



**IPAD-MD Expert Group Meeting on Standards
& Technology
Deliverable 6.3 (WP6)**

**Meeting Report on Forward Genetics Panel
Discussion**

**November 13th 2019
Rio Grande, Puerto Rico**

Reported by:
Dr. Asrar Ali Khan and Dr. Michael Raess



The IPAD-MD project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 653961

Table of Contents:

Introduction:	1
Panelists:	2
Panel Discussion:	4
<i>Contribution of forward genetics in understanding the genetic basis of human disease</i>	4
<i>Relevance of forward genetics in the post-genomic era</i>	4
<i>Advancements in forward genetics</i>	5
<i>Future of forward genetics</i>	6
<i>Challenges (technical and financial) in the field of forward genetics</i>	7
<i>Possible cooperative efforts from the forward genetics community</i>	7
<i>Input from the audience:</i>	8
Closing Remarks and Outcome:	9
Acknowledgements:	9

Introduction:

Forward genetics is an approach that identifies the genetic basis of a specific phenotype. In medicine, this translates to the discovery of mutations that are responsible for a genetic disease. Contrary to reverse genetics that starts with a specific gene and studies the effects of its altered expression on phenotype, forward genetics uncovers the genes behind a particular phenotype. Briefly, such an approach starts with a screen to identify a mutant phenotype that is either naturally occurring or artificially induced for example, by N-ethyl-N-nitrosourea (ENU) a potent mutagen. Next, the phenotype producing population is mapped followed by mapping of responsible candidate genes and sequencing to find the causative mutation. In the final step, the candidate mutation is validated using genetic engineering approaches.

Over the years, forward genetics has made significant contributions to human genetics research. For example, the etiology of several monogenic diseases like Rett syndrome¹ and Huntington's disease² have been successfully discovered using forward genetics approaches. Using model organisms like mice, the mutations responsible for complex phenotypes and disorders like obesity³ and circadian rhythm dysregulation⁴ were also successfully identified. The biggest advantage of this approach is its unbiased nature for establishing a clear relationship between a mutation and disease phenotype.

At a scientific review meeting of the Helmholtz Center Munich in February 2018, an interesting discussion was initiated about the current status and future directions of forward genetics. 'Where does forward genetics fit in other technologies in this post-genomic era and what is its relevance?'. This initial discussion led to the organization of the Forward Genetics Panel Discussion at the International Mammalian Genome Conference 2018 (IMGC 2018). The objective of the panel discussion was to explore the current state

¹ Amir et al. PMID: 10508514 DOI: [10.1038/13810](https://doi.org/10.1038/13810)

² MacDonald et al. PMID: 8458085 [https://doi.org/10.1016/0092-8674\(93\)90585-E](https://doi.org/10.1016/0092-8674(93)90585-E)

³ Zhang et al. PMID: 7984236 DOI: [10.1038/372425a0](https://doi.org/10.1038/372425a0)

⁴ Viaterna et al. PMID: 8171325 PMCID: [PMC3839659](https://pubmed.ncbi.nlm.nih.gov/PMC3839659/)

and future of forward genetics in the light of the recent developments in (functional) genomics and data-driven science. The panellists consisted of international leaders in the field of mouse genetics and pioneers in forward genetics approaches. The panel discussion was chaired by Prof. Dr. Martin Hrabě de Angelis.

The panel discussion was preceded by a lecture from Prof. Dr. Bruce Beutler (Nobel Laureate 2011) on his ongoing state-of-the-art forward genetics screens to uncover mutations leading to immunological phenotypes. This entire session on Forward Genetics at the IMGC 2018 was hosted by INFRAFRONTIER via the IPAD-MD project.

Panellists:

Prof. Dr. Bruce Beutler was awarded the Nobel Prize in Physiology or Medicine (2011) for his work on the activation of innate immunity. For more than a decade, Prof. Beutler has been one of the foremost leaders in the field of forward genetics and has made several key discoveries in immunology using this approach. He is the director of the Center for the Genetics of Host Defense at the University of Texas Southwestern Medical Center, where he and his team conduct robust and automated forward genetics screens to identify genes responsible for specific physiological processes like immunity, metabolism, developmental and neurobehavioral functions.

Prof. Dr. Monica Justice is the head of the Genetics and Genome Biology Program at the Hospital for Sick Children (SickKids). During her PhD, Prof. Justice pioneered the use of ENU-based chemical mutagenesis approaches in mice and still continues to lead this field. Via an initial forward genetic ENU suppressor screen, she has recently shown DNA damage response contributes to the pathology of Rett Syndrome.

Dr. Laura Reinholdt is an associate professor at the The Jackson Laboratory. Previously as a part of the Reprogenomics ENU program, Dr. Reinholdt led the cloning of several ENU alleles responsible for aberrant meiotic chromosome dynamics. Her group is widely interested in

the development and application of both forward and reverse genetic approaches for understanding the etiology of genome variation and its role in health and disease.

Prof. Dr. David Beier is the director of the Center for Developmental Biology and Regenerative Medicine at the Seattle Children's Research Institute. His lab has been on the forefront of several major developments in the genetic analysis of model organisms including the application of ENU mutagenesis for developmental investigation and for sequence-based analysis. A major focus of his lab has been screening ENU-mutagenized mice for defects in organ development.

Prof. Dr. Nadia Rosenthal is the scientific director of The Jackson Laboratory and a renowned expert in the use of mice for targeted mutagenesis in the study of muscle development, disease and repair. She was an integral part of EUCOMM, the European Conditional Mouse Mutagenesis Program, where she coordinated the selection and production of new Cre driver strains for the international mouse genetics community.

Dr. Ruth Arkell is an associate professor at the John Curtin School of Medical Research (Australian National University, ANU) where she heads the Early Mammalian Development Laboratory. Her group focuses on the genetic mechanisms that control mammalian gastrulation and the consequences of incorrect gastrulation. Dr. Arkell has extensive experience in conducting several forward genetics genome-wide ENU mutagenesis screens at MRC Harvell and ANU.

Prof. Dr. Martin Hrabě de Angelis is a professor at the Technical University Munich and the director of the European Mouse Mutant Archive (EMMA) and the Institute of Experimental Genetics at the Helmholtz Center Munich. Prof. Hrabě de Angelis is a strong proponent of forward genetics and aims to understand the complex mechanisms underlying the etiology of human diseases. He has made several prominent discoveries in the field of genetics using ENU mutagenesis forward genetics screens and has also made a large number of mouse models available to the global scientific research community via a systematic, genome-wide, mutagenesis screen.

Panel Discussion:

The following topics were discussed in the panel discussion highlighting the significance, current status and future prospects of forward genetics:

Contribution of forward genetics towards understanding the genetic basis of human disease

According to the panellists, there was no one particular forward genetics contribution that was the important towards the better understanding of human diseases and mammalian physiology. Some of the notable contributions of forward genetics were the discovery of Toll-like receptor (TLR)-4 as the lipopolysaccharide sensor⁵ and discovery of *Clock* gene as the central regulator of mammalian circadian rhythm⁶, discovery of early mammalian development genes⁷ and models for metabolic bone diseases⁸.

In their view, forward genetics is the re-evaluated application of classical mendelian genetics because it also deals with the heritability of quantifiable traits. It has gained more importance in the last few decades as the actual complexity of the genome has started to become evident.

Relevance of forward genetics in the post-genomic era

Forward genetics is still very relevant in the post-genomic era. Human geneticists are realising the importance of mouse models replicating the exact mutation found in human patients whereas previously they heavily relied on sequencing, association studies and

⁵ Poltorak et al. PMID: 9851930

⁶ Daxinger et al. PMID: 24025402 PMCID: [PMC4053835](#)

⁷ Anderson. PMID: 10689347

⁸ Sabrautski et al.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 653961.

reverse genetics to understand complex human diseases. The impact of environment and diet on diseases is widely acknowledged now. In addition, in most cases it is not a single gene but a network of genes that is responsible for a disease. Therefore, more sophisticated human diseases models are needed to accurately emulate complex human pathologies. Due to its unbiased nature, forward genetic approaches can determine which genetic regulatory networks have pathogenic consequences and also employ complex mouse models which would be immensely helpful to human geneticists.

Advancements in forward genetics

In the last few decades, several advancements have led to significant progression of forward genetic screens. Some of them are listed below:

Positional cloning: The process of positional cloning i.e. the identification of the causative mutation was expedited by the publication of the annotated mouse genome in 2002 and sequencing of whole mammalian exomes. Previously, such sequencing endeavours required more than 9 years to complete and now can be accomplished in a few weeks.

Genetic mapping: Another breakthrough was the ‘instant positional cloning’ technique that could resolve disease phenotypes almost instantaneously⁹ thereby removing the bottleneck of genetic mapping.

Mutant production: CRISPR-Cas9 genome editing has been used in forward genetics screens to create genome-wide mutant libraries because to its easier scalability. In addition unlike chemical mutagens or radiation, CRISPR allows the generation of mutant libraries with known mutation sites.

The development and use of inbred mice also made forward genetic screens easier. These strains possess a near overall homozygosity in their genetic loci making the identification of the causative mutation faster and cheaper.

⁹ Wang et al. PMID: 25605905 PMCID: [PMC4321302](https://pubmed.ncbi.nlm.nih.gov/25605905/)

Phenotype screening: Several new technologies have greatly improved the efficacy of phenotype screening methods used in forward genetics screens. These include induced pluripotent stem (iPS) cells, 3D-culture systems and organ-on-a-chip. In addition, the use of *in vivo* models like fruit flies, *C. elegans* and mice in phenotypic screens have opened up new avenues for forward genetics by standardising phenotyping pipelines. These technologies and advancements have enabled forward genetic screens to realistically recapitulate human disease biology.

Validation: The use of CRISPR also enabled efficient validation of disease mutations by the rapid generation of specific mouse models reproducing the disease phenotype.

Thus, the last few decades of advancements in genome editing, *in vitro* and *in vivo* models, and sequencing technologies collectively propelled forward genetic screens.

Future of forward genetics

Human geneticists primarily rely on mapping of genetic variants (genome wide association studies, GWAS) to determine pathogenic genetic changes. As mentioned previously, forward genetics is invaluable in this regard and can provide an efficient way to molecularly assess and validate such mutations thereby bridging the relationship between functional genetic variation and human diseases.

Forward genetics screens are promising classical genetics tools that are customizable and easy to use. This aspect is especially enticing for various research groups to undertake smaller customized screens that are specifically tailored to a scientific question. Subsequent services like cloning, phenotyping, genetic mapping and sequencing can be supported by larger core centres or infrastructures.

Challenges (technical and financial) in the field of forward genetics

As stated earlier, positional cloning was a major rate limiting step in forward genetics screen which has been overcome with the 'instant positional cloning' method. The speed and affordability of current-generation sequencing technologies has also greatly helped in this direction. However, these advancements have led to the generation of large amount of genotypic and phenotypic data increasing the demand for the mechanistic analyses needed to make sense of precisely how specific mutations lead to specific phenotypes.

CRISPR is a promising tool for forward genetics. However, its use in forward genetics can still be improved by minimising off-target effects, and by targeting isoforms or splice variants and non-coding sequences like regulatory elements.

One of the main challenges faced in forward genetics today is the competition for funding from human geneticists with funding agencies arguing that animal models like mice are not suitable tools to discover, validate and study disease causing mutations. As mentioned earlier, human geneticists require allele-specific models for translational research of human diseases that can also be used for preclinical studies. The generation of such 'synthetic complex disease models' is currently only possible in mice.

One aspect that still requires improvements is the automated handling of animal models in these screens as they involve breeding and housing of large number of mutant animals and their subsequent progeny which is labour-, time-, and cost-intensive.

Possible cooperative efforts from the forward genetics community

The panellists unanimously agreed that a collective effort is required to bring back forward genetics into the limelight and promote its use in present and future biomedical research. One such cooperative effort would be to develop a precision model generation and robust phenotyping pipeline for characterizing human functional genetic variation in mouse

models. This pipeline can be used by human geneticists to investigate pathogenic allelic variations and enable mouse geneticists to provide valuable and reliable support to human geneticists.

More focus should be placed on gene regulatory pathways and not on individual genes especially when involving model organisms. This would circumvent the problem of missing orthologs in humans. In addition, more effective ways need to be applied to translate molecular mechanisms to phenotypes.

Apart from deciphering the relationship between functional genetic variation and disease, it is also important to focus on the biology of the disease as a whole.

Input from the audience:

- Inform funders on the utility of mouse models for forward genetic screens.
- Use mouse models for validating GWAS studies.
- Maintain standards in mouse studies.
- Human geneticists and funding agencies should be informed about the advantages of forward genetics e.g., unbiased investigation, hypermorphic gain of function, productive approach, high efficiency.
- As stated previously, individual labs can independently apply forward genetics approaches to investigate specific scientific questions. The subsequent sequencing and bioinformatics services can be utilized from specialized core facilities, alleviating substantial financial and infrastructural burden on these investigators.
- A review article was suggested that would highlight the power of forward genetics in the post-genomic era.
- New technologies like advanced non-invasive phenotyping and machine learning-assisted phenotyping would propel the field of forward genetics.

Closing Remarks and Outcome:

It was clearly evident from the panel discussion that forward genetics is still valuable to human genetics and is needed to understand the genetic basis of human diseases. In addition, animal models (especially mouse models) hold enormous potential when combined with forward genetics screens and can efficiently complement human genetics research in the form of precise disease models. Recent advances in genome editing, sequencing technologies and mutant generation have made forward genetics screens accessible to the wider community of biomedical researchers. Consequently, this needs to be effectively communicated to the scientific community and policy officials. The panellists agreed that a review or commentary highlighting the power of forward genetics and a white paper informing the policy makers of its therapeutic potential is the immediate next step. Another tangible outcome was the renewed focus on bringing together and strengthening interactions between forward, mouse and human geneticists in upcoming INFRAFRONTIER stakeholder meetings and conferences.

Acknowledgements:

Special thanks to the panellists for their expert discussion and to the chair Prof. Hrabě de Angelis for moderating the session.

The IMGC 2018 organisers, especially Darla Miller and Ginger Shaw, helped immensely with setting up the panel discussion.