Next Generation Sequencing in Diagnosis: do we need a next-generation ethics?

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Genetic testing    Genetic counselling

Education and training
SUMMARY

1. Genomes in the news!
2. NGS in research
3. NGS in health care
4. Genetic testing and NGS
5. Ethical issues and dilemmas of NGS
1. Genomes in the news
"I was curious. Given the swimming pools of booze I've guzzled over the years – not to mention all of the cocaine, morphine, sleeping pills, cough syrup, LSD, Rohypnol...you name it – there's really no plausible medical reason why I should still be alive. Maybe my DNA could say why."

Ozzy Osbourne
NGS

454 Genome Sequencer
FLX System

The SOLiD™ System

Solexa Technology

Ion Torrent
NGS

454 Genome Sequencer FLX System

The SOLiD™ System

Ion Torrent

HiSeq

MiSeq

GS Junior System

Ion Proton

454 Genome Sequencer

FLX System
NGS

Sequencing Cost per Genome: 2001 to 2020

Rapid Progress
Average Progress
Current Progress
NGS - Next generation sequencing

<table>
<thead>
<tr>
<th></th>
<th>Life Technologies Ion Torrent</th>
<th>Roche 454</th>
<th>Illumina Solexa</th>
<th>LT Applied Biosystems SOLiD</th>
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<tr>
<td>Sequencing Chemistry</td>
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<td>Polymerase-based sequence-by-synthesis</td>
<td>Ligation-based sequencing</td>
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<td>Bridge amplification</td>
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*Sample preparation – 6 hours, sequencing time – 2 hours. *Data shown here represent the highest figures currently available on the company website and is highly likely to change by the time this article is published.
Conclusion 1.

NGS IS HERE TO STAY AND IS ALREADY ENTERING THE CLINICAL PRACTICE
2. NGS in research
**Most popular human cell in science gets sequenced**

The HeLa cell genome is riddled with errors, raising questions about its continued use.

**Ewen Callaway**

15 March 2013

The research world’s most famous human cell has had its genome decoded, and it’s a mess. German researchers this week report the genome sequence of the HeLa cell line, which originates from a deadly cervical tumour taken from a patient named Henrietta Lacks.
HeLa cells
HeLa cells

- Cervix cancer, Jan. 1951
- **Cells taken without her knowledge**
- 1st cell line immortalized *in vitro*
- The most used ever
- Initials of “Helen Lane” and “Helen Larson”
- Name of **Henrietta Lacks** disclosed in 1971
The Immortal Life of Henrietta Lacks

New York Times Bestseller

Rebecca Skloot

Doctors took her cells without asking. Those cells never died. They launched a medical revolution and a multimillion-dollar industry. More than twenty years later, her children found out. Their lives would never be the same.

The Immortal Black Woman

Henrietta Lacks

The Black History That’s Not Taught in School

The Mother of Modern Day Genetics

Created by Henrietta Lacks

Love and Adoration From Ted P

The Black History That’s Not Taught in School

The Mother of Modern Day Genetics
More cells in circulation than during life
Many important discoveries:
  • Anti-polio vaccine (Jonas Salk, 50’s)
  • Effects of zero gravity in space
  • Correct human chromosome number
  • >60,000 scientific articles
  • Contribution for ENCODE
  • Pharmacological assays
Multimilionary businesses
Some protests from her family
O genoma das células imortais de Henrietta Lacks foi sequenciado. A família não gostou de não ter sido consultada.

Há mais de 60 anos que a ciência usa as células de uma norte-americana, sem que ela ou a família tivessem dado autorização. A família já tinha ficado revoltada quando descobriu tudo. Agora, estas células relançam a discussão ética sobre os dados genéticos, que um laboratório europeu publicou sem pensar muito nisso – e retirou em seguida do domínio público, até chegar a acordo com a família.
HeLa publication brews bioethical storm

Genome of controversial cell line no longer public, but another sequence is in the works.

Ewen Callaway
27 March 2013

When Lars Steinmetz and his team published the genome of the world’s most famous human cell line earlier this month, they did not imagine that the work would become a bioethical lightning rod. He and his group at the European Molecular Biology Laboratory in Heidelberg, Germany, saw the HeLa cell genome as a helpful resource for their work examining how gene variants influence basic biological functions, and for the countless other scientists studying the same cell line.

But the descendants of Henrietta Lacks — whose cervical tumour gave rise to HeLa cells — saw otherwise, as did other scientists and bioethicists. They have criticized the decision to publish the sequence, noting that the HeLa cell line was established without Lacks’s consent (around the time she died in 1951) and that aspects of what Steinmetz and his team have published may disclose genetic traits borne by surviving family members.

In response, Steinmetz and his team pulled the genomic data from public databases.
The director of the US National Institutes of Health (NIH), Francis Collins met Lacks family members to discuss what should be done with genome data from their matriarch’s cell line.

On 8 August, Kate Hudson and F. Collins announced that the family has endorsed case-by-case release of the information, subject to approval by a committee that will include family members.

**Deal done over HeLa cell line**

**Family of Henrietta Lacks agrees to release genomic data.**

D

Stationary Lacks named Lacks. In 1974, she left behind a glowing obituary in The Baltimore Sun, where she wrote: "She was a woman of great strength and courage, with a kind spirit and a gentle touch. Her legacy will live on through her children, who were loved by all who knew her."

In 1951, Dr. David H. Geisinger, a virologist at Johns Hopkins University, received a sample of HeLa cells from Dr. Alfred Heim. The cells were derived from a cervical cancer sample obtained from a patient named Henrietta Lacks, who was diagnosed with cancer in 1951. The cells were named HeLa, and they rapidly became a staple in laboratories worldwide due to their ability to grow indefinitely in culture. Henrietta Lacks died of cancer later that year.

Many years later, in 1974, the National Institutes of Health (NIH) released the genome sequence of the HeLa cell line, which has become a cornerstone in the field of genomics. However, the decision to release the data was controversial, and the Lacks family has long been concerned about the use of their mother’s cells without their permission.

In 2013, the Lacks family reached an agreement with the NIH to release the genome data for the HeLa cell line, subject to a committee’s approval that will include family members.

The committee will review case-by-case releases of the genomic data, and the family has expressed satisfaction with the decision. The agreement ensures that the family’s concerns about the use of their mother’s cells are addressed, while providing access to important scientific research.
Conclusion 2.

BLURRING OF THE DIFFERENCE BETWEEN RESEARCH AND HEALTH CARE
3. NGS in health care
GWAs

- GWA studies identify **genetic variants** with small effects
- Need to **validate** findings and establish **clinical relevance**

- Insufficient evidence that genomic profiling is useful in measuring individual risks for common diseases, or developing personalised diet or lifestyle recommendations for disease prevention  
  (Janssens et al., Am J Hum Genet 2008; 82: 593-599)

- Only 2 out of 5 genes in ‘osteogenomic profiles’ were significantly associated with some disease, but none with bone disease  
  (Janssens et al., Am J Hum Genet 2008; 82: 593-599)

- This **genetic astrology** regarded as producing no more than entertaining horoscopes;  
  **potential for harm** and need to ensure these tests are evaluated and used appropriately  
  (Melzer et al., BMJ 2008; 336: 590-593)
DTC genetic testing

- direct-to-consumer (DTC) testing:
  - over the counter
  - over the web
- marketing strategies (*bypassing health professionals*)
- override medical indications
- suppress prior information and genetic counselling
- publicity creates *artificial needs* (unsubstantiated claims)
- change patients into consumers
Genetic horoscopes

Cassandra’s complex

“… a prophet of lies, a babbler from door to door?”

[Aeschylus, Agamemnon 1194]
DTC testing
(directly to the consumer)

• DTC often provides health-related information on genetic susceptibilities and recommendations for lifestyle changes

• Concerns include:
  (1) scientific and clinical validity of tests and their clinical utility (transparency of claims)
  (2) no medical indication (family history)
  (3) quality of the testing services
  (4) lack of appropriate genetic counselling (pre-test and interpretation of results)
  (5) appropriate regulatory and policy response
“Genetic astrology”

• at the reach of a sophisticated clairvoyant

• limited value (current state of knowledge / methods)
  • may change in the future
Conclusion 3.

HYPE ON ANALYTICALLY AND CLINICALLY UNVALIDATED GENETIC VARIANTS AND DTC GT
Genetic information: is it really different?

1. "Genetic exceptionalism" - all genetic information is special and needs exceptional means of protection

2. "Genetic inclusivism" - genetic information is not different from other medical information and should not be treated differently
The wide variation of definitions of genetic testing in international recommendations, guidelines and reports

Jorge Sequeiros • Milena Paneque • Bárbara Guimarães • Elina Rantanen • Poupaq Javaher • Irma Nippert • Jörg Schmidtke • Helena Kääriäinen • Ulf Kristoffersson • Jean-Jacques Cassiman

Definitions of genetic testing in European legal documents

Orsolya Varga • Sirpa Soini • Helena Kääriäinen • Jean-Jacques Cassiman • Irmgard Nippert • Wolf Rogowski • Herman Nys • Ulf Kristoffersson • Jörg Schmidtke • Jorge Sequeiros
### Context definitions of human and medical applications of genetic testing, and of clinical genetics testing

<table>
<thead>
<tr>
<th>NON-HUMAN</th>
<th>NON-MEDICAL</th>
<th>MEDICAL APPLICATIONS</th>
<th>BIOMEDICAL RESEARCH</th>
<th>PUBLIC HEALTH GENETICS</th>
<th>CLINICAL GENETICS TESTING</th>
<th>OTHER MEDICAL APPLICATIONS</th>
<th>ART:</th>
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<tr>
<td>Microbiology</td>
<td>Forensic testing (civil, criminal)</td>
<td>Heritable</td>
<td>Basic research</td>
<td>susceptibility testing (complex diseases)</td>
<td>Patient diagnosis: Diagnostic testing (confirmation, exclusion)</td>
<td>Pre-implantation genetic screening (aneuploidy screening)</td>
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<td>Plant</td>
<td>Ancestry testing</td>
<td>Genetic counseling</td>
<td>Clinical research</td>
<td>Genetic counselling: Presymptomatic testing (late-onset Mendelian diseases, non-cancer)</td>
<td>Genetic counselling: Pre-symptomatic testing (late-onset Mendelian diseases, non-cancer)</td>
<td>Pharmacogenetics testing (adrenal drug reactions, drug efficacy)</td>
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</table>

**Modified from:**
### Table 3. Context definitions of “genetics laboratory-based testing” and “genetic information”

**GENETIC INFORMATION**

**Genetics laboratory-based testing:**
- **Cytogenetics tests** (chromosomal-based) – identification of numerical or structural anomalies of individual chromosomes or chromosomal complement
- **Molecular genetics tests** (DNA or RNA-based) – identification at the nucleic acid level of sequence alterations in the DNA molecule and its functional significance, and epigenetic (non-covalent) changes
- **Biochemical genetics tests** (proteins, metabolites) – identification at the protein level of sequence alterations in the DNA molecule and its functional significance

**Other sources of genetic information:**
- Family history
- Personal history and physical examination
- Other laboratory exams (haematological, biochemical, physiological, image, functional) exams

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Two consensus definitions?

(1) Analysis of genetic material

(2) Genetic information

ECS, UM, Braga Postgrad. Course on NGS
Ethical concerns

• counselling of persons at-risk for Huntington disease since the mid 80s

• fears of genetic determinism surrounding the human genome project and the ELSI discussion

• genetic antidiscrimination laws enacted in the USA, in the early 90s
Genetic information

- Personal
  unlike a blood count or glucose
- Familial
- Powerful
  unlike cholesterol, or “pedigree”
- Permanent
- Highly predictive
- Potentially discriminatory

In the case of high penetrance monogenic diseases
Compelling to exorcize genetic exceptionalism

- in these modern times of sophisticated, high-throughput technologies, many policy documents began by clearly dismissing it
- many came to embrace “genetic inclusivism”
- some adding their rationale was that all medical information needs much better protection
Specificity of genetic information

• The diagnosis of a genetic disease may change the risk for relatives (who are not examined or tested and may never want to be!)

• The major aim may be “non-medical” (diagnosis, treatment or prevention of disease), but life decisions (e.g., reproductive choices, education, profession, change life styles)
PKD
polycystic kidney disease

- fluid-filled cysts
- blood in the urine
- edema
- renal failure
- dialysis
- transplant
Not all human genetic information is medical information

- paternity testing
- twin-studies
- civil identification
- criminal investigation

forensic applications

- results from human genetics research

- predictive genetic information for severe diseases with no cure, effective treatment or prevention
Conclusion 4.

BLURRING OF DISTINCTION BETWEEN GENETIC DATABASES AND BIOBANKS
Conclusion 5.

END OF PERSONAL PRIVACY AND CONFIDENTIALITY OF “ANONYMOUS” GENETIC DATA
4. Types of genetic testing
Not all health-related genetic information is equally sensitive

- **diagnostic** genetic information is different from **predictive** information

- not all predictive information is equally sensitive (e.g. pharmacogenetics)

- presymptomatic information for **Huntington disease** is different from predisposition to **hypertension**

*On the other hand,*

- some **non-genetic medical tests** (even a physical exam) may provide **sensitive genetic information**
3. Some genetic information is at least *special* and needs reinforced *protection*, as other sensitive medical information does, mainly in *healthy* persons.
Protection

- Privacy
- Confidenciality
- Informed Consent

- Guidelines for testing and counselling
- Quality assurance

- Data-protection
- Antidiscrimination legislation
  (insurance, employment, adoption)

- **Clinical validity and utility of MGTs**

- Verification
- Sanctions
Genetic testing

Components:

1. Information, consent, preparation
2. Laboratory assay
3. Interpretation, support
Presymptomatic testing for late-onset incurable diseases

- Genetic counselling and psychosocial evaluation before and support after testing!

Candidates for a presymptomatic test register for counselling, not for a mere blood test!
PST Protocol

Psychosocial Assessment
- Duration of contract, kinship, gender of transmitting parent (Anamnesis)
- Anxiety (SAS)
- Depression (BDI)

Social evaluation

Information

1st Blood sample

2nd Blood sample

Written Report

1st Genetic Counselling Session

2nd Genetic Counselling Session

Written Report

1st Psychological follow-up
- Anxiety (SAS)
- Depression (BDI)

2nd Psychological follow-up
- Anxiety (SAS)
- Depression (BDI)

3rd Psychological follow-up
- Anxiety (SAS)
- Depression (BDI)

Neurological Evaluation

Pre-test Sessions

Post-test Sessions
Children and adolescents

- Should not be tested for late-onset, incurable diseases!

- Loss of autonomy
- Discrimination
Susceptibility testing

• Common diseases - complex inheritance, i.e., mostly multifactorial, but also with a monogenic fraction (≈5%)

✓ many genes involved
✓ many non-genetic factors

• but, each with a very limited impact …!
Susceptibility testing

- professional enthusiasm (clinicians, scientists)
- urgent application to human health
- common diseases $\Rightarrow$ commercial pressure

(labs, biotechnology, insurance)
Susceptibility testing

Limitations:

• only small change in relative risk

• population diversity

• analytical and clinical validity

• doubtful benefits for population screening
Types of genetics tests

In the newborn, child, adult:

- **Diagnostic testing**
- Presymptomatic testing (AD)
- Carrier testing (AR, XR)
- Susceptibility testing (multifactorial)
- Pharmacogenetic testing

In the foetus or embryo:

- Prenatal diagnosis
- Pre-implantation diagnosis

In the population or populational group risk:

- Genetic screening
Conclusion 6.

**BLURRING OF THE DIFFERENCE BETWEEN PREDICTIVE AND DIAGNOSTIC TESTING**
5. Ethical issues and dilemmas with NGS
Applications of NGS

1. Whole-genome sequencing

2. Whole-exome sequencing

3. Panels of genes (heterogenous disorders)
1. NGS – ethical issues about testing for health care purposes

(1) Sequence all genome/exome (if cheaper than testing half a dozen of genes or even a single gene)?
   • answer only the clinical question?
   • what to do with all that information?
   • keep rest of info. for future, if further requests?
   • destroy and repeat the WGS (once it is cheap)?

(2) Do only gene panels for known diseases (filtering the required information)?
2. NGS – ethical issues about reporting results in health care

(1) What should/can be reported and what must not to requesting physicians?
- only disease-causing variants?
- variants of unknown clinical significance?
- normal variants?

(2) What to communicate to a patient/healthy person?
- everything found?
- incl. susceptibility variants of low predictive value?
- incl. variants with no or still undetermined clinical validity and utility?
- all pathogenic variants of current or future clinical relevance?
- only mutations for diseases with treatment or preventive measures?
- information about children?

(3) What process of informed consent *(opt-in, opt-out)*?
“Incidental findings”

• Once you perform a WGS/WES, you will \textit{necessarily} have “incidental” findings

• We should talk instead of \textit{unsolicited or unrequested findings}
Conclusion 7.

**CLINICAL APPLICATIONS OF NGS STILL A MATTER OF HOT ETHICAL DEBATE**
No choice for you
28/03/2013
Categories: ELSI
Written by: Caroline Wright and Guest Author

Guest Co-Author: Dr Anna Middleton is an Ethics Researcher and Registered Genetic Counsellor, based at the Welcome Trust Sanger Institute, UK.

The American College of Medical Genetics (ACMG) has recently published recommendations for reporting incidental findings (IFs) in clinical exome and genome sequencing. These advocate actively searching for a set of specific IFs unrelated to the condition under study. For example, a two year old child may have her (and her parents') exome sequenced to explore a diagnosis for intellectual disability and at the same time will be tested for a series of cancer and cardiac genetic variants. The ACMG feel it is unethical not to look for a series of incidental conditions while the genome is being interrogated, conditions that the patient or their family may be able to take steps to prevent. This flies in the face of multiple international guidelines that advise against testing children for adult onset conditions. The ACMG justify this as "a fiduciary duty to prevent harm by warning patients and their families". They conclude that "this principle supersedes concerns about autonomy", i.e. the duty of the clinician to perform opportunistic screening outweighs the patients right not to know about other genetic conditions and their right to be able to make autonomous decisions about testing.
ACMG Recommendations for Reporting of Incidental Findings in Clinical Exome and Genome Sequencing

Robert C. Green, MD, MPH1,2, Jonathan S. Berg, MD, PhD3, Wayne W. Grody, MD, PhD4,6, Sarah S. Kalia, ScM, CGC5, Bruce R. Korf, MD, PhD7, Christa L. Martin, PhD, FACMG8, Amy McGuire, JD, PhD9, Robert L. Nussbaum, MD10, Julienne M. O’Daniel, MS, CGC11, Kelly E. Ormond, MS, CGC12, Heidi L. Rehm, PhD, FACMG12,13, Michael S. Watson, MS, PhD, FACMG14, Marc S. Williams, MD, FACMG15, Leslie G. Biesecker, MD16

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ABSTRACT

• In clinical exome/genome sequencing, there is potential for the recognition and reporting of incidental or secondary findings unrelated to the indication for ordering the sequencing but of medical value for patient care.

• We recommend that laboratories performing clinical sequencing seek and report mutations of the specified classes or types in the genes listed.

• ...irrespective of age, but excluding fetal samples.

• We recognize that there are insufficient data on clinical utility to fully support these recommendations.
Primary Finding

This term is used to describe pathogenic alterations in a gene or genes that are relevant to the diagnostic indication for which the sequencing was ordered (e.g., a mutation in MECP2 in a girl with loss of developmental milestones).
Incidental Finding

This term has been used in a variety of clinical and research contexts to indicate unexpected positive findings. Other terms have been used to describe these findings, particularly when they are sought after (rather than being unexpectedly discovered). These terms include “serendipitous and iatrogenic” findings, “non-incidental secondary findings” “unanticipated findings”, and “off-target results”. We use “incidental findings” in this paper to indicate the results of a deliberate search for pathogenic or likely pathogenic alterations in genes that are not apparently relevant to a diagnostic indication for which the sequencing test was ordered.
RECOMMENDATIONS

1. Constitutional mutations found in the genes on the minimum list (see Table) should be reported by the laboratory, regardless of the indication for which the clinical sequencing was ordered.
   
a. Additional genes may be analyzed for incidental (secondary) variants, as deemed appropriate by the laboratory.

b. Incidental (secondary) variants should be reported regardless of the age of the patient.

c. Incidental (secondary) variants should be reported for any clinical sequencing conducted on a constitutional (but not tumor) tissue. This includes the normal sample of a tumor-normal sequenced dyad and unaffected members of a family trio.
RECOMMENDATIONS

2. The Working Group recommends that laboratories seek and report only the types of variants within these genes that we have delineated (see Table).

   a. For most genes, only variants that have been previously reported and are a recognized cause of the disorder or variants that are previously unreported but are of the type which is expected to cause the disorder, as defined by prior ACMG guidelines, should be reported.

   b. For some genes, predicted loss of function variants are not relevant (e.g., COL3A1 and most hypertrophic cardiomyopathy genes).

   c. For some genes (e.g., APOB), laboratories should only report variants for certain conditions.
RECOMMENDATIONS

3. It is the responsibility of the ordering clinician/team to provide comprehensive pre- and posttest counseling to the patient.
   a. Clinicians should be familiar with the basic attributes and limitations of clinical sequencing.
   b. Clinicians should alert patients to the possibility that clinical sequencing may generate incidental findings that could require further evaluation.
   c. Given the complexity of genomic information, the clinical geneticist should be consulted at the appropriate time that may include ordering, interpreting, and communicating genomic testing.
<table>
<thead>
<tr>
<th>Phenotype</th>
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<th>PMID - GeneReviews Entry</th>
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Jorge Sequeiros
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DISCLOSURES

LGB, JSB, WWG, CLM, AM, RLM, KEO, and HLR have grants related to genome sequencing.
LGB receives in kind research support from the Illumina Corp.
JSB and HLR are uncompensated members of Advisory Board members for Complete Genomics.
BRK, HLR, and MSWi are involved with clinical laboratories offering genome sequencing services.
AM, RLN, and JMO own stock in genome sequencing companies.
RLN provides compensated consulting to Complete Genomics.
JOD was employed by Illumina Corp. during the development of these recommendations.
Whole-genome sequencing in health care: recommendations of the European Society of Human Genetics

Carla G van El1, Martina C Cornel1,2,3, Pascal Borry4, Ros J Hastings5, Florence Fellmann6, Shirley V Hodgson7, Heidi C Howard8,9, Anne Cambon-Thomsen8,9, Bartha M Knoppers10, Hanne Meijers-Heijboer11, Hans Scheffer12, Lisbeth Tranebjærg13,14,15, Wybo Dondorp16,17, Guido MWR de Wert3,16,17 and ESHG Public and Professional Policy Committee

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website from 20 June to 1 August 2012 for comment by the membership. The final version was approved by the ESHG Board in December 2012.

CONSIDERATIONS
The changing landscape of diagnostic genetic testing in health care
Until recently, a diagnostic genetic test tended to focus on one specific question. In the case of a clinical suspicion of a monogenic condition, DNA analysis of one or a few specific genes was performed, whereas in cytogenetics, the whole genome was analysed at a relatively low resolution of 5–10Mb to answer a defined clinical question. Increasingly, however, diagnostic tests now look at a large panel of genes (eg, genes implicated in cardiovascular events) via microarrays, a relatively targeted approach. In addition, non-targeted high-resolution next-generation sequencing techniques may be applied, detecting mutations throughout the genome. Whole-genome- or exome sequencing (WGS, WES) generates an enormous amount of raw data requiring complex bioinformatic analyses to extract useful information. Depend-
Recommendations

- WGA requires a justification in terms of necessity (the need to solve a clinical problem) and proportionality (the balance of benefits and drawbacks for the patient).

- In the clinical setting, preferable to use a targeted approach first, to avoid unsolicited findings or findings that cannot be interpreted (limit the analysis to specific sets of genes).

- Known genetic variants with limited or no clinical utility should be filtered out (if possible, neither analyzed nor reported).
Additional Protocol concerning Genetic Testing for Health Purposes

- genetic tests for health purposes should only be carried out once their clinical utility has been proven, and under individualised medical supervision

7 May 2008
Recommendations

• Protocol should be in place for **guidance on the reporting of unsolicited findings**

• **Guidelines for informed consent** regarding diagnostic testing need be developed

• Clinicians should be aware and safeguard the patient’s position in the **potential crossover with research**
Recommendations

• In case of testing minors, guidelines are needed as to what unsolicited information should be disclosed, to balance autonomy and interests of the child and parental rights and needs for information that may be in the interest of the family.

• International collaboration to build sustainable databases on genotypic and phenotypic information of variants.

• Genetic education of health-care professionals.

• Inform the public and raise public awareness, enhance genetic literacy in patients and the lay public will help to involve wider society in this debate.
Amb l'entrada de força castellers nous i d'una canalla ben preparada ,...
A “next generation” bioethics?
effective, timely and responsible translation for the benefit of society!
“Henrietta Lacks”

Teresa Ricou