ed., p.1, inverted commas are mine.
16. *Ibid*., p. 10.
17. GARROD A. E., *Inborn factors of disease*. Oxford: Clarendon Press, 1931. In: HARPER P. S., ref. 5, p. 183.
18. HARPER P. S., ref. 5, p. 183.
19. GARROD A. E., *The Huxley Lecture on Diathesis*. British Medical Journal 1927; 2(3490): 967-71, p. 968.
20. *Ibid*., p. 970.
21. *Ibid*., p. 970.
22. HARRIS H., *Enzyme Polymorphisms in Man*. Proc R Soc Lond B Biol Sci 1966; 164(995): 298-310, p. 298.
23. The place of a specific DNA sequence on a chromosome.

24. HARRIS H., *Enzyme Variants in Human Populations*. Johns Hopkins Med J 1976; 138(6): 245-52, p. 245.
25. *Ibidem*.
26. BURGIO G. R., ref. 7.
27. MOTULSKY A. G., *Human Genetic Individuality and Risk Assessment*. In: MICHAEL A., BENDER AVRIL D., WOODHEAD ROBIN C. (ed), *Phenotypic Variation in Populations*. Leonard US, Springer US, 1988, pp.7-28, p. 8
28. *Ibidem*.
29. SCHANTZ J. W. and VON SCHANTZ M., *From Genes to Genomes*. UK, John Wiley & Sons Ltd., 2002, p. 210. BUSH W. S., and MOORE J. H., *Chapter 11: Genome-Wide Association Studies*. PLOS Comput Biol 2012, 8(12): 1002822.
30. VALLE D., *Genetics, Individuality, and Medicine in the 21st Century**. Am J Hum Genet 2004; 74(3): 374-81, p. 375, inverted commas are mine.
31. *Ibid*., p. 378.
32. OLSON M. V., *Human Genetic Individuality*. Annu Rev Genomics Hum Genet 2012; 13: 1-27, p. 2.
33. *Ibid*., p. 2.
34. One single gene defect has various phenotypic effects, involving diverse tissue, organs and systems.
35. Mutations at the same locus result in similar phenotypes.
36. Phenylketonuria is classified in ICD10 as *Disorders of aromatic amino-acid metabolism* (E70) and it is characterized by mutations of human *PAH* genic locus, responsible of the alteration of the enzyme phenylalanine hydroxylase able to transform phenylalanine (an essential aminoacid) into other products, consequently associated with the accumulation in the blood of the aminoacid (hyperphenylalaninemia) and mental retardation.
37. SCRIVER C. R., and WATERS P. J., *Monogenic Traits Are Not Simple: Lessons from Phenylketonuria*. Trends Genet 1999; 15(7): 267-72, p. 267.
38. *Ibidem*.
39. They perform analysis on a huge amount of data in short time and automatized way.
40. SCRIVER C. R., and WATERS P. J., ref. 37, p. 267.
41. Typically, DNA samples from people with the disease are compared with similar healthy people. They are placed on tiny chips and automatically scanned. Usually, “haplotypes” or groups of closely spaced and inherited SNPs are used as reference. If the frequency of certain SNPs is particularly significant in sick people, they could be considered as “associated” to a disease.

Generally, these variants are used as "post-it" of the genomic region including mutations causing the disease. Thus, the SNP *per se* may not directly cause the disease. Consequently, further genome analysis of the region is required (http://www.genome.gov/20019523). VISSCHER P. M., BROWN M. A., MCCARTHY M. I. et al., *Five Years of Gwas Discovery*. Am J Hum Genet 2012; 90(1): 7-24.

42. LANPHER B., BRUNETTI-PIERRI N. and LEE B., *Inborn Errors of Metabolism: The Flux from Mendelian to Complex Diseases*. Nat Rev Genet 2006; 7(6): 449-60. ADAMSKI J. *Genome-Wide Association Studies with Metabolomics*. Genome Med 2012; 4(4): 34.
43. Urea cycle disorders (UCDs) are caused by defects of several enzymes involved in the metabolism of waste nitrogen derived by the breakdown proteins and other nitrogen-containing molecules. It is classified in ICD10 as *Other disorders of amino-acid metabolism* (E72) and shows itself by cerebral edema and the related signs of lethargy, anorexia, hyper- or hypoventilation, hypothermia, seizures, neurologic posturing, and coma (Mew N.A. et al. http://www.ncbi.nlm.nih.gov/books/NBK1217/).
44. LANPHER B., BRUNETTI-PIERRI N. and LEE B., ref. 42. SUHRE K. and GIEGER C., *Genetic Variation in Metabolic Phenotypes: Study Designs and Applications*. Nat Rev Genet 2012; 13(11): 759-69, p. 762. ILLIG T., GIEGER C., ZHAI G. et al. *A Genome-Wide Perspective of Genetic Variation in Human Metabolism*. Nat Genet 2010; 42(2): 137-41, p. 137.
45. RAKYAN V. K., DOWN T. A., BALDING D. J. et al., *Epigenome-Wide Association Studies for Common Human Diseases*. Nat Rev Genet 2011, 12(8): 529-41.
46. FADISTA J., VIKMAN P., LAAKSO E. O. et al., *Global Genomic and Transcriptomic Analysis of Human Pancreatic Islets Reveals Novel Genes Influencing Glucose Metabolism*. Proc Natl Acad Sci U S A 2014; 111(38): 13924-9.
47. Briefly, serum blood metabolites concentrations are measured and genetic loci are sorted by GWAS according to specific parameters.
48. SUHRE K., and GIEGER C., ref. 45.
49. SUHRE K., SHIN S. Y., PETERSEN A. K. et al., *Human Metabolic Individuality in Biomedical and Pharmaceutical Research*. Nature 2011; 477(7362): pp.54-60. SUHRE K., and GIEGER C., ref. 45.
50. CHILDS B., ref. 2.
51. It was established in 1956 by the International Union of Biochemistry to standardize enzyme classification and nomenclature. In: MOSBY'S MEDICAL DICTIONARY, 8th ed. US: Elsevier, 2009.

52. HARRIS H., ref. 24.
53. CHAKRAVARTI A., *To a Future of Genetic Medicine*. Nature 2001; 409(6822): 822-3, 822.
54. It evolved in the more ambitious 1000 Genomes Projects born in 2008. PENNISI E., 2010, 1000 Genomes Project Gives New Map of Genetic Diversity. http://www.1000genomes.org.
55. http://hapmap.ncbi.nlm.nih.gov. INTERNATIONAL HAPMAP CONSORTIUM, *Integrating Ethics and Science in the International Hapmap Project*. Nat Rev Genet 2004; 5(6): 467-75.
56. STONEKING M., *Single Nucleotide Polymorphisms. From the Evolutionary Past*. Nature 2001; 409(6822): 821-2, 821.
57. WELLCOME TRUST CASE CONTROL CONSORTIUM, *Genome-Wide Association Study of 14,000 Cases of Seven Common Diseases and 3,000 Shared Controls*. Nature 2007; 447(7145): 661-78. VISSCHER P. M., BROWN M. A., MCCARTHY M. I. et al., ref. 41.
58. RECCHIA G. and PIRAZZOLI A., *Medicina Personalizzata: Attese, Rischi E Realtà*. In: MORETTO A., GIARETTA P., GENSINI G. F., TRABUCCHI M. (ed), *Filosofia Della Medicina. Metodo, Modelli, Cura Ed Errori*. Bologna, Il Mulino, 2009, pp.710, p. 206.. BUSH W. S. and MOORE J. H., ref. 29, p. 1.
59. HOOD L. and FRIEND S. H., *Predictive, Personalized, Preventive, Participatory (P4) Cancer Medicine*. Nat Rev Clin Oncol 2012; 8(3): 184-7, 184.
60. COLLINS F. S. and VARMUS H., *A New Initiative on Precision Medicine*. N Engl J Med 2015; 372(9): 793-5, 795. www.nih.gov/precisionmedicine. REARDON S., *Precision-Medicine Plan Raises Hopes*. Nature 2015; 517(7536): 540.
61. CHILDS B., ref. 2. CORBELLINI G., *Le Grammatiche Del Vivente*. Second ed. Bari, Laterza, 1999, p. 119.

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