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Applied Genomics in the Clinic

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

Laura Gribaldo, Sadiye Birep Aygun, Angela Brand, Jeremy Sujie Cao, Irena Drmic Hofman, Simona Dumitriu, Marco Fabbri, Francesca Romana Grati, Sibel Aylin Ugur Iseri , Leyla Kapur-Pojskić, Ilker Karacan , Chris Junnian Liu, Pinar Uysal Onganer, Ugur Özbek, Ewa Stepien, Ahmet Yesilyurt, Theodor Zamfirov

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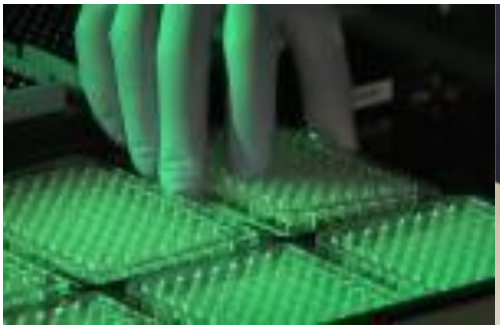

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"Applied Genomics in the Clinic"

Istanbul, Turkey, 17-19 October 2012

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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 Trysomy (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 Stęphień, 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 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.*

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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. 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**	a- BUL, POL, UK b- BUL c- TR, BH, CRO, POL, UK d- BUL, POL e- BUL f- RO
2. Which of the following new/emerging technology platforms are employed regularly in the clinical context in your country ? **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**	a- POL, UK b- TR, RO, UK c- POL, CRO, TR, RO, BUL, UK d- POL, TR, RO, BUL, UK e- UK f-
3. In your opinion which technology platform will penetrate fastest in to clinical applications? Please justify very briefly **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**	a- NONE b- TR, RO, BUL, UK c- POL, BOS, RO, UK d- CRO, BOS, RO e- TUR, BUL, RO
4. In your opinion which application area of genomic technologies will the clinic benefit from the earliest? Please justify very briefly **a. Cancer genotyping** **b. Prenatal testing** **c. Preimplantation genetic diagnosis** **d. Rare diseases** **e. Personalised medicine** **f. Some other area (please specify)**	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-

5. What is the scope of health/social security/ insurance system coverage in your country for : **a. Prenatal testing employing conventional karyotyping** **b. Prenatal testing employing molecular karyotyping (microarrays)** **c. Sequencing for disease diagnosis (common/rare)** **d. Next generation sequencing for disease diagnosis (common/rare)** **e. Pharmacogenomic testing for drug responsiveness** **f. Preimplantation genetic diagnosis**	a- CRO, POL, BOS, TR, RO, BUL, UK b- POL BY SPECIAL PROGRAMMES, RO, UK c- POL AS SUPPLEMENTARY DIAGNOSTICS, TR, BUL, UK d- UK e- CRO, POL COVERED AS PART OF CANCER GENOTYPING f- TR FOR DISORDERS WHICH CAN BE TREATED BY STEM CEL TRANPLANTATION, UK
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 **a. Prenatal testing employing conventional karyotyping** **b. Prenatal testing employing molecular karyotyping (microarrays)** **c. Sequencing for disease diagnosis (common/rare)** **d. Next generation sequencing for disease diagnosis (common/rare)** **e. Pharmacogenomic testing for drug responsiveness** **f. Preimplantation genetic diagnosis**	a- CRO SL/PL , POL SL/PL, BOS SL, TR SL/PL, RO SL/PL, BUL SL/PL, UK SL/PL b- POL SL/PL, TR PL, RO SL/PL, BUL PL, UK SL/PL c- POL SL/PL, BOS SL, TR SL/PL, BUL SL/PL, UK SL d- TR PL, BUL PL, UK SL e- CRO PL, POL SL/PL, TR PL, BUL PL f- POL PL, TR PL, BUL PL, UK PL
7. How often do you collaborate with a laboratory outside your own country for research / for diagnostic purposes? Please state scope (e.g. rare disease diagnosis, populations study, etc.)	**CRO** : RDD ONCE EVERY THREE TO SIX MONTHS **PO**L: RDD / RESEARCH ONCE OR TWICE A YEAR **BOS** : RDD UPTO 10-15 TIMES A YEAR **TUR** : RDD FEW TIMES A MONTH, RESEARCH ONCE A FEW YEARS **RO**: NO REPLY **BUL**: RDD ONCE A MONTH, RESEARCH ONCE EVERY 3 MONTHS
8. In your opinion what is the biggest obstacle in your country in particular for penetration of genomic applications in clinical services ?	**CRO**: ORGANISATION AND FINANCES **POL**: DISPERTION OF PROCEDURES OVER THE LIST OF GUARANTEED SERVICES (so called basket), LACK OF COMPREHENSIVE FINANCIAL /EDUCATIONAL PROGRAMS SUPPORTING RESEARCH IN CLINICAL GENETICS **BOS**: LACK OF POLICIES REGULATING GENETIC TESTING SERVICES IN FRAME OF MEDICAL

	DIAGNOSIS/ HEALTHCARE, LACK OF INVESTMENT IN THE FIELD BY PUBLIC HEALTH AUTHORITIES **TUR** : REIMBURSEMENT PROBLEM, RECOGNITION OF SERVICES BY THE SOCIAL SECURITY SYSTEM **RO**: POLITICAL WILL, ORGANISATION, EDUCATION **BUL** : SOCIAL SECURITY COVERAGE **UK**: FINANCES AND POLICIES
9. Is medical genetics recognised as a "medical specialty branch" in your country's education system?	**CRO**: ONLY FOR MEDICAL DOCTORS AS A CLINICAL SPECIALTY (NO LABORATORY SPECIALTY, NOT OFFERED TO NON MEDICINE ORIGIN STUDENTS) **POL**: YES, NOT AS A LABORATORY SPECIALTY **BOS**: NO **TUR:** YES, NOT AS A LABORATORY SPECIALTY **RO**: YES **BUL** : YES **UK**: YES
10. Name three measures at local authorities' level that will benefit incorporation of genomic applications in healthcare services offered in your country	**CRO**: 1. PRIORATISATION 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 MORST 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

	HEALTH IMPACT AND TECHNOLOGY ASSESMENT BY INDEPENDENT EXPERT PANELS 3. AWARENESS RAISING AMONG PHISIANS AND PATIENTS (CREATE PUBLIC DEMAND **RO:** 1. COMPLETION AND IMPLENEMATION OF THE NATIONAL PLAN FOR RARE DISEAES 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 Kiliclik 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.

European Commission

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

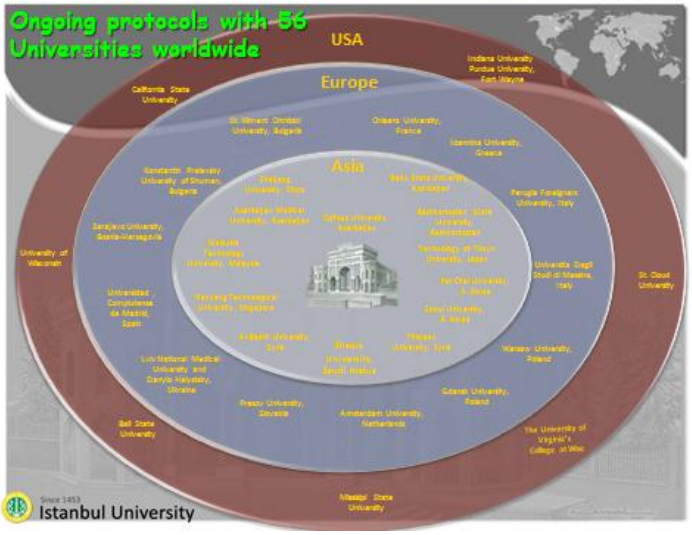
Improve communication

Strengthen networking

Final goal

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


ISTANBUL UNIVERSITY
Since 1453
✓20 Faculties
✓10 Vocational Schools
✓16 Research Institutes
✓60 Research Centers
✓ 74 000 Students
✓ 2600 Academics
✓ 10 000 Employees


Ongoing protocols with 56 Universities worldwide
USA
Europe
Asia
Istanbul University

HEALTH SERVICES

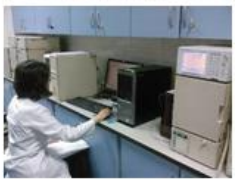
- 2 University Hospital
- Cardiology Institute
- Oncology Institute
- Faculty of Dentistry

- 3500 bed capacity
- 2.5 million outpatient/year
- 100.000 inpatient/year

European Commission

Istanbul University,
Institute of Experimental Medicine (DETAE),



Istanbul University has 7 Institutes having 492 postgraduate programmes. They are 254 master and 238 doctora programmes.

Institute of Business Economy	(11 Master)
Institute of Forensic Sciences	(3 Master, 3 Doctorate)
Institute of Marine Sciences and Management	(9 Master, 9 Doctorate)
Institute of Health Sciences	**(83 Master, 79 Doctorate)**
Institute of Basic and Applied Sciences	**(57 Master, 54 Doctorate)**
Institute of Social Sciences	(90 Master, 92 Doctorate)
Institute of Ataturk's Principles and Reforms	(1 Master, 1 Doctorate)

Istanbul University,
Institute for Experimental Medicine
(DETAE)

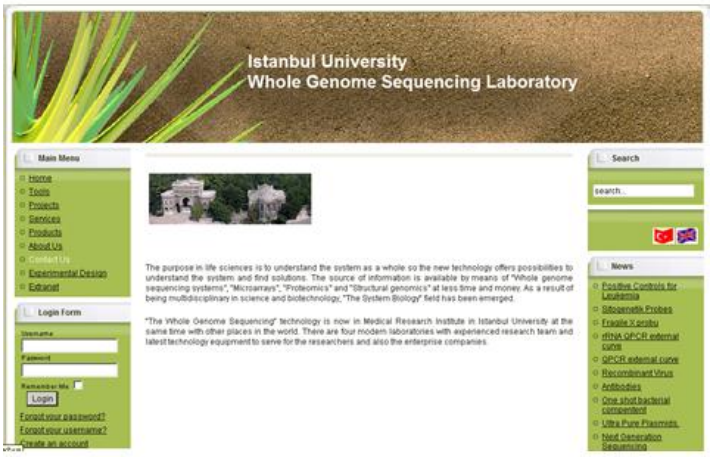
Five Departments
- Genetics
- Immunology
- Molecular Medicine
- Neurosciences
- Animal Model Organisms

European Commission

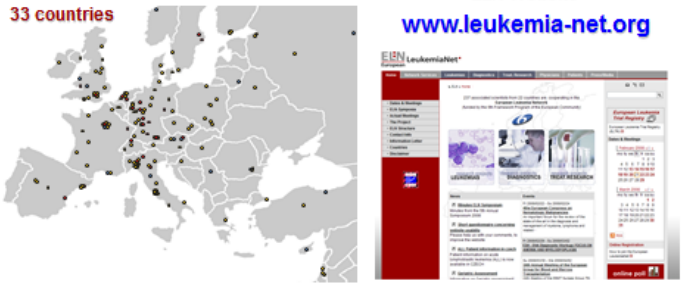
Istanbul University, Institute for Experimental Medicine (DETAE)

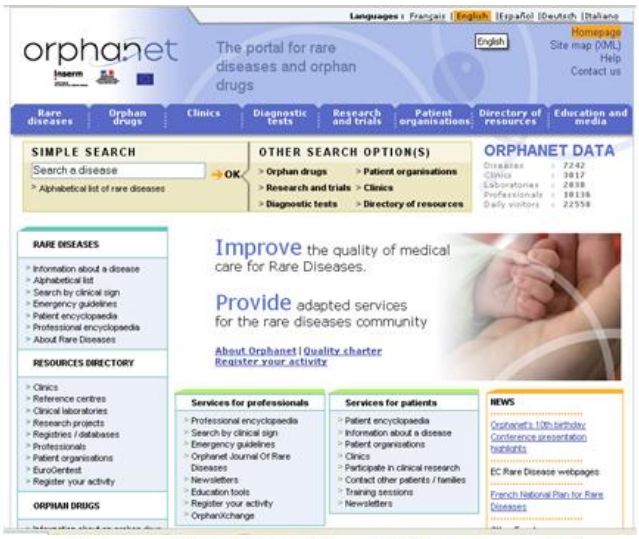
Five Units/Laboratories

- 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)

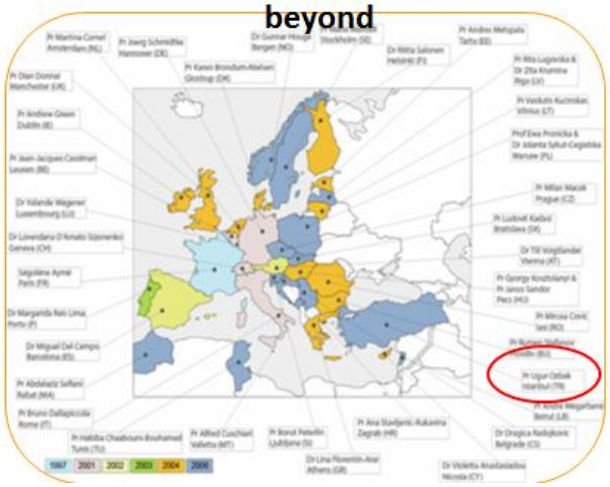



EPICURE
Functional Genomics and Neurobiology of Epilepsy: a Basis for New Therapeutic Strategies


33 countries
Austria, Belarus, Belgium, Croatia, Cypres, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Ireland, Israel, Italy, Lettland, Lithuania, Norway, Poland, Portugal, Romania, Russian Federation, Slovakia, Slovenia, Spain, Sweden, Switzerland, The Netherlands, Turkey, U.K., Ukraine
ELN-Website
www.leukemia-net.org
Information for physicians & patients
European Leukemia Trial Registry (ELTR)
Dates & Meetings, Links, Literature
Becoming member of the ELN



Data collection all over Europe and beyond

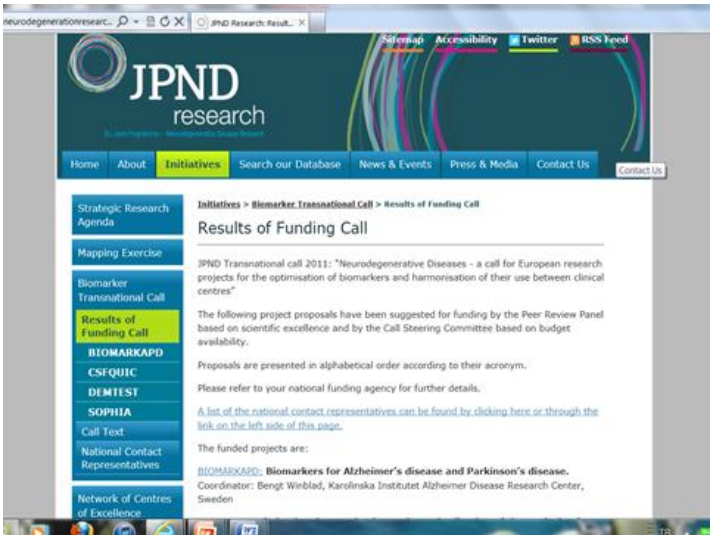


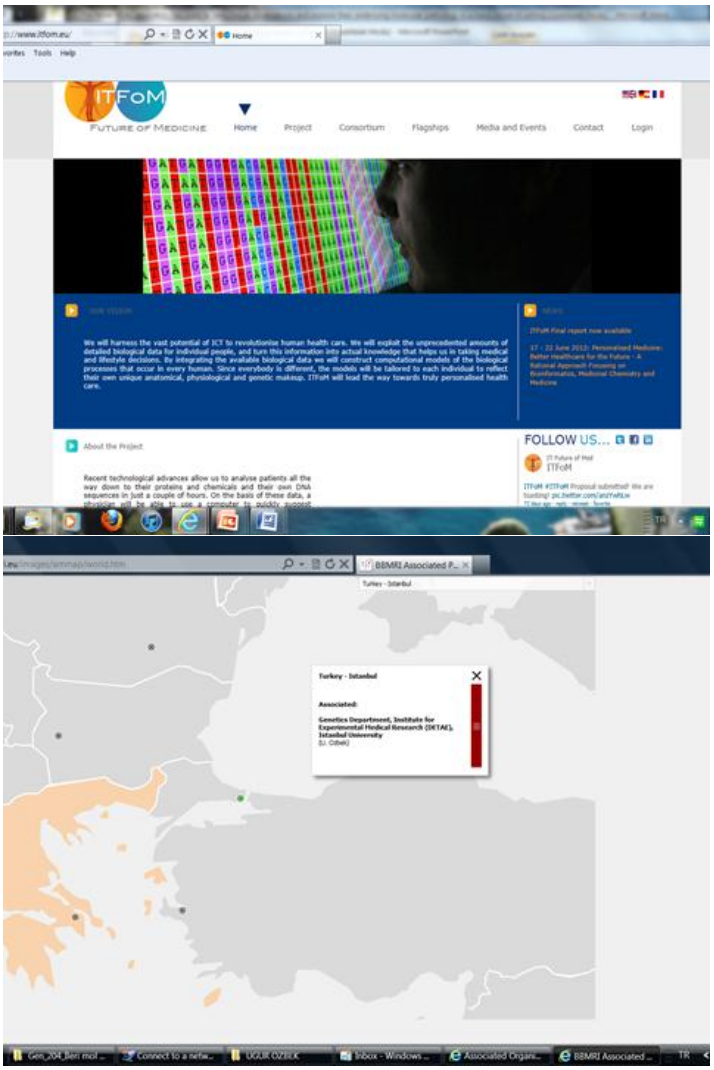
European Commission

MedGeNet

Euro-Mediterranean Network for Genetic Services

MEDGENET





In Summary...

- Tasks driven by DETAE:
  - Combining multidisciplinary reseach 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.

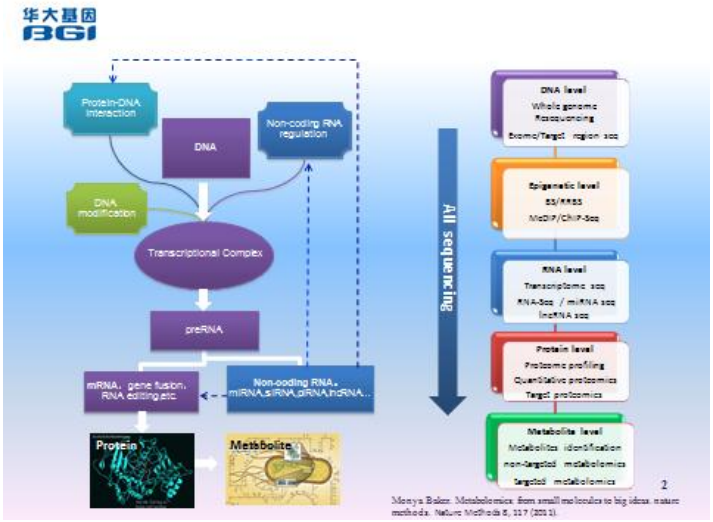
Application of Next Generation Sequencing in Human Disease Research and Clinical Application

Jeremy Sujie Cao

Oct. 17th

Workshop on "Applied Genomics in the Clinic"

www.genomics.cn BGI - Premier Scientific Partner

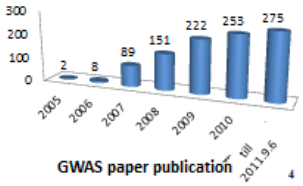


Haplotype Map of the Human Genome

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.

BGI--> 10%

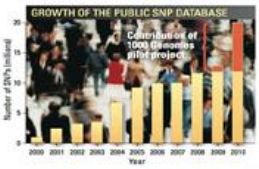
- **The first GWAS paper** about Age-related macular degeneration was published **in Science in 2005**
- **More than 1000** total publications until now
- **More than 4000 associated SNPs** related with common diseases such as diabetes, breast cancer, have been identified and replicated in GWAS.



1000 Genomes

A Deep Catalog of Human Genetic Variation

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



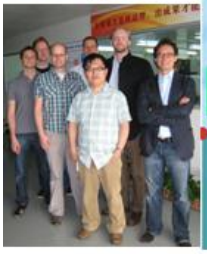
Premier Scientific Partner

wellcome trust sanger institute

genome.gov National Human Genome Research Institute

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

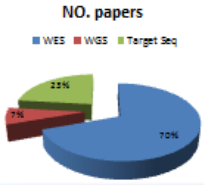


Are rare and novel variants more functional?

Extreme cases--Mendelian Disorders

Sequencing Technologies

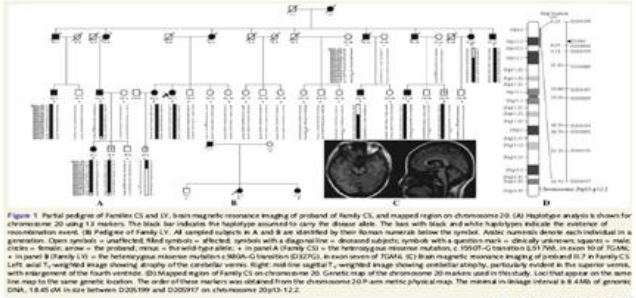
- **Whole genome resequencing**
  - Completeness
  - Long Insertions/deletions, Structural variations, CNVs detection
- **Whole Exome sequencing**
  - Cover majority of causative mutations of MDs
  - Cost-effective
- **Target capture sequencing**
  - Economical
  - Pre-knowledge of Candidate region



BRAIN

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

Jun Ling Wang,[1,2,*] Xu Yang,[3,*] Kun Xia,[2,*] Zheng Mao Hu,[2] Ling Weng,[1] Xin Jin,[3,4] Hong Jiang,[1,2] Peng Zhang,[2] Lu Shen,[1,5] Ji Feng Guo,[1,5] Nan Li,[1] Ying Rui Li,[2] Li Fang Lei,[1] Jie Zhou,[1] Juan Du,[1] Ya Fang Zhou,[1] Qian Pan,[2] Jian Wang,[3] Jun Wang,[3,4] Rui Qiang Li[3,4] and Bei Sha Tang[1,2,5]



Wang JL, *et al.*, 2010

Are rare variants more functional?

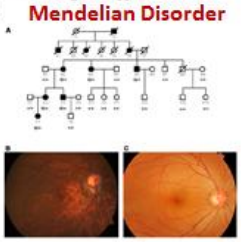
PLOS GENETICS

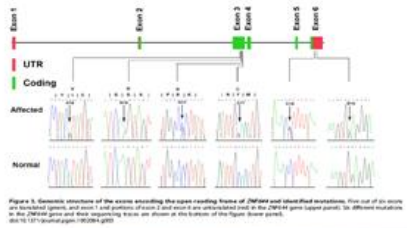
Exome Sequencing Identifies *ZNF644* Mutations in High Myopia

Yi Shi[1,2*], Yingrui Li[3*], Dingding Zhang[1,2], Hao Zhang[3], Yuanfeng Li[1], Fang Lu[1,2], Xiaoqi Liu[1,2], Fei He[1,2], Bo Gong[1,2], Li Cai[1], Ruiqiang Li[3], Shihuang Liao[4], Shi Ma[1,2], He Lin[1,2], Jing Cheng[1,2], Hancheng Zheng[3], Ying Shan[3], Bin Chen[4], Jianbin Hu[4], Xin Jin[3], Peiquan Zhao[4], Yiye Chen[4], Yong Zhang[3], Ying Lin[1,2], Xi Li[1], Yingchuan Fan[4], Huanming Yang[3], Jun Wang[3], Zhenglin Yang[1,2]

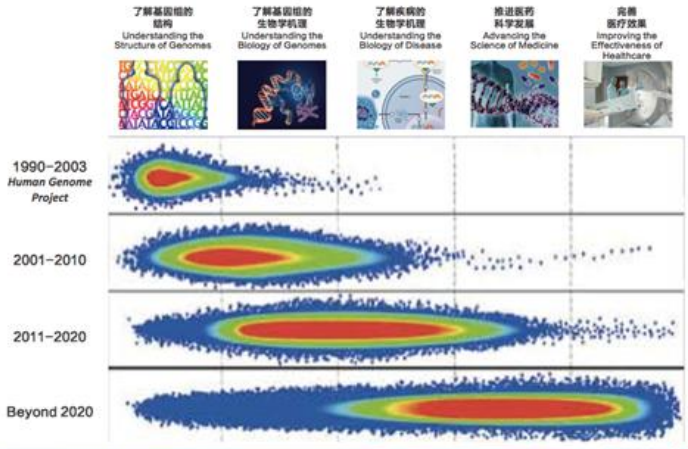
An extreme case to show that rare variants has stronger effect

Mendelian Disorder



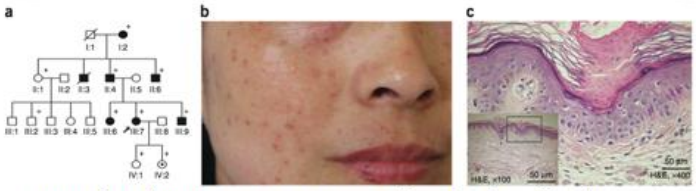


基因组发展与成就:从碱基对到身边应用
Genomic Accomplishments: Base Pairs to Bedside

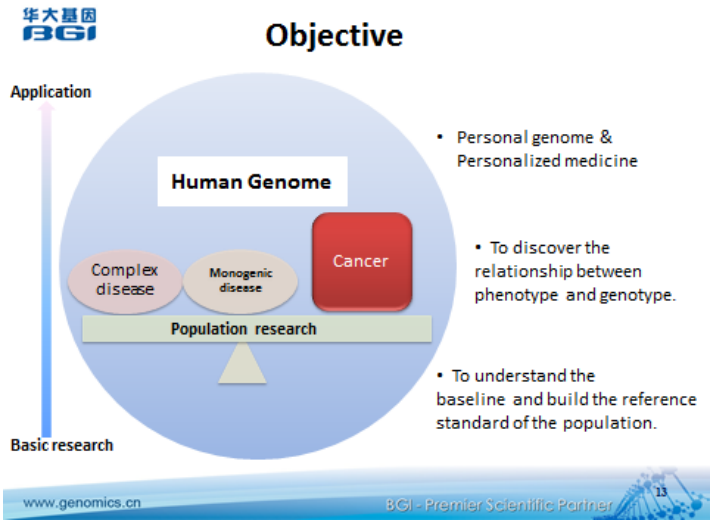


nature genetics

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 gene (MVK) emerged as the only candidate
- Sanger sequencing in 57 individuals with familial DSAP and 25 individuals with sporadic DSAP identified MVK mutations in 33% and 16% of these individuals (cases), respectively. All 14 MVK mutations identified in our study were absent in 676 individuals without DSAP.


Objective
Application
Human Genome
Complex disease
Monogenic disease
Cancer
Population research
Basic research
• Personal genome & Personalized medicine
• To discover the relationship between phenotype and genotype.
• To understand the baseline and build the reference standard of the population.
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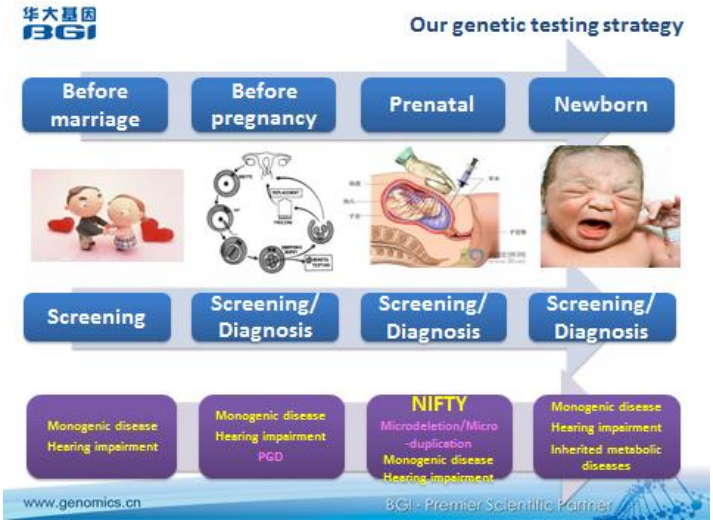
13


Our genetic testing strategy
Before marriage
Before pregnancy
Prenatal
Newborn
Screening
Screening/ Diagnosis
Screening/ Diagnosis
Screening/ Diagnosis
Monogenic disease
Hearing impairment
Monogenic disease
Hearing impairment
PGD
NIFTY
Microdeletion/Micro -duplication
Monogenic disease
Hearing impairment
Monogenic disease
Hearing impairment
Inherited metabolic diseases
www.genomics.cn
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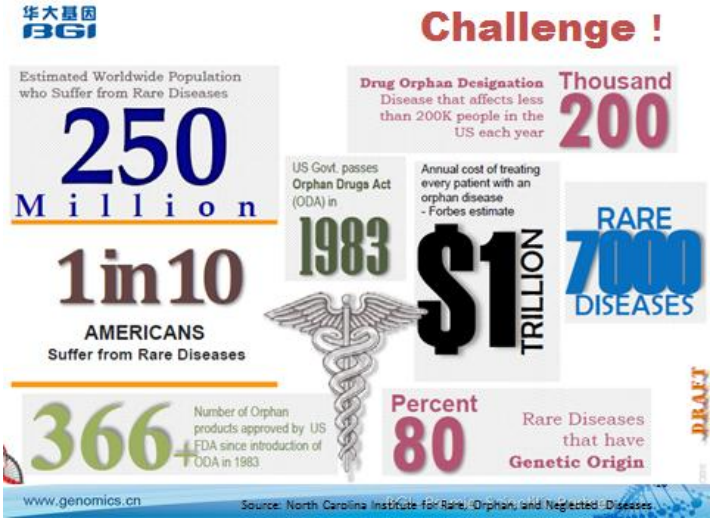
What is monogenic disease?

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

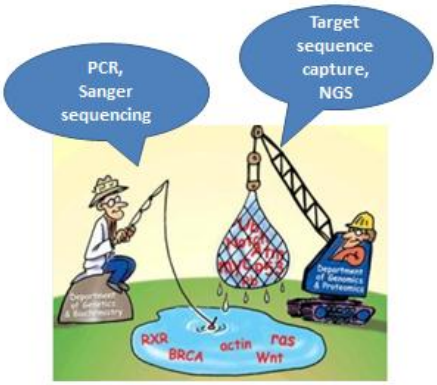
- Nucleic acid amplification（qPCR, RT-PCR, MLPA）
- Sequencing（Sanger, NGS）
- array CGH, SNP array

- 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

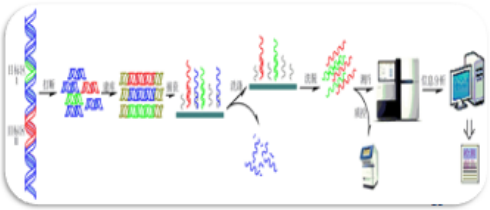
- Target sequence capture combined with NGS
- Exome/whole genome sequencing
- Sanger sequencing

Target sequence capture + NGS

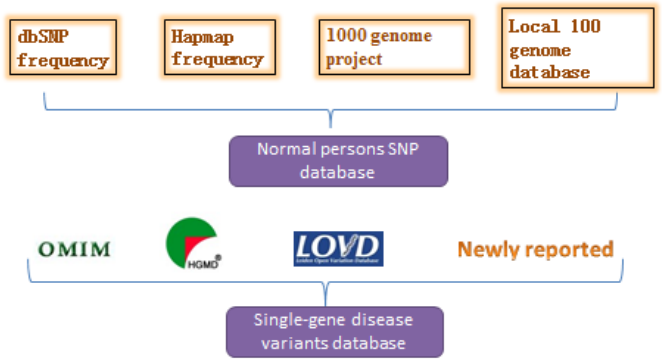


Technique principle

- 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



OMIM: online Mendelian inheritance in Men, http://www.ncbi.nlm.nih.gov/omim
HGMD: Human Gene Mutation Database. Cardiff University, UK http://www.hgmd.cf.ac.uk/ac/index.php
LOVD: Leiden Open Variation Database.

European Commission

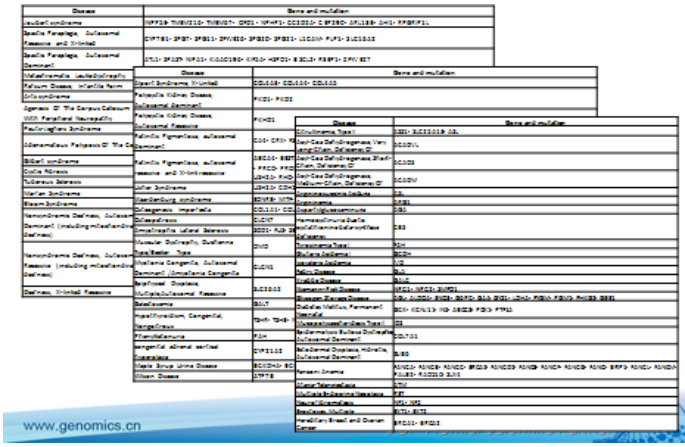
华大基因 BGI

Advantages

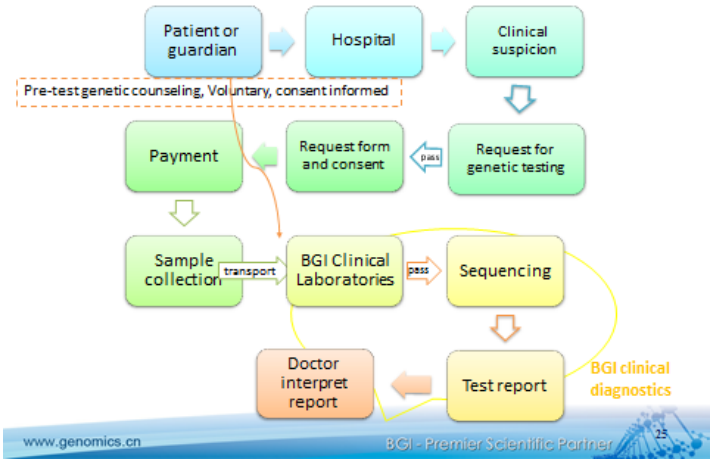
- **High throughput**
  Multiplex testing; detect 144 monogenic diseases (over 300 genes)
- **Wide detection scope**
  Simultaneously detect point mutation, minor insertion/deletion (<20bp), and large homozygous deletion/duplication; able to find novel mutation
- **Accurate and sensitive**
  Covers >95% target gene, sensitivity >99%
- **Automated bioinformatics analysis**
  Fast mutation identification; disease database and genetic polymorphism database available

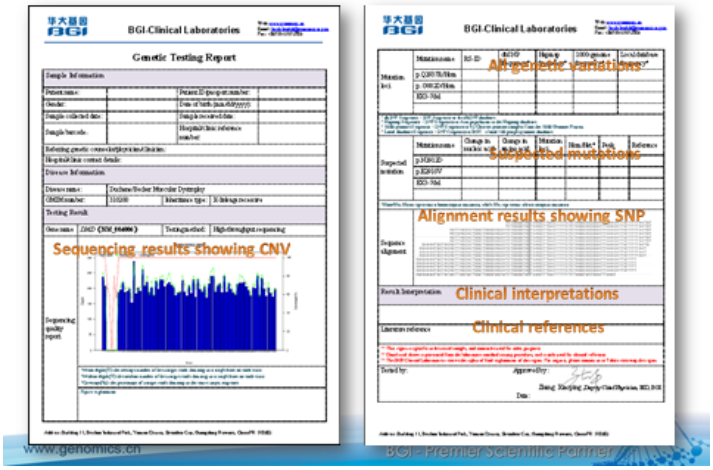
华大基因 BGI

Examples of diseases



Workflow





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?

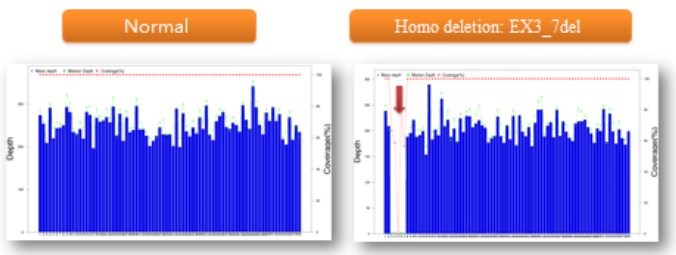
- Patients with clear phenotypes
- Patients with suspected disease but not sure
- People with no phenotypes but have family history
- Carrier screening

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

Case: Duchene/Becker Muscular Dystrophy

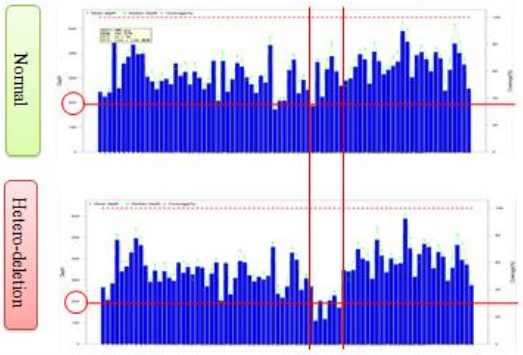
- 1 in 3500 live boy affected, 2/3 inherited from parents
- Muscle progressively dysfunction and sweeny. Sub-divide to DMD (OMIM 310200) and BMD (OMIM 310376).
- DMD onset at age 2-8, immobilize at 15, kill at 20s due to severe complications. X-recessive, mainly occur in boys.
- Mutation of DMD gene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

DMD homozygous deletion

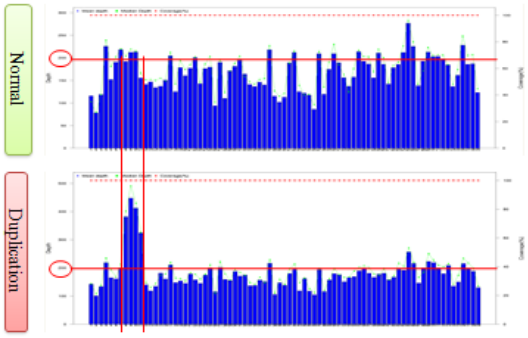


about 60% of DMD is caused by deletion. There are rare cases caused by micro-duplication (5-10%). The rest is caused by point mutation or frame shift which can take place in the entire coding region. Because DMD has very long and large numbers of exons, conventional methods such as Sanger sequencing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

DMD heterozygous deletion



DMD duplication



European Commission

Case: Gavin's Story

——Whole Exome Sequencing Finds Mystery Mutation by BGI

http://www.gavinsfoundation.org/whoisgavin.html

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

nature genetics

- We sequenced the exome of an individual with LCA and identified nonsense (c.507G>A, p.Trp169*) and missense (c.769G>A, p.Glu257Lys) 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.Glu257Lys variant.

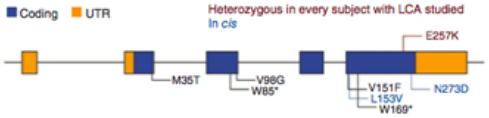
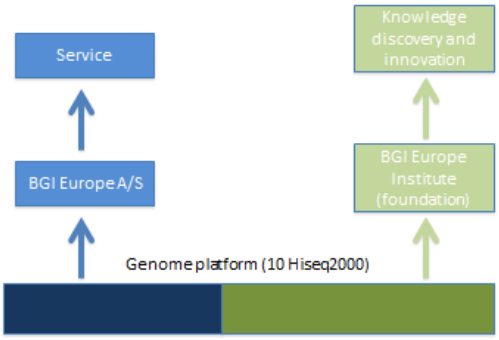


Figure 1 Schematic of the *NMNAT1* gene depicting NMNAT1 variants identified in individuals with LCA.

Disease list of Whole exome sequencing

Disease/condition	Number of genes	Note	Service type
Monogenic disease	400	(1) Detect a wide range of diseases/disorders with high sensitivity and efficiency; (2) Complete coverage of the target gene; (3) High-throughput results	Basic
Congenital tumour	54		
Hereditary malignant arrhythmia	48		
Pharmacogenetics	157		
Personal characteristics	54		Optional
Drug response for tumour targeted therapy	5	Medication guidance of certain anti-cancer drugs	
HLA high-resolution genetic typing	6	Human major histocompatibility complex testing required before hemopoietic stem cell or organ transplantation	
Leukemia fusion gene	4	Therapeutic assessment of leukemia treatment	
Personal genetic information storage	N/A	Storing personal genetic information for clients; information privacy will be guaranteed	
Result update	N/A	As scientific and medical research progress, more diseases and characteristics will be interpreted and analyzed	

BGI in Denmark



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

N ijmegen
U trecht
M aastricht

Making the joint lab work:

Platforms Knowledge Expertise

- Diagnostic questions
- Sample preparation
- analysis of pathogenic and non-pathogenic variants
- Sanger validation
- Clinical interpretation
- Diagnostic reporting
- Quality control

One space where people work together
One joint organisation

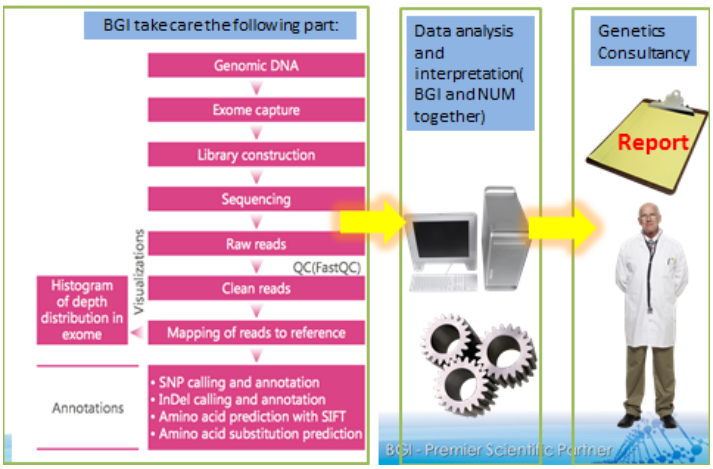
4.000 exomes in 2012-2013 for the Netherlands

Expanding in 2013-2015
Numbers > 10.000 exomes

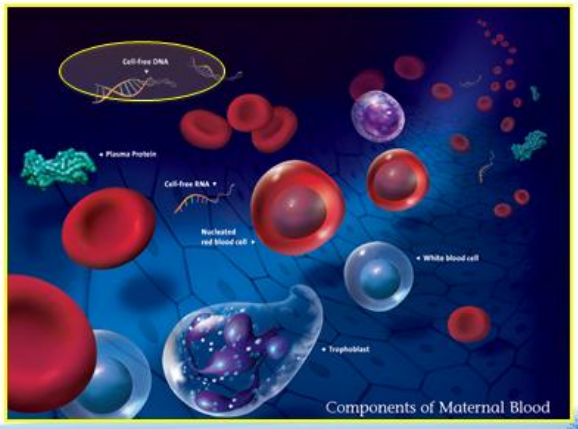
Platforms Knowledge Expertise

- High-throughput Sequencing
- Bioinformatics analysis and tools development
- Large scale data management
- Cloud computing

NUM-BGI Joined Lab Workflow



What's in maternal blood?



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

Clinical Validation Study（Phase 1, Double-blinded)

	NIFTY Test No.	Positive No.		
		T21	T18	T13
NIFTY Test No.	3464	189	64	10
Karyotyping No.	3464	188	63	10
False positive No.		1*	1*	0
False Negative No.		0	0	0
Detection Rate		100.00%	100.00%	100.00%
False Positive Rate		0.03%	0.029%	0.00%
Positive Predictive Rate		99.49%	98.44%	100.00%
Specificity		99.97%	99.97%	100%
False Negative Rate		0.00%	0.00%	0.00%

BGI Papers on NIFTY

Clinical Application of MPS-based Prenatal Noninvasive Fetal Trisomy (NIFTY) Test for Trisomy 21 and 18 in 5,853 Pregnancies with Mixed Risk Factors

Noninvasive Fetal Trisomy Test (NIFTY): An Advanced Noninvasive Prenatal Diagnosis Methodology for Fetal Autosomal and Heterosomal Aneuploidies

PLoS one

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

informa

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

Feature

High Accuracy	Digital sequencing with high throughput (Solexa sequencing)
Early Pregnancy Screening	Screening can be performed starting from the gestation period of 12 weeks, which allows early detection for a better decision and better health benefits
Non-invasive	Reduce mental stress of pregnant women, free of miscarriage risk and less risk of complications which may occur in antenatal diagnosis
High Precision	99% sensitivity, 99% specificity
Simple Sampling	Only 3~5mL of maternal peripheral blood are required

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BGI Certificates

Certificates:

Practice License of Medical Institution | Collaborative lab for CMDP | HLA EQA certificate | UCLA: HLA DNA exchange PT

ISO 17025

Clinical PCR

CLIA

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BGI Milestones
2001 BGI-Hongzhou
2009 BGI-Hong Kong
1999 BGI-Beijing
2007 BGI-Shenzhen
2010 BGI-Wuhan
2010 BGI-Europe
2010 BGI-Americans
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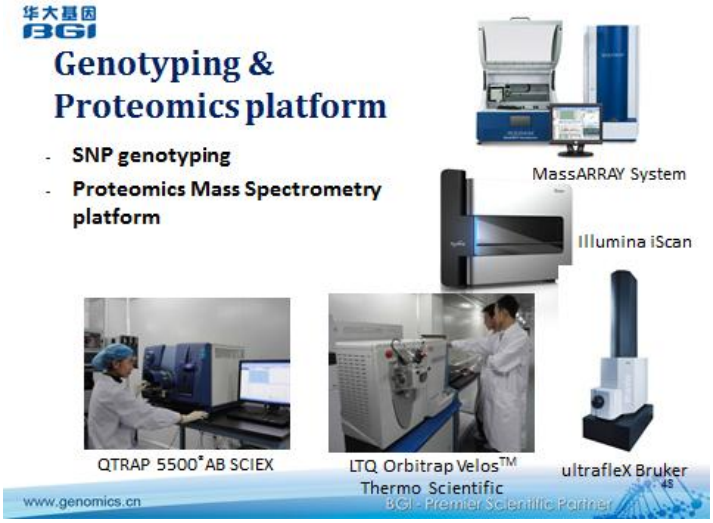
Genotyping &
Proteomics platform
- SNP genotyping
- Proteomics Mass Spectrometry platform
MassARRAY System
Illumina iScan
QTRAP 5500® AB SCIEX
LTQ Orbitrap Velos™
Thermo Scientific
ultrafleX Bruker
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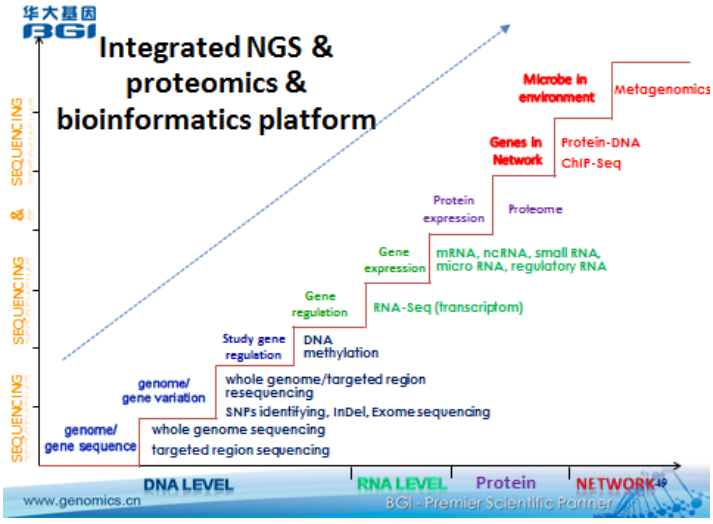
Integrated NGS &
proteomics &
bioinformatics platform
SEQUENCING
&
SEQUENCING
SEQUENCING
Microbe in environment
Metagenomics
Genes in Network
Protein-DNA
ChIP-Seq
Protein expression
Proteome
Gene expression
mRNA, ncRNA, small RNA, micro RNA, regulatory RNA
Gene regulation
RNA-Seq (transcriptom)
Study gene regulation
DNA methylation
genome/ gene variation
whole genome/targeted region resequencing
SNPs identifying, InDel, Exome sequencing
genome/ gene sequence
whole genome sequencing
targeted region sequencing
DNA LEVEL
RNA LEVEL
Protein
NETWORK
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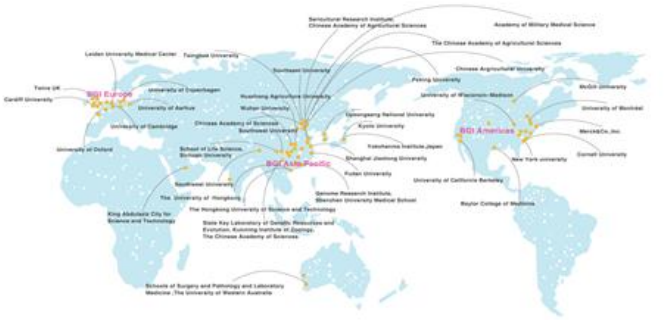
BGI dry lab-computing facilities

	# CPUS	Flops	RAM	Storage
2009.01	1,500	18T	4TB	2PB
2009.08	3,000	50T	10TB	5PB
2009.12	5,000	100T	20TB	10PB
2011.09	50,000	1,000T (1P)	200TB	1,000PB (1EB)

Acknowledgement



European Commission

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.

Redefining the trajectory of sequencing.	Powerful. Flexible. Scalable.	Two proven technologies. One powerful platform.	The most widely cited platform, now at half the price.	My Samples. My Study. MiSeq.
				
HiSeq 2000	HiSeq 1000	HiScanSQ	GA$_{IIx}$	MiSeq

HiSeq 2500

Combining innovation

- Clustering on-board
- Fast Chemistry
- Longer Reads

- Genome in a day
- Clustering on-board
- Complete walk-away workflow
- Longer 2x150 reads

- Data rate
- TDI scanning
- Larger flow cell

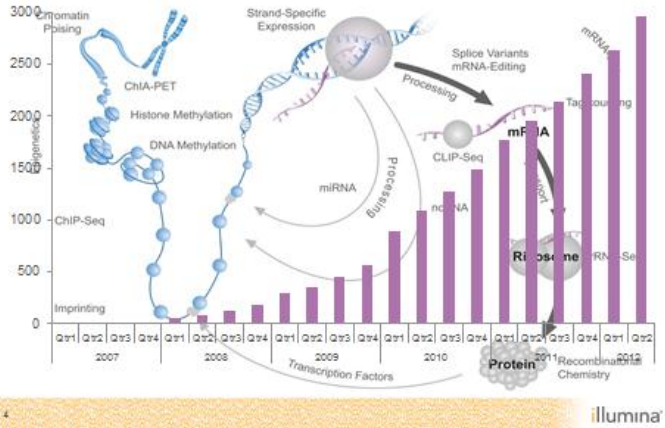
1 human genome in a day

3 500 gene diseases in Neonatal scr.

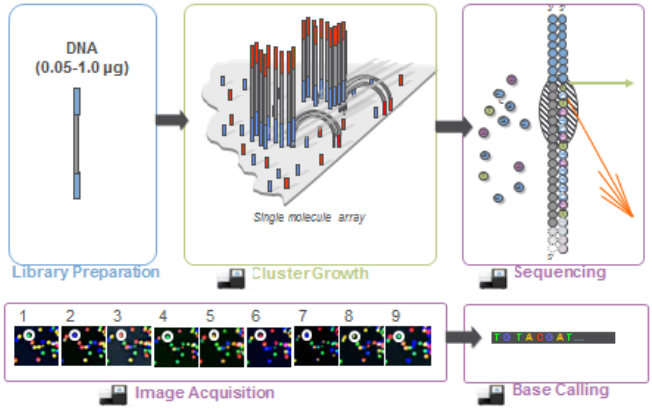
6 human genomes in 10.5 days

Fastest Publication Rate

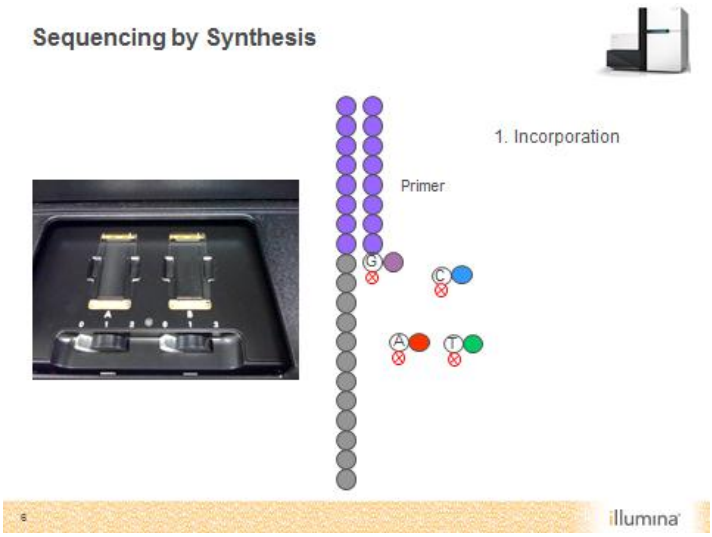
> 3000 peer-reviewed publications to date with Illumina sequencers



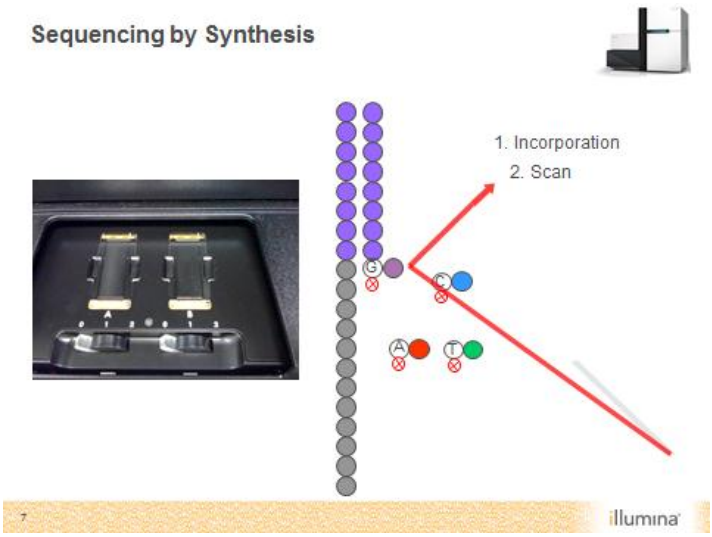
Illumina Sequencing Workflow



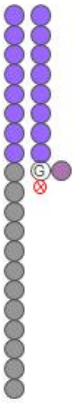
Sequencing by Synthesis



Sequencing by Synthesis

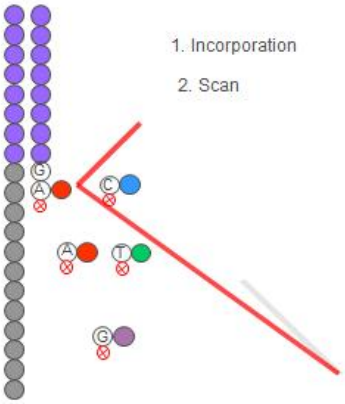


Sequencing by Synthesis



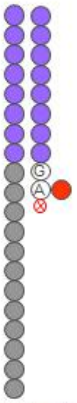
1. Incorporation
2. Scan
3. Cleavage

Sequencing by Synthesis



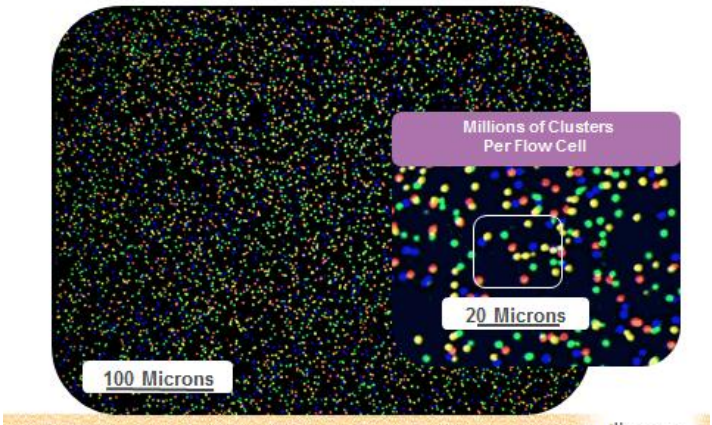
1. Incorporation
2. Scan

Sequencing by Synthesis



1. Incorporation
2. Scan
3. Cleavage

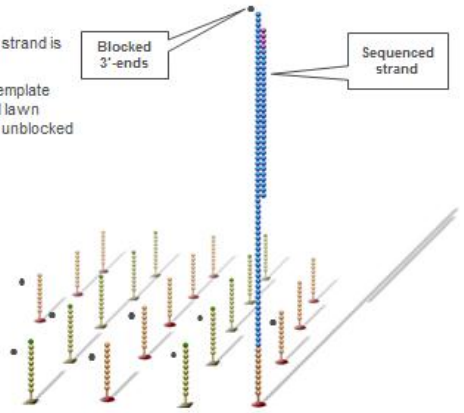
Sequencing



Paired end sequencing

Paired End Sequencing

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



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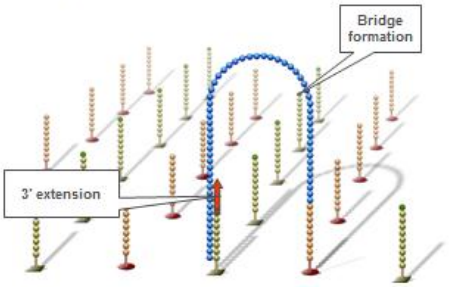
Paired End Sequencing

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



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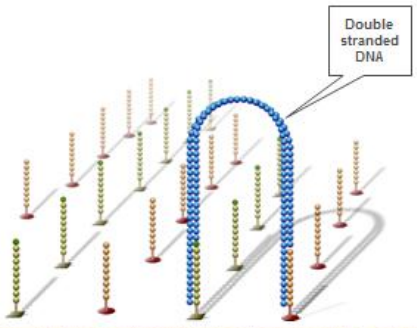
Paired End Sequencing



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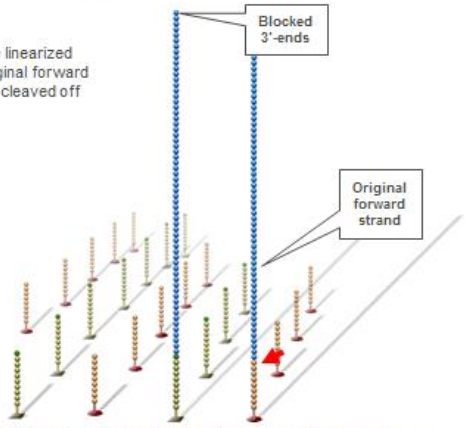
Paired End Sequencing

- Bridges are linearized and the original forward template is cleaved off



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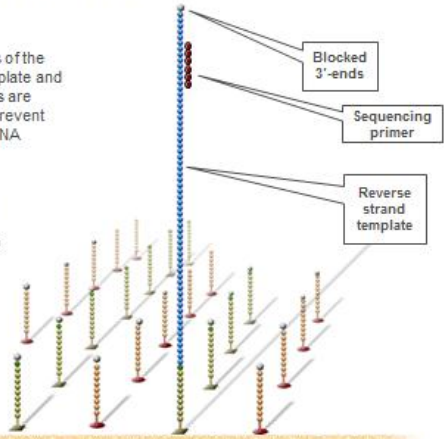
Paired end sequencing

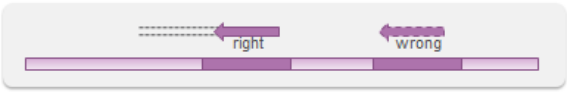
Sample Preparation → Cluster Generation → Sequencing

- Free 3' ends of the reverse template and lawn primers are blocked to prevent unwanted DNA priming
- Sequencing primer is hybridized to adapter 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 info not used: largest assembled fragment =128 Kb
- Pair info used: largest assembled fragment = 326 Kb

Key applications benefitting from paired end reads:
- Small genome de novo sequencing
- 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 Fluidics
Computing 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

Go

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MiSeq System update

System launched Sept 2011
Rapid Benchtop sequencer

The most accurate and highest throughput benchtop NGS machine

Loman et al 2012

Applications include:

- Amplicon sequencing
- Protocol development
- QC large scale runs
- Sequencing bacterial genomes
- Infectious disease

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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 .txt 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

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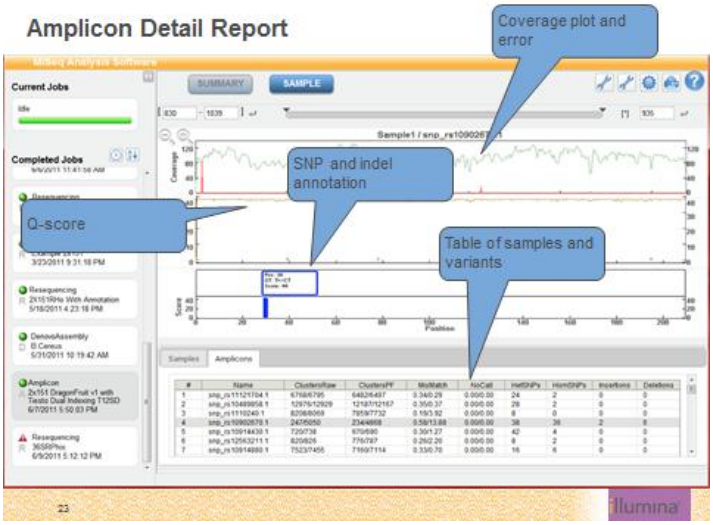
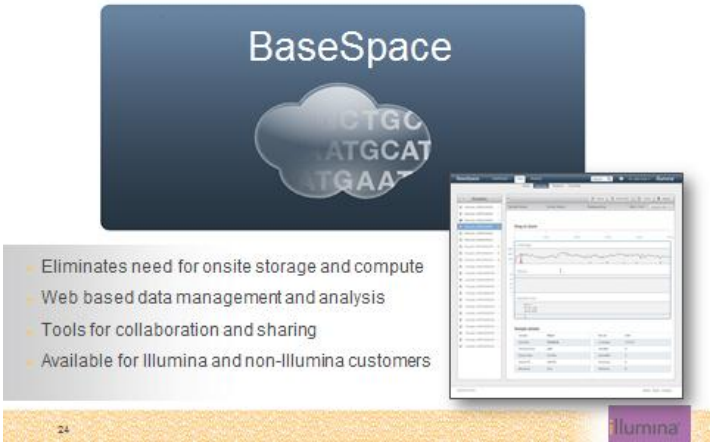
Amplicon Detail Report

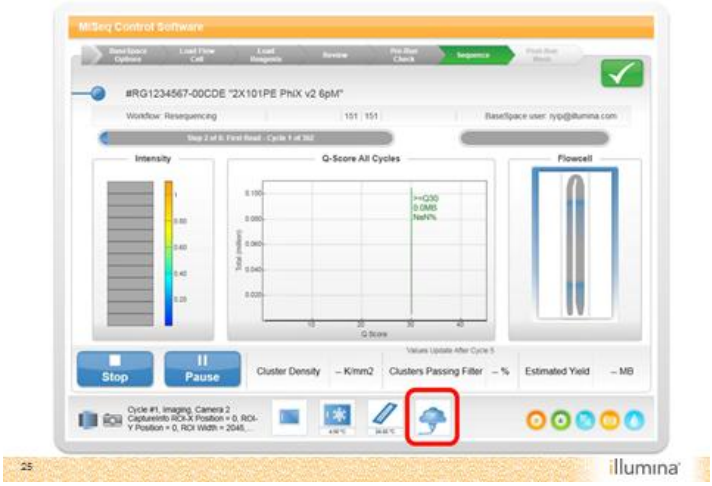


What?

BaseSpace is Illumina's genomic cloud computing environment



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

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BaseSpace Partners

Announcing Initial App Partners!

Knome · Strand Life Sciences · Omicia · Omicon · Integromics · Ingenuity · Xgenomatix · Diagnomics · Real Time Genomics · biomax · GenoLogics · Golden Helix · Spiral Genetics · Station X

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

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BaseSpace Apps

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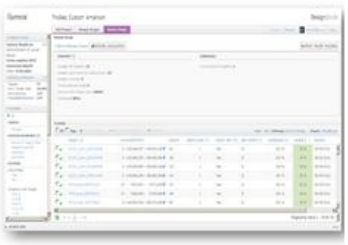
MiSeq Amplicon Viewer



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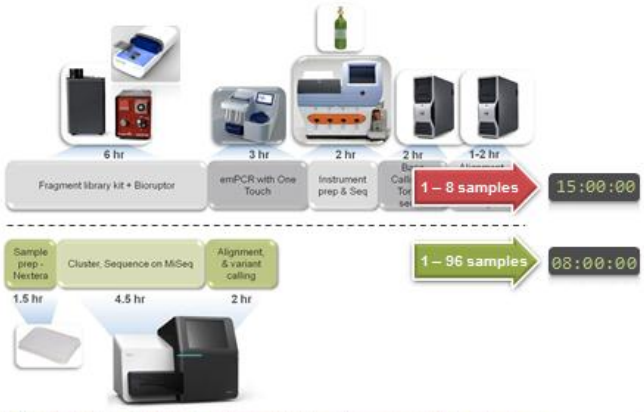
Nextera and MiSeq

Sequencing's fastest time to answer for rapid variant analysis



30 *Based on 1 x 36 bp reads illumina

Author's Performance Summary

MiSeq	• Lowest error rate • Highest throughput per run • Virtually non-existent homopolymer-associated indel errors • Simplest workflow
454 GS Jr	• Generated 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

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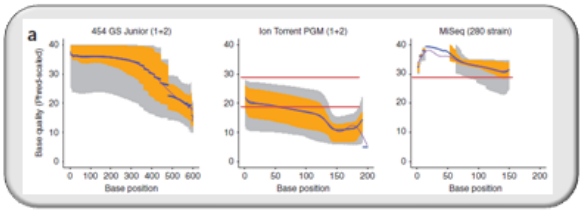
MiSeq Had the Highest Quality Data



- 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*

* InSequence 4/24/12 http://www.genomeweb.com/sequencing/comparison-desktop-sequencers-clarified-differentiates-no-single-platform-comes-out

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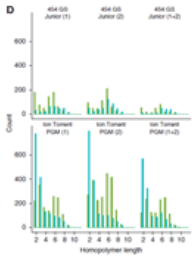
MiSeq Had Virtually No Discernable Homopolymer Associated Errors



- 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

Discerning true indels from homopolymer induced indels can never be fully addressed via software thus raising the potential for deleterious errors including frame shifts

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MiSeq at the Broad Institute

- Presentation from Shelia Fisher

- No hardware failures
- No chemistry failures

BROAD INSTITUTE

https://illumina.webex.com/illumina/lsr.php?AT=pb&SP=MC&rID=53939277&rKey=5ae4f946fa454bb5

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Nextera XT DNA Sample Prep

The fastest & easiest prep for small genomes, PCR amplicons and plasmids

- Rapid Prep
  - 90 min prep, only 15 min of hands on time
- Optimized for small genomes, PCR amplicons and plasmids
- Innovative sample normalization
  - No library quantification needed
- Fastest time to results
  - DNA to analyzed data in <8 hours with MiSeq
- Ultra low input
  - only a single nanogram of input DNA needed

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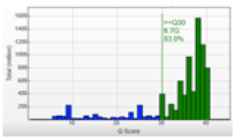
Extending MiSeq performance

	MiSeq
Yield	1.5-2G
Read Length	2x150
Number of Reads	5-7 Million clusters PF
Run time	27 Hours for 2x150
Quality at 2x150bp	75% bases ≥Q30

Improvements in Chemistry and Imaging

- Increased imaging area
  - Access second surface
- Improved SBS polymerase allowing faster kinetics
  - Chemistry cycle time of approx 2 mins (from 3.5mins)
  - Novel reagent formulation optimized for the MiSeq platform



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NGS Application –Life Science and Health Care
„The 21st Century vaccination“

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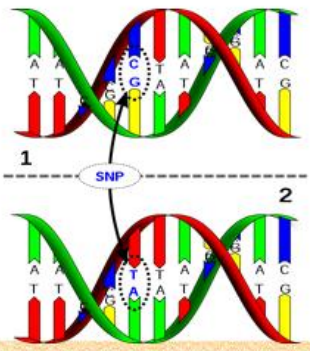
Did the Crack of Human DNA code are contribute to Medicine?

Predictive medicine



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

CFH gene – AMD high risk

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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, upd syndromes
- Preimplantation Genetic Screening - (PGS/PGD) Illumina + Bluegnome

1. Increase the number of successful pregnancy rates
2. 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 known syndromes)
Regions contain ~9000 genes

iScanDx System

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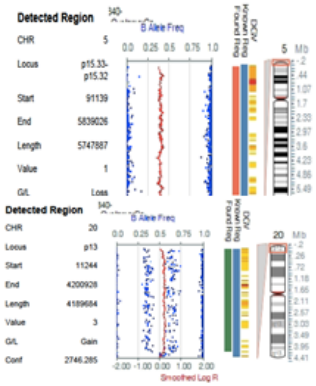
European Commission

Application of SNP array for rapid prenatal diagnosis: implementation, genetic counselling and diagnostic flow

Malgorzata Srebniak, Marjan Boter, Gre'tel Oudesluijs, Marieke Joosten, Lutgarde Govaerts, Diane Van Opstal, and Robert-Jan H Galjaard
European Journal of Human Genetics (2011), 1–8

- 64 samples to validate the Illumina platform using Human CytoSNP-12 (HCS)
  - (20 with a known (sub) microscopic chromosome abnormality, 5 with known maternal cell contamination (MCC) and 39 normal control samples).
- **No false-positive or false-negative results**.
- Prospective pilot study of 61 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.



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

Srebniak et.al., Molecular Cytogenetics 2012 Vol5:14 doi:10.1186/1755-8166-5-14

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

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MiSeq

Screening

- Germline carrier screen for mutations that cause Mendelian disease
  - Cystic fibrosis, Bloom syndrome, Canavan disease, Familial dysautonomia, Tay-Sachs disease, Gaucher's disease, Niemann-Pick disease type A, Fanconi anemia group C, Sickle cell anemia, β-thalassemia, α-thalassemia, Spinal muscular atrophy
- Larger panel of genes supported by literature may be included
- Forensic
- HLA Typing - Stanford University, Palo Alto, CA 94003
- IVF - PGD

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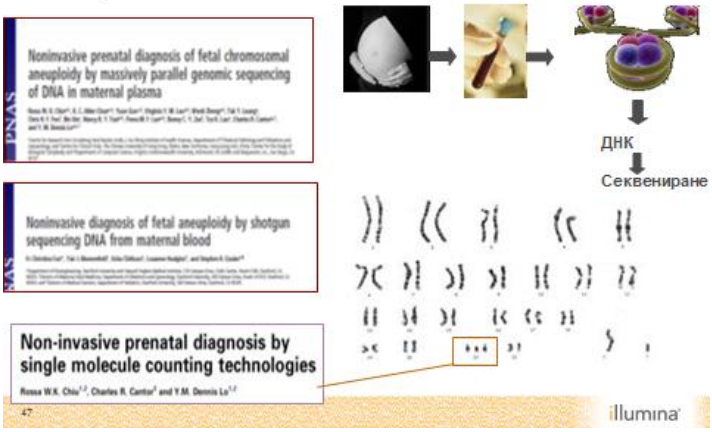
Non Invasive Chromosomal aberration such as trisomy 21 – Validated on Illumina HiSeq2000



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Prenatal Trisomy Testing

Sequenom | Aria Diagnostics | Verinata

LifeCodexx in Germany, 1st Sqnm licensee, obtained the CE-IVD Mark

Sequenom: $1,700
Verinata: $1,200
Ariosa Dx: $995
LifeCodexx (Germany): 1,250 EUR

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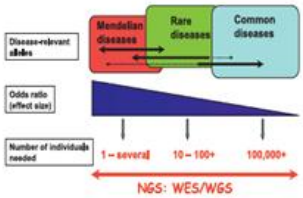
What can exome sequencing do for you?

- Identifying genetic variants
- Characterising monogenic (Mendelian) disorders
- Identification de novo mutations
- Characterising Complex trait disorders
- Characterising Cancer



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Whole Exome Sequencing help us diagnose correctly and solve Undiagnosed Mysteries

Nicholas Volker, a 6-year-old boy. The first year he was affected by extreme form of inflammatory bowel disease, characterised by multiple intestinal lesions, fistulas etc. 100+ operations. Not known the reason for that. Doctors ordered full exome sequencing to find the answer. Was find a specific genetic lesion in XIAP gene /basically associated with blood disorders/ that indicated the boy would respond to a bone marrow transplant.

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Nextera for PCR Amplicons

Quick workflow for long or short range PCR

Short Range - *CFTR*

7 Exons 255-416 bp range	90 min total 15 min hands on	12 patient samples	2x36 (6.5hrs) 95%>Q30

Long Range – *BRCA1/2*

22 Amplicons 1-8kb range 68.5kb total	90 min total 15 min hands on	5 patient samples	2x76(15hrs) 95%>Q30

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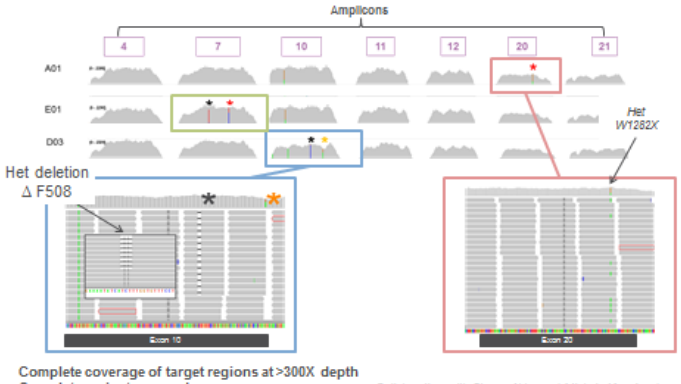
Nextera for PCR Amplicons

*Detection of mutations in CFTR with **short range** PCR*



Complete coverage of target regions at >300X depth
Complete variant concordance

Collaboration with Steven Abbs and Michale Yau, London

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Stages of Cancer

Risk Prediction | Screening | Early Detection | Diagnosis | Prognosis | Response to Therapy | Recurrence

- 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

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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 96 sample pooling on MiSeq
  - >90% 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

ABL1	EGFR	GNAS	MLH1	RET
AKT1	ERBB2	HNF1A	MPL	SMAD4
ALK	ERBB4	HRAS	NOTCH1	SMARCB1
APC	FBXW7	IDH1	NPM1	SMO
ATM	FGFR1	JAK2	NRAS	SRC
BRAF	FGFR2	JAK3	PDGFRA	STK11
CDH1	FGFR3	KDR	PIK3CA	TP53
CDKN2A	FLT3	KIT	PTEN	VHL
CSF1R	GNA11	KRAS	PTPN11	
CTNNB1	GNAQ	MET	RB1	

For research use only

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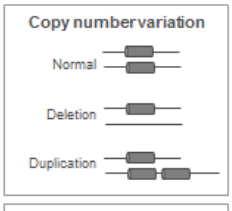
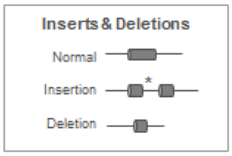
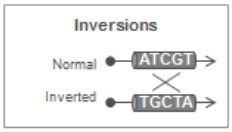
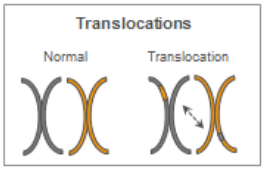
Types of Variation

- Single nucleotide variation (SNVs)
  - CGATTGCTAGGATCCACAGATA
  - CGATTGCTACGATCCACAGATA
  - CGATTGCTATGATCCACAGATA
- Structural variation (SVs)
  - Copy number variation
  - Inserts and deletions (indels)
  - Inversions
  - Translocations









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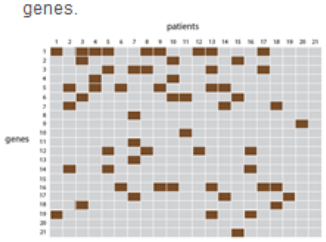
Targeted Resequencing

- 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.



Y-axis: cancer genes
X-axis: patients with the same type of cancer
Genes with mutations are highlighted.
Different mutation profiles can inform treatment decisions

TruSeq® Amplicon - Cancer Panel

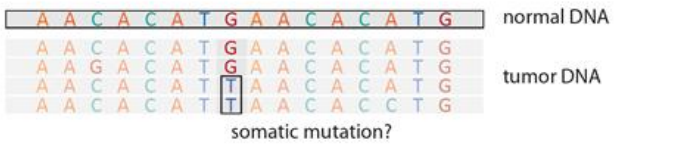
Rapidly detect hundreds of critical cancer mutations with the fastest and easiest multiplexed amplicon assay optimized for the MiSeq® system.

Table 1: TSACP Cancer-Related Genes

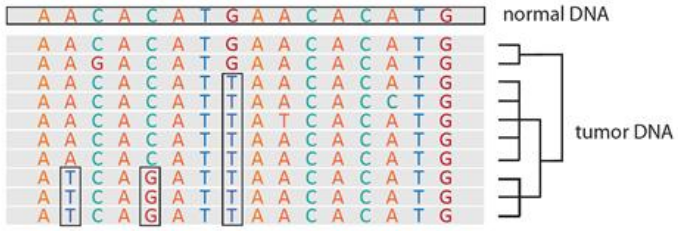
ABL1	EGFR	GNAS	MLH1	RET
AKT1	ERBB2	HNF1A	MPL	SMAD4
ALK	ERBB4	HRAS	NOTCH1	SMARCB1
APC	FBXW7	IDH1	NPM1	SMO
ATM	FGFR1	JAK2	NRAS	SRC
BRAF	FGFR2	JAK3	PDGFRA	STK11
CDH1	FGFR3	KDR	PIK3CA	TP53
CDKN2A	FLT3	KIT	PTEN	VHL
CSF1R	GNA11	KRAS	PTPN11	
CTNNB1	GNAQ	MET	RB1	

Cancer-related genes represented in the TSACP. For a full list of target regions, see the manifest file† (MyIllumina login required).

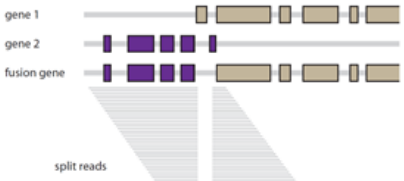
Deep Sequencing



somatic mutation?



RNA-Seq



Determine changes in cellular function
Expression levels of cancer-associated genes, such as genes involves in metastasis.
Discover or confirm fusion genes
Determine the exact structure of the fusion or mutation
Assess damage to RNA processing machinery, such as changes in splice variants

Fusion genes can be detected with a high level of accuracy and confidence
Based on Lee et al. Proc Natl Acad Sci U S A 109: 929-934

Others opportunities

- **Familial Hypercholesterolemia** (HF)
  - HF: 1/ 500 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 2x250pb
- **Leukemia:**
  - 50,000 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

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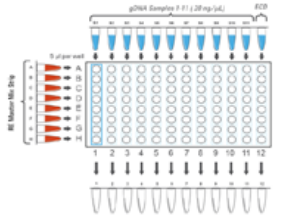
Haloplex current protocol

900ng per sample



- DNA digestion 4hr 20m
- Run gel of control lane
- Hybridise Haloplex probes 12hr 20m
- Capture target DNA >30m
- Ligate >20m
- Remove non-target DNA >1hr 20m + PCR amplify
- Purify >10m

Table 3 Thermal cycler program for HaloPlex restriction digestion

Step	Temperature	Time	Purpose
Step 1	37°C	4 hours	DNA digestion
Step 2	80°C	20 minutes	Enzyme inactivation
Step 3	4°C	Hold	Hold

Table 5 Thermal cycler program* for HaloPlex probe hybridization

Step	Temperature	Time
Step 1	95°C	10 minutes
Step 2	75°C	30 minutes
Step 3	68°C	30 minutes
Step 4	62°C	30 minutes
Step 5	55°C	30 minutes
Step 6	46°C	16 hours
Step 7	8°C	Hold*

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Metagenomics

Isolate DNA from single organism

Isolate community DNA

SEQUENCE

Genomics

Metagenomics

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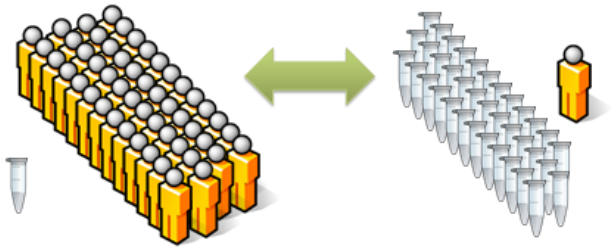
MiSeq flexibility – samples vs plexity

A Panel which looks at a single target for many samples

A Panel which looks at a many targets for a single sample



eg. Routine CF screening

eg. Cancer mutation panel

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Unprecedented Rate of Peer-Reviewed Publications

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ITFoM
Future of Medicine

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

Prof. Dr. Angela Brand MD PhD MPH, Director of the European Centre for Public Health Genomics (ECPHG), Coordinator of PHGEN (DG Senco)
Institute for Public Health Genomics@Maastricht University, The Netherlands

EC Workshop »Applied Genomics in the Clinic« (Istanbul, 17.10.2012)

European Commission

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Public Health Genomics (PHG):
translational research
„from cell to society"

"Public Health Genomics (PHG) is the responsible and effective translation of genome-based knowledge and technologies into public policy and health services for the benefit of population health."

[Bellagio Statement 2005: GRAPH*Int*, PHGEN, IPHG]

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Maastricht University Leading in Learning!

1. What do we need to translate?

2. How do we translate (innovations into healthcare systems)?

European Commission

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... genomics is a „moving target“ ...

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Maastricht University Leading in Learning!

... from

single and linear systems

to

non-linear networks in systems biology and systems medicine ...

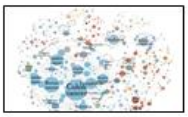
Maastricht University

Not only 4 P's ...

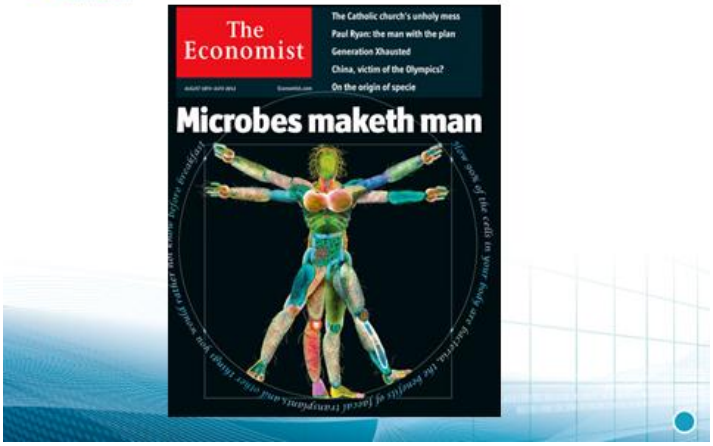
Maastricht University

... not only beyond the 4 P's, but also (A. Brand, 2008) ...

1. from common complex diseases to "multiple rare diseases"
2. from diseases to "diseasomes"
3. from risk factor to "risk pattern"
4. from clinical utility to "personal utility"




TIME
WHY YOUR DNA ISN'T YOUR DESTINY


The Economist
The Catholic church's unholy mess
Paul Ryan: the man with the plan
Generation Xhausted
China, victim of the Olympics?
On the origin of specie
Microbes maketh man

European Commission

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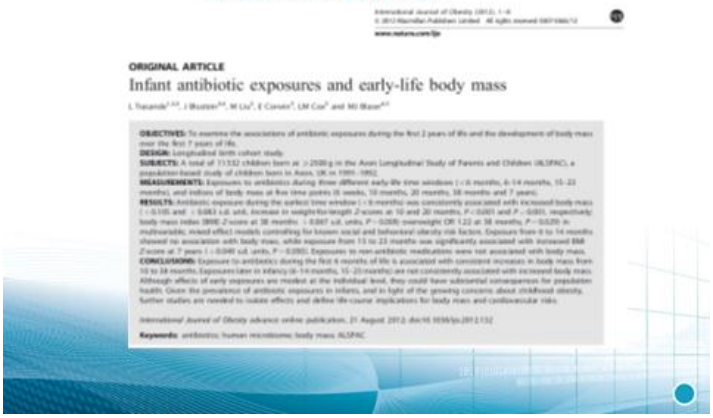
... obesity story (21.08.2012)



ORIGINAL ARTICLE

Infant antibiotic exposures and early-life body mass

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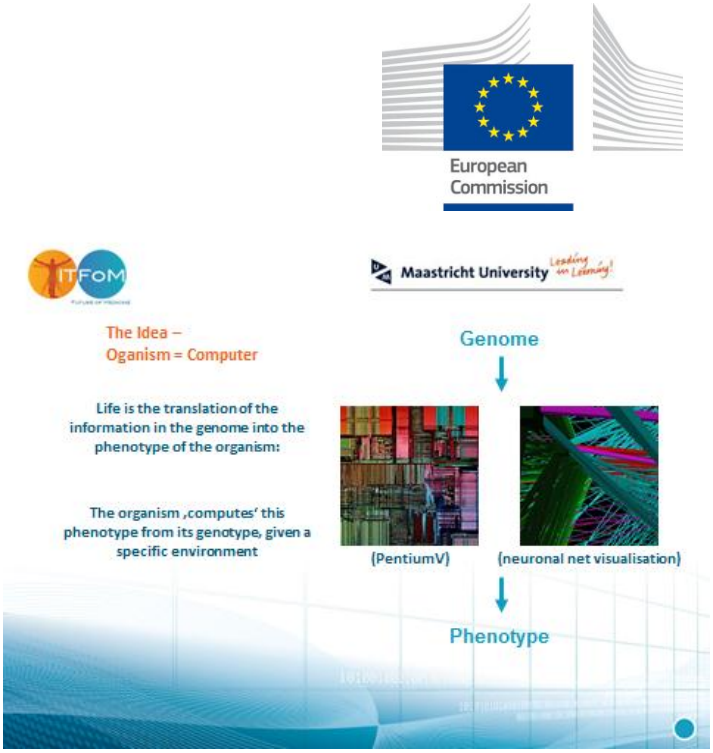
..... and also

(1) highly (in space & time) dynamic personal (health) information

(2) from statistical risks within groups to "individualized evidence"

(3) "virtual individual models"

ITFoM (www.itfom.eu) – "ICT for health & health for ICT":
a radically new vision for healthcare!



http://itfom_portal.nakijken.nl/

www.itfom.eu

European Commission

ITFoM

Maastricht University Leading in Learning!

"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.

ITFoM

Maastricht University Leading in Learning!

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

[David Steensma, DFCI, JCO, 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

European Commission

Maastricht University

Technology Transfer (TT)

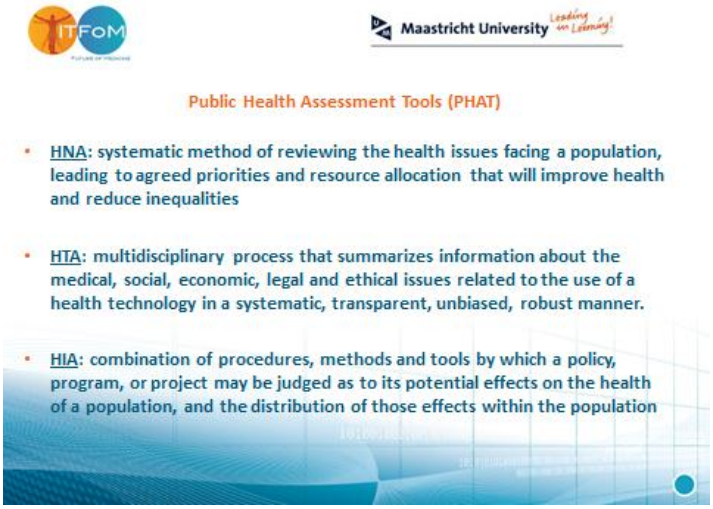
- Addresses 1st two phases
- Activity of the migration of academic discoveries to useful application in the development of marketable products or processes
- TTO or valorization office
- Most widely used activity in business development or academic research
- Process, technique, method, tool, activity

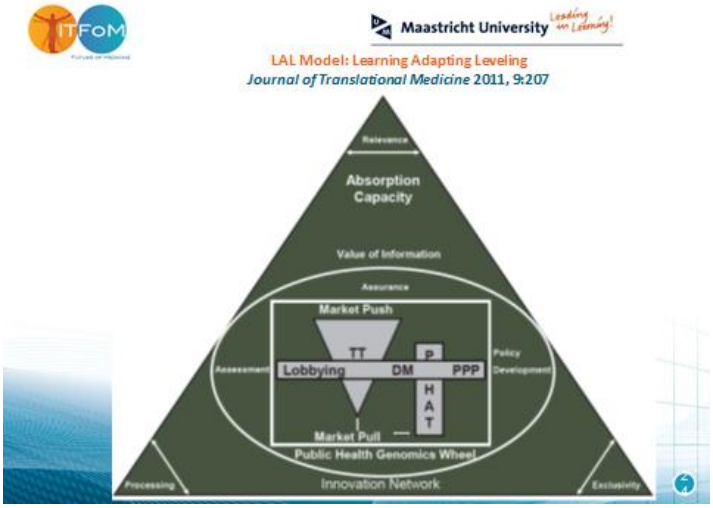
Maastricht University

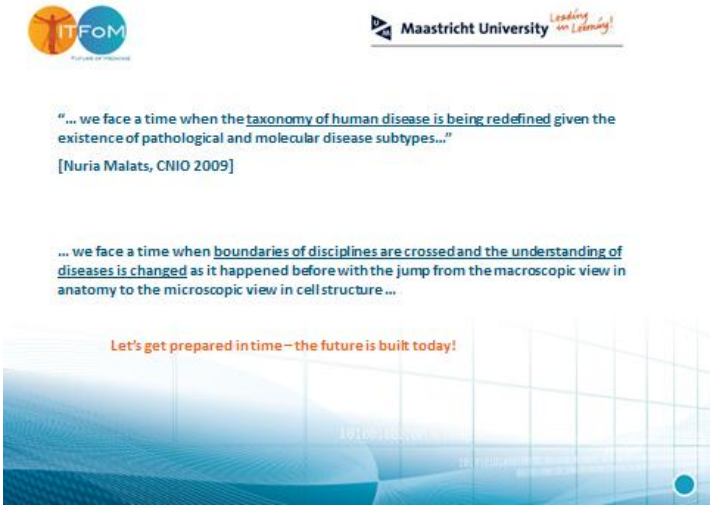
Public Health Trias (3rd phase)

[IOM, 1988]


Maastricht University
innovation & awareness & society
Medical benefit
• Outcome, Safety
• Willingness
Economic added value
• Financial sustainability
• ROI
Scientific knowledge
• Evidence and innovation
• New solutions
Ethical and social
• Fairness
• Acceptance
Legal frame-work
•Private protection
•Public interests


Maastricht University
Public Health Assessment Tools (PHAT)
HNA: systematic method of reviewing the health issues facing a population, leading to agreed priorities and resource allocation that will improve health and reduce inequalities
HTA: 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


Maastricht University
LAL Model: Learning Adapting Leveling
Journal of Translational Medicine 2011, 9:207
Relevance
Absorption Capacity
Value of Information
Assurance
Market Push
TT
P
Lobbying
DM
PPP
H
A
T
Market Pull
Public Health Genomics Wheel
Innovation Network
Processing
Exclusivity
Assessment
Policy Development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"... we face a time when the taxonomy of human disease is being redefined given the existence of pathological and molecular disease subtypes..."
[Nuria Malats, CNIO 2009]
... we face a time when boundaries of disciplines are crossed and the understanding of diseases is changed as it happened before with the jump from the macroscopic view in anatomy to the microscopic view in cell structure...
Let's get prepared in time – the future is built today!

European Commission

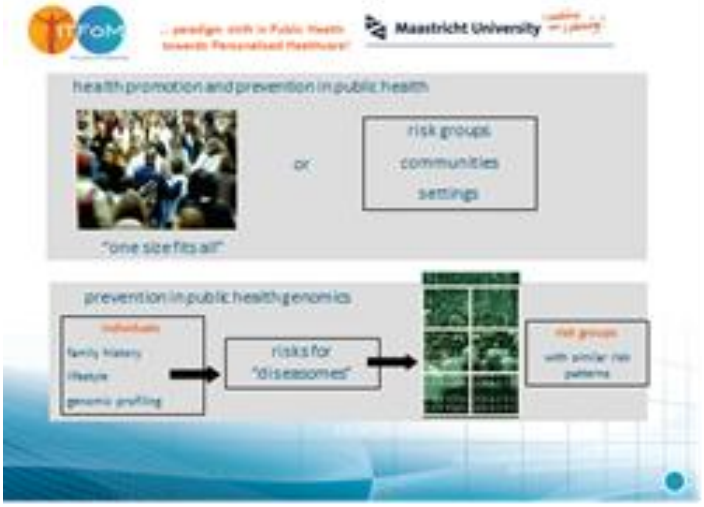
Public Health Genomics European Network (PHGEN)

public health genomics
European Network

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

Declaration of Rome - 19.04.2012


Maastricht University
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. „diseasomes" and „social networks"
• P4 medicine (predictive, preemtive, personalised, participatory):
„a change of view that changes everything"
• the changing roles of patients and doctors


Maastricht University
health promotion and prevention in public health
or
risk groups
communities
settings
"one size fits all"
prevention in public health genomics
risks for
"diseasomes"


European Best Practice Guidelines for Quality Assurance, Provision and Use of Genome-based Information and Technologies: the 2012 Declaration of Rome


Thanks for your attention!
"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 organizational changes ... in time."
Personalized Medicine

Applied Genomics in the Clinic

JRC Workshop

17-19 October 2012, Istanbul

Ewa Stępień PhD,

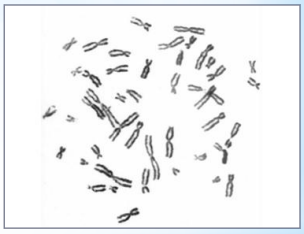
Department of Clinical Biochemistry, Division of Genetic Diagnostics and Nutrigenomics, Jagiellonian University Medical College, Krakow Poland

Giemsa magic

Professor Bogdan Kałużewski,

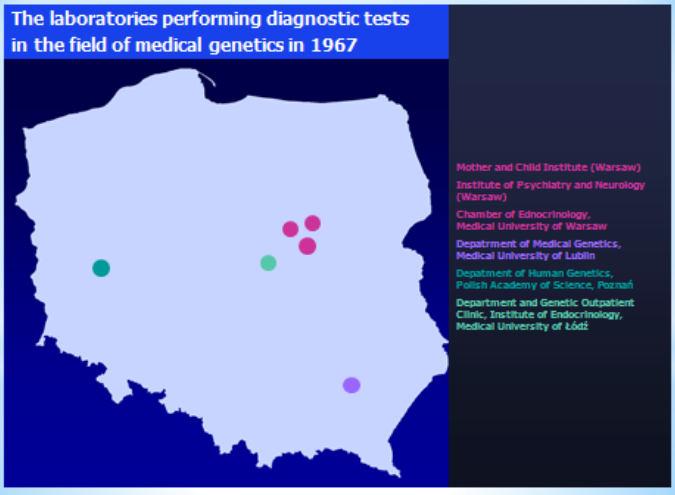
Medical University in Łódź, Poland

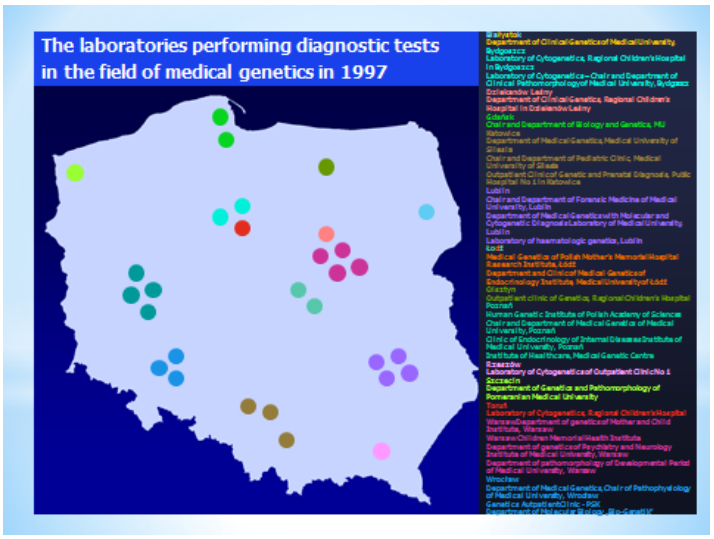
Chairman of the Medical Speciality Advisory Board of Ministry of Health for laboratory acreditation in the field of Medical Genetics

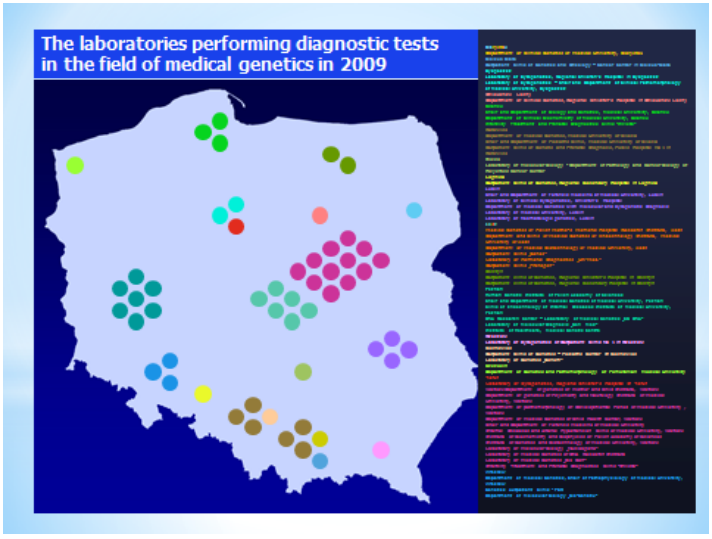


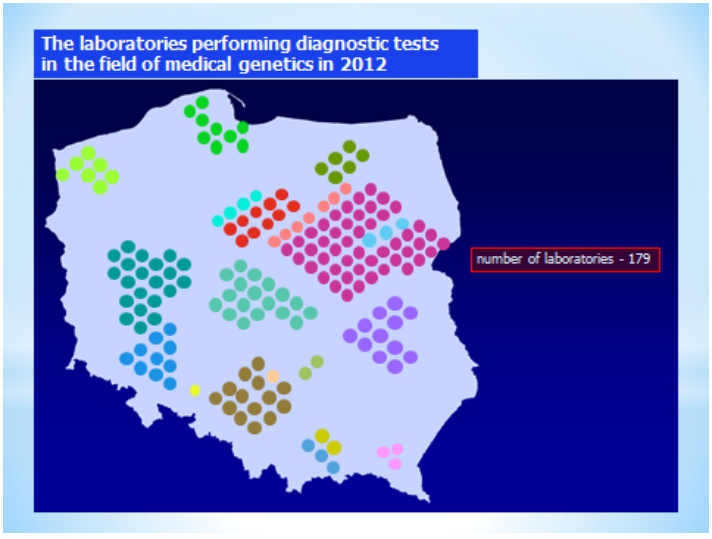
Metaphase chromosomes from human lymphocytes cell culture

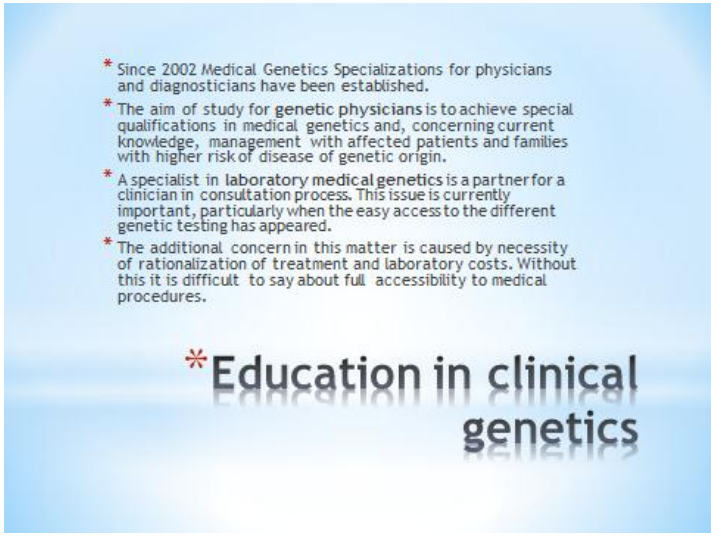
(Kałużewski, 1967)



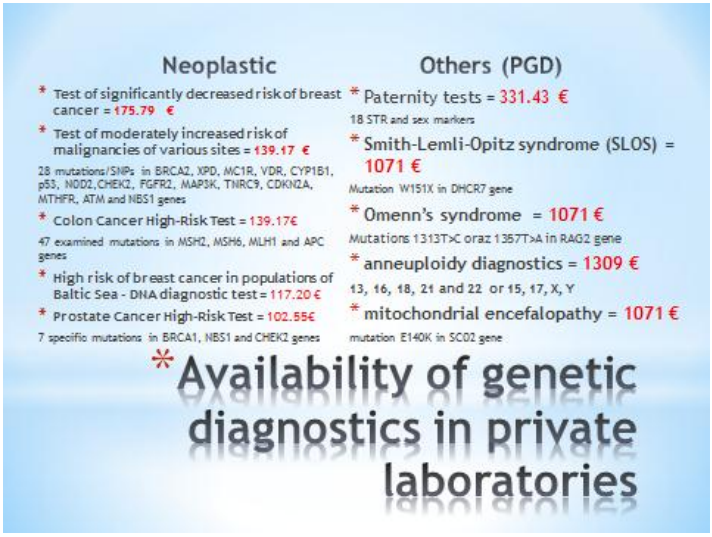

The laboratories performing diagnostic tests in the field of medical genetics in 1997


The laboratories performing diagnostic tests in the field of medical genetics in 2009


The laboratories performing diagnostic tests in the field of medical genetics in 2012
number of laboratories - 179

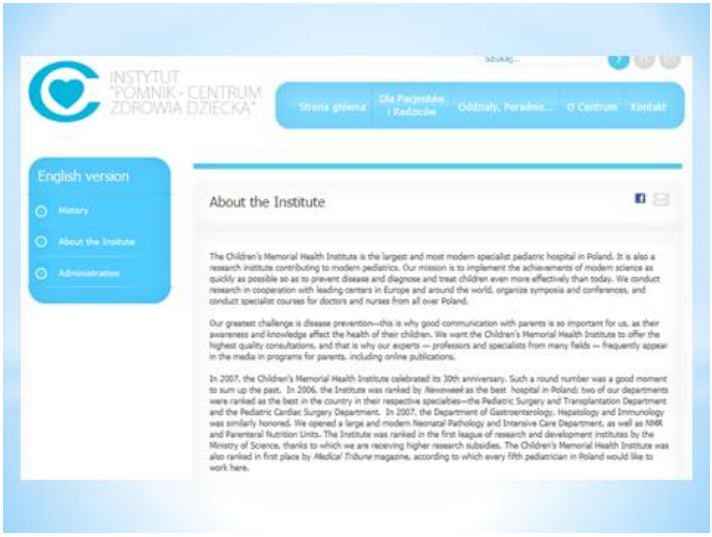

* Since 2002 Medical Genetics Specializations for physicians and diagnosticians have been established.
* The aim of study for genetic physicians is to achieve special qualifications in medical genetics and, concerning current knowledge, management with affected patients and families with higher risk 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 consultation:
* 9 points x 10.00 PLN = 90.00 PLN = 21.42 €
* Counselling in neoplasmatic disease:
* 4 points x 10.00 PLN = 40.00 PLN = 9.52 €
2. Specialistic Outpatient Care (High-cost diagnostic tests):
* 28 points x 9.00 PLN = 252.00 PLN = 60.00 €
3. Medical services enabled in separate contracts:
* Neoplasmatic diseases:
45 points x 11.20 PLN = 504.00 PLN = 120.00 €
* Non-neoplasmatic diseases:
90 points x 11.20 PLN = 1 008.00 PLN = 240.00 €
4. Profilactic programme of prenatal diagnostics:
* Genetic Counsel:
4 points x 11.00 PLN = 44.00 PLN = 10.48 €
* Utrasound testing:
30 points x 11.00 PLN = 330.00 PLN = 78.57 €
* Amniocentesis:
30 points x 11.00 PLN = 330.00 PLN = 78.57 €
* Cytogenetics and Biochemical tests:
120 points x 11.00 PLN = 1 320.00 PLN = 314.28 €
Total:
184 points x 11.00 = 2 024.00 PLN = 481.90 €
* Financial Issues of Medical Genetics in Poland - National Health Fund
1 € = 4.20 PLN


Neoplastic
* Test of significantly decreased risk of breast cancer = 175.79 €
* Test of moderately increased risk of malignancies of various sites = 139.17 €
28 mutations/SNPs in BRCA2, XPD, MC1R, VDR, CYP1B1, p53, NOD2, CHEK2, FGFR2, MAP3K, TNRC9, CDKN2A, MTHFR, ATM and NBS1 genes
* Colon Cancer High-Risk Test = 139.17€
47 examined mutations in MSH2, MSH6, MLH1 and APC genes
* High risk of breast cancer in populations of Baltic Sea - DNA diagnostic test = 117.20 €
* Prostate Cancer High-Risk Test = 102.55€
7 specific mutations in BRCA1, NBS1 and CHEK2 genes
Others (PGD)
* Paternity tests = 331.43 €
18 STR and sex markers
* Smith-Lemli-Opitz syndrome (SLOS) = 1071 €
Mutation W151X in DHCR7 gene
* Omenn's syndrome = 1071 €
Mutations 1313T>C oraz 1357T>A in RAG2 gene
* anneuploidy diagnostics = 1309 €
13, 16, 18, 21 and 22 or 15, 17, X, Y
* mitochondrial encefalopathy = 1071 €
mutation E140K in SCO2 gene
* Availability of genetic diagnostics in private laboratories


* Private genetic laboratories in Poland
INVICTA
* Laboraties at outpatients clinics (fertility clinics, infection diseases)
* Laboratory networks with easy access to tests (internet ordering)
* Collaboration with foreign laboratories
* Consultation of results after DNA examination
Bio Te 21


*Establishing of two committees dedicated for rare disease treatment:
*Operation Team for Rare Diseases
*Operation Team for Ultrarare 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


INSTYTUT "POMNIK - CENTRUM ZDROWIA DZIECKA"
English version
About the Institute


REGIONAL PROGRAMME
Małopolska REGION
EUROPEAN UNION EUROPEAN REGIONAL DEVELOPMENT FUND
EUROPEAN SOCIETY OF CARDIOLOGY
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
CENTRE for RARE CARDIOVASCULAR DISEASES


MALOPOLSKA
CENTRE OF BIOTECHNOLOGY
RESEARCH DEPARTMENTS
DEPARTMENT FOR GENETIC RESEARCH AND NUTRIGENOMICS
DEPARTMENT FOR STRUCTURAL BIOLOGY
THE NEUROBIOLOGY DEPARTMENT
THE BIOINFORMATICS DEPARTMENT
THE BIOREMEDIATION DEPARTMENT
DEPARTMENT FOR BIOTECHNOLOGY AND FOOD SAFETY


omicron
Enabling OMICs high-thROughput techNologies
at the Faculty of Medicine
Jagiellonian University Medical College
JAGIELLONIAN UNIVERSITY MEDICAL COLLEGE
Home
About us
Staff
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Work packages
Participating departments
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Department of Transplantation
Department of Internal and Agricultural Medicine
- Translational Vascular Biology Laboratory
Department of Pharmacology
Third Department of General Surgery
Fields of interest
Event calendar
Meetings
Workshops
Conferences
OMICRON Brochure
FAQ
About us • Project objectives
Project objectives
The overall objective of the project is to up-grade, stimulate, unlock, develop and extend the research potential of the Faculty of Medicine at the Jagiellonian University Medical College (JUMC).
The specific objectives are:
• to up-grade and extend the laboratory equipment and research performance at JUMC Faculty of Medicine allowing for most advanced genomic and proteomic research;
• to attract, develop and retain at the JUMC Faculty of Medicine the best research professionals of Polish origin and foreigners;
• to develop the research skills of the future leaders in the thematic domains concerned;
• to establish and expand networks across Europe to support high quality research, exchange of know-how and experience and ensure technology and knowledge transfer so that leading edge research can thrive at JUMC Faculty of Medicine;
• to increase the visibility of excellence of the JUMC Faculty of Medicine and ensure the setting-up of long-lasting strategic partnerships with leading centres in the respective priority fields;
• to increase the participation of beneficiaries in scientific fields covered by the EC FP7 and in future FPS.

Personalized medicine

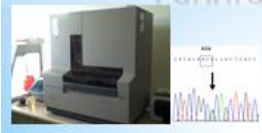
Patients selection for tailored therapy

- Colon cancer KRAS 100 per year
- Lung cancer EGFR 100 per year
- Melanoma
  - BRAF several per year
  - GIST about 20 per year
- CLM treatment monitoring 200 per year
- BRCA1 sequencing 1000 per year

Equipment

- PGM Ion Torrent
- 4-*capillary* 3130 Genetic Analyzer ABI
- Bio-Rad's QX100 Droplet Digital PCR system
- RotorGene Real-Time PCR machine

Laboratory of Molecular Biology Holycross Cancer Center Kielce, 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 private laboratories and companies dedicated to clinical genetics
- Increasing number of diagnostic centers equipped with highthroughput methods in genetics
- Establishing of the international co-operation for genetic diagnostics of rare diseases:
  - Incorporation (2011) of The Children's Memorial Health Institute in Warsaw to JOINT ACTION „Development of the European portal of rare disease and orphan drugs - ORPHANET Europe"
  - Establishing (2012) of Centre for Rare Cardiovascular Diseases in John Paul II in Krakow dedicated to rare cardiovascular diseases.

Main achievements in clinical genetic diagnostics in Poland

- Lack of comprehensive financial and education program 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.
- Limited availability to prenatal and preimplantation genetic diagnostics (high costs).

Main failures in clinical genetic diagnostics in Poland

European Commission

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. As 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.

European Commission

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 challenges creating an integrated approach for the EU action in the field of rare diseases. Adopted 11th November 2008.

Council Recommendation on a European action in the field of rare diseases recommending actions at national level to implement the EU action (e.g. National Plans for Rare Diseases). Adopted 8th 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, patient' s organisations, industry, FP Projects, Health Programme projects, etc. Adopted 30th November 2009.

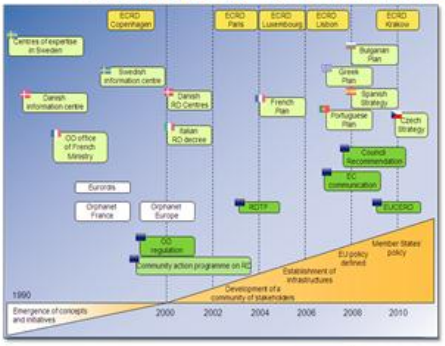
Legal basis for the developments of the EU Policy on rare diseases

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 23th of October of 2013.

Directive 95/46/EC of the European Parliament and of the Council of 24 October 1995 on the protection of individuals with regard to the processing of personal data on the free movement of such data. (Data Protection Directive).

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



From: Aymé 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.

European Commission

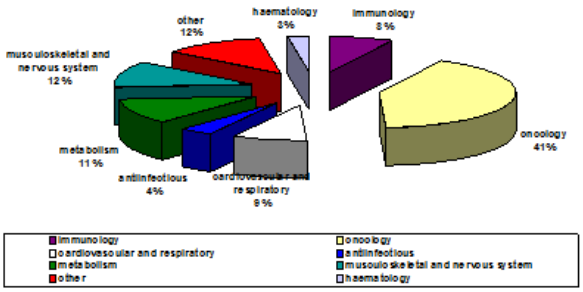
Why an orphan regulation?

- *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



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
Asks 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.

The Commission Communication and the Council Recommendation on rare diseases

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/

European Commission

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
ftp://ftp.cordis.europa.eu/pub/lifescihealth/docs/reprint_rec48300_rare_dis_060207.pdf

FP6 (2002-2006): 59 projects funded, € 230 million in total
http://cordis.europa.eu/lifescihealth/major/rare-diseases-projects-1.htm

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.

European Commission

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

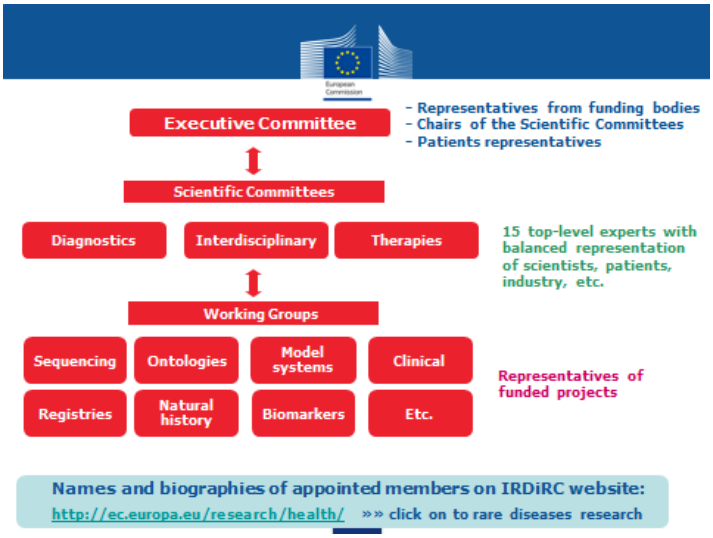
Priorities:

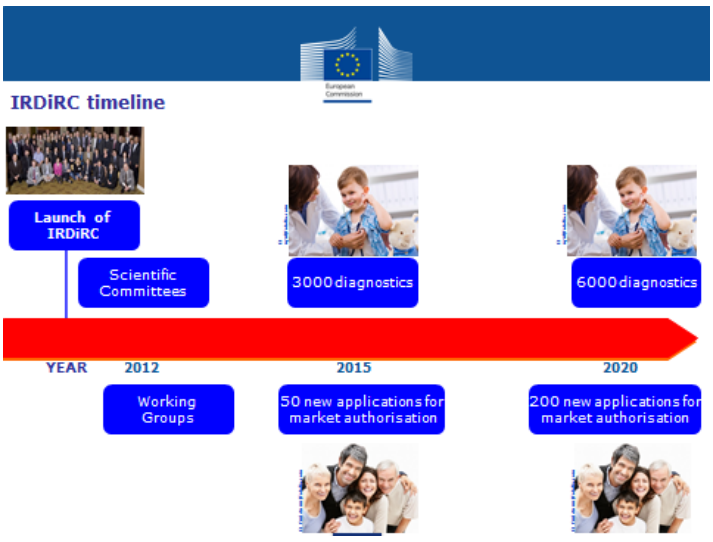
Excellent science
Industrial leadership
Societal challenges

International Rare Diseases Research Consortium (IRDiRC)

Recent development


Current 25 committed members
Europe
European Commission
German Federal Ministry of Education and research
Italian Higher Institute of Health Research
Italian Telethon Foundation
French Association against Myopathies
French National Research Agency
Netherlands Organisation for Health Research and Development
Lysogene (FR)
Prosensa (NL)
Spanish Carlos III Health Institute
UK National Institute for Health Research
Shire (IE)
North America
Canadian Institutes for Health Research (CA)
Genome Canada (CA)
Sanford Research (US)
Mendelian Disorders Genome Centres(US)
National Centre for Translational Therapeutics (US)
National Cancer Institute (US)
National Institute of Neurological Disorders and Stroke (US)
National Institute of Arthritis and Musculoskeletal and Skin Diseases (US)
National Institute of Child Health and Human Development (US)
National Eye Institute (US)
Office of Rare Diseases (US)
Food and Drug Administration (US)
Australia
Western Australian Department of Health


Executive Committee
- Representatives from funding bodies
- Chairs of the Scientific Committees
- Patients representatives
Scientific Committees
Diagnostics
Interdisciplinary
Therapies
15 top-level experts with balanced representation of scientists, patients, industry, etc.
Working Groups
Sequencing
Ontologies
Model systems
Clinical
Registries
Natural history
Biomarkers
Etc.
Representatives of funded projects
Names and biographies of appointed members on IRDiRC website:
http://ec.europa.eu/research/health/ »» click on to rare diseases research


IRDiRC timeline
Launch of IRDiRC
Scientific Committees
3000 diagnostics
6000 diagnostics
YEAR
2012
2015
2020
Working Groups
50 new applications for market authorisation
200 new applications for market authorisation

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

From: Aymé 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

Dyscerne: European Network of Centres of Reference for Dysmorphology (ended)

ECORN CF: European Centres of Reference Network for Cystic Fibrosis (ended)

PAAIR: Patient Associations and Alpha 1 International Registry (PAAIR) (ended)

EPNET: European Porphyria Network - providing better healthcare for patients and their families (ended)

EN-RBD: Establishment of a European Network of Rare Bleeding Disorders (ended)

Paediatric Hodgkins Lymphoma Network: European-wide organisation of quality controlled treatment (on-going)

NEUROPED: 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 Genodermatoses – TogetherAgainstGenodermatoses (on-going)

CARE NMD: Dissemination and Implementation of the Standards of Care for Duchene

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 goal 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.

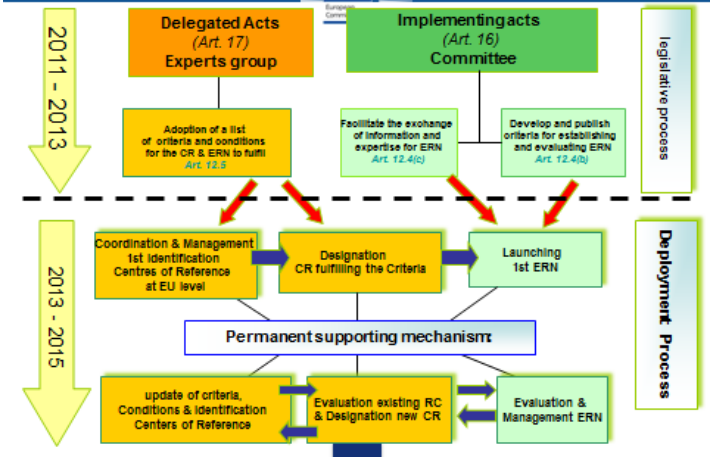
Article 12 : ERN

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

Milestones and timeline for the implementation (ERN)

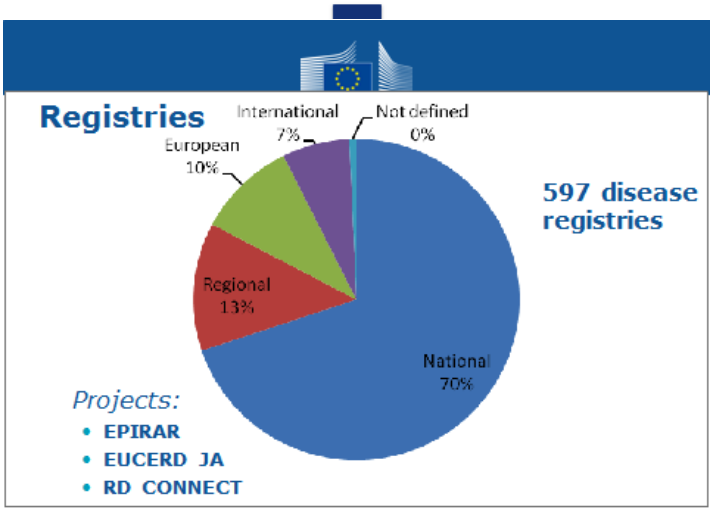


European Commission

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



Directorate for Health and Consumers priorities on rare diseases
Web site

Public health actions
http://ec.europa.eu/health/rare_diseases/policy/index_en.htm

Contact point at DG SANCO
antoni.montserrat@ec.europa.eu
jaroslaw.waligora@ec.europa.eu

CURRENT APPLICATIONS OF MEDICAL GENETICS IN TURKEY

DR. AHMET YESILYURT
ISTANBUL
OCTOBER, 2012

TURKEY

Turkey is 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

Certified Diagnosis center by The Turkish Minister of Health

	University	State	Private	Total
ANKARA	5	3	6	14
İZMİR	2		3	5
İSTANBUL	2	2	13	17
BURSA	1		1	2
ERZURUM	1			
ESKİŞEHİR	1			1
KAYSERİ	1	1	1	3
ANTALYA	1		1	2
KONYA	1		1	2
KOCAELİ	1			1
MALATYA			1	1
TRABZON		1		1
ADANA		1		1
DENİZLİ	1			1
Sum	16	8	27	52

WHAT WE ARE PERFORMING

IN CLINICAL GENETICS

-Dysmorhology

- Fetal examination

-Genetic counselling

-Management of congenital disorders

-Management of complex disorders

IN CYTOGENETICS

- Chromosome analysis
  - periferic blood samples
  - amniotic fluid
  - CVS
  - cord blood
  - skin biopsy
  - tumor tissues
- HRB, NOR, C, R etc. banding

-Tissue cultures

-FISH

IN MOLECULAR GENETICS

-Capillary electroforesis-based DNA sequencing
  - more than 200 single gene disorders

-STR analysis

- Real-Time PCR

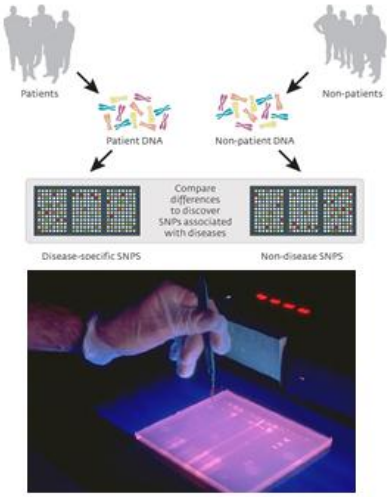
-QF-PCR

-MLPA

-Array CGH

-Molecular karyotyping in few center

-Next-gen based DNA sequencing in few center for routine diagnosis



REIMBURSEMENT FOR GENETIC TESTS

Social Security Institute (SGK) is responsible for reimbursement

Methodology-based reimbursement is using by SGK

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

PGD 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 genaration systems such as microarray, next-gen sequencing etc

BOTTLENECKS IN GENETICS APPLICATIONS IN TURKEY

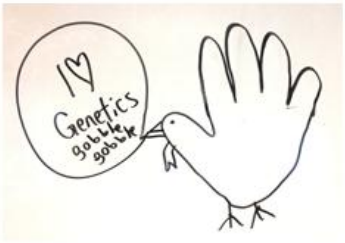
Lack or insufficieny of infrastructures for genetic laboratory in some university/state hospital

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

The education programme (4 year) of medical geneticist is not homogeneous

Educational activities are required to increase the knowledge in genetics applications

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



WHAT WE PERFORM

Diskapi Yildirim Beyazit Training and Research Hospital Medical Research School

Our main goal is perform translational studies from benchtop to bedside

- Regenerative medicine
- Stem cell research and applications

We are trying to make to implement new or rare genetic test such as single gene diabetes (MODY) in TURKEY

Pancreatic Islet Cell Research Center (PAHAM)

Department of Medical Genetics

*Single Gene Diabetes (MODY)

*Type 1-5

*Familial Hypocalciuric Hypercalcemia

*MEN1/MEN2 (RET)

*Konjonital Adrenal Hiperplazi

*Hereditary spastic paraplegia

*Cadasil (NOTCH3)

*BRAF, EGFR, KRAS

*SMA 1-2

*Parkinson

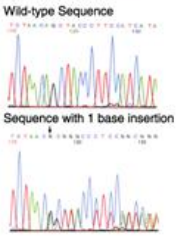
PARK2 (Parkin)

PINK1

PARK7

SNCA

LRRK2



PAHAM II

Cell Research Laboratory

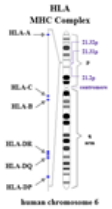
•Pancreatic islet isolation and transplantation laboratory

•Stemcell research laboratory

Proteomic Laboratory

•Protein characterisation and analysis for some disorders

HLA Typing Laboratory



- Low and High Resolution HLA Typing
  - HLA-A
  - HLA-B
  - HLA-C
  - HLA-DR
  - HLA-DQ
  - HLA-DP

Tests for transplantation

- PRA (Class I-II Tarama/Tanımlama)
- CDC
- Locus spesifik antikor Class I-II

Animal Lab. Facility

•There are some specific rodents such as spontaneous diabetic rat, obes, nude etc

THANKS FOR YOUR ATTENTION

- www.medicalresearchcenter.org

European Commission

CYTOGENETIC AND MOLECULAR DIAGNOSTIC IN CROATIA

Prof. dr. Irena Drmić Hofman
University Hospital Split
University of Split School of Medicine
CROATIA

Applied Genomics in the Clinics, 17-19 Oct 2012, Istanbul

CYTOGENETIC AND MOLECULAR TESTING IN CROATIA

4.5 million inhabitants, divided into 4 regions

CYTOGENETIC LABORATORIES IN CROATIA

Courtesy of Dr. Feodora Stipoljev

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 lymphocites, 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 lymphocites, 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, Gylbert sy)
- 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

Code	Short name	Full name	Sample type	Method recommended	Ref. genotype	Gene variation (gene variation testing)
[illegible]	CFTR (F508del)	CFTR (F508del), cystic fibrosis genotyping (CF)	EDTA, blood	1. "real-time" PCR 2. [illegible]	homozygote F508/F508	Allele: F508, F508del
[illegible]	CFTR (32 mutacije)	CFTR (32 mutacije), cystic fibrosis genotyping (CF)	EDTA, blood	[illegible]	normal/ normal	[illegible]

European Commission

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

Progress in Public Health Genetics

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

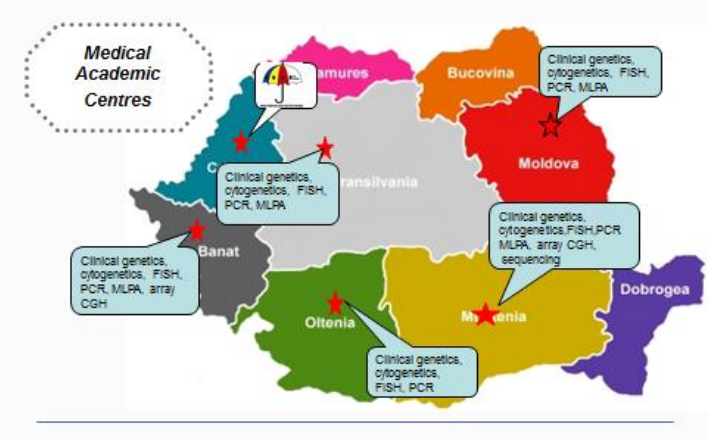
Romanian National Plan for Rare Diseases Strategies (cont)

- 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)

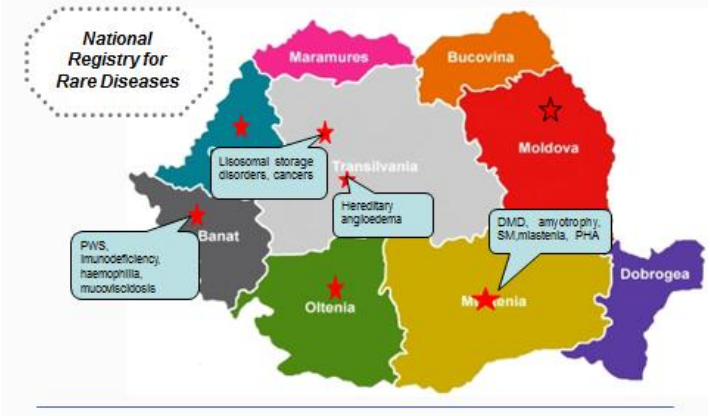
Implementation

- 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 (cont)



Implementation (cont)



Implementation (cont)

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 Centers network

Implementation
Regional Competence Centres

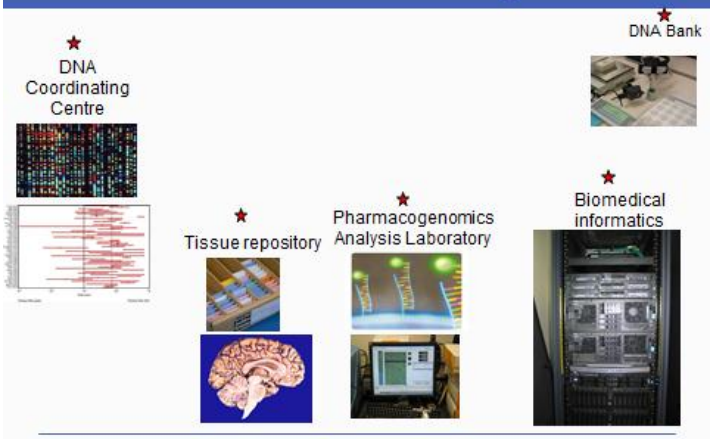
- Applying best practice guidelines agreed upon with all Centres of Reference
- Monitoring service delivery
- Information for Reference Centres and County Centres
- Organization / implementation of screening
- Setting up a database of reference specialists
- Prevention, diagnosis, treatment, recovery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

European Commission

Inviting you to see them!

THANK YOU!

Applied Genomics in Cancer: Sense and sensitivity

Dr Pinar Uysal-Onganer

University of Bedfordshire

Imperial College London

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

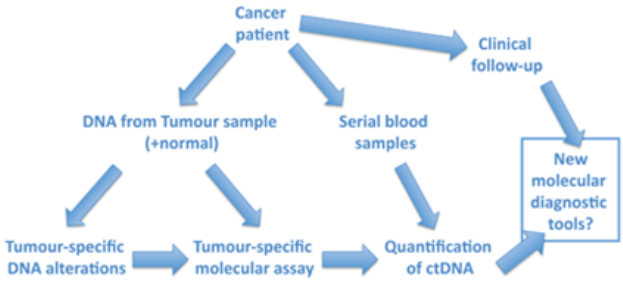
- Specific alterations in genes and the proteins they code for have been identified in many types of cancer
- Alongside this, scientists are using the latest microarray technologies to reveal genetic variations between cancers of the same type in different individuals
- The genetic signature of a person's 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

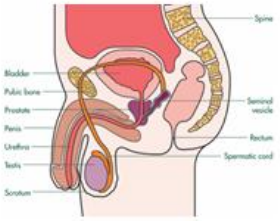
- is being funded, to the tune of £5.5million pounds, by CRUK, AstraZeneca, Pfizer and the government's Technology Strategy Board
- genetic testing labs with several hospitals involved, samples from patients diagnosed at these hospitals can be sent to one of these labs for high-quality genetic tests,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

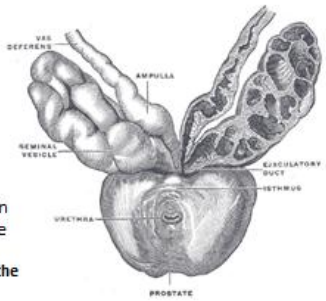
Which patients involved?

- breast cancer
- bowel cancer
- lung cancer
- prostate cancer
- ovarian cancer
- melanoma

Cancer types being tested	Rationale
KRAS in bowel and lung cancer; *NRAS* in bowel cancer and melanoma	Mutations in this family of genes have been found in these tumour types and the implications for treatment are the subject of ongoing research. Certain mutations in the KRAS gene have been found in people who don't respond to certain tyrosine kinase inhibitor drugs.
Bowel cancer, lung cancer, melanoma	This gene is frequently mutated in cancer, and is the focus of a lot of current research. Drugs that target mutant BRAF have also been used in clinical trials in the UK. A BRAF inhibitor drug has recently been approved in the United States for use in metastatic melanoma.
Bowel cancer, breast cancer, ovarian cancer	The p53 protein product of this gene has a pivotal role in detecting, stopping division of and programming self-destruction of potentially pre-cancerous cells with acquired abnormalities of DNA. As a result, this gene is the most commonly mutated gene in all cancer types and the subject of much ongoing research.
Lung cancer	Mutations in this gene can help predict response to certain tyrosine kinase inhibitor drugs.
Lung cancer, breast cancer, ovarian cancer, melanoma	Mutations in this gene are the focus of several labs worldwide, and there are a number of drugs that target the protein it makes – PI3 kinase – currently in clinical trials worldwide.
Melanoma	c-KIT gene mutations have been discovered in certain types of malignant melanoma and are the subject of ongoing research.
Breast cancer, prostate cancer, ovarian cancer	The tumour suppressing and growth regulating functions of the PTEN gene are commonly lost in many cancer types and this is an area of current research.
Prostate cancer	TMPRSS-ERG is the product of two genes (TMPRSS and ERG) that fuse together in some prostate cancers and is the subject of current research.
Lung cancer	This gene product is the result of the *ALK* and *EML4* genes joining together as part of a chromosomal rearrangement occurring in abnormal cells. Evidence is accumulating that patients with this genetic fault might benefit from certain drugs known as ALK inhibitors and an ALK inhibitor drug has recently been approved for use in the United States.

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

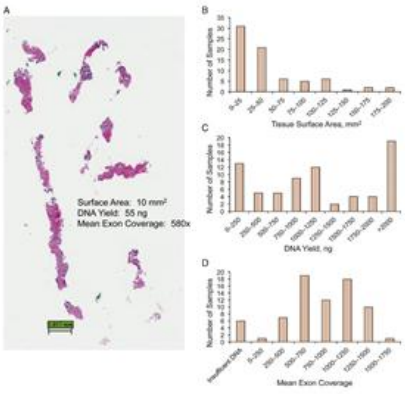
TMPRSS2-ERG fusion correlates with poor prognosis in PCa

- 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

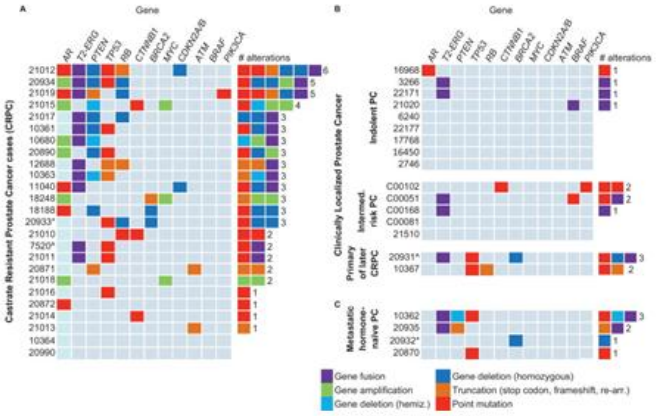
- assessment of the genomic landscape of advanced PCa is difficult (limited access to tissue and large amounts of DNA required)
- most patients with advanced PCa do not undergo biopsies of metastases as part of routine clinical care
- **NGS is a novel platform: requires little DNA and can use tissue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

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



Beltran et al., 2012

TMPRSS2:ERG fusion (44%)



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

European Commission

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

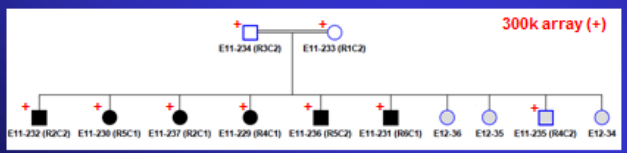
- Statistical analysis of the whole genome SNP array data using linkage software
  - Detect genotyping errors
  - Calculate two and multipoint lod scores
  - Constructing haplotypes
- Refinement of candidate loci with genotyping (additional microsatellites and/or SNPs)
- Candidate gene approach
- Exome-Targeted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

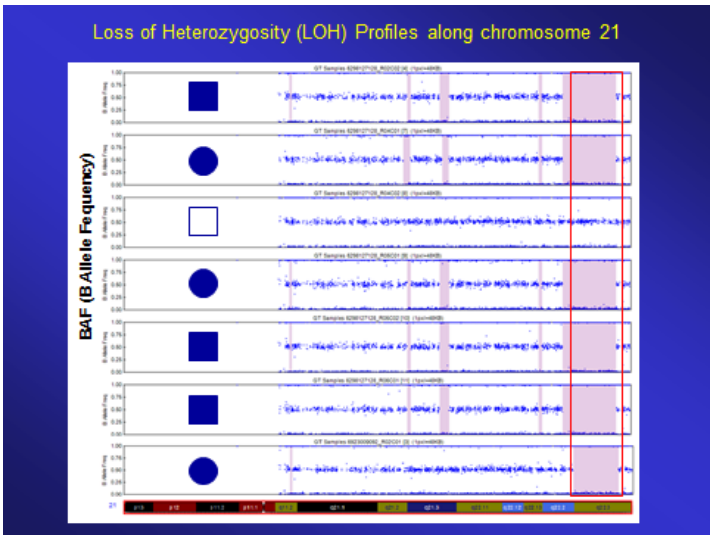
Disorders Analyzed

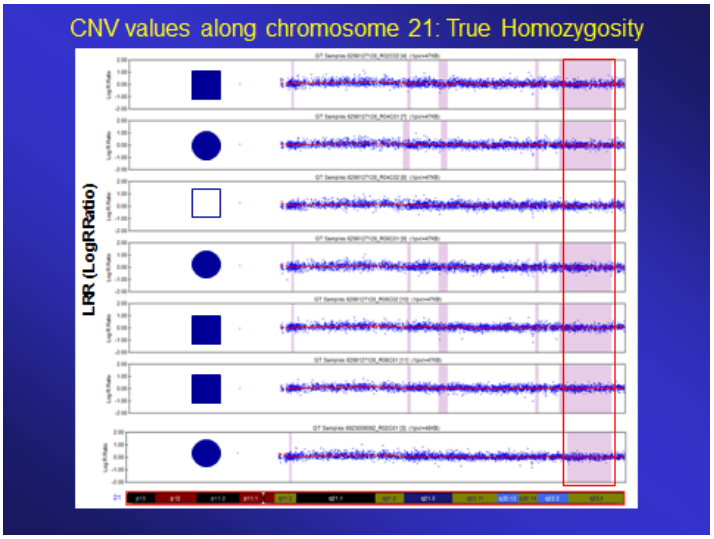
- Progressive Epilepsy
  - AR pedigree with multiple affected individuals
- Anophthalmia (absent eye) – Microphthalmia (small eye)
  - Developmental eye defect
  - 25 % of childhood visual impairment
  - Two AR pedigrees with similar malformations of the anterior eye

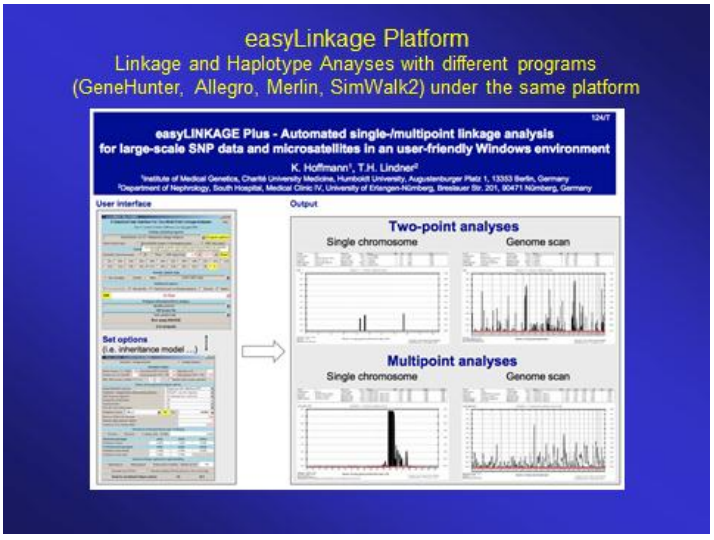
Progressive Epilepsy

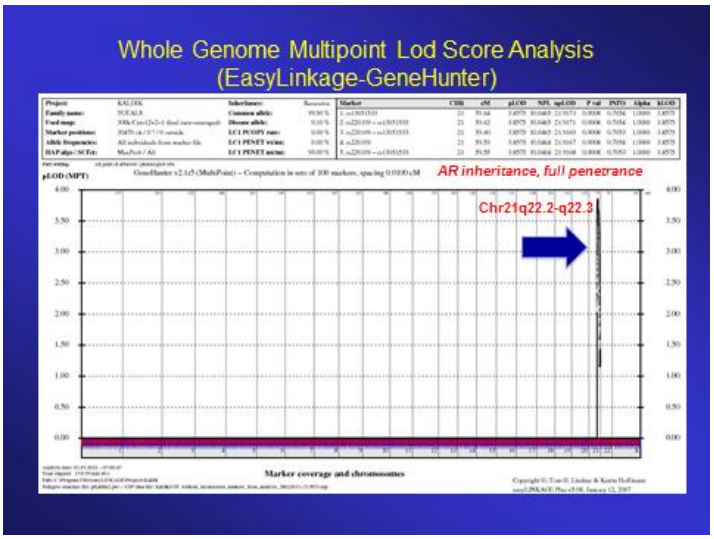
- Clinically undiagnosed form of progressive epilepsy
  - Tonic seizures starting at age 9
  - Progressive neurological dysfunction
- 300k Illumina array
  - Homozygosity mapping due to consanguinity
  - Lod score calculations
  - Candidate gene analysis

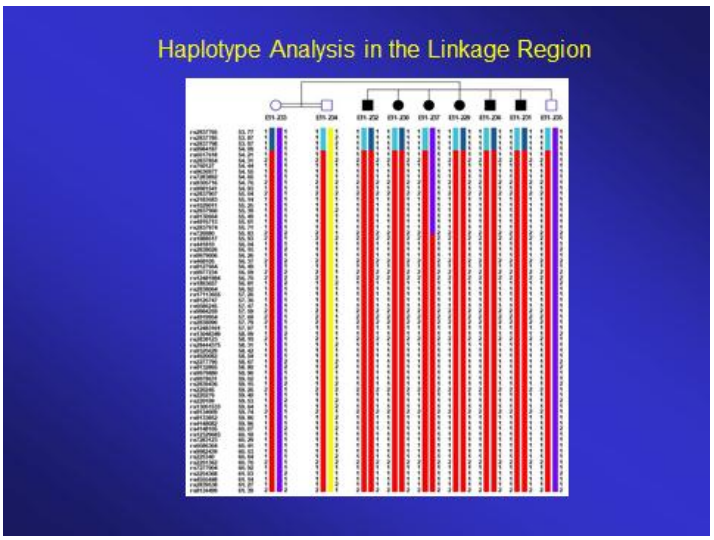


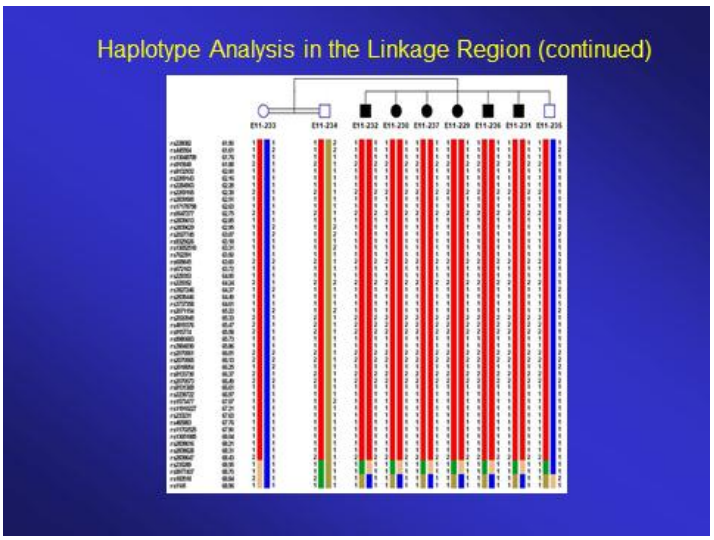

Loss of Heterozygosity (LOH) Profiles along chromosome 21
BAF (B Allele Frequency)


CNV values along chromosome 21: True Homozygosity
LRR (LogRRatio)


easyLinkage Platform
Linkage and Haplotype Anayses with different programs
(GeneHunter, Allegro, Merlin, SimWalk2) under the same platform
easyLINKAGE Plus - Automated single-/multipoint linkage analysis
for large-scale SNP data and microsatellites in an user-friendly Windows environment
K. Hoffmann, T.H. Lindner
User interface
Output
Set options
(i.e. inheritance model ...)
Two-point analyses
Single chromosome
Genome scan
Multipoint analyses
Single chromosome
Genome scan

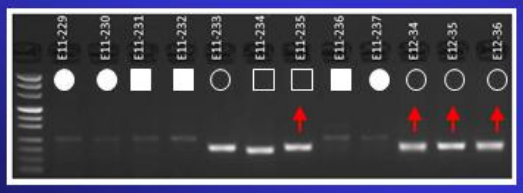

Whole Genome Multipoint Lod Score Analysis
(EasyLinkage-GeneHunter)
AR inheritance, full penetrance
Chr21q22.2-q22.3
Marker coverage and chromosomes


Haplotype Analysis in the Linkage Region


Haplotype Analysis in the Linkage Region (continued)

Genetics to Clinical Diagnosis

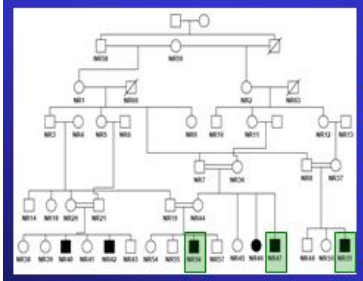
- ~ 5Mb Region, 100 genes
- *CSTB* encoding a protease inhibitor
  - Myoclonic epilepsy of Unverricht and Lundborg
- Dodectamer repeat expansion in the promoter



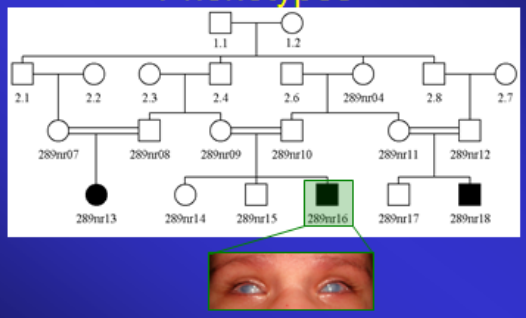
Anophthalmia-Microphthalmia

- 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



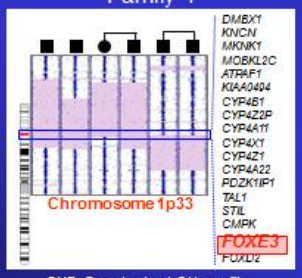
Family 2 Phenotypes



Microphthalmia, Sclerocornea, Aphakia

Genotyping and Statistical Analysis

Family 1

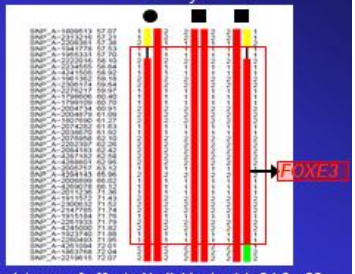


SNP Genotyping LOH profile

1.11 Mb, 24 genes

(Human 610-quad SNP chip, Illumina)

Family 2



Haplotypes of affected individuals at 1p34.3-p33

13.6 Mb, 226 genes

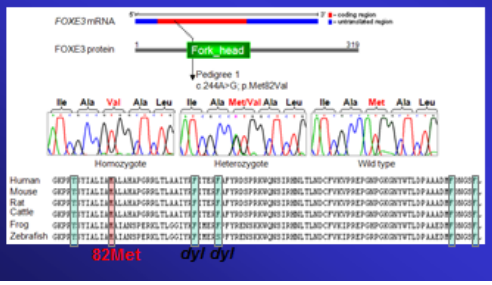
(250 K Array, Affymetrix)

FOXE3

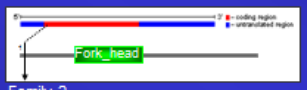
- Encodes a lens specific transcription factor
  - Forkhead domain: 110-amino-acid highly conserved DNA binding domain
- Two spontaneous *Foxe3* mutations cause dysgenetic lens phenotype in mice (*dyl* mice)
  - Connection between lens and cornea
  - Failure of the lens vesicle to separate from overlying ectoderm

Results: Family 1

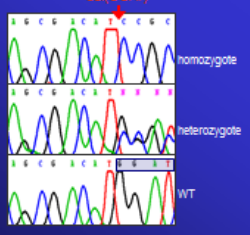
- c.224A>G: AR inheritance in 6 affected and 24 unaffected members of Family 1
- p.Met82Val resides in a Methionine-Aromatic Rosette
  - Hydrophobic sub-structure composed of a core methionine surrounded by five other conserved aromatic amino acids
- *dyl* mutations which abolish DNA binding also reside in this rosette
- Null allele predicted to prevent this assembly (EMSA)



Results: Family 2



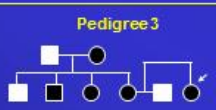
Family 2
c.21_24del; p.Met7IlefsX216



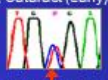
- c.21_24del: AR inheritance in 3 affected and 9 unaffected members of Family 2
- 4 bp deletion creating a frame shift and premature stop codon
- p.Met7IlefsX216
  - Abnormal and truncated protein after 7 residues
  - Null allele

Screening *FOXE3* in 236 Subjects: Dominant Inheritance

Pedigree 3

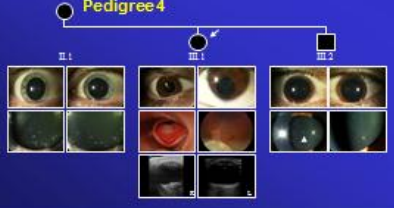


Microphthalmia, Sclerocornea, Peters' anomaly, 1° Aphakia, Cataract (early) and Coloboma

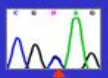


c.958T>C; p.X320ArgextX72
(abnormally enlarged protein)

Pedigree 4



Microphthalmia, Microcornea, Cerulean Cataracts (blue dot)



c.148G>C; p.Gly49Ala

Target gene for diagnostic screening in a broad spectrum of eye anomalies

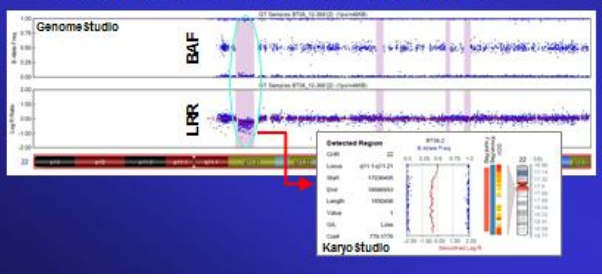
- Homozygous null *FOXE3* mutations in inbred pedigrees
  - Null mutation; abolishing DNA binding
  - Normal carrier status
- Heterozygous *FOXE3* mutations in two pedigrees with complete penetrance
  - Variable phenotypes and range of intrafamilial severity
  - Gain of function mutations?

Genomic Profiling of DNA CNVs with SNP arrays

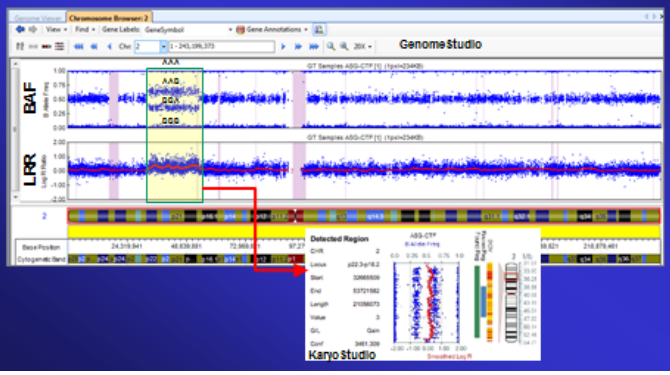
- Children with multiple congenital anomalies and mental retardation
- Illumina 300k array
- Scan the genome for CNVs
  - *Molecular Karyotyping with SNP array*
- Use of normalized BAF and LRR values
- GenomeStudio-KaryoStudio
  - cnvPartition
  - 3rd party programs (QuantiSNP, PennCNV, Nexus Copy Number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

İlker 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 cm^2)
- Automation (chip production, reagents)

Microarray

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

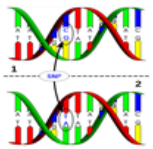
High-throughput system that can measure thousands of data simultaneously

SNP

- Definition: Variations in single base pairs that are randomly dispersed throughout the genome (every 100 to 300 bases along the 3-billion-base human genome)
- Act as measures of genetic diversity within the specie (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/)
- dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/)
- 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

Omni Whole-Genome Arrays

BeadChip	Array Format	Markers per Sample
HumanOmni5-Quad	4	~ 4.3 million
HumanOmni2.5S	8	~ 2.5 million
HumanOmni2.5-8	8	~ 2.5 million
HumanOmni1S	8	~ 1.25 million
HumanOmniExpress	12	~ 700,000
HumanCytoSNP-12	12	~ 300,000

Omni Semi-Custom Whole-Genome Arrays

BeadChip	Array Format	Markers per Sample
HumanOmni5-Quad+	4	~ 4.3 million (fixed) up to 500K (custom)
HumanOmni2.5S+	8	~2.5 million (fixed) up to 500K (custom)
HumanOmniExpress+	8	~700,000 (fixed) up to 200K (custom)

HumanCytoSNP-12 HD BeadChip
- 299,140 markers
- 12 samples per BeadChip
- 6,2 median marker spacing
- Reproducibility >99.9%
- Dense coverage of ~250 genomic regions (commonly studied in cytogenetics labs)
- Non-polymorphic probes
- Subtelomeric, pericentromeric and sex chromosome coverage
- ✓ Most cost effective BeadChip

SNP genotyping array applications

Genotype Analysis

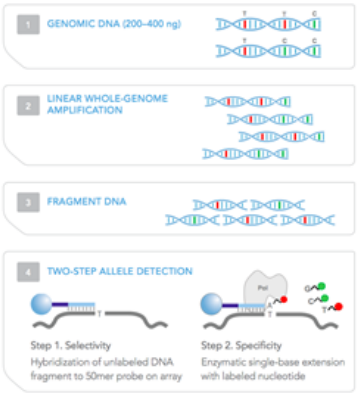
Linkage studies

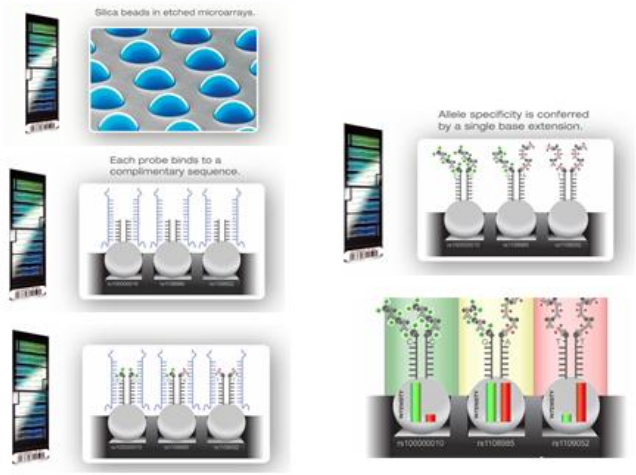
Whole-genome association

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 witho
- Hybridization
- Single base extension



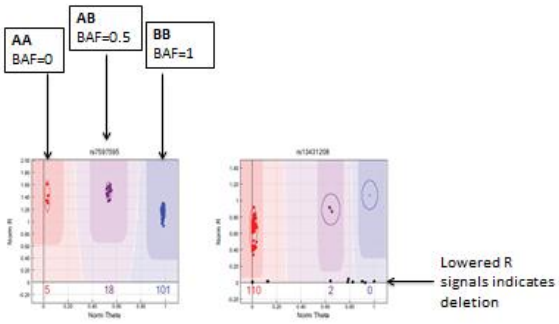


How the data generated

Two-color readout

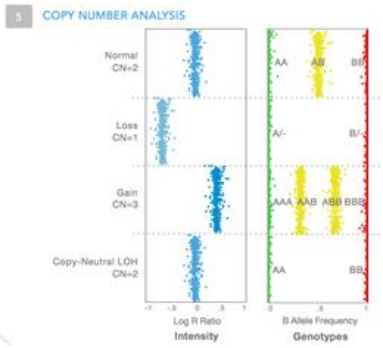
Normalized intensity values (R) and allelic intensity ratios (θ)

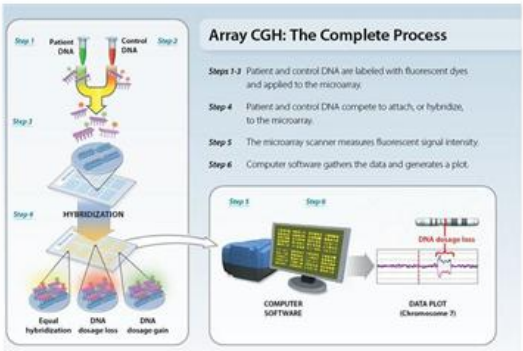
These values are used to calculate two metrics for each SNP marker in a sample LRR (Log R Ratio) and BAF (B Allele Frequency)



LRR is calculated from **R** value
BAF is calculated from **Theta** value

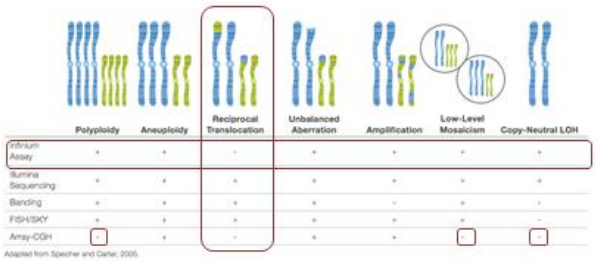
Outcome of a SNP array

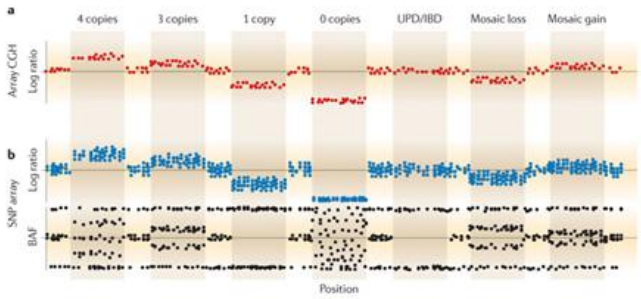




aCGH - SNP array

- **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

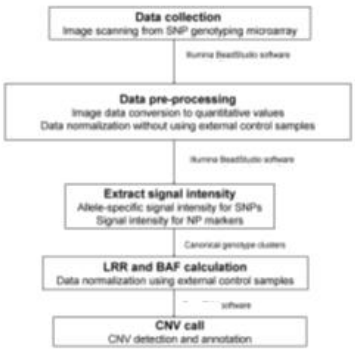
- Illumina software (BeadStudio, GenomeStudio)
- Commercial softwares (BioDiscovery, GoldenHelix, Partek etc.)
- Non-commercial softwares (PennCNV, QuantiSNP, CNVision etc.)

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

Am J Hum Genet. 2009 Feb;84(2):148-61. Epub 2009 Jan 22.

Population analysis of large copy number variants and hotspots of human genetic disease.

Ibarra A, Cooper GM, Baker C, Girirajan S, Li J, Absher D, Krauss RM, Myers RM, Ridker PM, Chasman DI, Mefford H, Ying P, Nickerson DA, Eichler EE.
Department of Genome Sciences, School of Medicine, University of Washington, Seattle, WA 98195, USA.

Abstract
Copy number variants (CNVs) contribute to human genetic and phenotypic diversity. However, the distribution of larger CNVs in the general population remains largely unexplored. We identify large variants in approximately 2500 individuals by using Illumina SNP data, with an emphasis on "hotspots" prone to recurrent mutations. We find variants larger than 500 kb in 5%-10% of individuals and variants greater than 1 Mb in 1%-2%. In contrast to previous studies, we find limited evidence for stratification of CNVs in geographically distinct human populations. Importantly, our sample size permits a robust distinction between truly rare and polymorphic but low-frequency copy number variation. We find that a significant fraction of individual CNVs larger than 100 kb are rare and that both gene density and size are strongly anticorrelated with allele frequency. Thus, although large CNVs commonly exist in normal individuals, which suggests that size alone can not be used as a predictor of pathogenicity, such variation is generally deleterious. Considering these observations, we combine our data with published CNVs from more than 12,000 individuals contrasting control and neurological disease collections. This analysis identifies known disease loci and highlights additional CNVs (e.g., 3q29, 16p12, and 15q25.2) for further investigation. This study provides one of the first analyses of large, rare (0.1%-1%) CNVs in the general population, with insights relevant to future analyses of genetic disease.

Genome arrays for the detection of copy number variations in idiopathic mental retardation, idiopathic generalized epilepsy and neuropsychiatric disorders: lessons for diagnostic workflow and research.

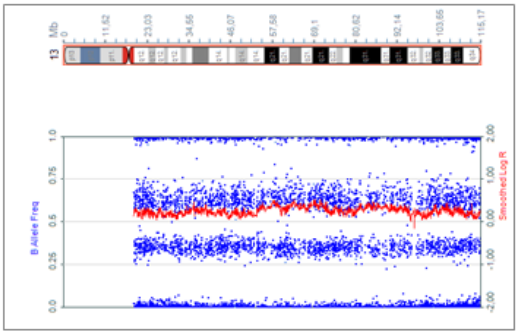
Hochstenbach R, Buizer-Voskamp JE, Vorstman JA, Ophoff RA.
Division of Biomedical Genetics, Department of Medical Genetics, University Medical Centre Utrecht, Utrecht, The Netherlands. phochste@umcutrecht.nl

Abstract
We review the contributions and limitations of genome-wide array-based identification of copy number variants (CNVs) in the clinical diagnostic evaluation of patients with mental retardation (MR) and other brain-related disorders. In unselected MR referrals a causative genomic gain or loss is detected in 14-18% of cases. Usually, such CNVs arise de novo, are not found in healthy subjects, and have a major impact on the phenotype by altering the dosage of multiple genes. This high diagnostic yield justifies array-based segmental aneuploidy screening as the initial genetic test in these patients. This also pertains to patients with autism (expected yield about 5-10% in nonsyndromic and 10-20% in syndromic patients) and schizophrenia (at least 5% yield). CNV studies in idiopathic generalized epilepsy, attention-deficit hyperactivity disorder, major depressive disorder and Tourette syndrome indicate that patients have, on average, a larger CNV burden as compared to controls. Collectively, the CNV studies suggest that a wide spectrum of disease-susceptibility variants exists, most of which are rare (<0.1%) and of variable and usually small effect. Notwithstanding, a rare CNV can have a major impact on the phenotype. Exome sequencing in MR and autism patients revealed de novo mutations in protein coding genes in 60 and 20% of cases, respectively. Therefore, it is likely that arrays will be supplanted by next-generation sequencing methods as the initial and perhaps ultimate diagnostic tool in patients with brain-related disorders, revealing both CNVs and mutations in a single test.

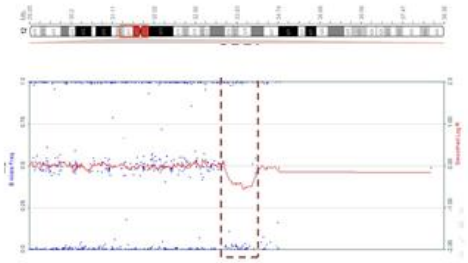
Molecular karyotying examples

- *Trisomies*
- *Deletions*
- *Duplications*
- *LOH regions*

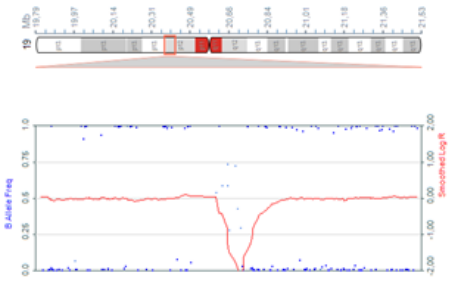
Trisomy 13



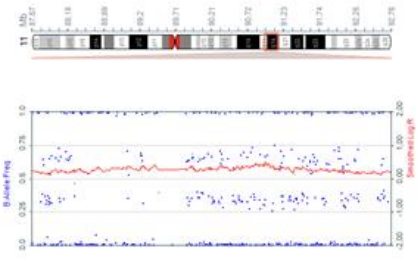
600kb Deletion on chr 12



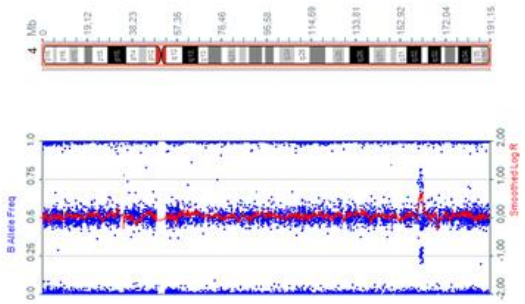
108kb deletion CN=0



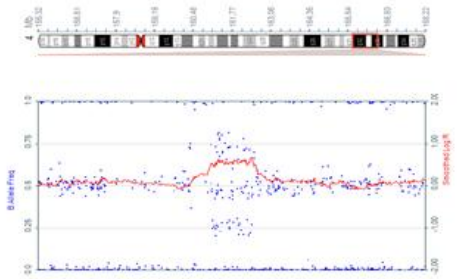
5Mb Duplication on chr 11



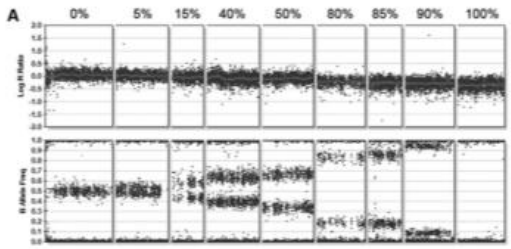
1.6Mb amplification on chr 4



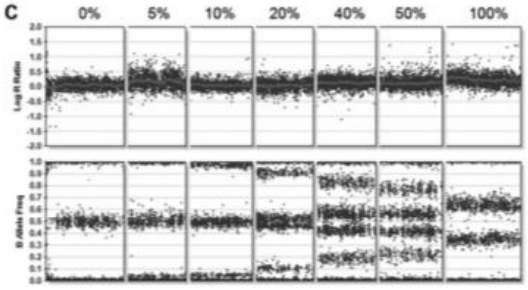
Copy number = 4



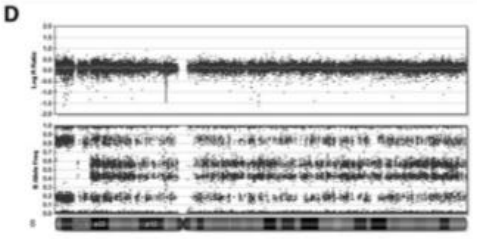
Mosaicism



nine patients with varying levels of mosaicism for deletions involving autosomes

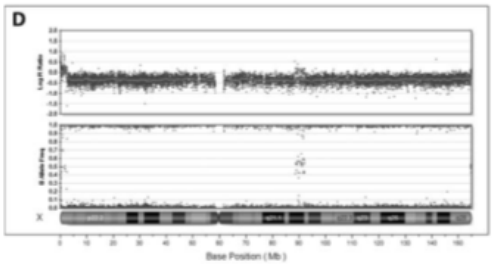


seven patients with varying levels of mosaicism for trisomies



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 pseudoautosomal regions appears similar to that seen with the autosomes.

Data interpretation

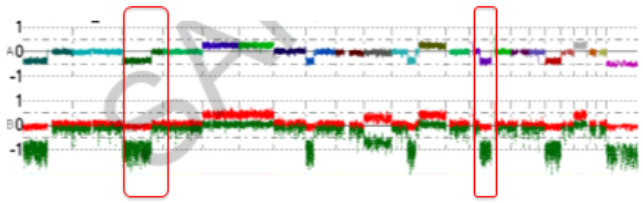
- dbVar (Database of genomic structural variation) http://www.ncbi.nlm.nih.gov/dbvar
- DGV (db of Genomic Variants) http://projects.tcag.ca/variation/
- DECIPHER http://decipher.sanger.ac.uk

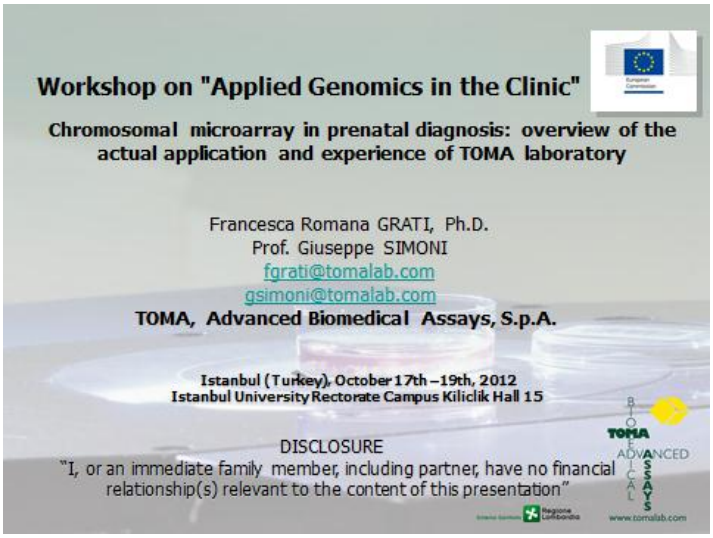
- DECIPHER tracks on UCSC GenomeBrowser

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





Overall incidence of chromosome abnormalities in prenatal samples detected by conventional karyotype (1994-2011; all indications are considered)

	n.	Abnormal karyotypes %
CVS	59.000	4,25
AF	135.000	2,12

TOMA lab cytogenetic audit

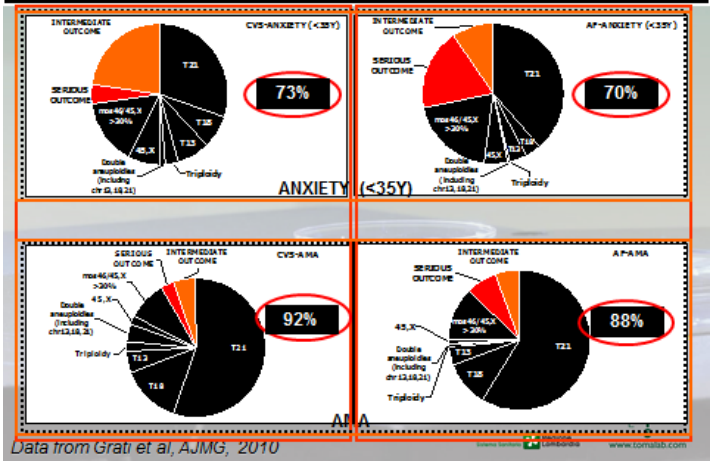
Detection rates of prenatal karyotyping according to the indication for invasive PD

(only chr abn leading to substantial phenotypic effect are reported)
Partial experience of TOMA lab on 84470 AF and 30658 CVS

INDICATION FOR INVASIVE PD	TYPE OF PRENATAL SAMPLE	
	CVS (%)	AF (%)
AMA (>35y)	2,42	1,42
Anxiety-no specific indication (<35y)	1,22	0,61
US fetal abnormality	27,86	11,46
Parent carrier of a chr abn	10,55	1,15
Previous affected child/fetus	2,13	0,72
Increased MSS for DS	7,61	2,28
Chr abn in a relative	1,21	0,88
Fetal demise	18,6	3,16
Other	0,76	1,13
Confirmatory amniocentesis after CVS abn.	---	5,06

Grati et al, 2010

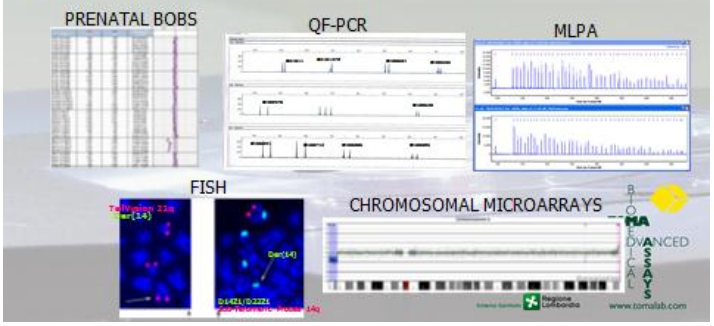
Incidences of T21, T18, T18, 45,X in PRENATAL SAMPLES out of all chromosome abn leading to substantial fetal phenotypic effect in the first and second trimester



Data from Grati et al, AJMG, 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-ier 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)

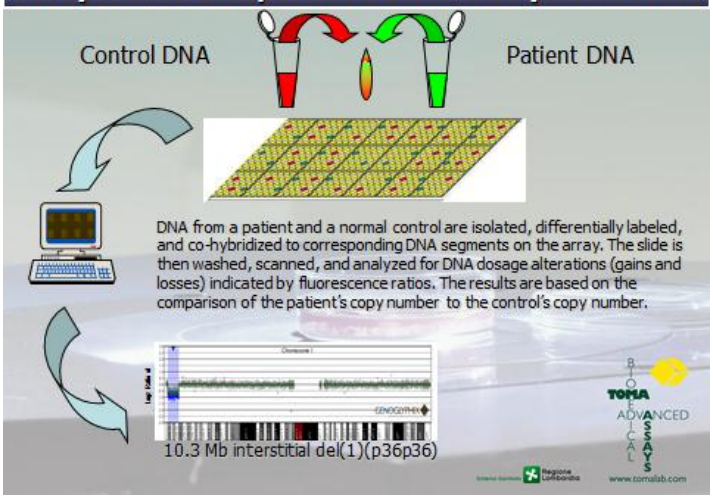
TOMA lab cytogenetic audit

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

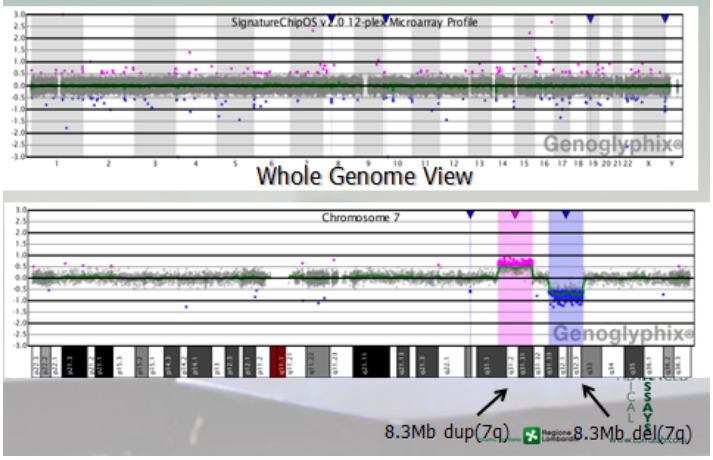
- **UPD condition exclusion on AF after a mosaic trisomy for an imprinted chr in CV:** in 0.5% of CVS analysed (~1/200) (Grati et al, 2006)
- **Increased risk of false negative result** due to the incompleteness of the combined cytogenetic analysis on CVS (STC+LTC) (Simoni et al, 1987; Lilford RJ et al., 1991; Ledbetter et al, 1992; Pittalis et al., 1994)
- **Apparently balanced '*de novo*' rearrangements:** 0.09% in AF and CVS (~1/800) (Giardino 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; Malvestiti et al, ISPD 2010 personal comunication)
- **Fetuses with US abnormalities and an apparently normal karyotype** (Gignac et al, 2006; Kjaergaard et al, 2010; Novelli et al, 2012)

Array-based Comparative Genomic Hybridization



Oligonucleotide-based Array CGH

Array CGH most often uses synthetic oligonucleotide (oligo) probes



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 through-put with 2~4 day turn-around time after DNA extraction
- ❑ Comprehensive – high resolution in density and genomic coverage

Microarray-based Cytogenetics

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

Kearney et al, 2011

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

❖ **An abnormal 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 (VOUS) 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**

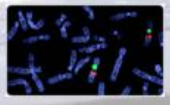
Interpretation of Copy Number Variation

- 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

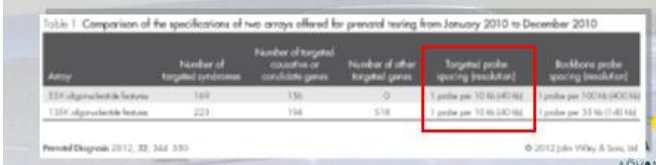
- Array resolution is a function of several factors:
  - the number of probes on an array,
  - the distance between probes, and
  - the statistical algorithms used to analyze array results.

- **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.**

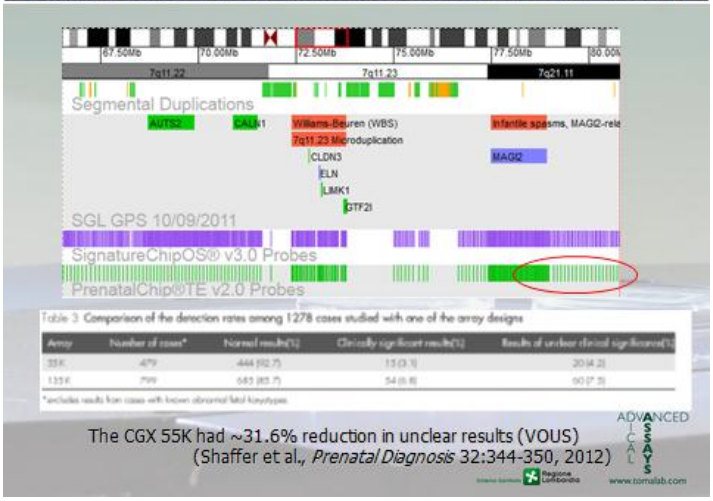
- A 400 kb threshold is recommended as the minimum genome-wide detection rate for arrays as the majority of copy variations below that level have been shown to be polymorphic in control populations. (Miller D, Adam M, Aradhya S, et al. Am J Hum Genet 2010; 86:749–764.)

NimbleGen CGX array 135K and 55K Designs (PerkinElmer)

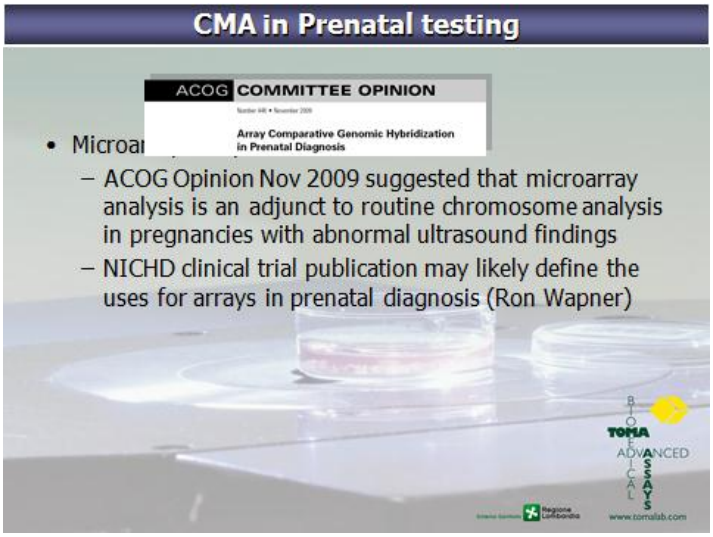
  - >245 known microdeletion/duplication syndromes
  - 41 subtelomere regions, 43 pericentromeric regions
- CGX **135K microarray** – higher density in backbone for abnormal ultrasound cases and de novo rearr
  - Backbone of 1 probe / 35 kb = 140 kb detection
- CGX **55K microarray** – lower density in backbone for other cases (low acceptance of VOUS)
  - Backbone of 1 probe / 100 kb = 400 kb detection

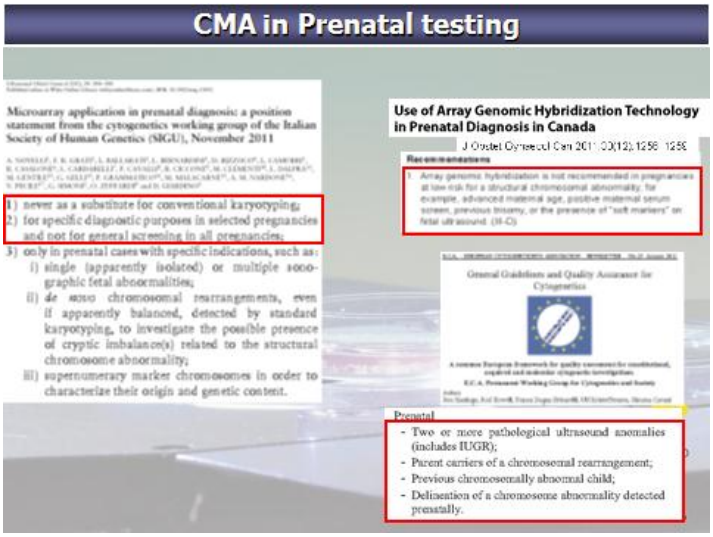


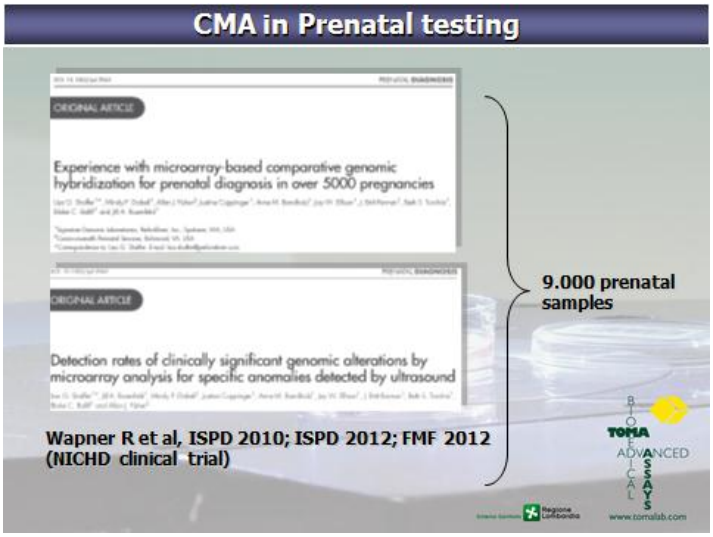
NimbleGen CGX array 135K and 55K Designs (PerkinElmer)



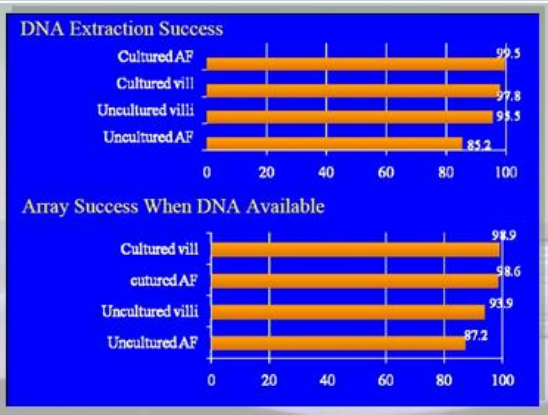
The CGX 55K had ~31.6% reduction in unclear results (VOUS)
(Shaffer et al., *Prenatal Diagnosis* 32:344-350, 2012)


CMA in Prenatal testing
ACOG COMMITTEE OPINION
Array Comparative Genomic Hybridization in Prenatal Diagnosis
• Microar
– 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
Microarray application in prenatal diagnosis: a position statement from the cytogenetics working group of the Italian Society of Human Genetics (SIGU), November 2011
1) never as a substitute for conventional karyotyping;
2) for specific diagnostic purposes in selected pregnancies and not for general screening in all pregnancies;
3) only in prenatal cases with specific indications, such as:
i) single (apparently isolated) or multiple sonographic fetal abnormalities;
ii) de novo chromosomal rearrangements, even if apparently balanced, detected by standard karyotyping, to investigate the possible presence of cryptic imbalance(s) related to the structural chromosome abnormality;
iii) supernumerary marker chromosomes in order to characterize their origin and genetic content.
Use of Array Genomic Hybridization Technology in Prenatal Diagnosis in Canada
Recommendations
1. Array genomic hybridization is not recommended in pregnancies at low risk for a structural chromosomal abnormality, for example, advanced maternal age, positive maternal serum screen, previous trisomy, or the presence of "soft markers" on fetal ultrasound. (III-D)
General Guidelines and Quality Assurance for Cytogenetics
Prenatal
- Two or more pathological ultrasound anomalies (includes IUGR);
- Parent carriers of a chromosomal rearrangement;
- Previous chromosomally abnormal child;
- Delineation of a chromosome abnormality detected prenatally.

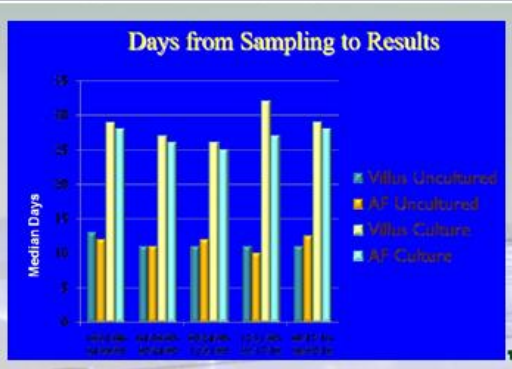

CMA in Prenatal testing
ORIGINAL ARTICLE
Experience with microarray-based comparative genomic hybridization for prenatal diagnosis in over 5000 pregnancies
ORIGINAL ARTICLE
Detection rates of clinically significant genomic alterations by microarray analysis for specific anomalies detected by ultrasound
9.000 prenatal samples
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



Slide from Ron Wapner

NICHD clinical trial - Days from sampling to results



Slide from Ron Wapner

NICHD clinical trial - Conclusions

aCNA Using Uncultured Villi And AF Is Feasible And Reliable

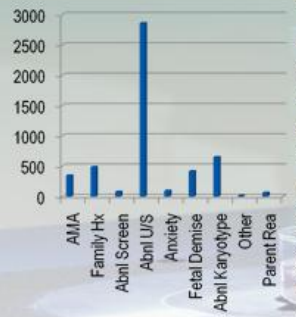
- Requires experience
  - AF DNA extraction
  - Running array with less DNA
- Villi More Reliable Than AF
  - More DNA
  - Higher Quality DNA

Discrepancies Between Uncultured And Cultured Analysis Occur

- More frequent with CVS
  - Biologic Differences: CPM
  - Technical Differences: Culture artifact, loss of small segments in culture

Slide from Ron Wapner

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	2,858
Parental Anxiety	95
Fetal Demise	417
Known Abnormal Fetal Karyotype	648
Other/Not Specified	13
Known Parental Rearrangement	61
Total	5,002

Shaffer et al, 2012

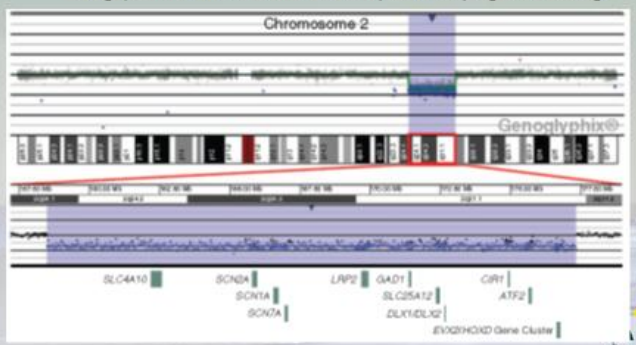
Stratification of the clinically significant results into the <10Mb and ≥10Mb categories (the reliable resolution for traditional karyotyping)

Abnormality	<10 Mb	≥10 Mb
Known microdeletion syndrome	35	NA
Known microduplication syndrome	3	NA
Microdeletion: reduced penetrance	46	NA
Microduplication: reduced penetrance	16	NA
Homozygous deletion	2	0
Terminal deletion	8	1
Terminal duplication	0	1
Other interstitial deletion	27	10
Other interstitial duplication	10	1
Unbalanced translocation	7	14
Insertion	2	0
Autosomal aneuploidy	NA	11
Sex chromosome aneuploidy	NA	6
XX male	NA	1
Polyploidy	NA	1
Complex rearrangements	12	7
Mosaic findings	4	1A
Total	172 (71%)	69 (29%)

~30% of clinically significant CNA were >10 Mb and were missed by karyotyping

Shaffer et al, 2012

A large, ~19Mb CNA 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%

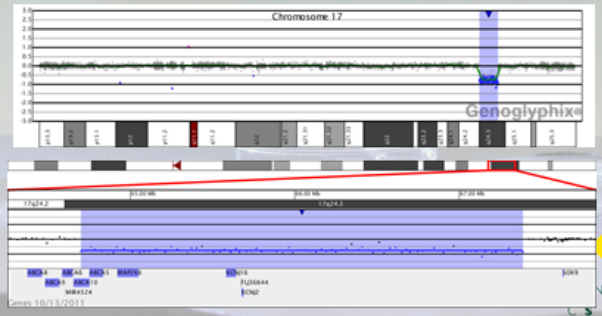
Table 5 Detection rates for cases with a known fetal chromosome abnormality in an on-going pregnancy or fetal demise, or a family history of a parental rearrangement

Karyotypic abnormality	Imbalance related to the known karyotype [%]	No imbalance [%]	Other unrelated finding, significant [%]	Other unrelated finding, unclear [%]	Total
Normocaic, apparently balanced rearrangement	19 (7.9)	207 (86.6)	4 (1.7)	9 (3.8)	239
Balanced translocation	15 (7.9)	166 (87.8)	4 (2.1)	4 (2.1)	189
Inversion	2 (4.5)	37 (84.1)	0 (0.0)	5 (11.4)	44
Insertion	2 (33.3)	4 (66.7)	0 (0.0)	0 (0.0)	6
Mosaic, apparently balanced rearrangement	0 (0.0)	5 (100.0)	0 (0.0)	0 (0.0)	5
Normocaic, apparently unbalanced rearrangement	183 (67.5)	82 (30.3)	3 (1.1)	3 (1.1)	271
Marker or ring chromosome	47 (53.4)	38 (43.2)	1 (1.1)	2 (2.3)	88
Suspected deletion	59 (84.3)	10 (14.3)	0 (0.0)	1 (1.4)	70
Suspected duplication	34 (57.6)	25 (42.4)	0 (0.0)	0 (0.0)	59
Complex rearrangements	15 (68.2)	5 (22.7)	2 (9.1)	0 (0.0)	22
Aneuploidy	16 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	16
Unbalanced translocation	12 (75.0)	4 (25.0)	0 (0.0)	0 (0.0)	16
Mosaic, apparently unbalanced rearrangement	44 (40.0)	62 (56.4)	2 (1.8)	2 (1.8)	110
Marker or ring chromosome	41 (44.6)	47 (51.1)	2 (2.2)	2 (2.2)	92
Other mosaic unbalanced karyotype	3 (16.7)	15 (83.3)	0 (0.0)	0 (0.0)	18
Variant	4 (28.6)	10 (71.4)	0 (0.0)	0 (0.0)	14
Mismatched genotypic and phenotypic sex	9 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	9
Total, abnormal karyotype	259 (40.0)	366 (56.5)	9 (1.4)	14 (2.2)	648
Family history of a chromosome rearrangement	33 (53.2)	27 (43.5)	0 (0.0)	2 (3.2)	62

Shaffer et al, 2012

Loss at a "Balanced" Translocation Breakpoint

- Referred for micrognathia and abnormal karyotype, 46,XX,t(16;17)(?q21;?q24)dn
- A 2.7Mb 17q24.3 deletion was identified.
- FISH studies confirmed that the deletion is at the breakpoint
- No copy number changes were seen on chromosome 16.



Shaffer et al, 2012

CMA in Definition of sSMCs and Rings

In cases with a homogeneous or mosaic sSMC or ring chr the detection rate by CMA for clinically significant abnormalities is ~50%

Table 5 Detection rates for cases with a known fetal chromosome abnormality in an on-going pregnancy or fetal demise, or a family history of a parental rearrangement

Karyotypic abnormality	Imbalance related to the known karyotype [%]	No imbalance [%]	Other unrelated finding, significant [%]	Other unrelated finding, unclear [%]	Total
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Suspected deletion	59 (84.3)	10 (14.3)	0 (0.0)	1 (1.4)	70
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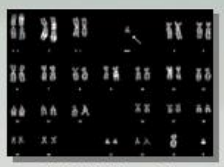
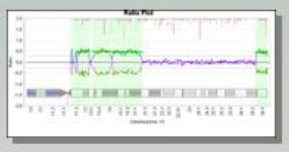
Other mosaic unbalanced karyotype	3 (16.7)	15 (83.3)	0 (0.0)	0 (0.0)	18
Variant	4 (28.6)	10 (71.4)	0 (0.0)	0 (0.0)	14
Mismatched genotypic and phenotypic sex	9 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	9
Total, abnormal karyotype	259 (40.0)	366 (56.5)	9 (1.4)	14 (2.2)	648
Family history of a chromosome rearrangement	33 (53.2)	27 (43.5)	0 (0.0)	2 (3.2)	62

Shaffer et al, 2012

CMA in Definition of sSMCs and Rings



47,XY,+der(15)del(15)(q21.1).ish der(15)(wcp15+,D15Z1+,SNRPN+,PML-)



arr cgh 15q11.2(18,748,501-18,928,999)x3,15q11.2(20,523,659-20,665,037)x3, 15q11.2q13.1(21,523,217-25,883,695)x3,15q13.1q14(28,918,525-34,527,040)x3, 15q14q21.2(35,776,624-47,502,899)x3, 15q26.2q26.3(95,833,747-100,170,013)x3

- chromosome origin
- BKP boundaries
- size
- genes content

Ballif et al, 2007; Gruchy et al, 2008; Tsuchiya et al, 2008; Malvestiti F et al, ISPD 2010; Vetro et al, 2012

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%)

Grati et al, AJMG, 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 IFS

Anomaly	Detection Rate
Single Anomaly	99/1772 (5.6%)
Anomalies in 2 or more organ systems	78/808 (9.6%)
Isolated abnormalities of growth	2/76 (2.6%)
One or more soft ultrasound markers*	2/78 (2.6%)

* Increased nuchal translucency excluded

Detection rates are significantly higher for multiple anomalies, compared to single systems or non-structural anomalies ($p<0.001$ for both, Fisher exact test).

Increased NT	Isolated	Other findings	Total
< 4mm	1/113 (0.9%)	1/7 (14.3%)	2/120 (1.7%)
≥4mm	6/96 (6.3%)	2/12 (16.7%)	8/108 (7.4%)
Total	**10/303 (3.3%)**	**6/49 (12.2%)**	**16/352 (4.5%)**

Detection rates in addition to those found by karyotyping

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

Anomalies in a Single Organ System or Single Anomaly

Organ System or Single Anomaly	Detection Rate
CNS	25/381 (6.6%)
Heart	6/237 (2.5%)
Facies (dysmorphism)	6/88 (6.8%)
Diaphragmatic hernia	4/48 (8.3%)
Omphalocele	4/49 (8.2%)
Musculoskeletal	18/203 (8.9%)
Genitourinary	7/115 (6.1%)
Nuchal or other body fluid accumulation	27/628 (4.3%)

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

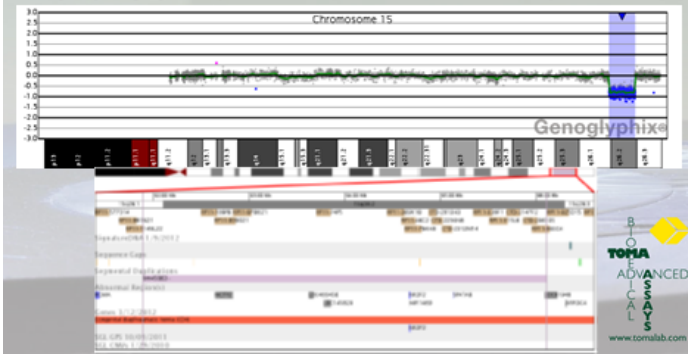
Anomaly	Detection Rate
Holoprosencephaly	9/85 (10.6%)
Posterior fossa defects	21/144 (14.6%)
Skeletal anomalies	15/140 (10.7%)
Ventricular septal defect	14/132 (10.6%)
Hypoplastic left heart	11/68 (16.2%)
Cleft lip/palate	14/136 (10.3%)

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 CMA IN A FETUS WITH HYDROPS AND INCREASED NT

- CVS: 35Y woman referred for NT 8mm and hydrops at 11wg
- Karyotype: 46,XX
- array-CGX 135K: De novo interstitial deletion of 4.2Mb in 15q26.1q26.2 involving the region CHD type 1 (MIM%142340).
- Between 17wg and 21wg: US and MR investigations confirm the presence of left CHD, hypoplasia 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

VOUS
- **inherited from a parent → benign**
- ***de novo* in origin → potential clinical relevance**

rates of reported VOUS

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 Anxiety)

Study	No. with significant abnormalities in AMA		No. with significant abnormalities in anxiety	
	# cases	DR (%)	# cases	DR (%)
Fiorentino et al, 2011	1/144	0,2		
Armengol et al, 2011	4/333	1,2		
Park et al. 2010	17/4073	0,3		
Lee et al, 2012	1/346	0,3	5/989	0,51
Breman et al., 2012 (personal communication)	3/394	0,76		
Shaffer LG, et al, 2012	1/346	0,3	0/95	0
Overall results	**27/5636**	**0,47**	**5/1084**	**0,46**

Novelli et al, 2012 commentary letter

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

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)

Indication for study (IFS)	Number with unclear variants	Rate
Abnormal ultrasound	138/2858	4.8%
Abnormal serum screening	5/77	6.5%
Family history	11/487	2.3%
AMA	8/346	2.3%
Anxiety	1/95	1.1%
Other/not specified	0/13	0.0%
TOTAL	**163/3876**	**4.2%**
Fetal demise	25/417	6.0%

Inherited variants	*De novo* variants	Parents not tested
119/3876 (3.1%)	15/3876 (0.39%)	29/3876 (0.75%)

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

Slide: courtesy of Shaffer LG (Signature Genomics dataset) ISPD 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

POINTS TO BE IMPROVED:

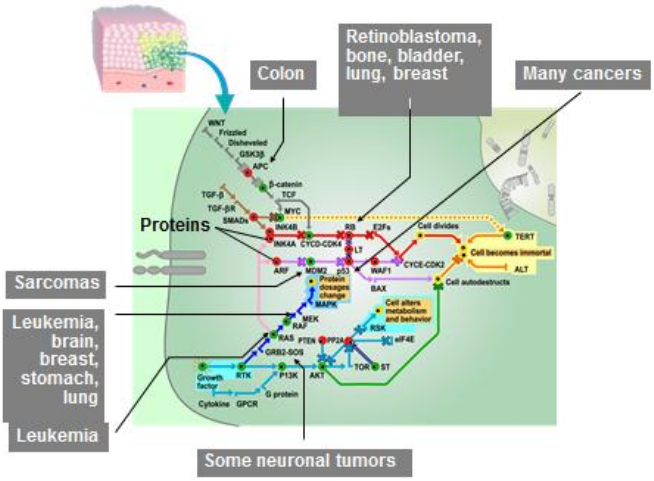
- **Knowledge on human genome architecture in normal population** *(Alkan et al, 2011; Cooper et al, 2011; Itsara et al, 2010; Itsara et al, 2009;...)*
- **Knowledge on entire phenotypic spectrum of microdeletion and microduplication syndromes and on uncertain variants** *(Cooper et al, 2011; Talkowski et al, 2011; Rosenfeld et al, 2010; Girirajan et al, 2010;...)*
- **pre- and post test counseling approach models for prenatal CMA**
  - ***reporting unsolicited findings related to late onset/cancer diseases?***
  - ***reporting VOUS?***
  - ***reporting pCNVs unrelated to the indication for CMA?***

(McGillivray et al, 2012; Wapner et al, 2012; Dondorp et al, 2012)

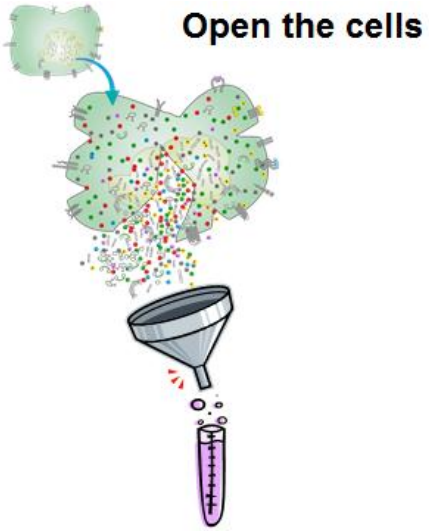
THE USE AND ANALYSIS OF EXPRESSION MICROARRAY DATA

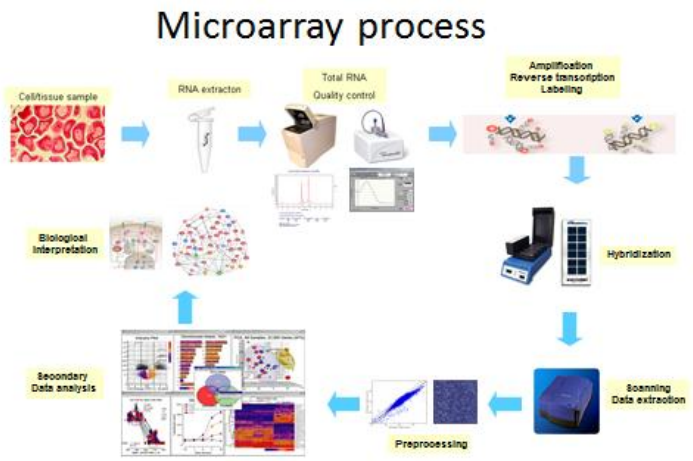
Marco Fabbri – JRC

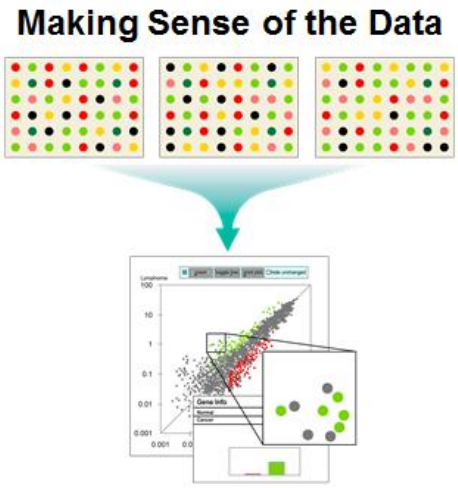
How to study a biological process?



Open the cells




Microarray process
Cell/tissue sample
RNA extracton
Total RNA
Quality control
Amplification
Reverse transcription
Labelling
Hybridization
Soanning
Data extraction
Preprocessing
Secondary
Data analysis
Biological
Interpretation


Making Sense of the Data

Analysis Tools

- Software

- Annotation

Type of Data Analysis

Class: characteristic shared by a group (e.g. cancer Vs. Normal)

- Identify differences at molecular levels between know class (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)

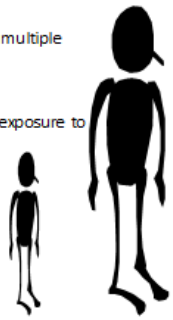
Class comparison

Differential expression analysis

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

What genes are up regulated 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.

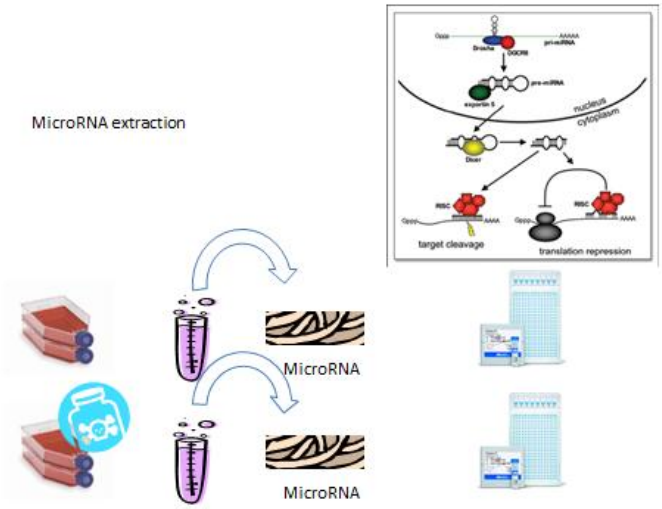
Gene upregulated (microarray)

KEGG enrichment analysis (David)

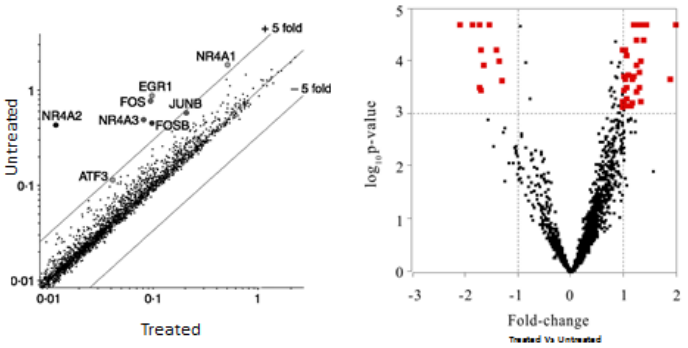
MicroRNA downregulated (HTqPCR)

Target prediction and KEGG enrichment analysis (Diana Mirpath)

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



Fold change: Not sufficient, needs statistics!



KEGG Entry	Name	Targets
hsa04510	Focal adhesion	67
hsa04010	MAPK signaling pathway	76
hsa05215	Prostate cancer	34
hsa05214	Glioma	27
hsa04310	Wnt signaling pathway	48
hsa04115	p53 signaling pathway	28
hsa04360	Axon guidance	41
hsa05211	Renal cell carcinoma	27
hsa05212	Pancreatic cancer	28
hsa04350	TGF-beta signaling pathway	32
hsa05030	Amyotrophic lateral sclerosis (ALS)	11
hsa05210	Colorectal cancer	30
hsa05218	Melanoma	26
hsa04520	Adherens junction	26
hsa05219	Bladder cancer	17

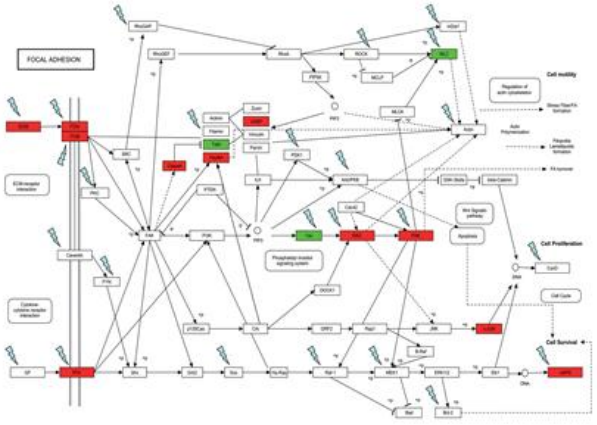
KEGG enrichment of targets of downregulated micoRNA

KEGG Entry	Name
hsa04510	Focal adhesion
hsa05200	Pathways in cancer
hsa04060	Cytokine-cytokine receptor interaction
hsa04010	MAPK signaling pathway
hsa04512	ECM-receptor interaction
hsa04360	Axon guidance
hsa05222	Small cell lung cancer

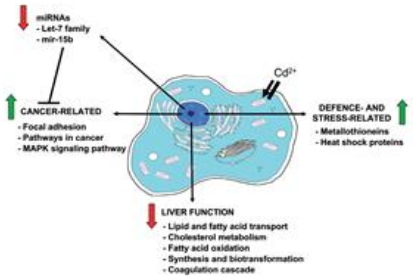
KEGG enrichment of upregulated mRNA

pathways downregulated by cadmium

KEGG Entry	Name	Genes	PValue
hsa04610	Complement and coagulation cascades	22	1.11E-14
hsa00260	Glycine, serine and threonine metabolism	11	8.50E-08
hsa00071	Fatty acid metabolism	11	9.41E-07
hsa00650	Butanoate metabolism	9	1.89E-05
hsa00100	Steroid biosynthesis	7	2.09E-05
hsa00280	Valine, leucine and isoleucine degradation	10	2.47E-05
hsa00380	Tryptophan metabolism	9	8.40E-05
hsa00330	Arginine and proline metabolism	10	1.16E-04
hsa00900	Terpenoid backbone biosynthesis	6	1.46E-04
hsa00980	Metabolism of xenobiotics by cytochrome P450	10	2.71E-04
hsa00010	Glycolysis / Gluconeogenesis	10	2.71E-04
hsa00982	Drug metabolism	10	3.98E-04
hsa03320	PPAR signaling pathway	10	7.98E-04



Schematic model of Cd activity



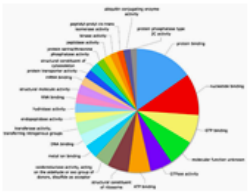
Whole Genome Analysis and MicroRNAs Regulation in HepG2 Cells Exposed to Cadmium

Marco Falcioni[1], Chiara Urani[1*], Maria Grazia Sacco[1], Claudio Provaccionti[2], and Laura Gribaldo[1]*

[1]Institute for Health and Cutaneous Protection, Molecular Biology and Genomics Unit, Joint Research Center, Ispra (VA), Italy; [2]Environmental Sciences Department, University of Milano Bicocca, Milano, Italy

The Three Gene Ontologies

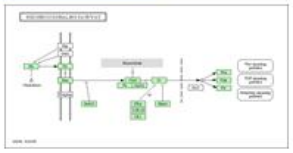
- Molecular function
  - The tasks performed by individual gene products
- Biological process
  - Broad biological goal or objective that are accomplished by ordered assemblies of molecular functions
- Cellular component
  - Subcellular structures, locations, and macromolecular complexes
- A gene product may be part of several different ontologies!



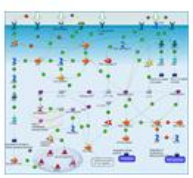
Pathway Analysis

- Discover relationships between the annotated genes

REACTOME



gene INGENUITY SYSTEMS

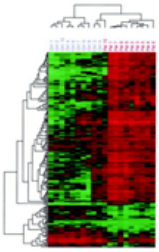


Class discovery

- 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 toxic insult (Huang et al., Toxicol Sci, 2001)

Class Discovery

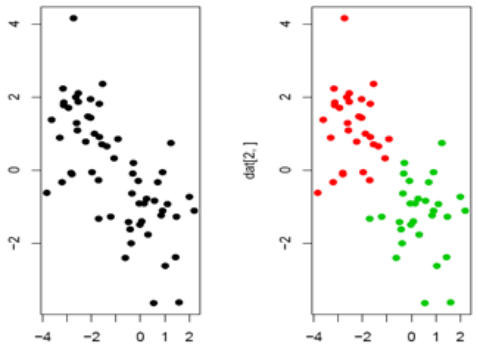
- Objective?
  - Can data tell us which classes are similar?
  - Are there subgroups?
- Methods
  - Cluster analysis
  - K-means,
  - Principal Component Analysis (PCA)
  - Self-organizing maps (SOM)
    - Class Id's are not known to the algorithm
      - For example, does not know which one is cancer or non cancer
      - Do the expression values differentiate, does it discover new classes



Aim of clustering: Group objects according to their similarity

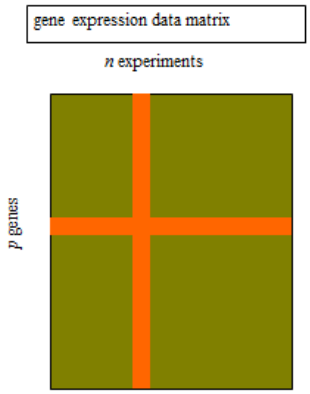
Cluster:
a set of objects that are similar to each other and separated from the other objects.

Example: green/ 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 $\mathbf{R}^p$ or $\mathbf{R}^n$).



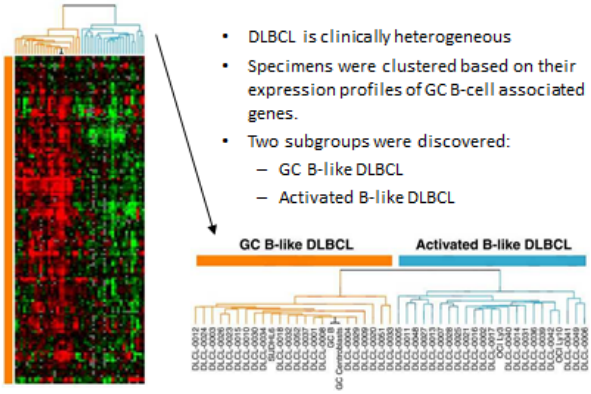
Why cluster genes?

- 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 **broad clinical application**

.

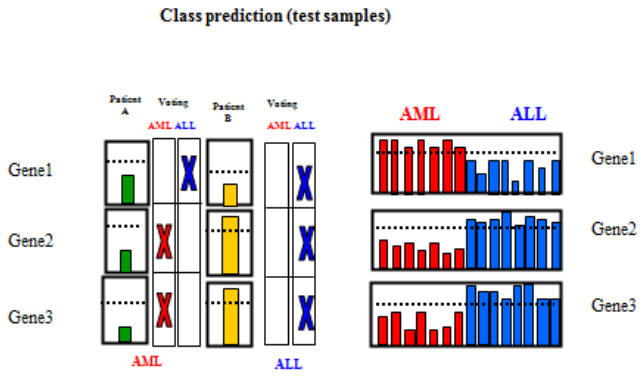
Gene selection

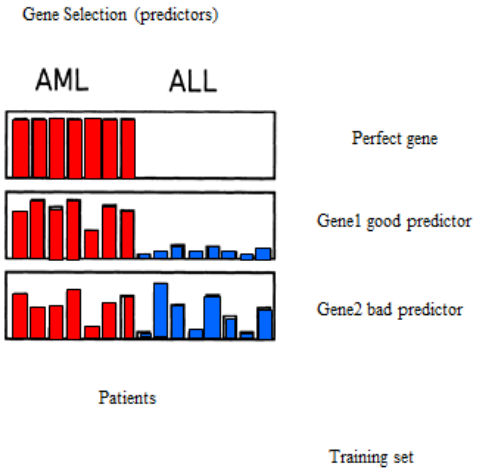
Most classifiers do not use all of the genes whose expression is measured. Consequently, **one step** in developing a classifier is determining which genes to include.

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 ("**noise 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

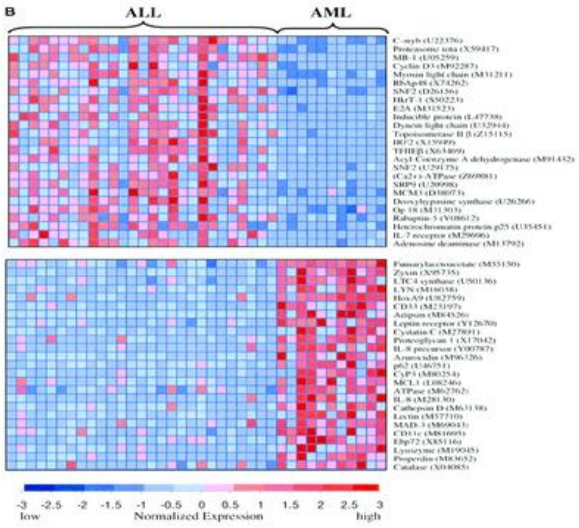




Results

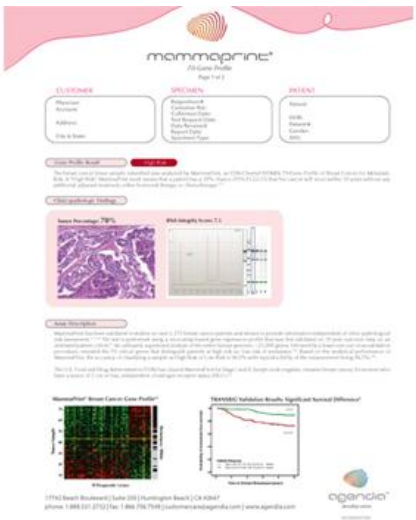
How good are the predictors?

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



European Commission

Description of evaluated studies

Study reference	Cancer type	Clinical endpoint	Sample size	Number of events (%)	Number of channels (type)	Number of genes after filtration*
2	Non-Hodgkin lymphoma	Survival	240	138 (58%)	2 (Lymphochip)	6931
3	Acute lymphocytic leukaemia	Relapse-free survival	233	32 (14%)	1 (Affymetrix)	12 226
4	Breast cancer	5-year metastasis-free survival	97	46 (47%)	2 (Agilent)	4948
5	Lung adenocarcinoma	Survival	86	24 (28%)	1 (Affymetrix)	9132
6,7	Lung adenocarcinoma	4-year survival	621	23 (3·9%)	1 (Affymetrix)	5491
8	Medulloblastoma	Survival	60	21 (35%)	1 (Affymetrix)	6718
9	Hepatocellular carcinoma	1-year recurrence-free survival	60	29 (32%)	1 (Affymetrix)	4851

*For the data of van 't Veer and colleagues,[1] the same filter was used as in the original publication. For other studies, genes with little variation in expression were excluded. †Only patients with clinical follow-up of at least 4 years after surgical resection were analysed.

Table: Description of eligible studies ordered by sample size

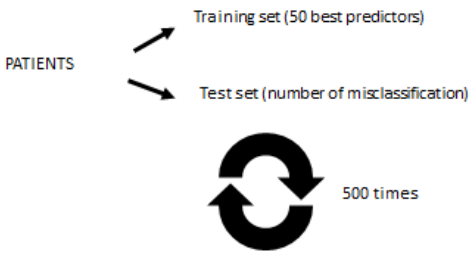
Can we trust those studies?

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

Lancet 2005; 365: 488–92 Stefan Michiels, Serge Koscielny, Catherine Hill

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

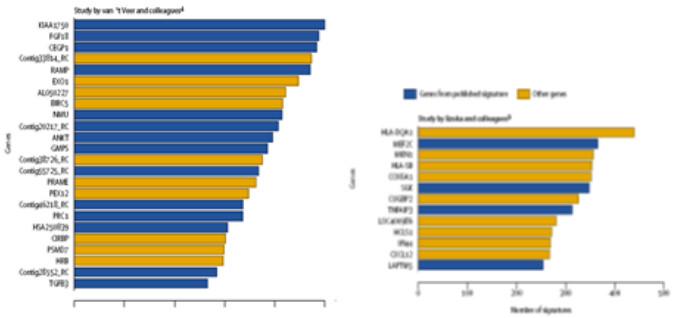
Random validation strategy



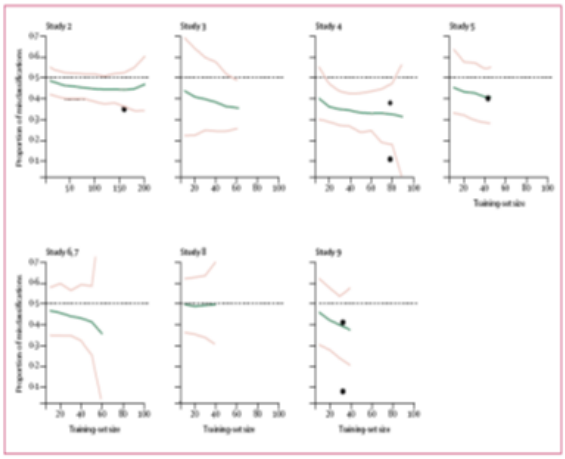
- evaluation of the misclassification rate
- 50 best predictors are selected

(Entire process is repeated with different size of the training set)

Genes included in at least 250 of 500 molecular signatures for two of the studies



Proportion of misclassifications in validation sets as a function of corresponding training-set sizes



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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.

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