Applied Genomics in the Clinic

Report and recommendations of a JRC workshop within the context of JRC Enlargement and Integration Activities (E&IA)

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1. Abstract

The workshop "Applied Genomics in the Clinic" was organised in Istanbul on 17-19 October 2012 within the context of JRC Enlargement and Integration Activities (E&IA). The main aim of the workshop was to get an overview of the state of the art of applied genomics in the clinical context in accession and candidate countries, as well as new members, to share best practices in EU and to evaluate these in the light of a public health perspective. There is a clear divide behind the genomic services offered in a country and the awareness among research scientists of the available genomic applications and the future impact of genomic technologies on health services and clinical approaches. In all countries there are a number of common obstacles that delay penetration of genomic technologies in clinical applications: lack of recognised experts (medical genetics has to be recognised as a medical specialty) lack of a regulatory framework that involves political determination of decision makers, lack of common databases on methods and experts, lack of on-going education for physicians and most importantly reimbursement of testing. Stronger connections and collaborations with the EU for research and technology transfer will function as leverage for these countries in adopting genomic tools and harmonising the quality of healthcare services they offer. It is very important to establish recognized objective state of the art guidelines for application of genomic technologies in clinical practice. Such guidelines adopted by countries could contribute to form the basis of reimbursement policies at national and cross border levels. In addition establishing reliable, not for profit, open access databases for building reference datasets for correct and efficient interpretation of complex data generated by advanced genomic technologies will speed up adoption of the technology in the clinic.

2. Executive Summary

In the coming decade, advanced genomic technologies are expected to have a substantial impact on, and even change current frames of public health. Disease classification and taxonomy, molecular diagnostics, drug development processes, stratified and personalized medicine as well as lifestyle and nutrition choices are some of the areas that will be directly affected. While the cost of whole genome sequencing reduces and test specificity and sensitivities improve, huge amounts of data are being generated that require proper management, guided and monitored validation, extensive analyses and customised clinical interpretation to help serve the medical community and health of individual patients in general. Technology has evolved at such a rapid pace that today a consumer can have his or her entire genome sequenced by a single company in a matter of days for less than $ 5,000, though the addition of interpretation may extend this timeframe. With the next-generation sequencing technologies currently being developed, the cost is projected to continue to decline significantly over the next few years, to the point that large-scale genome sequencing is expected to become comparable in cost to a single gene test or to a diagnostic imaging test such as a computed tomography (CT) scan (Mardis, 2006). Given the rapid technological advances, the potential effect on
the lives of patients and the increasing use of genomic information in clinical care, it is important to address how genomics data can be integrated into the clinical setting. Genetic tests are already used to assess the risk of breast and ovarian cancers, to diagnose recessive diseases such as cystic fibrosis, to determine drug dosages based on individual patient metabolism, and to identify therapeutic options for treating lung and breast tumours, melanoma, and leukaemia.

Recent studies have also demonstrated the usefulness of genomics for diagnosing disease and guiding treatment in the clinic. For example, genetic testing of the relatives of patients newly diagnosed with colon cancer has suggested a prevention strategy for identifying individuals with Lynch syndrome (Coates et al., 2011). Genomics data have been used to provide definitive diagnoses for patients with neuropathy, inflammatory bowel disease, and Proteus syndrome as well as to guide therapeutic care for patients with arterial calcifications, movement disorders, and Miller syndrome (Bainbridge et al., 2011; Lindhurst et al., 2011; Lupski et al., 2010; Ng et al, 2010; St. Hilaire et al., 2011; Worthey et al., 2011). Although applications of genomics technologies are currently limited in number, their number will only continue to increase. Thus, it is important to determine how genomic data can best be integrated with clinical practice so as to maximize patient benefit.

It is becoming increasingly clear that large-scale genomic information would be integrated more fully into clinical practice, which meant that issues related to implementing this change needed to be addressed. On the other hand, most patients and health care providers have not yet realized just how broad an effect genomic discovery is likely to have on treatment course and health.

The main aim of the workshop was to get an overview of the state of the art of applied genomics in the clinical context in accession and candidate countries, as well as new members, to share best practices in EU and to evaluate these in the light of a public health perspective. Experts from target countries attending the meeting as country representatives presented a summary of the molecular genetic/genomic services available in their respective countries both from the clinical and research environment perspective. Selected speakers presented examples of current applications of diverse genomic technologies in healthcare services. The European Best Practice Guidelines for Genome-based Information and Technologies and the EU policy on rare diseases, including a summary on the legal basis for the developments of the EU Policy on rare diseases were also shared with the attendants. The European Project for Rare Diseases National Plans Development (EUROPLAN) is a project co-funded by the EU Commission (DG-SANCO) to promote and implement National Plans or Strategies to tackle rare diseases, to share relevant experiences within Countries, linking national efforts with a common strategy at European level. Participants also presented the situation in their respective countries regarding the elaboration and the implementation of Rare Diseases National Plans/Strategies.
3. Presentations and session highlights

The morning session of the first day started with the welcome address given by Dr Laura Gribaldo from the European Commission’s Joint Research Centre. She introduced the role of the Institute for Health and Consumer Protection (IHCP) at the JRC, as provider of scientific and technical support to the EU policies for the protection of European citizens in the areas of food, consumer products, chemicals and public health. Furthermore Dr. Gribaldo summarized the role of DG Enlargement in managing the process whereby countries join the European Union. The concepts of sharing knowledge, improve communication and strengthen networking have been identified as the basis of any harmonised effort to enlarge Europe.

A general introduction on the University of Istanbul was given by Prof Ugur Ozbek, the local host. This University counts 20 faculties and 74 000 students, it has on going protocols with 56 Universities worldwide and 483 Erasmus Agreements. In terms of health services it counts 2 University Hospitals, a Cardiology Institute, an Oncology Institute and a Faculty of Dentistry, with a total of 3500 bed capacity, 2.5 million outpatient/year and 100.000 in patient/year. The Institute of Experimental Medicine (DETAE) which co-hosted the meeting is dedicated solely to medical research, and it is one of the first and largest biomedical research institutes in Turkey established in 1992. Five departments belong to the Institute: Genetics, Immunology, Molecular Medicine, Neurosciences, and Animal Model Organisms. In the Genetics Department there are five units/laboratories: Tuberculosis Molecular Epidemiology unit, Diabetes research and application unit, Molecular Andrology unit, Whole genome sequencing laboratory (FLX-454-Ion torrent), Whole genome expression microarray laboratory (Illumina). They are partners in a number of European initiatives including Orphanet, ELN, MedGeNet (Euro-Mediterranean Network for Genetic Services), ITFOM and the FP7 project Epicure.

In summary, they combine multidisciplinary research and education, coordination of the postgraduate programs on Immunology, Genetics, Molecular Medicine, Neuroscience and conduct competitive international projects in medical sciences through development of novel approaches for the prevention and diagnosis of common human diseases. Their strengths are: enthusiastic young scientists eager to learn new techniques, and expand their vision, established strong infrastructure for varying projects (a unique whole genome analysis laboratory in operation in Turkey), a suitable environment for collaborations on a complex disease like diabetes (diabetes centres, immunology and genetics departments all in one place).

The first scientific session focused on new technologies like Single Nucleotide Polymorphism (SNP) Microarrays for Genotyping and Next Generation Sequencing (NGS).
Dr Jeremy Sujie Cao from BGI, one of the largest genomic organizations in the world, gave a presentation on "Application of Next Generation Sequencing in Human Disease Research and Clinical Application", showing how the BGI Healthcare Platform provides a series of hereditary disease testing services. Monogenic disease and hearing impairment tests are available at all life stage. At the prenatal stage, Non-Invasive Fetal Tryptsomy (NIFTY) test is significantly better than other conventional prenatal screening methods available at the present time. For a new born, inherited metabolic disease screening can be applied. Non-invasive and invasive test of chromosomal microdeletion/microduplication and Pre-implantation Genetic Diagnosis (PGD) technologies are still in research, but are coming soon and they are expected to be better strategies than the existing tests.

Dr. Teodor Zamfirov, representative of Illumina Inc. from Bulgaria introduced us to which technological applications find way nowadays in the health care system, how the technology revolutionizes our deep understanding of the pathology process for many diseases, what is the prevention capacity employing genomic technology in the field of public health and medical conditions such as: Metabolic disorders, rare disease diagnostics, oncology, cardiology, personalized medicine, reproductive genetics, inherited diseases, pre and postnatal diagnosis and more. He exemplified various applications of the Illumina Inc. SNP microarrays in healthcare. Further in his presentation he described the latest developments regarding Illumina’s personal next generation sequencer - The MiSeq. This presentation was in depth and covered capacity, pricing, and the perspectives for further development of an entire new medical discipline - Personalized Medicine, based on the information coming out of the individual genome of each patient.

In the second session of the first day, Prof. Angela Brand, Director of the European Centre for Public Health Genomics at the Institute for Public Health Genomics in Maastricht University, gave a presentation on European Best Practice Guidelines for Genome-based Information and Technologies – The Public Health Genomics European Network (PHGEN ) Declaration of Rome, a document endorsed by experts from the field of public health genomics representing key European and national organizations from policy making, academia and private sector. The on-going success of genome wide association studies (GWAS) followed by chromosomal microarrays and eventually Whole Genome Shotgun (WGS ) in uncovering genetic risk factors for many common diseases has fuelled expectations of a new era of health care based on personalized treatment, early detection, and disease prevention. An optimal process is needed for appropriate translation of these new genomic discoveries into practice. The process should include mechanisms for developing an understanding of the relationship between these newly discovered factors and clinical outcomes (clinical validity), and the costs, benefits, and harms of genome-based technologies in real world settings (clinical utility). Furthermore, the process should facilitate the development of evidence-based guidelines for the use of genomic applications; and appropriate implementation of these applications in practice, including protection of individuals and communities against discrimination based on genetic information. The application of genome-based technologies and
informatics with the aim of combating diseases of public health significance brings a slew of ethical and social issues that challenge the normative frameworks used in clinical genetics until now.

In the same session Dr Gribaldo summarised the EU policy on rare diseases, from the legal basis for the development of the EU Policy on rare diseases, to the emergence of concepts and initiatives surrounding rare diseases in Europe, the content of the Commission Communication and the Council Recommendation, and the history of support of rare diseases research at the European level, as well as the future way forward.

In the afternoon session the country representatives discussed the state of the art of genomics penetration in clinical services in their respective countries. Country information ranging from the distribution of Genetic Diagnosis Centres, to the tests performed in different areas in a given country, availability of genetic testing in public and private (if any) laboratories has been presented. The state of medical genetics as a medical specialty, availability of genetic counselling and education for genetic counsellors, current coverage of rare diseases were among covered topics. The most frequently mentioned and underscored clinical applications within context were: dysmorphology testing, foetal examination, genetic counselling, management of congenital disorders, translation of research on complex disorders in Clinical genetics, chromosome analysis on peripheral blood samples, amniotic fluid, Chorionic Villus Sampling (CVS) or cord blood, Fluorescence In Situ Hybridization (FISH) in cytogenetic, and Capillary electrophoresis-based DNA sequencing Short Tandem Repeat (STR) analysis, Real-Time Polimerase Chain Reaction (RT-PCR), Quantitative Fluorescence Polimerase Chain Reaction (QF-PCR), Multiplex Ligation-dependent Probe Amplification (MLPA) and Array Comparative Genomic Hybridization (CGH) in molecular genetics. SNP based or chromosomal microarrays and NGS currently are not offered in any of the target countries of the workshop although there is awareness among researcher clinicians.

Dr Ewa Stępień, from the Department of Clinical Biochemistry, Jagiellonian University in Krakow, gave an overview on the reorganisation of Health System in Poland with procedures in medical genetics for physicians and laboratory diagnosticians, with special emphasis on educational and financial issues. She underscored new initiatives like the new Centre for rare cardiovascular diseases in Krakow) and international co-operation for genetic diagnostics of rare diseases that has been created with the incorporation (2011) of The Children’s Memorial Health Institute in Warsaw to the JOINT ACTION "Development of the European portal of rare disease and orphan drugs – ORPHANET Europe". Main achievements and main failures in clinical genetic diagnostics in Poland have been presented over a timeline. Organization of genetic counselling in Poland covered by National Health Fund is one of the achievements, like the education program in Medical Genetics (Specializations) for physicians and laboratory geneticists. New private laboratories and companies dedicated to clinical genetics have been established, and there is increasing number of diagnostic centres equipped with high throughput methods in
genetics. The main obstacles to the penetration of new technologies were presented as lack of comprehensive financial and education programs supporting development of scientific research in clinical genetics, dispersion of procedures over the list of guaranteed services (so-called “basket”), lack of interest in introduction of quality control system in genetic laboratories and limited availability to prenatal and pre-implantation genetic diagnostics (high costs).

Dr Ahmet Yesilyurt from the University of Ankara, made an overview of current applications of medical genetics in Turkey. The Social Security Institute (SGK) is responsible for reimbursement; the frequency of the genetic test of each genetic centre can be followed with a global system called “MEDULLA” and PGD can be charged for just some disorders which can be treated via Human Leukocyte Antigen (HLA) typing compatible bone marrow transplantation from siblings. The most challenging problem presented was introducing a new test using next-generation sequencing since new technology hardly gains coverage by the social security system. Bottlenecks in genetic applications in Turkey were stated as: lack or insufficiency of infrastructures for genetic testing laboratories in some university/state hospitals, lack of well-educated staff to perform complex genetic tests, not homogeneous education programme (4 year) of medical geneticist, and insufficiency of bioinformaticians to evaluate complex and huge data from high throughput systems.

Dr. Lejla Kapur-Pojskić presented data on Bosnia. Apparently at the moment any regulation is still lacking in Bosnia, as well as any official role for medical genetics. Laboratories are basically equipped with PCR facilities and RT PCR analysis are conducted on 30-40 patients per year, to detect leukaemia/lymphoma markers, as well as for Huntington diseases. Medical genetics is not recognised as specialty. Prenatal diagnosis is covered by insurances. There seems to be a lot of cross border patients'sample traffic to neighbouring Croatia where a special bilateral agreement for reimbursement of tests exists.

Dr. Irena Drmic Hofman represented Croatia, as Chief of the Department of Pathology and Department of Biochemistry at the University Hospital Split and University of Split School of Medicine. Cytogenetics in Croatia is conducted in three hospitals: namely in the University Hospital “Rebro” Zagreb, Pediatric Clinic, Clinical Hospital Center “Sisters of Mercy,” and Clinical Hospital “Holly Spirit”, Clinic of Obstetrics & Gynecology. The services offered are mainly cytogenetic analysis of foetal and peripheral blood lymphocytes, amniotic fluid and spontaneous abortions, FISH analysis of bone marrow, FISH analysis for enumeration, microdeletion and microduplication syndromes, and whole chromosome painting, subtelomere analysis. In the Clinical Hospital Centre Sisters of Mercy Zagreb, molecular analysis of non-syndromic deafness, acondroplasia and hypocondroplasia, Rett syndrome and MLPA are carried out. The diagnostics focuses on monogenic diseases, leukaemia and lymphoma, tumour tissues, infectious diseases, risk factors, HLA typing and transfusion testing as well as molecular testing in Forensic Medicine.
Dr. Simona Dimitriu from the University of Medicine and Pharmacy "Victor Babes" in Timisoara, especially pointed out the consented effort in Romania for establishing a national plan for rare diseases which, she thinks, could serve as basis for a regulated, quality assured, up-to-date genetic testing environment in general. In Romania there seems to be a divide in the technology level among regions, and research and university settings. Chromosomal Microarray Analysis (CMAs) and NGS are finding way into the clinic through private laboratories.

The day was ended with an informal round table discussion on the state of research in the represented countries and notes were taken that served as basis for the SWOT analysis of the last day.

The first session of the second day was dedicated to the state-of-the-art applications of genomic technologies for clinical purposes. Dr. Francesca Grati from TOMA, Italy, gave a presentation on "Chromosomal microarrays in prenatal diagnosis: overview of the actual application and experiences". With the development of advanced genome-wide or targeted techniques for interrogating the human genome, new methodologies are becoming available for prenatal screening and diagnosis, and the implementation of these methodologies into healthcare provisions will soon be changing the landscape of prenatal diagnosis. It is widely accepted that this technology can be considered as unique if not mandatory in challenging prenatal cases to clarify the pathogenicity of cytogenetic abnormalities and their prognosis.

These challenging prenatal cases are: cases requiring a paternal uniparental disomy (UPD) condition exclusion on AF upon a mosaic trisomy for an imprinted chromosome is found in Chorionic Villi, cases having a high risk of false negative result due to the incompleteness of the combined cytogenetic analysis on CVS, cases with an apparently balanced ‘de novo’ rearrangement and in foetuses with US abnormalities and an apparently normal karyotype.

On the other hand, it is still necessary to improve knowledge on human genome architecture in ‘normal’ considered populations, on the entire phenotypic spectrum of microdeletion and microduplication syndromes and on uncertain variants, as well as pre- and post- test counselling approach models for prenatal CMA.

Dr S. Birep Aygun presented a current overview of the state-of-the-art in non-invasive prenatal diagnosis, and a case study on non-invasive foetal Y chromosome detection from maternal plasma via RT-PCR. Non-invasive molecular techniques include genetic analysis on foetal cells or on free foetal DNA or RNA isolated from maternal blood. Non-invasive genetic testing for Anti-D and foetal gender when mother’s genetic status is indicative is already common practice in some EU countries e.g. UK, the Netherlands. Non-invasive genetic tests for common aneuploidies like Down syndrome, Trisomy 18, and Trisomy 13 foetal DNA present in maternal blood are already in the market and simultaneously under development. If an elevated risk of chromosomal or genetic abnormality is indicated
by a non-invasive screening test, a more invasive technique may be employed to gather additional information. The case study presented demonstrates the feasibility and reproducibility of a biomarker system for non-invasive Y chromosome determination via real time PCR with 100% specificity.

Dr Uysal-Onganer from the Department of Surgery & Cancer, Faculty of Medicine, Imperial College, London, discussed what has been done till now in UK in "Applied Genomics in Cancer". She underlined the fact that CMA and NGS studies have led to significant advances in our understanding of the cancer genome of several tumour types. Furthermore, current efforts are aimed at bringing sequencing discoveries into the clinic in the form of biomarkers (diagnostic, prognostic, and predictive) and biomarker-designed clinical trials. However, the new discipline of public health genomics, which seeks to evaluate the use of emerging genomics information effectively and responsibly to improve the health of individuals and populations, is essential. New diagnostic and predictive markers are still needed; pros and cons are still an issue, however a lot has been achieved considering drug response and tumour recurrence in certain cancer types. The stratified medicine programme by Cancer Research UK (CRUK) was highly praised.

At the end of the second day a complete session was dedicated to Bioinformatics: Dr S. İşeri from Istanbul University, Dr M. Fabbri from IHCP and Mr. Ilker Karacan from DONE Genetics, a local genomics and bioinformatics company, presented various case studies that served as examples of data analysis and result interpretation in the field of gene expression analysis, karyotyping, linkage and Copy Number Variation (CNV) analysis.

On the last day of the workshop a whole session was devoted to Strengths, Weaknesses, Opportunities, and Threats (SWOT) analysis on penetration of Genomic Technologies into the Clinical Services. Contributions of all participants were noted and results were deduced in the afternoon by Dr. Gribaldo and Dr. Aygun to be disseminated to the participants for review, further contribution and discussion. The results of the SWOT analysis serve as the basis for the recommendations presented in this report.

4. General Conclusions

- There is a clear divide between the genomic services offered in a country and the awareness among research scientists of the available genomic applications and the future impact of genomic technologies on health services and clinical approaches.

- Medical specialists of no / minor genetic / genomic background may generally overlook available technologies and influence local decision makers in certain ways that leads to a lag in adoption of genomic tools.
Establishment of the necessary infrastructure for generation, storage, transfer and interpretation of genomic data may be a heavy cost burden for target countries.

In all countries there are a number of common obstacles that delay penetration of genomic technologies in clinical applications: lack of recognised experts, lack of a regulatory framework that involves political determination of decision-makers, lack of common databases on methods and experts, lack of on-going education for physicians and most importantly reimbursement of testing, lag of local health impact / health technology assessors behind technological advancements in the field.

There is a unanimous opinion that public health in the near future is going to be shaped by data generated via genomic technologies. A general agreed upon universal definition for clinical utility and its demonstration will facilitate this process.

The two and a half days agenda was in general considered sufficient and satisfying as seen in the post event evaluation forms. There was a unanimous request for a next workshop.

5. SURVEY RESULTS

Prior to the workshop all participants had received a copy of the country expert survey and where kindly asked to present relevant data. The survey results are summarised in the Table 1 of the Annex I.

The roundtable discussion following country presentations and presentations of invited speakers yielded the two technologies expected to penetrate into clinical services the fastest were agreed upon to be Chromosomal Microarrays and Next Generation Sequencing. The two areas where genomic applications are estimated to enter fastest into clinical services came out to be prenatal diagnosis and cancer genotyping/treatment.

Penetration of Genomic Technologies into the Clinical Services- HOW?

At the end of the workshop a whole session was dedicated to an open SWOT analysis on penetration of Genomic Technologies into the Clinical Services with contributions from all participants.

SWOT Outcome

Participants agreed that genomic medicine may not be cost-effective today but said that it may become cost-effective soon as costs go down and efficacy goes up. Furthermore, when people (be it a physician, patient, citizen or family member of a patient) are well informed on the availability of such approaches, they are usually
interested in the technology. Probably the correct best approach for scientists and clinicians would be to work closely and define the mode(s) of utilising the technology in such ways that are relevant, significantly contributing to the health of the patient and involve minimum risk or harm.

**Strengths:** existing research environment, international collaborations, awareness of researchers, already trained academic personnel

**Weaknesses:** lack of regulation, existing regulation stopping or lagging new advancements, lack of political will, lack of trained physicians, lack of hardware software infrastructure to maintain data, lack of trained bioinformaticians to analyse generated data, interpretation of results.

**Opportunities:** awareness in the society (patient demand), existing bilateral, pan European and international networks for data and experience sharing, technology becoming more readily available.

**Threats:** Technology platforms still too expensive, testing usually reimbursed on basis of price may lead to outsourcing to biotechnology company run laboratories, resistance of old school physicians, inability to generate reference open access databases.

**Factors most effective:**

- Genomic Medicine/ Personalized Medicine not always the same thing
- Data interpretation
- Data storage
- Clinical implication of data
- Education of physicians
- Availability of open access/ public databases
- Drug/therapy design (individual response?) one size does not fit all
- Who pays for the test?
- Technology advancing rapidly
- Research
- Legal/ Ethical Issues
- Clinical Implementation
- The Industry
- Research Funding
- Political will/ regulation/ reimbursement
- A number of studies already underscore the rapidly shifting landscape for genomic tools in the diagnostic setting. The results could influence decision makers to revisit guidelines concerning [prenatal] genetic testing. Chromosomal microarrays can provide additional clinically relevant information to traditional karyotyping, and will probably become a standard approach in prenatal diagnostics going forward.
- Research which employs whole-genome sequencing for the clinical diagnosis of prenatal samples highlights how sequencing is being used increasingly for prenatal testing. A number of biotechnology firms, such as Sequenom,
Ariosa, LifeCodexx have already launched commercial tests that use sequencing to noninvasively detect aneuploidies in prenatal samples. Research results demonstrate the possibility of mapping foetal balanced chromosomal translocations via next generation sequencing.

- Chromosomal microarrays will most probably be applied also in the area of cancer genomics, mainly covering the issue of patient dependent drug response and development of cancer type specific expression panels. Such disease specific panels are more affordable and comparatively quick yielding when there are no means of NGS.

- Next generation sequencing will also play a major role in the field of cancer research and diagnosis, by most probably allowing cheaper and faster profiling.

- Stronger connections and collaborations with the EU for research and technology transfer could function as leverage for these countries in adopting genomic tools and harmonising the quality of healthcare services they offer.

- Regulations to control cross-border movement of samples should take into account that the patient himself is also the sample and can move freely, any regulation should allow if not promote ease for finding an expert centre for a given condition/disease.

- The study of rare diseases offers a way of implementing the tools and procedures that will later be used in more widespread applications of genomic medicine.

**Analytical validity and clinical utility**

- Increasing the sensitivity of sequencing.

- There needs to be agreement upon standards for both analytical and clinical validation.

- Clinical actions need to be determined through collaborative efforts involving physicians, patients, their families, and laboratories.

- For genomic testing to be accepted, it should have not only analytical validity but also clinical and social utility.

- Genomic testing should be used as a tool that is integrated with traditional tests for making a disease diagnosis and guiding therapy.

  Human genetic diversity and genetic differences between maternally and paternally derived chromosomes need to be considered when interpreting genomic data.

- Pharmacogenetics results can be important for patient care, but data need to be carefully integrated into patient records and care processes.
Sequencing devices, interpretation software packages, and testing laboratories will all need to meet stricter proficiency standards as genomic medicine progresses.

**Ethical issues**

- Patients’ genomic information should always be obtained within the confines of a doctor–patient relationship.

- If patients are empowered to make their privacy preferences available to caregivers and researchers, the delivery of care and the use of patient data for research could both be enhanced.

- Health care providers have a responsibility to provide patients with clinically significant genomic information but not necessarily other less clearly actionable information.

- Patients’ concerns about confidentiality cannot be completely resolved with technological approaches.

- Assuring patient privacy.

**Education and training**

- Education and training should focus on competencies. For a non-specialist health care provider, these competencies may include recognizing when a genomic diagnostic test is needed or how pharmacogenomics testing can guide decisions about therapy.

- Genetics and genomics should be integrated into health professional education from undergraduate study through to maintenance of certification.

- Collaborative efforts among health professionals will be essential in implementing genomic medicine.

**Databases and repositories**

- Genomic data should be put into meaningful formats in order to be most useful to health care providers.

- Clinical data will need to be linked to genomic databases in order to further understanding of the phenotypic effects of genetic variants.
Many laboratories do not have the resources to place their data in the public domain. Grant support may be necessary to move data into the public domain so that experts can be engaged to curate it.

Establishing a curated genomic-variant database: who is going to curate it and whether the database is clinically validated.

A clinical-grade genome sequence and phenotype repository is needed first, and the curating at that point will revolve around collecting the proper information about the data being deposited. A clinical variant database can then be derived from those data by grading and assessing the sets of sequence and phenotype information in order to build decision-support tools.

Databases for genetic variants involved in cancer may be quicker to achieve, considering the more wide spread efforts for cancer inventories and registries. However cancer variants raise somewhat different issues than maintaining databases for germ line variants. Sequencing cancer genomes also uncovers germ line sequence information, but in sequencing cancer genomes there tends to be a much more direct link between acquired mutations and the disease. These might be interrogated specifically for interactions with drugs, the ability to treat, or even to prevent the development of tumours.

Including cancer genomes in a master database could be problematic unless it is possible to create very definitive (CANCER) subsets among general data. Data should be clearly annotated to specify whether a variant has somatic effects, germ line effects, or both.

6. ORGANISER IMPRESSIONS

The most difficult part in organising this workshop was to spot, find and contact experts from the target countries. Tools for professional networking which, allow direct access to the expert need to be devised and promoted.

A SWOT analysis being held during the workshop, together with the survey results presented by the country experts served as seed for this report intended to serve as a recommendation for target countries.

A social media connection has been formed for maintaining and continuing contact among all participants which, is also open for new members to join in order to establish a networked community.

Regional and International cooperation should be further enhanced, and guided, if possible by the EU, since this allows stronger acceptance by the local authorities/decision makers in implementing new policies regarding public health.
7. RECOMMENDATIONS

- It is very important to establish recognized objective state-of-the-art guidelines for application of genomic technologies in clinical practice. Such guidelines adopted by countries will form the basis of reimbursement policies at national and cross border levels.

- It is very important to establish reliable, not for profit, open access databases for building reference datasets for correct and efficient interpretation of complex data generated by advanced genomic technologies.

- Medical genetics has to be recognised as a medical specialty both at clinic and laboratory levels.

- The genomics field should take a systems approach* especially to whole-genome sequencing, which will require important changes by government, healthcare providers, and patients.

- There should be more collaboration between clinical entities and laboratories, a greater emphasis on the fact that some parts of the genome will remain refractory to analysis, and public to laboratories to establish databases that can be used to refine and deliver genomic medicine.

- Informatics capabilities should be leveraged to create clinical genotype–phenotype databases, education should be improved, and reimbursement should be set at levels that make it possible for the healthcare system to do analytical thinking about how best to serve patients.

- There should be greater interoperability of medical records systems (Electronic Health Records, EHR) so that information relevant to health care follows people throughout life and that genomic information is always accessible for further innovation.

- A universal healthcare information technology system should be established that includes both genetic and clinical information, and barriers to data sharing should be reduced.

- There should be funding for education, novel research to explore gene–phenotype relationships, and improved sequencing technologies.

- More emphasis should be placed on genetics and genomics in medical schools.

* Systems approach is defined as an interdisciplinary method of study that involves consideration of all the components involved in a process and their interactions with each other.
REFERENCES

Mardis ER., Anticipating the 1,000 dollar genome, Genome Biol. 2006; 7(7):112.


### 8. ANNEX 1

#### Table 1 Results of the survey

| 1. Which of the following new/emerging technology platforms do you employ in your laboratory? | a- BUL, POL, UK  
|                                                                                         | b- BUL  
|                                                                                         | c- TR, BH, CRO, POL, UK  
|                                                                                         | d- BUL, POL  
|                                                                                         | e- BUL  
|                                                                                         | f- RO  
| a. Microarrays for expression profiling mRNA/miRNA  
| b. Next Generation Sequencing  
| c. Real-Time PCR  
| d. Array CGH  
| e. More than one of these  
| f. None of these  

| 2. Which of the following new/emerging technology platforms are employed regularly in the clinical context in your country? | a- POL, UK  
|                                                                                         | b- TR, RO, UK  
|                                                                                         | c- POL, CRO, TR, RO, BUL, UK  
|                                                                                         | d- POL, TR, RO, BUL, UK  
|                                                                                         | e- UK  
|                                                                                         | f-  
| a. Microarrays for expression profiling mRNA/miRNA  
| b. Next Generation Sequencing  
| c. Real-Time PCR  
| d. Array CGH  
| e. Chromosomal microarrays  
| f. None of these  

| 3. In your opinion which technology platform will penetrate fastest into clinical applications? Please justify very briefly | a- NONE  
|                                                                                         | b- TR, RO, BUL, UK  
|                                                                                         | c- POL, BOS, RO, UK  
|                                                                                         | d- CRO, BOS, RO  
|                                                                                         | e- TUR, BUL, RO  
| a. Microarrays for expression profiling mRNA/miRNA  
| b. Next Generation Sequencing  
| c. Real-Time PCR  
| d. Array CGH  
| e. Chromosomal Microarrays  
| f. None of these  

| 4. In your opinion which application area of genomic technologies will the clinic benefit from the earliest? Please justify very briefly | a- CRO, POL, BOS, RO, BUL, UK  
|                                                                                         | b- CRO, BOS, RO, BUL, UK, TR  
|                                                                                         | c- POL, BUL, UK, TR  
|                                                                                         | d- POL, RO, UK  
|                                                                                         | e- TR, BUL  
|                                                                                         | f-  
| a. Cancer genotyping  
| b. Prenatal testing  
| c. Preimplantation genetic diagnosis  
| d. Rare diseases  
| e. Personalised medicine  
| f. Some other area (please specify)  

---

**Note:** The tables are structured with headers indicating the survey questions and the options provided, followed by the corresponding responses from different countries.
5. What is the scope of health/social security/insurance system coverage in your country for:

<table>
<thead>
<tr>
<th>a. Prenatal testing employing conventional karyotyping</th>
<th>a- CRO, POL, BOS, TR, RO, BUL, UK</th>
</tr>
</thead>
<tbody>
<tr>
<td>b. Prenatal testing employing molecular karyotyping (microarrays)</td>
<td>b- POL BY SPECIAL PROGRAMMES, RO, UK</td>
</tr>
<tr>
<td>c. Sequencing for disease diagnosis (common/rare)</td>
<td>c- POL AS SUPPLEMENTARY DIAGNOSTICS, TR, BUL, UK</td>
</tr>
<tr>
<td>d. Next generation sequencing for disease diagnosis (common/rare)</td>
<td>d- UK</td>
</tr>
<tr>
<td>e. Pharmacogenomic testing for drug responsiveness</td>
<td>e- CRO, POL COVERED AS PART OF CANCER GENOTYPING</td>
</tr>
<tr>
<td>f. Preimplantation genetic diagnosis</td>
<td>f- TR FOR DISORDERS WHICH CAN BE TREATED BY STEM CEL TRANPLANTATION, UK</td>
</tr>
</tbody>
</table>

6. Which type of testing is offered as a regular service by a state/private laboratory in your country, please indicate as SL or PL

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>b. Prenatal testing employing molecular karyotyping (microarrays)</td>
<td>b- POL SL/PL, TR PL, RO SL/PL, BUL PL, UK SL/PL</td>
</tr>
<tr>
<td>c. Sequencing for disease diagnosis (common/rare)</td>
<td>c- POL SL/PL, BOS SL, TR SL/PL, BUL SL/PL, UK SL</td>
</tr>
<tr>
<td>d. Next generation sequencing for disease diagnosis (common/rare)</td>
<td>d- TR PL, BUL PL, UK SL</td>
</tr>
<tr>
<td>e. Pharmacogenomic testing for drug responsiveness</td>
<td>e- CRO PL, POL SL/PL, TR PL, BUL PL</td>
</tr>
<tr>
<td>f. Preimplantation genetic diagnosis</td>
<td>f- POL PL, TR PL, BUL PL, UK PL</td>
</tr>
</tbody>
</table>

7. How often do you collaborate with a laboratory outside your own country for research/diagnostic purposes? Please state scope (e.g. rare disease diagnosis, populations study, etc.)

<table>
<thead>
<tr>
<th>CRO: RDD ONCE EVERY THREE TO SIX MONTHS</th>
</tr>
</thead>
<tbody>
<tr>
<td>POL: RDD / RESEARCH ONCE OR TWICE A YEAR</td>
</tr>
<tr>
<td>BOS: RDD UPTO 10-15 TIMES A YEAR</td>
</tr>
<tr>
<td>TUR: RDD FEW TIMES A MONTH, RESEARCH ONCE A FEW YEARS</td>
</tr>
<tr>
<td>RO: NO REPLY</td>
</tr>
<tr>
<td>BUL: RDD ONCE A MONTH, RESEARCH ONCE EVERY 3 MONTHS</td>
</tr>
</tbody>
</table>

8. In your opinion what is the biggest obstacle in your country in particular for penetration of genomic applications in clinical services?

<table>
<thead>
<tr>
<th>CRO: ORGANISATION AND FINANCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>POL: DISPERSION OF PROCEDURES OVER THE LIST OF GUARANTEED SERVICES (so called basket), LACK OF COMPREHENSIVE FINANCIAL/EDUCATIONAL PROGRAMS SUPPORTING RESEARCH IN CLINICAL GENETICS</td>
</tr>
<tr>
<td>BOS: LACK OF POLICIES REGULATING GENETIC TESTING SERVICES IN FRAME OF MEDICAL</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th><strong>9. Is medical genetics recognised as a “medical specialty branch” in your country’s education system?</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CRO:</strong> ONLY FOR MEDICAL DOCTORS AS A CLINICAL SPECIALITY (NO LABORATORY SPECIALITY, NOT OFFERED TO NON MEDICINE ORIGIN STUDENTS)</td>
</tr>
<tr>
<td><strong>POL:</strong> YES, NOT AS A LABORATORY SPECIALTY</td>
</tr>
<tr>
<td><strong>BOS:</strong> NO</td>
</tr>
<tr>
<td><strong>TUR:</strong> YES, NOT AS A LABORATORY SPECIALTY</td>
</tr>
<tr>
<td><strong>UK:</strong> YES</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>10. Name three measures at local authorities’ level that will benefit incorporation of genomic applications in healthcare services offered in your country</strong></th>
</tr>
</thead>
</table>
| **CRO:** 1. PRIORITISATION FOR INCLUSION BACKED UP BY MAPPING OF COMPETENCES, METHODS AND SOURCES  
2. STANDARDISATION AND HARMONISATION OF SERVICES (QUALITY ASSURANCE)  
3. EDUCATION AND DISSEMINATION OF KNOWLEDGE |
| **POL:** 1. INTRODUCTION OF A REGULATED AND HARMONISED QUALITY CONTROL SYSTEM  
2. LEGAL REGULATIONS THAT ALLOW MORE COMMON USE OF PRENATAL AND PREIMPLANTATION DIAGNOSTICS AND STATE COVERAGE  
3. MESURES TO IMPROVE COMMUNICATION BETWEEN THE CLINIC AND RESEARCH LABORATORIES |
| **BOS:** 1. DEVELOPMENT OF A LIST OF MOST FREQUENTLY REQUIRED TYPES OF GENETIC TESTING AND RECOGNITION FOR PUBLIC HEALTH COVERAGE  
2. DEVELOPMENT OF EXPERT PANEL(S) FOR GENETIC COUNSELING SERVICES  
3. POLICY REGULATING QUALITY AND HARMONISATION OF GENETIC TESTING SERVICES FOR ALL LOCAL AND INTERNATIONALLY FUNCTIONING LABORATORIES |
| **TUR:** 1. STANDARDISATION AND HARMONISATION OF QUALITY OF SERVICES VIA A REGISTRY  
2. RECOGNITION OF AVAILABLE TESTING AND PUBLIC HEALTH COVERAGE, UPTODATE |
<table>
<thead>
<tr>
<th>HEALTH IMPACT AND TECHNOLOGY ASSESSMENT BY INDEPENDENT EXPERT PANELS</th>
</tr>
</thead>
<tbody>
<tr>
<td>3. AWARENESS RAISING AMONG PHYSICIANS AND PATIENTS (CREATE PUBLIC DEMAND)</td>
</tr>
</tbody>
</table>

**RO:**
1. COMPLETION AND IMPLEMENTATION OF THE NATIONAL PLAN FOR RARE DISEASES
2. DEFINITION AND INVENTORY FOR CENTERS OF EXPERTISE
3. HARMONISED QUALITY ASSURANCE

**BUL:** NO REPLY

**UK:** STANDARDISATION, PUBLIC HEALTH, BETTER BUDGETTING (CUSTOMISED CANCER DRUGS)
ANNEX II

Applied Genomics in the Clinic
Istanbul University Rectorate Campus Kiliçk Hall

17-19 October 2012

IHCP-JRC Enlargement and Integration Activities (E&IA)
Our mission is to provide scientific and technical support to the EU policies for the protection of the interests and health of European citizens in the areas of food, consumer products, chemicals and public health.
DG Enlargement policy

The EU’s enlargement policy, as enshrined in the Treaty on European Union, is the response to the legitimate aspiration of people of our continent to join the endeavour of a unified Europe.

- Manage the process whereby countries join the European Union, under the guidance of the Commissioner for Enlargement.
- Assist candidate countries and potential candidates in meeting the Copenhagen criteria, monitor their progress and embracing the objectives of the Europe 2020 strategy.
- Define and implement the EU's stabilisation and association policy in the Western Balkans.
- Manage the Commission’s information and communication policy relating to enlargement in candidate countries and potential candidates.

How to get a successful meeting

- Apply win-to-win concept
- Sharing knowledge
- Improve communication
- Strengthen networking

Final goal

Produce a report with the outcome of the workshop, develop recommendations by participants countries
HEALTH SERVICES

- 2 University Hospital
- Cardiology Institute
- Oncology Institute
- Faculty of Dentistry

- 3500 bed capacity
- 2.5 million outpatient/year
- 100,000 inpatient/year
Istanbul University has 7 Institutes having 492 postgraduate programmes. They are 254 master and 238 doctorate programmes.

- Institute of Business Economy
- Institute of Forensic Sciences
- Institute of Marine Sciences and Management
- Institute of Health Sciences
- Institute of Basic and Applied Sciences
- Institute of Social Sciences
- Institute of Atatürk’s Principles and Reforms

(11 Master)
(3 Master, 3 Doctorate)
(9 Master, 9 Doctorate)
(83 Master, 79 Doctorate)
(57 Master, 54 Doctorate)
(90 Master, 92 Doctorate)
(1 Master, 1 Doctorate)

Istanbul University, Institute for Experimental Medicine (DETAE)

Five Departments
- Genetics
- Immunology
- Molecular Medicine
- Neurosciences
- Animal Model Organisms
Istanbul University, Institute for Experimental Medicine (DETAE)

Five Units/Laboratories
- Tuberculosis molecular epidemiology unit
- Diabetes research and application unit
- Molecular andrology unit
- Whole genome sequence lab (FLX-454-Ion torrent)
- Whole genome expression array unit (Illumina)
Results of Funding Call

The following project proposals have been suggested for funding by the Thematic Panel based on scientific excellence and by the Call Screening Committee based on budget availability.

The funded projects are:

- Alzheimer’s Disease and Parkinson’s Disease

Network of Centres of Excellence

- Alzheimer’s Disease and Parkinson’s Disease

Coordinator: Bengt Winblad, Karolinska Institute Alzheimer Disease Research Center, Sweden
In Summary...

- Tasks driven by DETAE:
  - Combining multidisciplinary research and education under one ceiling.
  - Coordination of the postgraduate programs on Immunology, Genetics, Molecular Medicine, Neuroscience of the Istanbul University
  - Conducting competitive international projects in medical sciences through development of novel approaches for the prevention and diagnosis of common human diseases.
Application of Next Generation Sequencing in Human Disease Research and Clinical Application

Jeremy Sujie Cao

Oct. 17th
Workshop on “Applied Genomics in the Clinic”
Haplotype Map of the Human Genome

The goal of the International HapMap Project is to compare the genetic sequences of different individuals to identify chromosomal regions where genetic variants are shared.

- The first GWAS paper about age-related macular degeneration was published in Science in 2005.
- More than 1,000 total publications until now.
- More than 4,000 associated SNPs related with common diseases such as diabetes, breast cancer have been identified and replicated in GWAS.

www.genomesci.cn

1080 Genomes
A Decade Catalogue of Human Genetic Variation

International project to construct a next generation baseline data set for human genetics

- 45ms
  - Find 95% accessible SNPs at allele frequencies above 1%, down towards 0.1% in coding regions
  - Genotyping them and place on haplotype backgrounds
  - Also discover and characterize indels, structural variants

www.genomesci.cn

Genome of the Netherlands

In the next few months, the genomes in 750 samples from Dutch biobanks will be analysed by BGI, the Beijing Genomics Institute, based in Shenzhen, which today has the best experience in high throughput sequencing. The

Unique in-depth perspective on regional genetic variants
Are rare and novel variants more functional?

*Extreme cases--Mendelian Disorders*

**Sequencing Technologies**

- **Whole genome resequencing**
  - Completeness
  - Long insertions/deletions, structural variations, CNVs detection
- **Whole Exome sequencing**
  - Cover majority of causative mutations of MDs
  - Cost-effective
- **Target capture sequencing**
  - Economical
  - Pre-knowledge of Candidate region

---

**BRAIN**

TGM6 identified as a novel causative gene of spinocerebellar ataxias using exome sequencing

Wang IL, et al., 2010
Are rare variants more functional?

Exome Sequencing Identifies ZNF644 Mutations in High Myopia

An extreme case to show that rare variants have stronger effects in Mendelian Disorder

Gene Accompishments: Base Pairs to Bedside

Exome sequencing identifies MVK mutations in disseminated superficial actinic porokeratosis

- We performed exome sequencing in one unaffected and two affected individuals from a DSAP family. The mevalonate kinase (MVK) emerged as the only candidate.
- Sanger sequencing in 57 individuals with familial DSAP and 25 individuals with sporadic DSAP identified MVK mutations in 58% and 16% of these individuals (cases), respectively. All 14 MVK mutations identified in our study were absent in 676 individuals without DSAP.
Objective

- Personal genome & Personalized medicine
- To discover the relationship between phenotype and genotype.
- To understand the baseline and build the reference standard of the population.

Our genetic testing strategy

- Before marriage
- Before pregnancy
- Prenatal
- Newborn

- Screening
- Screening/Diagnosis
- Screening/Diagnosis
- Screening/Diagnosis
What is monogenic disease?

You may also heard single-gene disease, rare disease, Mendelian disease

Background

- a single mutated gene, Mendelian pattern of inheritance
- point mutation, deletion, insertion, frame shift
- Autosomal dominant/recessive; X-linked dominant/recessive; Y-linked
- >7000 monogenetic diseases, 10-50 more every year.
- 1 in every 200 newborns
- Genetic testing is the gold standard
Genetic test methods

- Nucleic acid amplification (qPCR, RT-PCR, MLPA)
- Sequencing (Sanger, NGS)
- array CGH, SNP array

- PCR and Sanger sequencing are the two main techniques used in domestic market; screening ability is poor, diseases available for testing are limited
- Lack of genetic disease database, poor in analysis ability
- Very few specialized hospitals and major general hospitals can perform monogenetic disease genetic testing

Technical strategy at BGI

- Target sequence capture combined with NGS
- Exome/whole genome sequencing
- Sanger sequencing
Target sequence capture + NGS

Technique principle

- A customized chip containing a series of oligonucleotide probes that specifically recognize the interested sequence is generated. DNA sequence of target area can then be captured by hybridizing to the probes on chip, following the NGS and bioinformatics analysis.

Databases

- dbSNP frequency
- Hapmap frequency
- 1000 genome project
- Local 100 genome database
- Normal persons SNP database
- OMIM
- LOVD
- Newly reported
- Single-gene disease variants database


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Advantages

• High throughput
  Multiple testing, detect 144 monogenic diseases (over 300 genes)

• Wide detection scope
  Simultaneously detect point mutation, minor insertion/deletion (<20bp),
  and large homoygous deletion/duplication; able to find novel mutation

• Accurate and sensitive
  Covers >95% target gene, sensitivity >99%

• Automated bioinformatics analysis
  Fast mutation identification; disease database and genetic polymorphism
database available

Examples of diseases

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute Myeloid Leukemia</td>
<td>Leukemia is a type of cancer that starts in the blood-forming tissues such as bone marrow.</td>
</tr>
<tr>
<td>Chronic Myeloid Leukemia</td>
<td>Leukemia is a type of cancer that starts in the blood-forming tissues such as bone marrow.</td>
</tr>
<tr>
<td>Myelodysplastic Syndrome</td>
<td>Leukemia is a type of cancer that starts in the blood-forming tissues such as bone marrow.</td>
</tr>
<tr>
<td>Polycythemia Vera</td>
<td>Leukemia is a type of cancer that starts in the blood-forming tissues such as bone marrow.</td>
</tr>
<tr>
<td>Essential Hemolytic Anemia</td>
<td>Leukemia is a type of cancer that starts in the blood-forming tissues such as bone marrow.</td>
</tr>
</tbody>
</table>
Report

- 30-50 days
- Sequencing quality assessment (depth, coverage)
- Variation sites including deletion/duplications and single base variations (dbSNP frequency, HapMap frequency, 1000 genome frequency, local database frequency)
- Possible mutations (nucleotide change – amino acid change)
- Interpretations
- Reference
Who will have the test?

- Patients with clear phenotypes
- Patients with suspected disease but not sure
- People with no phenotypes but have family history
- Carrier screening

- Assisting diagnosis: provide genetic proof for uncertain phenotypes
- Research collaboration: deeper understanding of genetics, human mutations

Case: Duchenne/BeckerMuscular Dystrophy

- 1 in 3500 live boy affected, 2/3 inherited from parents
- Muscle progressively dysfunction and sweeney. Sub-divide to DMD (OMIM 310200) and BMD (OMIM 310376).
- DMD onset at age 2-8, immobilize at 15, kill at 20s due to severe complications. X-recessive, mainly occur in boys.
- Mutation of DMD gene (Xp21.2-Xp21.3, 76 exons) causes Dystrophin change

DMD homozygous deletion

- About 60% of DMD is caused by deletion. There are rare cases caused by micro-duplication (5-10%). The rest is caused by point mutation or frame shift which can take place in the entire coding region. Because DMD has very long and large numbers of exons, conventional methods such as Sanger sequencing is too costly and time-consuming.
DMD heterozygous deletion

DMD duplication
Case: Gavin’s Story
Whole Exome Sequencing Finds Mystery Mutation by BGI

With the Whole Exome Sequencing, BGI found a new LCA mutation in 3 samples of Gavin’s family.

Exome sequencing identifies NMNAT1 mutations as a cause of Leber congenital amaurosis

- We sequenced the exome of an individual with LCA and identified nonsense (c.5076A>T, p.Tyr1695*) and missense (c.7696G>A, p.Glu257Asp) mutations in NMNAT1, which encodes an enzyme in the nicotinamide adenine dinucleotide (NAD+) biosynthesis pathway implicated in protection against axonal degeneration.
- We also found NMNAT1 mutations in ten other individuals with LCA, all of whom carry the p.Glu257Asp variant.

Figure 1. Schematic of the NMNAT1 gene depicting NMNAT1 variants identified in individuals with LCA.

Disease list of Whole exome sequencing

<table>
<thead>
<tr>
<th>Disorder/condition</th>
<th>Number of genes</th>
<th>Note</th>
<th>Service type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monogenic disease</td>
<td>405</td>
<td>(1) Detect a wide range of diseases/disorders with high sensitivity and efficiency: Complete coverage of the target gene: High-throughput results</td>
<td></td>
</tr>
<tr>
<td>Congenital tumour</td>
<td>54</td>
<td>Basic</td>
<td></td>
</tr>
<tr>
<td>Hereditary malignant</td>
<td>48</td>
<td>(2)</td>
<td></td>
</tr>
<tr>
<td>Polyneuropathy</td>
<td>157</td>
<td>(3)</td>
<td></td>
</tr>
<tr>
<td>Pharmacogenetics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Personal characteristics</td>
<td>54</td>
<td>Optional</td>
<td></td>
</tr>
<tr>
<td>Drug response of tumour targeted therapy</td>
<td>5</td>
<td>Medication guidance of certain anti-cancer drugs</td>
<td></td>
</tr>
<tr>
<td>High-resolution genetic typing</td>
<td>6</td>
<td>Human major histocompatibility complex testing required before hematopoietic stem cell or organ transplantation</td>
<td></td>
</tr>
<tr>
<td>Leukaemia fusion gene</td>
<td>4</td>
<td>Therapeutic assessment of leukemia treatment</td>
<td></td>
</tr>
<tr>
<td>Personal genetic information storage</td>
<td>N/A</td>
<td>Storing personal genetic information for clinical information privacy will be guaranteed</td>
<td></td>
</tr>
<tr>
<td>Result update</td>
<td>N/A</td>
<td>As scientific and medical research progress, more diseases and characteristics will be interpreted and analyzed</td>
<td></td>
</tr>
</tbody>
</table>
**BGI in Denmark**

- **Service**
- **Knowledge Discovery and Innovation**
- **BGI Europe A/S**
- **BGI Europe Institute (Foundation)**
- **Genome platform (10 Hiseq2000)**

---

**Nijmegen adopt exome sequencing as part of their routine diagnostics practical**

- In the past two years Nijmegen have built the experience in analyzing over 1000 clinical exomes and the unprecedented power of Next Generation Sequencing (NGS) applied to clinical genetics hold the promise of changing the current paradigm of genetic testing.

- Nijmegen have recently (December, 2011) acquired accreditation by the Dutch accreditation Council, accepting whole exome sequencing as a clinical diagnostic test.

---

**The NUM-BGI joint lab**

- **Making the joint lab work:**
- **Diagnostic questions**
- **Sample preparation**
- **Analysis of pathogenic and non-pathogenic variants**
- **Variant validation**
- **Clinical interpretation**
- **Diagnostic reporting**
- **Quality control**

**Platforms**

**Knowledge**

**Expertise**

**One space where people work together**

The joint organization

- 4,000 exomes in 2013-2015 for the Netherlands

**Expanding in 2018-2019**

**Numbers ≠ 10,000 exomes**
NUM-BGI Joined Lab Workflow

Data analysis and interpretation (BGI and NUM together)

Genetics Consultancy

Report

What’s in maternal blood?

Components of Maternal Blood

Establishment of the Non-invasive fetal Trisomy test (NIFTY) using maternal plasma

1997
Cell-free DNA in maternal blood

2007
NGS Platform

2008
Technical pipeline development and QAQC

2009-2010
Phase I: Clinical trial

2011
Phase II: Clinical validation
### Clinical Validation Study (Phase 1, Double-blinded)

<table>
<thead>
<tr>
<th>NIFTY Test</th>
<th>Positive No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>T21</td>
</tr>
<tr>
<td>NIFTY Test No.</td>
<td>3464</td>
</tr>
<tr>
<td>Karyotyping No.</td>
<td>3464</td>
</tr>
<tr>
<td>False positive No.</td>
<td>1*</td>
</tr>
<tr>
<td>False Negative No.</td>
<td>0</td>
</tr>
<tr>
<td>Detection Rate</td>
<td>100.00%</td>
</tr>
<tr>
<td>False Positive Rate</td>
<td>0.03%</td>
</tr>
<tr>
<td>Positive Predictive Rate</td>
<td>95.42%</td>
</tr>
<tr>
<td>Specificity</td>
<td>99.97%</td>
</tr>
<tr>
<td>False Negative Rate</td>
<td>0.00%</td>
</tr>
</tbody>
</table>

### BGI Papers on NIFTY

**Noninvasive Fetal Trisomy Test (NIFTY): An Advanced Noninvasive Prenatal Diagnosis Methodology for Fatal Autosomal and Heterosamal Aneuploides**

- [Paper Link](#)

**Prenatal Detection of Aneuploidy and Imbalanced Chromosomal Arrangements by Massively Parallel Sequencing**

- [Publication](#)

**Noninvasive prenatal diagnosis of common fetal chromosomal aneuploidies by maternal plasma DNA sequencing**

- [Article](#)

---

*Note: The above references may need to be expanded for a comprehensive understanding.*
<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Accuracy</td>
<td>Digital sequencing with high throughput (Solexa sequencing)</td>
</tr>
<tr>
<td>Early Pregnancy Screening</td>
<td>Screening can be performed starting from the gestation period of 12 weeks, which allows early detection for a better decision and better health benefits.</td>
</tr>
<tr>
<td>Non-invasive</td>
<td>Reduce mental stress of pregnant women, free of miscarriage risk and less risk of complications which may occur in antenatal diagnosis.</td>
</tr>
<tr>
<td>High Precision</td>
<td>99% sensitivity, 99% specificity</td>
</tr>
<tr>
<td>Simple Sampling</td>
<td>Only 3-5 mL of maternal peripheral blood are required</td>
</tr>
</tbody>
</table>

BGI Certificates

- Practice License of Medical Institution
- Collaborative lab for CMOP
- HLA EQA certificate
- UCLA HLA DNA exchange PT
- 60 TRIS
- Client PIC
- ESOP
**BGI dry lab-computing facilities**

<table>
<thead>
<tr>
<th>Year</th>
<th># CPUs</th>
<th>Flops</th>
<th>RAM</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006.01</td>
<td>1,500</td>
<td>18T</td>
<td>4TB</td>
<td>2PB</td>
</tr>
<tr>
<td>2006.08</td>
<td>3,000</td>
<td>50T</td>
<td>10TB</td>
<td>5PB</td>
</tr>
<tr>
<td>2006.12</td>
<td>5,000</td>
<td>100T</td>
<td>20TB</td>
<td>10PB</td>
</tr>
<tr>
<td>2011.09</td>
<td>50,000</td>
<td>1,000T (1P)</td>
<td>200TB</td>
<td>1,000PB (1EB)</td>
</tr>
</tbody>
</table>

**Acknowledgement**

**NEXT GENERATION SEQUENCING**

*Made it easy by Illumina*

**ELTA 90**

Trained specialists in San Diego and Cambridge employed by our own company
A Sequencer for Every Need. Every Budget. Every Lab.
The Market Leader in Life Science and Clinical Approach
NGS Terms: Coverage, Uniformity, Sensitivity, Throughput, Capacity, Cluster
density, Raw accuracy, Q scores, Deep Sequencing, Number of reads,
Alignments, Bioinformatics Data Analyses, Homopolymer indel errors etc.

HiSeq 2500
Combining innovation

- Clustering on-board
- Fast Chemistry
- Longer Reads

HiSeq 2000

- Genome in a day
- Clustering on-board
- Complete walk-away workflow
- Longer 2x150 reads
- Data rate
- TG1 scanning
- Larger flow cell

Illumina

1 human genome in a day
3,500 gene diseases in Neonatal screen
6 human genomes in 10.5 days

Fastest Publication Rate

> 3000 peer-reviewed publications to date with Illumina sequencers
Illumina Sequencing Workflow

1. DNA (0.05-1.0 μg)
   - Library Preparation
   - Cluster Growth
   - Sequencing
   - Image Acquisition
   - Base Calling

Sequencing by Synthesis

1. Incorporation
   - Primer

Sequencing by Synthesis

1. Incorporation
2. Scan
Sequencing

Paired end sequencing

Paired End Sequencing

- Sequenced strand is stripped off
- 3'-ends of template strands and lawn primers are unblocked

Blocked 3'-ends

Sequenced strand
Paired End Sequencing

- Single-stranded template loops over to form a bridge by hybridizing with a lawn primer.
- $3'$-ends of lawn primer is extended.

Paired End Sequencing

- Double-stranded DNA.

Paired End Sequencing

- Bridges are linearized and the original forward template is cleaved off.
Paired end sequencing

- Free 3’ ends of the reverse template and lawn primers are blocked to prevent unwanted DNA priming.
- Sequencing primer is hybridized to adaptor sequence.

Paired-End Sequencing
Critical for a Broad Range of Applications

Sequence both ends of DNA fragment
Unique placement of one end can resolve ambiguous placement of other
De novo assembly of E.coli bacterium
  - Pair inferred: largest assembled fragment ~120 kb
  - Pair inferred: largest assembled fragment ~320 kb
Key applications benefitting from paired end reads:
  - Structural variant detection and screening
  - Overlapping reads for applications needing ultra-high sensitivity

MiSeq – Single Instrument Workflow
The World’s Most Widely Adopted Sequencing Technology Just Got Personal

2011: MiSeq launch, NGS in routine

Included On Instrument:
- Cluster Generation
- Paired-End Fluctics
- Composing for Primary and Secondary Analysis
**MiSeq Instrument**
*Next-Gen Made Simple: Load & Go*

*DESIGNED FOR THE WAY YOU WANT TO WORK*
- Preloaded single-use reagent cartridge
- Positive consumables tracking
- Auto flow cell positioning
- Walkaway automation

**MiSeq System update**

System launched Sept 2011
Rapid Benchtop sequencer

*The most accurate and highest throughput benchtop NGS machine*

Loman et al 2012

**MiSeq Reporter**
*Built-in, walkaway bioinformatics*

- Simple on-premise bioinformatics computer built into the instrument
- No user intervention required from sample loading to report generation
- Custom bioinformatics reports for
  - Resequencing
  - Amplicon and Cancer panel resequencing
  - Small RNA
  - De novo assembly
  - 16S metagenomics
  - Library QC
- Outputs in .fastq and .bf format for maximum flexibility in downstream data analysis
- Outputs .bam and .vcf for maximum compatibility with any tertiary analysis solution
- Simple to read graphical reports accessible via any browser
What?
BaseSpace is Illumina’s genomic cloud computing environment

- Eliminates need for on-site storage and compute
- Web-based data management and analysis
- Tools for collaboration and sharing
- Available for Illumina and non-Illumina customers

MiSeq Pushes Data to BaseSpace
BaseSpace
The Best Place to Store Your NGS Data

- Automatic push from MiSeq
- Secure and reliable
- Simple to use

BaseSpace

Reads and qualities
Sample and experiment descriptions
Analysis results
variants
expression
methylation
covariance
intensity
without errors
now...

BaseSpace Partners

Announcing Initial App Partners:

- Key Vendors in clinical interpretation, annotation, and visualization.
- Differing data models (thick client, web services, hybrid)
- Differing data usage (fastq, vcf, BAM)
- Twenty additional vendors working to deploy in BaseSpace soon.

BaseSpace Apps

Top 10 Apps

- Avadis NGS
- Know the limits
- iSeq 1000
- i7
- i5
- KROMA
- i5/i7
- i5/i8
- i5/mi10
- i5/mi20
MiSeq Amplicon Viewer

Nextera and MiSeq
Sequencing’s fastest time to answer for rapid variant analysis
**Author's Performance Summary**

- **MiSeq**
  - Lowest error rate
  - Highest throughput per run
  - Virtually non-existent homopolymer-associated indel errors
  - Simplest workflow

- **454 GS Jr**
  - Generous longest reads
  - Most contiguous assemblies
  - 0.38 homopolymer-associated indel errors per 100 bp

- **IT PGM**
  - Fastest, hourly throughput
  - 1.5 homopolymer-associated indel errors per 100 bp

---

**MiSeq Had the Highest Quality Data**

- MiSeq generated high quality data greater than Q30 along the length of the read
- The PGM yielded no data above Q30 and a significant fraction below Q20
- The use of a reference based quality scoring system allows for direct comparison of data from each system

> While NGS data can always be confirmed by Sanger sequencing, “it’s much nicer if you can put the sample in and walk away with a strong answer without having to do too much extra validation.” Nick Loman

---

**MiSeq Had Virtually No Discernable Homopolymer Associated Errors**

- MiSeq had <0.001 indel errors per 100bp
- Serial addition chemistries suffered higher rates of homopolymer associated indel errors: 0.38 for GS Junior and 1.5 for PGM per 100bp

Discarding true indels from homopolymer-induced indels can never be fully addressed via software thus raising the potential for debatable errors including frame shifts.
MiSeq at the Broad Institute

- Presentation from Sheila Fisher
- No hardware failures
- No chemistry failures

Nextera XT DNA Sample Prep
The fastest & easiest prep for small genomes, PCR amplicons and plasmids

- Rapid Prep
  - 50 min prep, only 15 min hands-on time
- Optimized for small genomes, PCR amplicons and plasmids
- Innovative sample normalization
  - No library quantification needed
- Fastest time to results
  - DNA analyzed data in 4 hours with MiSeq
- Ultra low input
  - only a single nanogram of input DNA needed

Extending MiSeq performance

<table>
<thead>
<tr>
<th>MiSeq</th>
<th>Yield</th>
<th>Read Length</th>
<th>Number of Reads</th>
<th>Run time</th>
<th>Quality at 2x150bp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.5-2G</td>
<td>2x150</td>
<td>5-7 Million clusters PF</td>
<td>27 Hours for 2x150</td>
<td>75% bases ≥ Q30</td>
</tr>
</tbody>
</table>

Improvements in Chemistry and Imaging
- Increased imaging area
  - Acqix® second surface
- Improved SBS polymerase allowing faster kinetics
  - Chemistry cycle time of app. 2 mins (from 3 mins)
- Novel reagent formulation optimized for the MiSeq platform
NGS Application – Life Science and Health Care

„The 21st Century vaccination“

Did the Crack of Human DNA code are contribute to Medicine?

Predictive medicine

Which loci are responsible for developing of Cardio or other chronic diseases – GWAS studies

CPH gene – AMD high risk

SNP arrays – utility in clinical cytogenetics

- Constitutional –
  - ISCA and ACMG recommend testing with microarray first for following referrals:
    - Mental retardation (MR), Multiple congenital abnormalities (MCA), autism spectrum disorders (ASD), suspected microdeletion/duplication syndrome, uT syndromes
- Preimplantation Genetic Screening (PGS/PGD) Illumina = Bluegnome
  - Increase the number of successful pregnancy rates
  - Avoid inheritance of Mendelian disorders
  - Higher density in 447 “disease genes

All pericentromeres and subtelomeres
Sex chromosomes
Common regions of interest (e.g., associated with skeletal syndromes)
Regions contain ~9000 genes
Application of SNP array for rapid prenatal diagnosis: implementation, genetic counselling and diagnostic flow

M. J. D. Smith, M. J. D. N. Smith, J. D. M. Smith, J. D. N. Smith
European Journal of Human Genetics (2011), 1-3

- 64 samples to validate the Illumina platform using Human CytoSNP-12 (HCS)
  - 93 with known (sub)microscopic chromosome anomalies, 5 with known maternal cell contamination (MCC) and 29 normal controls samples.
- No false-positive or false-negative results.
- Prospective pilot study of 81 fetuses with ultrasound abnormalities and a normal karyotype tested with HCS
  - In 4 out of 61 (6.5%) fetuses, a clinically relevant abnormality was detected.

Genomic SNP array as a gold standard for prenatal diagnosis of foetal ultrasound abnormalities, Now in Erasmus Medical University

- Replaced karyotyping by Human CytoSNP12 array in referrals of foetal UDS abnormalities because.
- HCS detects all clinically relevant unbalanced chromosome abnormalities also detected by karyotyping (including trisomy).
- HCS has 25-50X higher resolution than karyotyping -> genome wide screening for microdeletions and duplications
- Employ on uncultured tissue (Fetal)
- Faster than karyotyping (most result in one week)
- Risk of undetected low level mosaicism is supported
- SNP analysis now the preferred technique

MiSeq
Screening

- Germ line carrier screen for mutations that cause Mendel disease
  - Cystic fibrosis, Bloom syndrome, Caravan disease, Fanconi anemia, Neurofibromatosis, Thalassaemia disease, Gaucher’s disease, Niemann-Pick disease type A, Peutz-Jeghers syndrome group C, Sick cell anemia, Beta thalassaemia, Beta thalassaemia, Spinal muscular atrophy
- Larger panel of genes supported by literature may be included
- Forensic
- HLA Typing - Stanford University, Palo Alto, CA 94303
  - NPS-202
Non Invasive Chromosomal aberration such as trisomy 21 - Validated on Illumina HiSeq2000

Non-invasive prenatal diagnosis by single molecule counting technologies

Prenatal Trisomy Testing

Sequenom, Arta Diagnostics, Verinata

LifeCodexx in Germany, 1st Step licensee, obtained the CE-IVD Mark
Sequenom: $1,700
Verinata: $1,200
Arata Diagnostics: $995
LifeCodexx (Germany): 1,250 EUR

What can exome sequencing do for you?

- Identifying genetic variants
- Characterizing monogenic (Mendelian) disorders
- Identification of novel mutations
- Characterizing Complex trait disorders
- Characterizing Cancer
Whole Exome Sequencing help us diagnose correctly and solve Undiagnosed Mysteries

Nicholas Volkov, a 5-year-old boy. The first year he was affected by extreme form of inflammatory bowel disease, characterised by multiple intestinal lesions. Frustrated after 100+ operations. Not known the reason for that. Doctors ordered full exome sequencing to find the answer. Was find a specific genetic lesion in IAP gene (basically associated with blood disorders) that indicated the boy would respond to a bone marrow transplant.

Nextera for PCR Amplicons
Quick workflow for long or short range PCR

Nextera for PCR Amplicons
Detection of mutations in CFTR with short range PCR

Complete coverage of target regions at >100X depth
Complete variant concordance

Collaboration with Steven Atts and Michelle Yeo, London
Stages of Cancer

- Cancer is a disease of the genome
- Next-generation sequencing can potentially impact every step of cancer management
- There are 3 components to every cancer study:
  
  patient + cancer + technology

TruSeq® Amplicon – Cancer Panel
Hundreds of loci. Rapid prep. FFPE-ready.

- Comprehensive Content
  - >35 kb total including oncogenes such as BRAF, KRAS & EGFR
  - 212 amplicons in one tube, 48 genes
- Unrivaled Multiplexing
  - Up to 98 sample pooling on MiSeq
  - >99% specificity and uniformity
- Detect low frequency variants (<5%)
- Unparalleled Workflow
  - FFPE-enabled with sample QC kit
  - No qPCR quant needed for normalization
  - Automated paired end sequencing with MiSeq
  - Pre-configured, automated data analysis

Types of Variation

- Single nucleotide variation (SNVs)
  - CGATTGCTA/GATCCCCAGATA
  - CGATTGCTA/GATCCCCAGATA
  - CGATTGCTA/GATCCCCAGATA
- Structural variation (SVs)
  - Copy number variation
  - Insertions and deletions (indels)
  - Inversions
  - Translocations

Copy number variation
- Normal
- Deletion

Insertions & Deletions
- Normal
- Insertion
- Deletion

Inversions
- Normal
- Inverted
Targeted Resequencing

- Focused on a restricted set of genes, selected based on prior knowledge.
- By selecting genes, results are easier to interpret, can focus on actionable genes.

Deep Sequencing

RNA-Seq

RNA-Seq can be detected with a high level of accuracy and confidence.

Based on Lee et al. Proc Natl Acad Sci USA 109: 9293-9298
Others opportunities

- Familial Hypercholesterolemia (FH)
  - FH: 1.5 million people suffer this disease - Estimated 10 Million affected worldwide
  - Soon in Europe
  - Have already CE/IVD experience
  - Wants to transfer their assay onto MiSeq: CE/IVD marking, Cost, NO emulsion PCR
  - Ongoing pilot study using 2x250bp

- Leukemia:
  - 59,000 samples in Leukemia – largest center in Germany
  - Reference Lab in Europe heading NGS consortium (IRON project)
  - Successfully switched from Roche to MiSeq
  - Have developed a Gene panel to analyze acute myeloid leukemia and myelodysplastic syndrome

August 15, 2012

**MLL Developing RainDance, MiSeq Gene Panel for Myeloid Malignancies**

---

**Haloplex current protocol**

- DNA digestion 4hr 20m
- Run gel of control lanes
- Hybridise Haloplex probes 12hr 20m
- Capture target DNA >50m
- Ligate >20m
- Remove non-target DNA >1hr 20m + PCR amplify
- Purify >10m

---

**Metagenomics**

Isolate DNA from single organism

<table>
<thead>
<tr>
<th>Isolate DNA from single organism</th>
<th>Isolate community DNA</th>
</tr>
</thead>
</table>

Sequence

Genomics

Metagenomics
MiSeq flexibility – samples vsplexity

A panel which looks at a single target for many samples

eg. Routine CF screening

A panel which looks at many targets for a single sample

eg. Cancer mutation panel

Unprecedented Rate of Peer-Reviewed Publications

ITFoM
Future of Medicine

European Best Practice Guidelines for Genome-based Information and Technologies – The PHENOME Declaration of Rome

Prof. Dr. Angela Brusa MD PhD MRI, Director of the European Centre for Public Health Genetics (ECPHG), Chairman of PHENOME (ECPHG) Institute for Public Health Genetics, Maastricht University, The Netherlands

© Workshop entitled Genomics in the Clinic (Heidelberg, 17-20, 2013)
1. What do we need to translate?

2. How do we translate (innovations into healthcare systems)?
... genomics is a "moving target"... from single and linear systems to non-linear networks in systems biology and systems medicine ...
... not only beyond the 4 P’s, but also [6, 55, 56, 208] ... 

1. from common complex diseases to “multiple rare diseases”

2. from diseases to “diseasomes”

3. from risk factor to “risk patterns”

4. from clinical utility to “personal utility”
... obesity story [21.08.2012]

ORIGONAL ARTICLE
Infant antibiotic exposures and early-life body mass

... and also

(1) highly (in space & time) dynamic personal (health) information
(2) from statistical risks within groups to "individualized evidence"
(3) "virtual individual models"

IT-toAll (www.itfom.eu) – "ICT for health & health for ICT": a radically new vision for healthcare!
“From Stratified Medicine to truly Individualized Medicine”

- No existing groups, only individuals
  - Every test will be part of treatment. No result can be transferred to another patient.
  - Every therapy is unique, not reproducible.
- No existing method on how to evaluate the new kind of technology
  - How can we fulfill the hierarchy of evidence, the golden standard to prove the efficacy of a treatment?
- The patient is not only consumer of the technology, but also part of it
  - There is no boundary between patient and treatment. The patient is a unique part of the technology itself.

“...The contemporary clinical trials development process is like a duck-billed platypus, an organism that no rational person would have designed a priori.”

[D. Steensma, EFCL, 2009]
2. How do we translate innovations into healthcare systems?

Translation in daily life

- Direct / timely implementation in healthcare quite low
  (Literature, Patents, Market data)

- Identify 3 phases:
  - Lab → Industrial application
  - Industrial application → Market
  - Market → Healthcare integration

- Focus generally on first two phases
Technology Transfer (TT)

- Addresses 1st two phases
- Activity of the migration of academic discoveries to useful application in the development of marketable products or processes
- TTO or valorization office
- Most widely used activity in business development or academic research
- Process, technique, method, tool, activity

Public Health Trials (3rd phase)
Public Health Assessment Tools (PHAT)

- **HIA**: systematic method of reviewing the health issues facing a population, leading to agreed priorities and resource allocation that will improve health and reduce inequalities.

- **HIA**: multidisciplinary process that summarizes information about the medical, social, economic, legal and ethical issues related to the use of a health technology in a systematic, transparent, unbiased, robust manner.

- **HIA**: combination of procedures, methods and tools by which a policy, program, or project may be judged as to its potential effects on the health of a population, and the distribution of those effects within the population.
"...we face a time when the taxonomy of human disease is being redefined given the existence of pathological and molecular disease subtypes..."

[Harrie Mele, CNIO 2009]

"...we face a time when boundaries of disciplines are crossed and the understanding of disease is changing as it happened before with the jump from the macroscopic view in anatomy to the microscopic view in cullstructure..."

Let's get prepared in time – the future is built today!
Public Health Genomics European Network (PHGEN)

"European Best Practice Guidelines for Quality Assurance, Provision and Use of Genome-based Information and Technologies"

www.phgen.eu
We have to define today what kind of (policy)『guidelines』 we need for tomorrow?

... taking into account e.g.

- **dynamics** of the field: genomics is a „moving target“ (from HG to PG)
- **genome-environment interactions**, changing permanently over time and space (incl. epigenomics: „from cell to society to cell“)
- **health information** instead of biomarkers
- **systems network thinking** of biomedicine and environment (incl. social environment): e.g. „diseases“ and „social networks“
- **E4 medicine** (predictive, preventive, personal, participatory): „a change of view that changes everything“
- **the changing roles of patients and doctors**

---

**Prevention in public health**

- **risk groups**
  - communities
  - settings

**Prevention in public health: genomics**

- **risk groups** with similar risk patterns
European Best Practice Guidelines for Quality Assurance, Provision and Use of Genome-based Information and Technologies: the 2012 Declaration of Rome

1. Introduction

- Need to develop efficient and effective health information systems that can support personalized medicine and healthcare.
- Importance of integrating genome-based information into clinical practice.
- Need for standardization and interoperability in genomic data sharing and analysis.
- Importance of privacy and ethics in genomic research.

2. Applications

- Personalized medicine: Tailoring treatments to individual genetic make-up.
- Disease prevention: Identifying genetic predispositions to diseases.
- Public health: Tracking disease outbreaks and managing infectious diseases.
- Genetic testing: Providing genetic information for diagnostic and preventive purposes.

3. Challenges

- Data privacy and security: Protecting patient data from unauthorized access.
- Data quality and reliability: Ensuring the accuracy and integrity of genomic data.
- Data sharing and collaboration: Building a network for sharing genomic data and research.
- Economic sustainability: Ensuring the long-term viability of genomic research and healthcare.

4. Conclusion

- Need for continued investment in genomic research and healthcare.
- Importance of international collaboration and standardization.
- Continued efforts to address the challenges faced in genomic research and healthcare.

[End of Page]
"Future perspective
Personal health drives a fundamental change not just in what is known, but also in how we think of ourselves and the way we are living, thus redefining our society. The political will is there, but we have to prepare for all the various organisational changes... in time."

Personalised Medicine
March 2011, Vol. 8, No. 2, Pages 119-128 DOI: 10.2217/pme.11.08
Public health perspective from personalized medicine to personal health
Simone Casoni, Ben van Doolen, Núria Martas, Reif Zoubi, Hans Cattenhead & Angel Brand
* Applied Genomics in the Clinic
JRC Workshop
17-19 October 2012, Istanbul

Ewa Stępień PhD,
Department of Clinical Biochemistry,
Division of Genetic Diagnostics and
Nutrigenomics, Jagiellonian University
Medical College, Krakow Poland

*Giemsia magic*

Professor Bogdan Kałużewski,
Medical University in Łódź, Poland
Chairman of the Medical Speciality Advisory Board of Ministry of Health laboratory accreditation in the field of Medical Genetics

Metaphase chromosomes
from human lymphocytes cell culture
(Kałużewski, 1967)

The laboratories performing diagnostic tests in the field of medical genetics in 1967

Mother and Child Institute (Warsaw)
Institute of Psychiatry and Neurology (Warsaw)
Institute of Dermatology, Medical University of Warsaw
Institute of Parasitology, Medical University of Warsaw
Institute of Forensic Medicine (Warsaw)
Institute of Paediatrics, Medical University of Silesia
Institute of Paediatrics, Medical University of Białystok
Institute of Paediatrics, Medical University of Lublin
Institute of Paediatrics, Medical University of Katowice
Institute of Paediatrics, Medical University of Lodz
Since 2002, medical genetics specializations for physicians and diagnosticians have been established.

The aim of study for genetic physicians is to achieve special qualifications in medical genetics and, concerning current knowledge, management with affected patients and families with higher risk of disease of genetic origin.

A specialist in laboratory medical genetics is a partner for a clinician in consultation process. This issue is currently important, particularly when the easy access to the different genetic testing has appeared.

The additional concern in this matter is caused by necessity of rationalization of treatment and laboratory costs. Without this it is difficult to say about full accessibility to medical procedures.

*Education in clinical genetics*

1. Specialistic Outpatient Care:
   * Complex genetic consultations:
     * 9 points x 10.00 PLN = 90.00 PLN = 21.42 €
   * Counseling in neoplastic diseases:
     * 4 points x 10.00 PLN = 40.00 PLN = 9.53 €

2. Specialistic Outpatient Care (High-cost diagnostic tests):
   * 28 points x 9.00 PLN = 252.00 PLN = 60.00 €

*Financial Issues of Medical Genetics in Poland - National Health Fund*
Neoplastic

- Test of significantly decreased risk of breast cancer = 178.79 €
- Test of moderately increased risk of malignancies of various sites = 129.17 €
- 22 mutations/MM in BRCA1, BRCA2, ATM, CHEK2, CDH1, RAD51, TP53, CDKN1A, CDKN2A, FHM, MTHFR, XRCC3, XRCC4, XRCC5, ATM, FANCA, FANCC, FANCF, FANCG, FANCI, FANCJ, FANCN, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FANCQ, FANCP, FAN...
Establishing of two committees dedicated for rare disease treatment:
  * Operation Team for Rare Diseases
  * Operation Team for Ultra-rare Diseases
under the auspices of Polish Ministry of Healthcare and with the cooperation of Polish National Health Fund. The main scope for these teams is increasing the availability of diagnostics and treatment of rare diseases.

* Clinical genomics
New initiatives in Poland

English version:

About the Institute

The Children's Memorial Health Institute is a large and more modern specialty hospital. Formerly, it was a research institute conducting studies on pediatric patients. Now it is one of the few centers in Europe where modern medical research is carried out. The Children's Memorial Health Institute has been a leader in the field of pediatric medicine for many years. It conducts clinical research in cooperation with local centers in Poland and the world, organizes seminars and conferences, and provides specialized care for children and families from all over Poland.

Our greatest challenge is to prevent orms and further actions. Our goal is to provide the best care and treatment for children with rare diseases. We work with the Children's Memorial Health Institute to offer the best possible care for children suspected of having rare diseases. We also provide support for research and development from many fields. The Institute participates in international programs for patients, including online publications.

In 2011, the Children's Memorial Health Institute celebrated its 50th anniversary. This event was a great moment for the Institute. The Director of the Institute, Dr. Jan Paweł, highlighted the importance of research in pediatric medicine, particularly in the area of rare diseases. He also praised the contributions of the Institute to the development of modern medical research in Poland and the world. The Children's Memorial Health Institute has been a leader in the field of pediatric medicine for many years. It conducts clinical research in cooperation with local centers in Poland and the world, organizes seminars and conferences, and provides specialized care for children and families from all over Poland.

Objectives

* To increase the availability of comprehensive care for patients with rare cardiovascular diseases
* To increase our knowledge and experience in management of rare cardiovascular diseases

* Centre for Rare Cardiovascular Diseases in John Paul II Hospital in Krakow
Personalized medicine

Patients selection for tailored therapy
- Colon cancer: KRAS 500 per year
- Lung cancer: EGFR 100 per year
- Melanoma
  - BRAF 500 per year
  - G691T about 20 per year
- CLL: treatment monitoring 100 per year
- BRCAT sequencing 1000 per year

Equipment:
- Masson Torrent
- 4-cartridge 3130 Genetic Analyzer ABI
- 60-base pyrosequencing digital system
- Ion Torrent Proton 3™ machine

Laboratory of Molecular Biology
Holy Cross Cancer Center
Kielce, Poland

Main achievements in clinical genetic diagnostics in Poland

- Organization of genetic counseling in Poland covered by National Health Fund
- Starting education program in medical genetics (specializations) for physicians and diagnosticians
- Establishing new orlace laboratories and companies dedicated to clinical genetics
- Increasing number of diagnostic centers equipped with high-throughput methods in genetics
- Establishing of the international cooperation for genetic diagnostics of rare diseases
- Incorporation (part 1) of the Children's Memorial Health Institute in Warsaw into the Joint Action „Development of the European portal of rare diseases and rare phenotypes – SHARED/Europe”
- Establishing (part 1) of centers for rare cardiovascular diseases in Joint Action II on Allowable costs for rare cardiovascular diseases.

Main failures in clinical genetic diagnostics in Poland

- Lack of comprehensive financial and education program supporting development of scientific research in clinical genetics
- Dispersal of procedures over the list of guaranteed services (so-called „jewel”)
- Lack of interest in introduction of quality control system in genetic laboratories
- Limited availability to prenatal and preimplantation genetic diagnostics (high costs)
EU Policy on Rare Diseases

Health information unit,
DG Health and Consumers, European Commission

The Commission Communication and the Council Recommendation on rare diseases

There is probably no other area in public health in which 27 national approaches could benefit so much from collaboration at EU level. The reduced number of patients for these diseases and the need to mobilise resources require a co-ordinated European approach to be efficient.

Legal basis for the developments of the EU Policy on rare diseases

A Community action programme on Rare Diseases, including genetic diseases, was adopted for the period of 1 January 1999 to 31 December 2003 with the aim of ensuring a high level of health protection in relation to RD. At the first EU effort in this area, specific attention was given to improving knowledge and facilitating access to information about these diseases.

Orphan Medicinal Product Regulation (Regulation (EC) No 141/2000 of the European Parliament and of the Council of 16 December 1999 on orphan medicinal products, was proposed to set up the criteria for orphan designation in the EU and describes the incentives (e.g. 10-year market exclusivity, protocol assistance, access to the Centralised Procedure for Marketing Authorisation) to encourage the research, development and marketing of medicines to treat, prevent or diagnose rare diseases.
Legal basis for the developments of the EU Policy on rare diseases

Commission Communication COM (2008) 679/2 to the European Parliament, the Council, the Economic and Social Committee and the Committee of the Regions on Rare diseases: Europe’s challenge: creating an integrated approach for the EU action in the field of rare diseases. Adopted 16th November 2008.

Council Recommendation on a European action in the field of rare diseases, recommending actions at national levels to implement the EU action (e.g. National Plans for Rare Diseases). Adopted 16th June 2009.

Decision of the Commission creating a European Union Committee of Experts on Rare Diseases during 2009. To be composed by 51 members representing Member States, patients organisations, industry, FP Projects, Health Programme projects, etc. Adopted 30th November 2009.

Legal basis for the developments of the EU Policy on rare diseases

Directive of the European Parliament and of the Council of 9 March 2011 on the application of patients’ rights in cross-border healthcare (2011/24/EU) provides for the development of European reference networks (ERNs) by Commission and Member States. The ERN can improve the access to diagnosis and the provision of high-quality healthcare to patients who have conditions requiring a particular concentration of resources or expertise, especially for rare diseases. Deadline for transposition the 22nd of October of 2013.


Directive 2005/28/EC laying down principles and detailed guidelines for good clinical practice as regards investigational medicinal products for human use, as well as the requirements for authorisation of the manufacturing or importation of such products (“clinical trials”).

Emergence of concepts and initiatives surrounding rare diseases in Europe

Why an orphan regulation?

- Rare diseases -> developing and marketing cost would not be recovered by the expected sales
- Persons suffering from rare conditions deserve same quality of treatment as other patients
- Pharmaceutical Industry does not develop medicines for rare diseases under normal market conditions
- Objective:
  - provide incentives that stimulate research and development (push)
  - modify market conditions (pull)

Main incentives for orphan designation

- Economic / marketing
- Fee reduction / exemption
- Extended incentives for SMEs (post authorisation)
- Market exclusivity
- Product development
- Protocol assistance
- Community marketing authorisation
- National incentives (EC inventory)

Distribution of Opinions

[Bar and pie charts showing the distribution of opinions]
The Commission Communication and the Council Recommendation on rare diseases – Main priorities

I. Plans and strategies in the field of rare diseases
   Calls on the MS to elaborate and adopt a plan or strategy by the end of 2013.

II. Adequate definition, codification and inventorying of rare diseases
   Evokes the common definition of a rare disease as a condition affecting no more than 5 per 10,000 persons, aims to ensure that rare diseases are adequately coded and traceable in all health information systems based on the ICD and in respect of national procedures and encourages MS to contribute actively to the inventory of rare diseases based on the Orphanet network.

III. Research on rare diseases
   Calls for the identification and fostering of rare disease research at all levels.

IV. Centres of expertise and European reference networks for rare diseases
   Ask the MS to identify and facilitate networks of expertise based on a multidisciplinary approach to care, and foster the diffusion and mobility of expertise and knowledge.

V. Gathering the expertise on rare diseases at European level
   MS should share best practices, develop medical training relevant to the diagnosis and management of rare diseases, coordinate European guidelines, and, to minimise the delay in access to orphan drugs, MS should share clinical/therapeutic added-value assessment reports at the Community level.

VI. Empowerment of patient organisations
   MS should consult patient representatives on policy development; facilitate patient access to updated information on rare diseases; promote patient organisation activities.

VII. Sustainability
   Long-term sustainability in the field of information, research and healthcare of infrastructures must be ensured.

**EUCERD**

The Commission is assisted by an EU Committee of Experts on Rare Diseases (EUCERD) to advise on implementation of the Communication and the Recommendation.

The Committee is assisted by a Scientific Secretariat, supported through the Health Programme.

Composed by 51 members representing Member States, patient’s organisations, pharmaceutical industry, FP Projects, Health Programme projects and ECDC + 12 Commission representatives (SANCO, RTD, ENTR, EMA, COMP).

http://www.eucerd.eu/
Adequate definition, codification and inventorying of rare diseases

ICD-10 revision

Information for patients and professionals

Orphanet

- accessed by 20,000 users each day from over 200 countries. Still correct?

Rare diseases research
History of support at the European level

EU has invested in research on rare diseases for more than 2 decades

FP5 (1998-2002): 47 projects funded, €64 million in total
is_056207.pdf

FP6 (2002-2006): 59 projects funded, €230 million in total

EC support to rare diseases research
FP7 Health Theme 2007-2013

66 ongoing projects: EC support around €325 million

- Europe-wide studies of natural history and pathophysiology: development of in vitro/in vivo models, registries and biobanks, identification of biomarkers etc.

- Development of preventative, diagnostic and therapeutic interventions, including pharmacological approaches and innovative approaches such as cell and gene therapies, and regenerative medicine.

- In most diseases areas: neurology, immunology, cancer, pneumology, dermatology, uro-gynaecology, metabolism etc.
Work Programme 2012 for Health Theme

€ 108 million earmarked for the following topics:

- Support for international rare diseases research
- Clinical utility of -omics for better diagnosis of rare diseases
- Databases, biobanks and clinical “bio-informatics” hub for rare diseases
- Preclinical and/or clinical development of substances with a clear potential as orphan drugs
- Observational trials in rare diseases
- Best practice and knowledge sharing in the clinical management of rare diseases

Horizon 2020: The next Framework Programme for research and innovation

Proposed budget: €80bn, a 46% increase compared to FP7

Priorities: Excellent science
- Industrial leadership
- Societal challenges

International Rare Diseases Research Consortium (IRDiRC)

Recent development
Countries in Europe with a national alliance for rare disease patient organizations

From: Ayimé S., Rodwell C., eds., *2011 Report on the State of the Art of Rare Disease Activities in Europe of the European Union Committee of Experts on Rare Diseases - Part I: Overview of Rare Disease Activities in Europe and Key Developments in 2010*, July 2011.

Pilot European Reference networks

**DISCERN** European Network of Centres of Reference for Dysmorphology (ended)
**ECdfIN** CF: European Centres of Reference Network for Cystic Fibrosis (ended)
**PAARIR** Patient Associations and Affiliated International Registry (PAARIR) (ended)
**EPET** European Porphyria Network - providing better healthcare for patients and their families (ended)
**EN-RBD** Establishment of a European Network of Rare Blood Disorders (ended)
**Pneumon* Hodgkin Lymphoma Network**: European-wide organisation of quality controlled treatment (on-going)
**NEUROED**: European Network of Reference for Rare Paediatric Neurological Diseases (ended)
**EURO HISTIO NET**: A reference network for Langerhans cell histiocytosis and associated syndrome in EU (on-going)
**TAG**: Improving Health Care and Social Support for Patients and Family affected by Severe Congenital Diseases - Together against Ricer Difficulties (on-going)
**CARE MND**: Dissemination and Implementation of the Standards of Care for Duchenne muscular dystrophy in European and Associated Countries (on-going)

Directive on the application of patients’ rights in cross-border healthcare

The Directive intends to clarify patients’ rights to access safe and good quality healthcare in another Member State (MS), and be reimbursed for it.

Increase transparency by making mandatory for MS and healthcare providers to make public comprehensive and accurate information on the services, the possible treatment options, the prices, and the quality and safety of the services they provide.

This Directive will increase cooperation between national health authorities:

National Contact Points
Cross-border recognition of prescriptions
EU structures to implement projects on European reference, eHealth and health technology assessment networks
Art 12. ERN

Art. 12 of the Directive notably foresees enhanced cooperation of Member States in the area of European reference networks (ERN).

Main goals is to facilitate improvements in the diagnosis and treatment of certain diseases of conditions across the EU:

- By the delivery of high-quality, accessible and cost-effective healthcare

for patients suffering of medical conditions which could require a particular concentration of expertise or resources, particularly in medical domains where expertise is rare.

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Article 12 : ERN

The Commission shall support MS in the development of ERN between healthcare providers and Centres of expertise in the Member States.

Participation in the ERN shall be voluntary. Its members shall participate and contribute to the networks’ activities in accordance with the MS legislation where the members are established.

ERN shall be open to new healthcare providers which might wish to join them, provided that such healthcare providers fulfil all the required conditions and criteria.
EUCERD recommendation

Recommendations for Centres of Expertise adopted unanimously by the European Union Committee of Experts on Rare Diseases

Adopted on 24 of October 2011

Registries

Projects:
- EPIBAR
- EUCERD JA
- RD CONNECT

597 disease registries

Directorate for Health and Consumers priorities on rare diseases

Public health actions

Contact point at DG SANCO
antoniet.montserrat@ec.europa.eu
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CURRENT APPLICATIONS OF MEDICAL GENETICS IN TURKEY

DR. AHMET YESILYURT
ISTANBUL
OCTOBER, 2012

TURKEY

Turkey is a rapidly growing country with a population of 75,000,000.

There are some different cultural and genetic diversity in Turkish population.

This mosaic background makes the Turkey very amazing country as well as some difficulties in genetic studies.

Our knowledge about genetic background of Turkish population still insufficient.

| Distribution of Genetic Diagnosis Centers in TURKEY |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | 2013 | 2012 | 2011 | 2010 |
| ANKARA         | 2    | 2    | 16   | 14   |
| ISTANBUL       | 2    | 2    | 17   | 11   |
| DUSKA          | 1    | 1    | 2    | 2    |
| BESBUN        | 1    | 1    | 1    | 1    |
| KÜKÜŞOY       | 1    | 1    | 1    | 1    |
| MARMARIS       | 1    | 1    | 1    | 1    |
| MALATYA        | 1    | 1    | 2    | 2    |
| AMPHIA         | 1    | 1    | 3    | 3    |
| ANKARA         | 1    | 1    | 3    | 3    |
| BESBUN         | 1    | 1    | 2    | 2    |
| BESBUN         | 1    | 1    | 1    | 1    |
| MALATYA        | 1    | 1    | 1    | 1    |
| MARMARIS       | 1    | 1    | 1    | 1    |
| KÜKÜŞOY       | 1    | 1    | 1    | 1    |
| ANKARA         | 1    | 1    | 1    | 1    |
| MARMARIS       | 1    | 1    | 1    | 1    |
| MALATYA        | 1    | 1    | 1    | 1    |
| ANKARA         | 1    | 1    | 1    | 1    |
| BESBUN         | 1    | 1    | 1    | 1    |

Certified Diagnosis center by the Turkish Ministry of Health
WHAT WE ARE PERFORMING
IN CLINICAL GENETICS
- Dysmorphology
- Fetal examination
- Genetic counseling
- Management of congenital disorders
- Management of complex disorders

IN CYTOGENETICS
- Chromosome analysis
- Genomic blood samples
- amniotic fluid
- CVS
- cord blood
- skin biopsy
- tumor tissue
- HIV, NMR, C, R etc. banding
- Tissue cultures

IN MOLECULAR GENETICS
- Cell-based molecular-based DNA sequencing
  - more than 100 single-gene disorders
- qPCR analysis
- Real-Time PCR
- GAPD
- mRNA
- Array CGH
- Molecular haplotyping in few centers
- Exome-based DNA sequencing in few centers for routine diagnosis
REIMBURSEMENT FOR GENETIC TESTS

Social Security Institute (SSI) is responsible for reimbursement.

Methodology-based reimbursement is using by SSI.

The frequency of the genetic test of each genetic center can be followed with a global system called "MEDICUS".

PCR can be charged for just some disorders which can be treated: HLA typing compatible bone marrow transplantation from siblings.

The most challenging problem is to set a new test using with next-generation systems such as microarray, next-gen sequencing etc.

BOTTLENECKS IN GENETICS APPLICATIONS IN TURKEY

Lack or insufficiency of infrastructure for genetic laboratory in some universities/hospitals.

There is no enough well-educated staff to perform complex genetic tests.

The education programme (4 year) of medical genetics is not homogenous.

Educational activities are required to increase the knowledge in genetics applications.

Bioinformaticians are not sufficient to evaluate complex and huge data from highthroughput systems.

WHAT WE PERFORM

Oncology Vitamin Research Training and Research Hospital Medical Research School.

Our main goal is to perform translational studies from benchtop to bedside:
- Regenerative medicine
- Stem cell research and applications

We are trying to make to implement new or new genetic test such as single gene diabetes (MODY) in TURKEY.
Pancreatic islet cell Research Center (PAHAMS)
Department of Medical Genetics
Staphylopoulos, S. (2001)

- Transplantation genetics
- Inflammatory bowel disease

PAHAMS II
Cell Research Laboratory
- Pancreatic and cardiovascular transplantation laboratory
- Stem cell research laboratory
- Prosthetic laboratory
- Protein characterization and analysis
- For some disorders
H.LA Typing Laboratory

- Low and High Resolution HLA Typing
  - HLAA
  - HLAB
  - HLAC
  - HLADR
  - HLADQ
  - HLADP

Tests for transplantation
- PRA (Class II Tarama/Tamamlama)
- CDO
- Locus spesifik antikor Class II

Animal Lab. Facility

- There are some specific rodents such as spontaneous diabetic rat, etc.

THANKS FOR YOUR ATTENTION

- www.medicalresearchcenter.org
Clinical Hospital Center Sisters of Mercy Zagreb

- Cytogenetic analysis of peripheral blood lymphocites
- FISH analysis for enumeration, microdeletion and microduplication syndromes, whole chromosome painting, subtelomere analysis
- Molecular analysis of nonsyndromic deafness, ahondroplasia and hypohondroplasia, Rett syndrome
- MLPA

Clinical Hospital Holly Spirit, Zagreb

- Cytogenetic analysis of fetal and peripheral blood lymphocites
- chorionic villi,
- amniotic fluid and
- spontaneous abortions

Medical Faculty Osijek

- Cytogenetic analysis of peripheral blood lymphocites, and spontaneous abortions
- FISH analysis for enumeration, microdeletion and microduplication syndromes
- Molecular analysis of AZFs in male sterility, congenital deafness, UPD15
• Medical Faculty Rijeka

• Cytogenetic analysis of peripheral blood lymphocytes, amniotic fluid and spontaneous abortions
• FISH analysis for enumeration, microdeletion and microduplication syndromes

MOLECULAR TESTING IN CROATIA

• Molecular tests for Monogenic Diseases
• Molecular tests for Leukemia and Lymphoma
• Molecular tests for Tumor Tissue
• Molecular tests for Risk Factors
• Molecular tests for Infectious Diseases
• HLA typing and Transfusion testing
• Molecular testing in Forensic Medicine

• University Hospital Split

• Cytogenetic analysis of peripheral blood lymphocytes, amniotic fluid and spontaneous abortions
• Molecular analysis
MOLECULAR TESTING IN CROATIA

- Molecular tests for Leukemia and Lymphoma (AML, CML and childhood ALL panels, Lymphoma clonality testing, ABL mutation sequencing) - ELN Referal center: Zagreb
- PCR, QRT-PCR and conventional sequencing (Rijeka, & Split)

MOLECULAR TESTING IN CROATIA

- Molecular tests for Infectious Diseases (HBV, HCV, HGV, HIV, EBV, CMV, HSV, Chlamydia, Borrelia...)
- PCR, QRT-PCR and conventional sequencing
- Rijeka, Zagreb & Split

MOLECULAR TESTING IN CROATIA

- Molecular tests for Risk Factors (thrombophilia, stroke and myocardial infarction, recurrent abortion)
- Rijeka, Zagreb & Split
MOLECULAR TESTING IN CROATIA

- Molecular tests for Tumor Tissue (Rijeka, Zagreb and Split)
- K-RAS, B-RAF, EGFR, c-kit, PDGFR, p53, soft tissue tumor panel (sarcoma)
- PCR, QRT-PCR, conventional sequencing and pyrosequencing

MOLECULAR TESTING IN CROATIA

- Molecular tests for Monogenic Diseases (CF, AZF, neurodegenerative diseases, MD1, FRAXA, Wilson, HFE, AAT, Gylbertsy)
- University Hospital Zagreb

MOLECULAR TESTING IN CROATIA

- Molecular testing in Forensic Medicine (DNA identification, paternity and maternity testing)
MOLECULAR TESTING IN CROATIA

- HLA typing (high resolution DNA testing) and Transfusion testing (ABO, Rh, HPA, HNA testing)
- Croatian Institute for Transfusion Medicine Zagreb

LABORATORY HARMONISATION 2010

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<th>Short name</th>
<th>Full name</th>
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<td>CC, CT, TT</td>
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Genetics / Genomics

Genetics
The study of genes and of their effects.

Genomics
"The study of functions and interactions of all the genes in the genome, including their interactions with environmental factors."

Progress in Public Health Genetics

Rare diseases:
- Single gene disorders

Common diseases:
- Multiple genes
- Gene / environment interactions

Activities
- Newborn screening
- Reproductive health
- Genetic services
- Chronic diseases
- Infectious diseases
- Environmental health
- Epidemiology
Romanian National Plan for Rare Diseases
Strategies

- Define reference centre and competence centres
- Encourage participation in European networks
- Adequate standards for authorization / recognition

Romanian National Plan for Rare Diseases
Strategies (cont)

- Encourage multidisciplinary approach
- Provide national network of screening and policies in the field of rare diseases
- Support the use of information and communication technologies (telemedicine)

Implementation

- As a first step, identification of reference centres on national level as leaders in the fields of genetics research and medical genetics, genomic technology, health information technology, healthcare delivery, policy, program administration, legal counsel
- All these come from the public and the Academia
Implementation
National Reference Centres

- Uniqueness in the country for a disease or group of diseases
- Development of the National Registry for the diseases under their attention
- Clinical diagnostic and specialty performance investigation, treatment initiation, development of guidelines on monitoring and treatment of patients in competence centres
- Best practice guidelines
- Management of health programs on disease group
- Multidisciplinary approach
- Linking research
- Protocols for screening programs
- Report of prescription of Orphan Drugs results
- Information / education
- Collaboration with the European Centre specialized in rare diseases
- Participation in the European Reference Centres network

Implementation
Regional Competence Centres

- Applying best practice guidelines agreed upon with all Centres of Reference
- Monitoring service delivery
- Information for Reference Centres and County Centres
- Organization / implementation of screening
- Setting up a database of reference specialists
- Prevention, diagnosis, treatment, recovery
- Collaboration with European programs, etc.

Implementation
County Centres

- Screening implementation
- Detection, diagnosis and monitoring
- Referral of complex cases to the centres of competence
- Informing and educating patients, families, population
- Establishing and maintaining relationship with patient
- Implementation, monitoring treatment and recovery procedures and integration
- Evidence of patients and resources
Implementation
County Centres (cont)

Infrastructure Development

Infrastructure Development (cont)

Projected model:
- Data collection and retention
- Data analysis
- Hypothesis generation
- Interdisciplinarity
- Re-use of data
Infrastructure Development (cont)

- Central institutional review board / National Committee (ensure continuity)
- Reorganization and sustaining the entities designed to realize statistical and epidemiological studies
- Development of educational programs and tools for physicians and other health professionals
- Computer infrastructure development

Interactions among Organizations

Hoping for results
Applied Genomics in Cancer:
Sense and sensitivity

Dr Pinar Uysal-Oganer

What is the biggest challenge facing biology in the 21st century?

The need to deal with its incredible complexity
Cancer is a leading cause of disease worldwide

- 12.7 million new cancer cases occurring in 2008
- will increase to 22.2 million new cases each year by 2030
- a leading cause of death worldwide, with 7.6 million deaths (around 13% of all deaths) in 2008
- Tobacco is by far the single most important risk factor for cancer and caused 22% of all cancer deaths and 71% of lung cancer

New diagnostic and predictive markers needed

- Specific alterations in genes and the proteins they code for have been identified in many types of cancer
- Alongside this, scientists are using the latest microarray technologies to reveal genetic variations between cancers of the same type in different individuals
- The genetic signature of a person's tumour may influence the outcome of radiotherapy, drug or hormone treatment
- Increasingly, this information will be translated into the clinic, allowing doctors to tailor treatment to the individual patient

Advances in cancer genomics and molecular technologies are opening new possibilities for diagnostics
What has been done in UK?

- Rational clinical decisions on the management and treatment of cancer rely on accurate diagnostic information.
- Molecular analysis of tumour samples has been used to predict prognosis or response to treatment, but should be complemented by non-invasive methods for monitoring disease progression or dynamics.

Circulating DNA in plasma and serum include tumour-specific sequences that are a promising source of diagnostic information.

Pros and Cons of ctDNA measurements

- The mechanisms through which tumour DNA reaches blood circulation are unclear.
- ctDNA are higher in cancer patients compared with healthy controls, but these differences are not consistent enough for robust diagnostic tools.
- ctDNA can be measured by trying together genomic and molecular techniques.
- These assays must be applied to body fluid samples such as blood plasma that have been carefully collected and processed to extract ctDNA.
- ctDNA may be useful for identifying the presence of cancer mutations, for detecting systemic or residual tumour burden, or for non-invasive monitoring of tumour changes.
Stratified Medicine Programme by CRUK

- When breast cancer drug trastuzumab (Herceptin) became available to the NHS in 2006, many hospital pathology labs were caught on the hop
- Trastuzumab is designed to treat women whose tumours contain high levels of a protein called Her2, but having to routinely, reliably and accurately test a tumour’s Her2 levels, as part of ‘business-as-usual’, was uncharted territory for many pathologists

Stratified Medicine Programme is partnership

- is being funded, to the tune of £5.5million pounds, by CRUK, AstraZeneca, Pfizer and the government’s Technology Strategy Board
- genetic testing labs with several hospitals involved, samples from patients diagnosed at these hospitals can be sent to one of these labs for high-quality genetic tests, and the results sent back electronically

Which patients involved?

- breast cancer
- bowel cancer
- lung cancer
- prostate cancer
- ovarian cancer
- melanoma
Prostate is the size of a walnut and surrounds the first part of the tube (urethra) which carries urine from the bladder to the penis.

It produces a thick white fluid called semen that mixes with the sperm produced by the testes. It also produces a protein called prostate-specific antigen (PSA) that turns the semen into liquid.

Prostate cancer

Generally affects men over 50, and is rarely found in younger men.

It is the commonest type of cancer in men.

Environmental and dietary factors are likely to be involved.

Initially tumours are androgen-dependent and treated by androgen deprivation.

However, cancer often recurs in an androgen-independent form.

We are currently unable to predict which patients may or may not respond to a specific drug.
TMPRSS2-ERG fusion correlates with poor prognosis in PCa

- ETS-related gene (ERG) is important in hematopoiesis, angiogenesis, vascular and bone development
- TMPRSS2-ERG fusion is found in 40-60% of prostate tumours.

Next-generation Sequencing (NGS) and PCa

- assessment of the genomic landscape of advanced PCa is difficult (limited access to tissue and large amounts of DNA required)
- most patients with advanced PCa do not undergo biopsies of metastases as part of routine clinical care
- NGS is a novel platform: requires little DNA and can use tissue that is formalin-fixed and embedded in paraffin

182 genes sequenced across entire coding sequence and 14 genes sequenced across selected introns

Beirne et al., 2012
NGS provides new insight into genomic alterations

- some molecular alterations arise early and persist during disease progression:
  - they may be driving events
  - potential biomarkers to use cancer diagnosis and guide the course of patients’ therapy
- FFPE tissue, including needle biopsy material can be used
- little amount of DNA is enough to achieve deep sequence coverage
- step toward designing targeted assays to detect driving mutations
- has potential to lead to find new biomarkers, drug targets to guide the development of future therapies

with some controversial debates

- should we test and pre-treat potential cancer patients? (breast and ovarian cancer examples)
- should we keep (NHS) patient records to be available for research?

*primum non nocere*
Conclusion

NGS studies have led to significant advances in our understanding of the cancer genome of several tumor types. Current efforts are aimed toward bringing sequencing discoveries into the clinic in the form of biomarkers (diagnostic, prognostic, and predictive) and biomarker-designed clinical trials.

A new era of personalized medicine is on the horizon, however, the new discipline of public health genomics, which seeks to evaluate the use of emerging genomics information effectively and responsibly to improve the health of individuals and populations is essential.

Closing remark

It has been 10 years since the Human Genome Project was drafted, and we are still asking how genomes will help healthcare.
Analysis of Genomic Data: Linkage and CNV Analyses using whole genome SNP data

Sibel A. Uğur İşeri, PhD
Istanbul University, Institute of Experimental Medicine (DETAE)

Workshop on ‘Applied Genomics in the Clinic’
18.10.2012

- Disease gene identification through linkage analysis of whole genome SNP data in families with rare recessive disorders
- Genomic profiling of copy number variations (CNVs) with SNP arrays

From Genome Scan to Disease Gene Identification

- Locus and gene analyses starting from the initial genome scan data
- SNP data generated on Illumina platform
  - Whole genome SNP array genotyping in extended pedigrees with AR inheritance
The Strategy

- Statistical analysis of the whole genome SNP array data using linkage software
  - Detect genotyping errors
  - Calculate two and multipoint lod scores
  - Constructing haplotypes
- Refinement of candidate loci with genotyping (additional microsatellites and/or SNPs)
- Candidate gene approach
- Exome-Targeted sequencing

Disorders Analyzed

- Progressive Epilepsy
  - AR pedigree with multiple affected individuals
- Anophthalmia (absent eye) – Microphthalmia (small eye)
  - Developmental eye defect
  - 25% of childhood visual impairment
  - Two AR pedigrees with similar malformations of the anterior eye

Progressive Epilepsy

- Clinically undiagnosed form of progressive epilepsy
  - Tonic seizures starting at age 9
  - Progressive neurological dysfunction
- 300k Illumina array
  - Homozygosity mapping due to consanguinity
  - Lod score calculations
  - Candidate gene analysis
Genetics to Clinical Diagnosis

- 5Mb Region, 100 genes
- CST7 encoding a protease inhibitor
  - Myoclonic epilepsy of Unverricht and Lundborg
- Dodecamer repeat expansion in the promoter

Anophthalmia-Microphthalmia

- Whole genome SNP array genotyping in two extended pedigrees with AR malformations of the anterior eye
- Candidate gene analysis
  - AR FOXE3 mutations, full penetrance

Family 1 Phenotypes

- Aphakia, Sclerocornea
- Microphthalmia
- Sclerocornea, Glaucoma
Family 2 Phenotypes

Genotyping and Statistical Analysis

FOXE3

- Encodes a lens specific transcription factor
  - Forkhead domain: 110-amino-acid highly conserved DNA binding domain
- Two spontaneous Foxe3 mutations cause dysgenetic lens phenotype in mice (dyI mice)
  - Connection between lens and cornea
  - Failure of the lens vesicle to separate from overlying ectoderm
Results: Family 1
- c.224A>C: AR inheritance in 6 affected and 24 unaffected members of Family 1
- p.Met7Val resides in a Methionine-Aromatic Rosette
  - Hydrophobic sub-structure composed of a core methionine surrounded by five other conserved aromatic amino acids
- dyl mutations which abolish DNA binding also reside in this rosette
- Null allele predicted to prevent this assembly (EMSA)

Results: Family 2
- c.21_24del: AR inheritance in 3 affected and 9 unaffected members of Family 2
  - 4 bp deletion creating a frame shift and premature stop codon
- p.Met11lefsX216
  - Abnormal and truncated protein after 7 residues
  - Null allele

Screening FOXE3 in 236 Subjects: Dominant Inheritance

Pedigree 3
- Microphthalmia, Exomma, Protein anamolies, 1st Avala, Calvaria (early) and Coloboma
- c.907T>C; p.K303Argx072
  (abnormally elongated protein)

Pedigree 4
- Microphthalmia, Microsoma, Convex Calvariae (blue dot)
- c.1489C>T; p.96y/Glu
Target gene for diagnostic screening in a broad spectrum of eye anomalies

- Homozygous null FOXE3 mutations in inbred pedigrees
  - Null mutation, abolishing DNA binding
  - Normal carrier status
- Heterozygous FOXE3 mutations in two pedigrees with complete penetrance
  - Variable phenotypes and range of intrafamilial severity
  - Gain of function mutations?

Genomic Profiling of DNA CNVs with SNP arrays

- Children with multiple congenital anomalies and mental retardation
- Illumina 300k array
- Scan the genome for CNVs
  - Molecular Karyotyping with SNP array
- Use of normalized BAF and LRR values
- GenomeStudio-KaryoStudio
  - cnvPartition
  - 3rd party programs (QuantiSNP, PennCNV, Nexus Copy Number etc)

SNP array detects a microdeletion

- A child with microcephaly and ocular findings
- Normal karyotype
- 1.65 Mb deletion on 22q11 confirmed by FISH
21 Mb Amplification on chromosome 2p

To Sum up,

- Versatility of SNP array genotyping
- Spots regions of the genome associated with the phenotype
- Small CNV events, unknown regions
  - Databases, in-house data
  - Parental testing, de novo events?
  - Related genes in region?

Applied Genomics in the Clinic Workshop
Istanbul University, Turkey
17-19 October 2012

SNP Genotyping Microarrays, Data Analysis Basics
&
Data Interpretation

Ilker Karacan
Done Genetik
Outline

- Background
  - Microarray
  - SNP
- SNP microarray technology
- Outcome of a SNP array - One SNP analysis
- Data analysis
- CNV
Microarray

- Base-pairing hybridization
- Parallelism (more than one test)
- Multiplexing (more than one sample)
- Miniaturization (a few cm²)
- Automation (chip production, reagents)

Microarray

✓ Availability of whole genome sequences
✓ Advances in micro-nano technology
✓ Advances in computer science

High-throughput system that can measure thousands of data simultaneously

SNP

- Definition: Variations in single base pairs that are randomly dispersed throughout the genome (every 200 to 300 bases along the 3-billion-base human genome)
- Act as measures of genetic diversity within the species (i.e., 90% of human genetic variation)

- SNPs can occur in both coding (genes) and non-coding regions of the genome
- Many SNPs have no effect on cell function, but others could predispose people to disease or influence their response to a drug or other factor

SNP databases:
- HAPMAP (http://hapmap.ncbi.nlm.nih.gov/)
- Ensembl (http://www.ensembl.org/)
HapMap

- Large project to identify SNPs in humans.
- A catalog of common genetic variants that occur in human beings.

- What these variants are, where they occur in our DNA, and how often they are distributed among people within populations and among populations in different parts of the world.

- HapMap project opened door to whole genome genotyping platforms

Snp detection platforms

- Taqman assay—Applied Biosystems
- SNPStream assay—Orchid Cellmark/Beckman Coulter
- iPLEX assay—Sequenom

- GoldenGate genotyping microarray—Illumina
- Infinium genotyping microarray—Illumina
- GeneChip microarray—Affymetrix

Illumina Infinium Arrays

- Human660Quad
  - 96 samples per BeadChip
  - 660,000 markers
  - 6.7 median marker spacing
  - Reproducibility=99.9%
  - Denser coverage of ~250 genomic regions (commonly studied in cytogenetics lab)
  - Non-polymorphic probes
  - Subtelomeric, pericentromeric, sex chromosome coverage
  - Most cost effective BeadChip

- Human1M-Duo
  - 96 samples per BeadChip
  - 1 million markers
  - 6.7 median marker spacing
  - Reproducibility=99.9%
  - Denser coverage of ~250 genomic regions (commonly studied in cytogenetics lab)
  - Non-polymorphic probes
  - Subtelomeric, pericentromeric, sex chromosome coverage
  - Most cost effective BeadChip
SNP genotyping array applications

Genotype Analysis

Linkage studies

Whole-genome association

Whole genome LOH / copy number variation analysis

Illumina Infinium technology

- Low amount of DNA required (200ng genomic DNA)
- Single tube sample preparation
- Whole genome amplification with
- Hybridization
- Single base extension
How the data generated

Two-color readout

Normalized intensity values ($R$) and allelic intensity ratios ($\theta$)

These values are used to calculate two metrics for each SNP marker in a sample: LRR ($\log R$ Ratio) and BAF (B Allele Frequency)
LRR is calculated from R value
BAF is calculated from Theta value

Outcome of a SNP array
aCGH - SNP array

- Both platforms have reduced sensitivity in detection of duplications (3 CN) compared with deletions (1 CN) when using signal intensities.

- However, SNP arrays offer an additional metric (BAF) that enables a more accurate detection of copy number than aCGH does.

- BAF is also very informative to detect LOH and UPD regions.
Data analysis

Types of softwares

- Illumina software (BeadStudio, GenomeStudio)
- Commercial softwares (BioDiscovery, GoldenHelix, Partek etc.)
- Non-commercial softwares (PennCNV, QuantiSNP, CNVision etc.)

Data analysis

Three types of possible analysis

- Genotyping analysis
- Specific SNPs sets analysis (= genotyping)
- Copy number analysis
- LOH analysis

Data processing flowchart
Copy Number Variations

DNA sequence which is differently represented among individuals based on its deletion or duplication.

Cnv detection

Methods to detect structural variation
1. Experimental methods
   • Hybridization-based approaches (SNP microarrays and aCGH)
   • Single-molecule analysis (optical mapping)
   • PCR-based techniques

2. Computational methods (NGS)

CNVs are common
Molecular karyotyping examples

- Trisomies
- Deletions
- Duplications
- LOH regions

Trisomy 13

![Trisomy 13](image_url)
600kb Deletion on chr 12

108kb deletion CN=0

5Mb Duplication on chr 11
1.6Mb amplification on chr 4

Copy number = 4

Mosaicism

nine patients with varying levels of mosaicism for deletions involving autosomes
seven patients with varying levels of mosaicism for trisomies

Mosaic trisomy 8(40%) with an altered pattern near the telomere of the p-arm demonstrates UPD

* X chromosome reveals only a single genotype at all loci

Note that the log R ratio reflects a 20% increase for the normal levels expected in a male and the B allele frequency of pseudautosomal regions appears similar to that seen with the autosomes.
Data interpretation

- dbVar (Database of genomic structural variation) http://www.ncbi.nlm.nih.gov/dbvar
- DGV (db of Genomic Variants) http://projects.tcag.ca/variation/
- DECIPHER http://decipher.sanger.ac.uk

- DECIPHER tracks on UCSC GenomeBrowser
  ex:12q14.2

Renal Cell Carcinoma Example

- Nearly 100% of RCC have loss of 3p
- Loss of 9p → independent predictor of poor survival in patients
- Loss of 14q → associated with higher grade and stage

Workshop on "Applied Genomics in the Clinic"

Chromosomal microarray in prenatal diagnosis: overview of the actual application and experience of TOMA laboratory

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Istanbul (Turkey), October 12th – 19th, 2012
Istanbul University/Istanbul Campus Kilicli Hall 15

DECLARATION
"I, or an immediate family member, including partner, have no financial relationship(s) relevant to the content of this presentation"
Overall incidence of chromosome abnormalities in prenatal samples detected by conventional karyotype (1994–2011; all indications are considered)

Abnormal karyotypes

<table>
<thead>
<tr>
<th></th>
<th>n.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CVS</td>
<td>59,000</td>
</tr>
<tr>
<td></td>
<td>AF</td>
<td>135,000</td>
</tr>
</tbody>
</table>

Detection rates of prenatal karyotyping according to the indication for invasive PD

(only chr abn leading to substantial phenotypic effect are reported)

Partial experience of TOMA lab on 64470 AF and 30658 CVS

<table>
<thead>
<tr>
<th>INDICATION FOR INVASIVE PD</th>
<th>TYPE OF PRENATAL SAMPLE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CVS (%)</td>
</tr>
<tr>
<td>13MA (≥35y)</td>
<td>2.42</td>
</tr>
<tr>
<td>Anxiety-no specific indication (&lt;35y)</td>
<td>1.22</td>
</tr>
<tr>
<td>US fetal abnormality</td>
<td>27.65</td>
</tr>
<tr>
<td>Parent carrier of a chr abn</td>
<td>10.55</td>
</tr>
<tr>
<td>Previous affected child/ectus</td>
<td>2.13</td>
</tr>
<tr>
<td>Increased HGS for DS</td>
<td>7.61</td>
</tr>
<tr>
<td>Chr abn in a relative</td>
<td>1.21</td>
</tr>
<tr>
<td>- Fetal demise</td>
<td>15.5</td>
</tr>
<tr>
<td>Other</td>
<td>0.76</td>
</tr>
<tr>
<td>Confirmatory amniocentesis after CVS abn</td>
<td>--</td>
</tr>
</tbody>
</table>

Grati et al. 2010

Incidences of T21, T18, T16, 45,X in PRENATAL SAMPLES out of all chromosome abn leading to substantial fetal phenotypic effect in the first and second trimester

- 73%
- 70%
- 92%
- 88%

Data from Grati et al. 2010
New technologies for genetic diagnosis on CV and AF samples

Through the development of advanced genome-wide or targeted techniques to interrogate the human genome, new methodologies are becoming available for prenatal diagnosis, and the implementation of these methodologies into healthcare provision is changing the landscape of prenatal diagnosis.

Frequency of supplementary investigations after a first-ter karyotyping in “high risk” pregnancies

- **Molecular tests on amnio & CVS: 1.2%**
  (FISH, MLPA, CMA, QF-PCR, UPD test, QF-PCR, etc...)

- **Amnio after CVS: 2.05 %**
  (mosaic in CV)

Their application is mandatory in challenging prenatal cases and efforts have to be done to clarify their pathogenicity and prognosis.
**Supplementary investigations in high risk pregnancies**

- **UPD condition exclusion on AF after a mosaic trisomy for an imprinted chr in CVS**: in 0.5% of CVS analysed (~1/200) (Greti et al, 2006)

- **Increased risk of false negative result due to the incompleteness of the combined cytogenetic analysis on CVS (STC+LTC)** (Simon et al, 1987; Lilford RJ et al., 1991; Ledbetter et al, 1992; Pittalis et al., 1994)

- **Apparent balanced ‘de novo’ rearrangements**: 0.09% in AF and CVS (~1/1000) (Gandino et al, 2009)

- **Marker chromosomes**: 0.1% (~1/1000) in CVS and 0.06% (~1/1600) in AF (Liehr & Weise, 2007; Dalprà et al, 2005; Melvesteii et al, ISPD 2010 personal communication)

- ** Fetuses with US abnormalities and an apparently normal karyotype**: (Signac et al, 2006; Kjaergaard et al, 2010; Novelli et al, 2012)

---

**Array-based Comparative Genomic Hybridization**

DNA from a patient and a normal control are isolated, differentially labeled, and co-hybridized to corresponding DNA segments on the array. The slide is then washed, scanned, and analyzed for DNA dosage alterations (gains and losses) indicated by fluorescent ratios. The results are based on the comparison of the patient’s copy number to the control’s copy number.
**Microarray-based Cytogenetics**

### Advantages:
- Simultaneous and comprehensive identification of both microscopic and submicroscopic unbalanced abnormalities
- Essentially a simultaneous FISH experiment with thousands or millions of probes
- Objective; gains or losses easily identified, genomic location known
- Very high throughput with 2–4 day turn-around time after DNA extraction
- Comprehensive – high resolution in density and genomic coverage

### Disadvantages:
- Will not identify balanced rearrangements
- May uncover unwanted information:
  - Adult on-set condition in a prenatal setting
  - Consanguinity (SNP arrays)
- May identify regions of unclear clinical significance
  - Counseling dilemmas and parental anxiety
Interpretation of Copy Number Variation

Pathogenic CNV (pCNV) is clinically relevant to the proband's phenotype:
- contains dosage-sensitive, disease-causing genes
- occurs within a region of the genome known to be involved in chromosomal syndromes
- are statistically enriched in patient populations as compared to controls

- The CNV is documented as clinically significant in multiple peer-reviewed publications, even if penetrance and expressivity of the CNV are known to be variable
  - A normal result necessitates follow-up tests on the patient/fetus to confirm the diagnosis and/or learn the mechanism of the rearrangement, and parental testing to determine whether the patient's CNV is inherited or de novo. Parental test results will inform recurrence risk estimates.

Interpretation of Copy Number Variation

Benign CNV (bCNV) is not thought to cause an abnormal phenotype:
- is found in both the patient population and control populations in statistically equal frequencies
- Maybe ethnic-specific or found widely in most populations

The CNV has been reported in multiple peer-reviewed publications or curated databases as a benign variant, particularly if the nature of the copy number variation has been well characterized (e.g., copy number variation of the salivary amylase gene) and/or the CNV represents a common polymorphism (CNV should be documented in 1% of the population)

Interpretation of Copy Number Variation

CNV of uncertain clinical significance (VUS) include findings that are later demonstrated to be either clearly pathogenic or clearly benign, however, at the time of reporting, insufficient evidence is available for unequivocal determination of clinical significance and the CNV meets the reporting criteria established by the laboratory:
- is sufficiently large (contains one or more genes) to be of concern but
- does not contain any known disease-causing genes
- has not been seen before in the laboratory, not reported in the medical literature, or not found in available databases

- An uncertain finding calls for testing parents to further inform the diagnosis.
**Interpretation of Copy Number Variation**

- UCSC Genome Browser
- DECIPHER website
- Online Mendelian Inheritance in Man database
- Database of Genomic Variants
- ISCA Consortium database

All can help you interpret the clinical significance of CNVs.

---

**Visualization of Microarray Results**

- Multiple methods exist to confirm array result abnormalities:
  - Fluorescence-probe-based FISH (fluorescence in situ hybridization)
  - qPCR (real-time quantitative polymerase chain reaction)
  - MLPA (multiplex ligation-dependent probe amplification).

- FISH is the only one of these tests, however, that can provide information about the nature and cause of an imbalance, which is especially relevant to determining whether parents carry a balanced rearrangement (an important factor when assessing recurrence risk).

- In Toma lab FISH is performed on abnormal and unclear microarray results whenever possible.

- Chromosome visualization is essential for:
  - Identifying the type of rearrangement
  - Assessing additional family members
  - Providing accurate genetic counseling
Platform resolution

- Array resolution is a function of several factors:
  - the number of probes on an array,
  - the distance between probes, and
  - the statistical algorithms used to analyze array results.

- A higher number of probes does not necessarily mean a higher resolution: an oligo array with fewer probes can have similar resolution to that of a SNP array because the oligo array software requires fewer probes to make an accurate call.

- A 400 kb threshold is recommended as the minimum genome-wide detection rate for arrays, as the majority of copy variations below that level have been shown to be polymorphic in control populations. (Miller D, Adam M, Aradhya S, et al. Am J Hum Genet 2010; 86:749–764.)
CMA in Prenatal testing

- ACOG Opinion Nov 2009 suggested that microarray analysis is an adjunct to routine chromosome analysis in pregnancies with abnormal ultrasound findings.
- NICHD clinical trial publication may likely define the uses for arrays in prenatal diagnosis (Ron Wapner)

CMA in Prenatal testing

Use of Array Genomic Hybridization Technology in Prenatal Diagnosis in Canada

1. Serve as a substitute for conventional karyotyping, especially for specific diagnostic purposes in selected populations and not for general screening in all pregnancies.
2. able to provide results for single or multiple aneuploidies.
3. less prone to technical artifacts than conventional cytogenetics.
4. no requirement for optimal metaphase quality.
5. no requirement for a specific cell line.

CMA in Prenatal testing

Experience with microarray-based comparative genomic hybridization for prenatal diagnosis in over 5000 pregnancies

- Detection rates of clinically significant genomic alterations by microarray analysis for specific anomalies detected by ultrasound
- Wapner R et al, ISPD 2010; ISPD 2012; FMF 2012 (NICHD clinical trial)
NICHD clinical trial - Success rate: CVS vs AF; cultured Vs uncultured samples

DNA Extraction Success

- Cultured: 82.2%
- AF: 77.8%
- Cultured villi: 83.3%
- AF villi: 81.5%
- Uncultured villi: 78.3%
- Uncultured AF: 75.3%

Array Success When DNA Available

- Cultured villi: 96.5%
- AF villi: 95.9%
- Cultured AF: 95.9%
- Uncultured villi: 72.3%
- Uncultured AF: 62.2%

NICHD clinical trial - Days from sampling to results

Days from Sampling to Results

NICHD clinical trial - Conclusions

- aCNA Using Uncultured Villi And AF Is Feasible And Reliable
- Requires experience
- AF DNA extraction
- Running array with less DNA
- Villi More Reliable Than AF
- More DNA
- Higher Quality DNA
- Discrepancies Between Uncultured And Cultured Analysis Occur
- More frequent with CVS
- Biologic Differences: CPM
- Technical Differences: Culture artifact, loss of small segments in culture
Signature’s study - Indications for Study

- Indication for Study
- Number of Cases
  - Advanced Maternal Age: 346
  - Family History: 487
  - Abnormal Maternal Screen: 77
  - Abnormal Ultrasound Findings: 2,859
  - Parental Anxiety: 95
  - Fetoplacental Profile: 417
  - Known Abnormal Fetal Membrane: 649
  - Other: 13
  - Known Parental Rearrangement: 61
  - Total: 5,002

Shaffer et al, 2012

Stratification of the clinically significant results into the <1Mb and >1Mb categories (the reliable resolution for traditional karyotyping)

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>&lt;1Mb</th>
<th>&gt;1Mb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe microdeletion syndrome</td>
<td></td>
<td>1A</td>
</tr>
<tr>
<td>Severe microduplication syndrome</td>
<td>3</td>
<td>1A</td>
</tr>
<tr>
<td>Microdeletion, reduced penetrance</td>
<td>40</td>
<td>1A</td>
</tr>
<tr>
<td>Microduplication, reduced penetrance</td>
<td>15</td>
<td>1A</td>
</tr>
<tr>
<td>Nontelomere deletion</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Terminal deletion</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Terminal duplication</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Other reduced deletions</td>
<td>37</td>
<td>10</td>
</tr>
<tr>
<td>Other interstitial deletion</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Unbalanced translocation</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Deletion</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Autosome rearrangement</td>
<td>1A</td>
<td>11</td>
</tr>
<tr>
<td>Sex chromosome aneuploidy</td>
<td>1A</td>
<td>6</td>
</tr>
<tr>
<td>X-null</td>
<td>1A</td>
<td>1</td>
</tr>
<tr>
<td>Y-null</td>
<td>1A</td>
<td>1</td>
</tr>
<tr>
<td>Polysomy</td>
<td>1A</td>
<td>1</td>
</tr>
<tr>
<td>Complex rearrangements</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>Missing findings</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>172</td>
<td>111</td>
</tr>
</tbody>
</table>

Shaffer et al, 2012

A large, >1Mb deletion that was missed by routine cytogenetic testing

Shaffer et al, 2012
CMA in known abnormal fetal karyotypes

In cases with a known fetal balanced rearrangement the detection rate for clinically significant cryptic abnormalities is 10%.

<table>
<thead>
<tr>
<th>Karyotype Abnormality</th>
<th>Inversion (R)</th>
<th>Translocation (R)</th>
<th>Insertion (R)</th>
<th>Deletion (R)</th>
<th>Other Causes (R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balanced translocation</td>
<td>30.9%</td>
<td>40.5%</td>
<td>40.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inversion</td>
<td>10.0%</td>
<td>10.0%</td>
<td>10.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Translocation</td>
<td>20.0%</td>
<td>20.0%</td>
<td>20.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insertion</td>
<td>10.0%</td>
<td>10.0%</td>
<td>10.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deletion</td>
<td>10.0%</td>
<td>10.0%</td>
<td>10.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other Causes</td>
<td>10.0%</td>
<td>10.0%</td>
<td>10.0%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Shaffer et al., 2012

Loss at a “Balanced” Translocation Breakpoint

- Referred for micrognathia and abnormal karyotype, 46,XX,t(15;17)(q21;q25)dn
- A 2.7 Mb 17q24.3 deletion was identified.
- FISH studies confirmed that the deletion is at the breakpoint.
- No copy number changes were seen on chromosome 15.

CMA in Definition of sSMCs and Rings

In cases with a homogenous or mosaic sSMC or ring chr the detection rate by CMA for clinically significant abnormalities is 10%.

<table>
<thead>
<tr>
<th>Karyotype Abnormality</th>
<th>Inversion (R)</th>
<th>Translocation (R)</th>
<th>Insertion (R)</th>
<th>Deletion (R)</th>
<th>Other Causes (R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balanced translocation</td>
<td>30.9%</td>
<td>40.5%</td>
<td>40.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inversion</td>
<td>10.0%</td>
<td>10.0%</td>
<td>10.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Translocation</td>
<td>20.0%</td>
<td>20.0%</td>
<td>20.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insertion</td>
<td>10.0%</td>
<td>10.0%</td>
<td>10.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deletion</td>
<td>10.0%</td>
<td>10.0%</td>
<td>10.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other Causes</td>
<td>10.0%</td>
<td>10.0%</td>
<td>10.0%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Shaffer et al., 2012
CMA in Definition of sSMCs and Rings

- Chromosome origin
- BKP boundaries
- Size
- Genes content

CMA in Fetuses with US abnormalities and apparently normal karyotype:

- Detection rate of karyotype in fetuses with US abnormalities is ~28% in CVS and ~12% in AF (in average 20%)
  
  Grant et al., JMG, 2010

- With the use of a karyotype-only approach, a relevant portion of clinically significant cryptic variations is not detected

Clinically Significant Copy Number Alterations by IHS

<table>
<thead>
<tr>
<th>Anomaly</th>
<th>Detection Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Anomaly</td>
<td>99/1722 (5.6%)</td>
</tr>
<tr>
<td>Anomalies in 2 or more organ systems</td>
<td>78/819 (9.5%)</td>
</tr>
<tr>
<td>Isolated abnormalities of growth</td>
<td>2/76 (2.6%)</td>
</tr>
<tr>
<td>One or more soft ultrasound markers*</td>
<td>2/78 (2.5%)</td>
</tr>
</tbody>
</table>

* Increased nuchal translucency excluded

Detection rates are significantly higher for multiple anomalies, compared to single systems or non-structural anomalies (5.0%) for both, Fisher exact test.

<table>
<thead>
<tr>
<th>Increased NT</th>
<th>Isolated</th>
<th>Other findings</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 4mm</td>
<td>1/13 (0.9%)</td>
<td>1/7 (14.3%)</td>
<td>2/120 (1.7%)</td>
</tr>
<tr>
<td>≥ 4mm</td>
<td>6/96 (6.3%)</td>
<td>2/12 (16.7%)</td>
<td>8/198 (4.1%)</td>
</tr>
<tr>
<td>Total</td>
<td>7/103 (6.8%)</td>
<td>6/45 (12.8%)</td>
<td>16/352 (4.6%)</td>
</tr>
</tbody>
</table>

Detection rates in addition to those found by karyotyping

Slides courtesy of Shaffer LG (Signature Genomics dataset). ISPD 2012: Paper submitted.
### Anomalies in a Single Organ System or Single Anomaly

<table>
<thead>
<tr>
<th>Organ System or Single Anomaly</th>
<th>Detection Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNS</td>
<td>25/381 (6.6%)</td>
</tr>
<tr>
<td>Heart</td>
<td>6/237 (2.5%)</td>
</tr>
<tr>
<td>Facial (dysmorphism)</td>
<td>6/88 (6.8%)</td>
</tr>
<tr>
<td>Diaphragmatic hernia</td>
<td>4/48 (8.4%)</td>
</tr>
<tr>
<td>Omphalocele</td>
<td>4/49 (8.2%)</td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>10/203 (5.0%)</td>
</tr>
<tr>
<td>Genitourinary</td>
<td>7/115 (6.1%)</td>
</tr>
<tr>
<td>Nuchal or other body fluid accumulation</td>
<td>27/828 (4.3%)</td>
</tr>
</tbody>
</table>

Detection rates in addition to those found by karyotyping

*Slide courtesy of Shaffer LG (Signature Genomics dataset) ISPD 2012; Paper submitted*

### Anomalies in Isolation or with Multiple Findings

<table>
<thead>
<tr>
<th>Anomaly</th>
<th>Detection Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Holoprosencephaly</td>
<td>9/85 (10.6%)</td>
</tr>
<tr>
<td>Posterior fossa defects</td>
<td>21/144 (14.5%)</td>
</tr>
<tr>
<td>Skeletal anomalies</td>
<td>15/140 (10.7%)</td>
</tr>
<tr>
<td>Ventricular septal defect</td>
<td>14/132 (10.6%)</td>
</tr>
<tr>
<td>Hypoplastic left heart</td>
<td>11/68 (16.2%)</td>
</tr>
<tr>
<td>Cleft lip/palate</td>
<td>14/136 (10.3%)</td>
</tr>
</tbody>
</table>

Detection rates in addition to those found by karyotyping

*Slide courtesy of Shaffer LG (Signature Genomics dataset) ISPD 2012; Paper submitted*

### Congenital Diaphragmatic Hernia (CHD) Identified by CMN in a Fetus with Hydrops and Increased NT

- CVS 35 weeks referred for NT 8mm and hydrops at 12+6w
- karyotype 46,XX
- array-CGH 1350 probe (maternal del 8q23) and 8q22.3.2 involving the region CHD type I (chr8:42236490)
- Sensitivities of CHD and 8q22.3.2 US and MRI investigations confirm the presence of left CHD, hypoplastic of the right lung and cardiac heart disease
- Pregnancy is ongoing and couple has been sent to a reference centre for the delivery and newborn surgery
Anomalies in Isolation or with Multiple Findings

These results fully justify the use of microarray testing in trying to identify the etiology of the clinical phenotypes, thus microarray should be:

- Considered as the first test after a RAD test (i.e., QF-PCR) to exclude common aneuploidies (more cost-efficient) (Vetro et al, 2012)
- Used concurrently with conventional karyotyping

Incidence of variations of unclear significance (VOUS)

With the increased ability to detect cryptic imbalances with microarrays, VOUS can be identified

- *Inherited* from a parent —— benign
- *De novo* in origin —— potential clinical relevance

Inheritance from a parent often does not help assign the clinical relevance of the alteration because of the possibility of incomplete penetrance or variable expressivity.

Reporting criteria seem to differ between laboratories regarding VOUS

CMA AS A SUBSTITUTE OF KARYOTYPE IN ALL PREGNANCIES (AMA and Anxiet)

<table>
<thead>
<tr>
<th>Study</th>
<th>No. with significant abnormalities in AMA # cases</th>
<th>DR (%)</th>
<th>No. with significant abnormalities in anxiety # cases</th>
<th>DR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Florentino et al 2011</td>
<td>1/144</td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amengual et al 2011</td>
<td>4/333</td>
<td>1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Park et al 2010</td>
<td>12/1072</td>
<td>0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lee et al 2012</td>
<td>1/346</td>
<td>0.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brennan et al. 2012 (personal communication)</td>
<td>3/194</td>
<td>0.76</td>
<td>5/988</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Overall results: 27/2636 (0.47) 2/1094 (0.19)

Combining Signature Genomics’ data with that of 5 other studies, ~0.5% of pregnancies with AMA or with anxiety have abnormalities detected by array that would not be detectable by karyotyping.
Incidence of variations of unclear significance (VOUS)

<table>
<thead>
<tr>
<th>Indication for study (IFS)</th>
<th>Number with unclear variants</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal ultrasound</td>
<td>1382958</td>
<td>4.8%</td>
</tr>
<tr>
<td>Abnormal serum screening</td>
<td>577</td>
<td>6.5%</td>
</tr>
<tr>
<td>Family history</td>
<td>11467</td>
<td>2.3%</td>
</tr>
<tr>
<td>AMA</td>
<td>8346</td>
<td>2.3%</td>
</tr>
<tr>
<td>Anxiety</td>
<td>105</td>
<td>1.1%</td>
</tr>
<tr>
<td>Otherwise specified</td>
<td>0/13</td>
<td>0.9%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>1630876</td>
<td>4.2%</td>
</tr>
</tbody>
</table>

*1.1% of cases had unclear variants that were de novo or of unknown inheritance.

SRS: courtesy of Shaffer LG (Signature Genomics dataset) ISPA 2012 Paper submitted

CMA in AMA and Anxiety as substitute of karyotype

The analysis of the proportion of VOUS compared to clinically significant cryptic unbalances shows that using CMA technology in prenatal setting without a specific clinical indication as a substitute for conventional karyotype can provide more unclear than clinically significant results

Pathogenic CNVs: 0.5%

AMA & ANXIETY INDICATIONS

Points to be improved:
- Knowledge on human genome architecture in normal population (Alloin et al., 2011; Cooper et al., 2011; Ianna et al., 2010; Ianna et al., 2005;...)
- Knowledge on entire phenotypic spectrum of microdeletion and microduplication syndromes and on uncertain variants (Cooper et al., 2011; Ianna et al., 2010; Soder et al., 2010;...)
- Pre- and post-test counseling approach models for prenatal CMA
  - Reporting unclassified findings related to late onset/uncertain diseases?
  - Reporting VOUS?
  - Reporting pathologic CNVs unrelated to any indication for CMA?
    (McEvoy et al., 2012; Wagner et al., 2012; Dorsos et al., 2012)
THE USE AND ANALYSIS OF EXPRESSION MICROARRAY DATA

Marco Fabbri – JRC
How to study a biological process?

Open the cells
Microarray process

Making Sense of the Data
Analysis Tools

- Software
  - MeV
  - GeneSpring GX
  - Partek

- Annotation
  - GeneGo
  - AmiGO
  - GeneAtlas

Type of Data Analysis

Class: characteristic shared by a group (e.g., cancer vs. normal)

- Identify differences at molecular levels between known classes (class comparison)
- Diagnose or predict to which class a new sample belongs (class prediction)
- Divide samples into reproducible classes that have similar behavior or properties (class discovery)
**Class comparison**

Differential expression analysis

**Goal:** Identify genes differentially expressed among predefined classes of samples.

**What genes are upregulated between control and test or multiple test conditions?**
- Normal vs. tumor or Treated vs. untreated

**Example:** Measure gene products before and after toxic exposure to identify mechanisms of action of toxicant.

---

**Whole genome analysis and microRNAs regulation in HepG2 cells exposed to cadmium.**

- Gene upregulated (microarray)
- KEGG enrichment analysis (David)
- MicroRNA downregulated (HTqPCR)
  - Target prediction and KEGG enrichment analysis (Diana Mirpath)
Whole genome analysis and microRNAs regulation in HepG2 cells exposed to cadmium.

MicroRNA extraction

Fold change: Not sufficient, needs statistics!
pathways downregulated by cadmium
The Three Gene Ontologies

- **Molecular function**
  - The tasks performed by individual gene products

- **Biological process**
  - Broad biological goal or objective that are accomplished by ordered assemblies of molecular functions

- **Cellular component**
  - Subcellular structures, locations, and macromolecular complexes

  A gene product may be part of several different ontologies

Pathway Analysis

- Discover relationships between the annotated genes

---

Schematic model of Cd activity

Whole Genome Analysis and MicroRNAs Regulation in HepG2 Cells Exposed to Cadmium

Pathway analysis tools:

- **Reactome**
- **Ingenuity**
Class discovery

- Goal: Identify sets of genes or samples that cluster together.
- Example: Cluster temporal gene expression patterns to get insight into genetic regulation in response to tonic insult (Huang et al., Toxicol Sci, 2001)

Class Discovery

- Objective?
  - Can data tell us which classes are similar?
  - Are there subgroups?
- Methods
  - Cluster analysis
  - K-means
  - Principal Component Analysis (PCA)
  - Self-organizing maps (SOM)
  - Class IDs are not known to the algorithm
  - For example, does not know which one is cancer or non-cancer?
  - Do the expression values differentiate, class is distinct now clustered?
Aim of clustering: Group objects according to their similarity

Cluster: a set of objects that are similar to each other and separated from the other objects.

Example: green and red data points were generated from two different normal distributions.

Clustering microarray data

- Genes and experiments/samples are given as the row and column vectors of a gene expression data matrix.
- Clustering may be applied either to genes or experiments (regarded as vectors in $\mathbb{R}^n$ or $\mathbb{R}^m$).

Why cluster genes?

- Identify groups of possibly co-regulated genes (e.g. in conjunction with sequence data).
- Identify typical temporal or spatial gene expression patterns (e.g. cell cycle data).
- Arrange a set of genes in a linear order that is at least not totally meaningless.
Why cluster experiments/samples?

- Quality control: Detect experimental artifacts/bad hybridizations
- Check whether samples are grouped according to known categories (though this might be better addressed using a supervised approach: statistical tests, classification)
- Identify new classes of biological samples (e.g. tumor subtypes)

Example of Class Discovery:
Distinct Types of Diffuse Large B-Cell Lymphoma

- DLBCL is clinically heterogeneous
- Specimens were clustered based on their expression profiles of GC B-cell associated genes.
- Two subgroups were discovered:
  - GC B-like DLBCL
  - Activated B-like DLBCL

(Figures and information taken from Alizadeh et al., Nature 403:503-11, 2000)

Class prediction
Goal: Develop multi-gene predictor of class membership.
Diagnose or predict to which class a new sample belongs
Example:
Molecular Classification of AML and ALL by Gene Expression Monitoring
Therapeutic relevant genomic Classifiers

Oncologists need improved tools for selecting treatments for individual patients. Most cancer treatments benefit only a minority of the patients to whom they are administered.

Expression profiling new technology to identify classifiers for tailoring treatments to patients.

Method: Microarray

- RNA prepared from cells was hybridized to high-density oligonucleotide Affymetrix microarrays containing probes for 6817 human genes;

- Samples were subjected to a priori quality control standards regarding the amount of labeled RNA and the quality of the scanned microarray image.

A MULTIGENE CLASSIFIER

A multigene expression signature classifier is a function that provides a classification of a tumor based on the expression levels of the component genes.

Split the samples in two groups (training set and a test set).

Gene selection in the training set (good predictors)

Application of the voting procedure in the test set and the error evaluation.
Split-Sample

The most straightforward method of estimating the accuracy of future prediction is the split-sample validation method of partitioning the set of samples into a training set and a test set.

Training set: 38 bone marrow samples (27 ALL, 11 AML) obtained from acute leukemia patients at the time of diagnosis.

Test set: 34 leukemia samples (24 bone marrow and 10 peripheral blood samples).

This internal validation should not, however, be confused with the kind of external validation of the classifier in a setting simulating a clinical application.

Gene selection

Most classifiers do not use all of the genes whose expression is measured. Consequently, one step in developing a classifier is determining which genes to include.

The number of genes that are actually differentially expressed between the classes ("informative genes") is usually small compared to the number of genes that are not differentially expressed ("redundant genes").

Voting scheme

- Compare expression of genes of patients in the test set.
- Each gene of the patient is assigned to the class with an expression more similar.
- The patient is assigned to the more voted class and error rate is evaluated.
Results

How good are the predictors?

Independent test: The 50-gene predictor was applied to an independent collection of 34 leukemia samples. The predictor made assigned 29 of the 34 samples, and the accuracy was 100%;
Mammaprint

- Gene signature derived from selected retrospective review
  - 78 node negative breast cancer patients not treated with adjuvant therapy
    - Supervised top-down approach
  - Two outcomes — "Low Risk" or "High Risk" of disease recurrence without adjuvant therapy
  - Uses fresh or frozen tumor, not formalin-fixed paraffin-embedded
  - 70 gene cDNA microarray
  - FDA approved
Description of evaluated studies

Can we trust those studies?

**Prediction of cancer outcome with microarrays: a multiple random validation strategy**

Class prediction suffers of one major limitation over fitting.
The algorithm performs well on the samples from which it was built but poorly on independent samples.

**Random validation strategy**

![Diagram of random validation strategy]

- Training set (50 best predictors)
- Test set (number of misclassification)
- 500 times
- Evaluation of the misclassification rate
- 50 best predictors are selected

(Entire process is repeated with different size of the training set)
Genes included in at least 150 of 500 molecular signatures for two of the studies

Proportion of misclassifications in validation sets as a function of corresponding training-set sizes
Abstract
Within the context of JRC Enlargement and Integration Activities (E&IA), the workshop “Applied genomics in the Clinic” was organised in Istanbul on 17-19 October 2012. The main aim of the workshop was to get an overview of the state of the art of applied genomics in the clinical context in accession and candidate countries, as well as new members, to share best practices in EU and to evaluate these in the light of a public health perspective. There is a clear divide behind the genomic services offered in a country and the awareness among research scientists of the available genomic applications and the future impact of genomic technologies on health services and clinical approaches. In all countries there are a number of common obstacles that delay penetration of genomic technologies in clinical applications: lack of recognised experts (medical genetics has to be recognised as a medical specialty) lack of a regulatory framework that involves political determination of decision makers, lack of common databases on methods and experts, lack of ongoing education for physicians and most importantly reimbursement of testing. Stronger connections and collaborations with the EU for research and technology transfer will function as a leverage for these countries in adopting genomic tools and harmonising the quality of healthcare services they offer. It is very important to establish recognized objective state of the art guidelines for application of genomic technologies in clinical practice. Such guidelines adopted by countries will form the basis of reimbursement policies at national and cross border levels. In addition establishing reliable, not for profit, open access databases for building reference datasets for correct and efficient interpretation of complex data generated by advanced genomic technologies will speed up adoption of the technology in the clinic.
As the Commission’s in-house science service, the Joint Research Centre’s mission is to provide EU policies with independent, evidence-based scientific and technical support throughout the whole policy cycle.

Working in close cooperation with policy Directorates-General, the JRC addresses key societal challenges while stimulating innovation through developing new standards, methods and tools, and sharing and transferring its know-how to the Member States and international community.

Key policy areas include: environment and climate change; energy and transport; agriculture and food security; health and consumer protection; information society and digital agenda; safety and security including nuclear; all supported through a cross-cutting and multi-disciplinary approach.