Handy Resources for Genetics

Textbooks

Smith's Recognizable Patterns of Human Malformation

Available in the library

Recommended for Pediatric residents as the photos tend to crop up on exams

Management of Genetic Syndromes

Borrow from one of us – primarily helpful for Geneticists

Handbook of Physical Measurements

Useful for all those odd things we check – borrow PRN

Web-based

OMIM http://www.ncbi.nlm.nih.gov/sites/entrez?db=omim&TabCmd=Limits

*Good for searching out potential genetic causes for anomalies

*Results not always pertinent to clinical questions

GeneTests http://www.ncbi.nlm.nih.gov/sites/GeneTests/?db=GeneTests

*Great source of relatively update information and management guidelines (GeneReviews)

*Provides list of all testing laboratories for specific disorders

*Compiled links under Resources allows single handout for patients/families

*Check out the Illustrated Glossary!

Unique http://www.rarechromo.org/html/home.asp

*The only place to find one-of-a-kind chromosomal disorders

*Printable booklets with private & published information on select disorders

Orphanet http://www.orpha.net/consor/cgi-bin/index.php

*Good source of literature on really rare disorders

Genetic Alliance - http://www.geneticalliance.org/publications

*Great for patients and non-Genetic providers regarding understanding genetics

Univ of Florida http://www.peds.ufl.edu/divisions/genetics/teaching-resources.htm

*A great learning resource about so many things Genetic

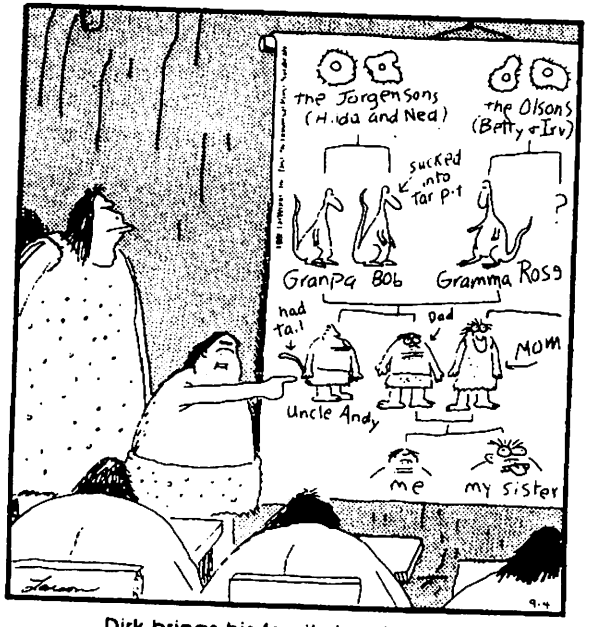
(Thanks to Mo Alom, PL-3 for pointing us to this site)

Other resources: http://www.genome.gov/10000464

3

Getting to the Roots: Recording the Family Tree

A complete pedigree is often a work of great labour, and in its finished form is frequently a work of art.
—Karl Pearson, 1912 (from Resta, 1993)



Dirk brings his family tree to class

CREATING A MEDICAL PEDIGREE: GETTING STARTED

The basic medical pedigree is a graphic depiction of how family members are biologically and legally related to one another, from one generation to the next. Each family member is represented by a square (male), or a circle (female), and they are connected to each other by relationship lines. This family "map" is meaningless if the symbols cannot be interpreted from clinician to clinician. The symbols outlined here are from the 1995 recommendations of the National Society of Genetic Counselors Pedigree Standardization Task Force (Bennett et al., 1995). For personal use, you may photocopy Appendix A.1 for a handy, double-sided "cheat sheet" containing common pedigree symbols, the basic information to include on a pedigree, and a prototype imaginary pedigree using all the standardized symbols. The pedigree icons in and of themselves provide scant information; the power of a pedigree lies within the associative network of symbols.

Generally, the pedigree is taken in a face-to-face interview with the patient, before the physical examination. A patient is usually more comfortable sharing the intimate details of his or her personal and family life while fully clothed rather than wearing one of the highly fashionable rear-exposure gowns that are available in most examination rooms.

I find it useful to take a preliminary pedigree on the telephone. Many patients have limited knowledge of the health of their extended relatives. By asking medical-family history questions in advance of the appointment, the patient can do the homework of contacting the appropriate family members to get more accurate details. The patient can also help to arrange to obtain medical records (see Chapter 6). At the appointment, the pedigree that was taken by telephone can be verified with the patient. Medical-family history questionnaires can be a useful tool to collect pertinent information before the patient's appointment. However, a questionnaire should not substitute for an actual pedigree. Sample genetic family history questionnaires for cancer and a child being placed for adoption are included in Appendices A.3 and A.4, respectively.

The *consultand* is the individual seeking genetic counseling and/or testing . This person is identified on the pedigree by an arrow, so that he or she can be easily identified when referring to the pedigree. If more than one person (consultands) come to the appointment (for example, a parent and child, or two sisters), identify each person with an arrow on the pedigree. The consultand can be a healthy person or a person with a medical condition.

The *proband* is the *affected* individual that brings the family to medical attention (Marazita, 1995). Identifying the proband is important in genetic mapping studies and research. Some researchers use the term propositus (plural is propositi) interchangeably with proband(s). The *index case* is a term used in genetic research to describe the first affected person to be studied in the family. Sometimes an individual is both a proband and the consultand.

Even with the use of standardized pedigree symbols, a *key* or *legend* is essential for any pedigree. The main purpose of the key is to define the shading (or hatching) of symbols that indicate who is affected on the pedigree. The key is also used to ex-

TABLE 3.1 Essential Information to Record on Family Members in a Pedigree

Age, birth date, or year of birth
Age at death (year if known)
Cause of death
Full sibs versus half sibs
Relevant health information (e.g., height, weight)
Age at diagnosis
Affected/unaffected status (define shading of symbol in key/legend)
Personally evaluated or medically documented (*)
Testing status ("E" is used for evaluation on pedigree and defined in key/legend)
Pregnancies with gestational age noted LMP or EDD (estimated date of delivery)
Pregnancy complications with gestational age noted (e.g., 6 wk, 34 wk): miscarriage (SAB), stillbirth (SB), pregnancy termination (TOP), ectopic (ECT)
Infertility vs. no children by choice
Ethnic background for each grandparent
Use a "?" if family history is unknown/unavailable
Consanguinity (note degree of relationship if not implicit in pedigree)
Family names (if appropriate)
Date pedigree taken or updated
Name of person who took pedigree, and credentials (M.D., R.N., M.S., C.G.C.)
Key/legend

plain any infrequently used symbols (such as adoption or artificial insemination) or uncommon abbreviations.

Table 3.1 serves as a quick reference to the information essential to record on a pedigree. Remember to document on the pedigree your name and credentials (such as R.N., M.D., M.S.), and the name of the consultand (the person who has the appointment). It is also helpful to record the name of the *historian* (the person giving the information). For example, a foster or adoptive parent may not have access to accurate history about the biological family of the child. Remember to date the pedigree. This is particularly important if ages rather than birth dates are recorded for family members on the pedigree. Was the pedigree taken yesterday or 10 years ago?

Use abbreviations sparingly and define them in the key. For example, CP may be short for cleft palate or cerebral palsy; MVA may mean motor vehicle accident or multiple vascular accidents; SB may be interpreted as stillbirth, spina bifida, or even shortness of breath!

Because the pedigree is part of the patient's medical record, it should be drawn with permanent ink. Using a black pen is best because blue ink may be faint if the record is microfilmed. It is acceptable to draft a pedigree in pencil; just be wary of errors in transcription. My favorite pedigree drawing tool is a correction pen that quickly obliterates my frequent drawing errors.

Draw the pedigree on your institution's medical progress notepaper (if available). A sample pedigree form is included for your use in Appendix A.2. A standardized pedigree form has the advantage that you can include common pedigree symbols as a reference on the form. This fill-in-the-blanks approach serves as a reminder to

document easily overlooked family history information (such as family ethnicity and whether or not there is consanguinity). These forms are limited in that pedigrees of large families may be difficult to squeeze onto the page.

Plastic drawing templates of various-sized circles, squares, triangles, diamonds, and arrows, are helpful for keeping the pedigree symbols neat and of uniform size. Such templates are available at most art and office supply stores.

Computer software programs for drawing pedigrees are reviewed in Appendix A.5: The Genetics Library. Most of the currently available programs are not practical for drawing a quick pedigree in a clinical setting. These drawing programs are efficacious for large research pedigrees, patient registries, or in preparing a pedigree for professional publication.

Laying The Foundation—Pedigree Line Definitions

Pedigrees can become quite complicated when they include multiple generations. Add to this the common occurrence of a person having children with multiple partners, and a pedigree soon looks like a television-wiring diagram! There are four main "line definitions" which form the "trunk" and "branches" of a medical family tree (Fig. 3.1). Here are some simple rules to remember:

- A *relationship line* is a horizontal line between two partners; a slash or break in this line documents a separation or divorce.
- If possible, the male partner should be to the left of the female partner. (See Chapter 8, Fig. 8.1 for how to draw relationships between same-sex partners.)
- A couple who is *consanguineous* (meaning they are biological relatives such as cousins) should be connected by a *double* relationship line (Figs. 3.10 and 3.11).

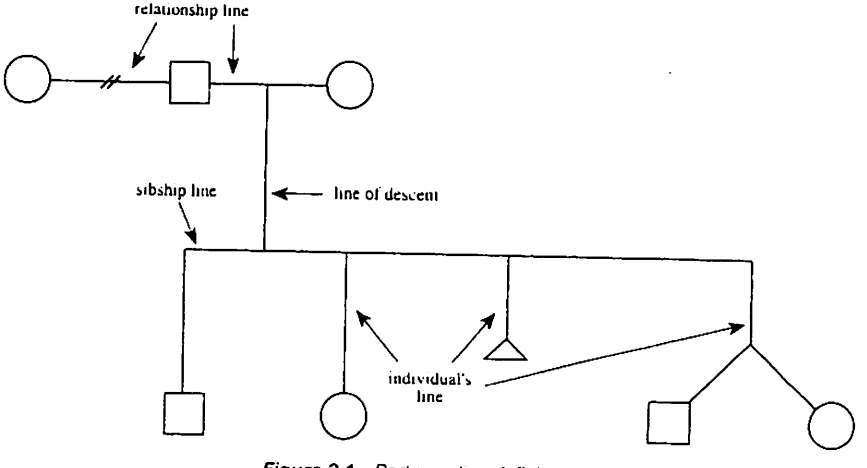


Figure 3.1 Pedigree line definitions.

- The *sibship line* is a horizontal line connecting brothers and sisters ("sibs").
- Each sibling has a vertical *individual's line* attached to the horizontal sibship line.
- *Twins* (fraternal/dizygotic or identical/monozygotic) share one vertical individual's line, attached to the horizontal sibship line.
- For pregnancies not carried to term, the individual's line is shorter (Fig. 3.5).
- The *line of descent* is a vertical bridge connecting the horizontal sibship line to the horizontal relationship line.

Remembering the applications of these line definitions is important in the pedigree symbolization of adoption (Fig. 3.7), and in symbolizing assisted reproductive technologies or ART (see Chapter 8 and Fig. 8.1).

Keeping Track of Who is Who on the Pedigree

Begin by asking the consultand (or a parent if the consultand is a child):

"Do you have a partner, or are you married?"

"How many biological brothers and sisters do you have?"

"How many children do you have?"

The answers to these questions give you an idea of how much room you will need on the paper to draw the pedigree.

Always ask if siblings share the same mother and father—people often do not distinguish an adopted sibling or a stepsibling from biological kin. Remind the historian that you are also interested in information about deceased relatives. For example, an adult may forget to tell you about a sibling who died as an infant 25 years ago.

If you are interviewing an elderly person, you may be taking a five-generation pedigree (e.g., grandparents, parents, aunts and uncles, cousins, siblings, nieces and nephews, children and grandchildren). If you begin your pedigree in the middle of the page, it is easy to extend your pedigree "up" and "down." If the consultand is a child, or a pregnant mother, usually it is easier to start the pedigree toward the bottom of the page and extend your pedigree "up" toward the top of the paper as you inquire about prior generations. Large pedigrees are often easier to record with the paper in a "landscape" or "lengthwise" orientation, versus a "portrait" or "up-and-down" orientation.

When possible, draw siblings in birth order. Recording the ages (or years of birth) of the siblings is an obvious way of showing when they are *not* represented on the pedigree in birth order. It is not necessary to draw each partner or spouse of a sibling on the pedigree, particularly if the siblings do not have children. It may be important to record the partner if an offspring has a significant medical history. This is particularly important when a family history for a common medical disorder, such as cancer, is identified.

Each generation in a pedigree should be on the same horizontal plane. For example, a person's siblings and cousins are drawn on the same horizontal axis; the parents, aunts, and uncles are drawn on the same horizontal line. In pedigrees used for publication or research, usually each generation is defined by a Roman numeral (e.g., I, II, III), and each person in the generation is given an Arabic number, from left to right (e.g., I-3, I-4, II-3). This makes it easy to refer to family members in the pedigree by number, and thus protects family confidentiality (Fig. 3.2). In clinical pedigrees, names are usually recorded on the pedigree (parallel or next to the individual's line). The family surname is placed above the sibship line, or above the relationship line. Of course, if names are recorded on the pedigree, care must be taken to preserve the confidentiality of the pedigree.

How Many Generations Are Included In A Pedigree?

A basic pedigree usually includes three generations—the consultand's *first-degree relatives* (parents, children, siblings) and *second-degree relatives* (half siblings, grandparents, aunts and uncles, grandchildren). *Third-degree relatives*, particularly cousins, are often included, if only to note that they "exist." For example, one can place a diamond with a "3" inside to show that an aunt or uncle has three children. Figure 3.3 shows the pedigree framework for denoting a relative's relationship to the proband (for example, a first cousin is a third-degree relative to the proband).

If a health problem of significance is identified, the pedigree is extended back as far as possible (Refer to Table 4.1 for clues to family history features suggestive of a genetic disorder). For example, if a 60-year-old woman with breast cancer is interested in genetic risk assessment for her two daughters, you would ask her about any cancer in her parents, grandparents, uncles and aunts, cousins, children, and grandchildren. You would inquire about great-aunts and great-uncles and great-grandpar-

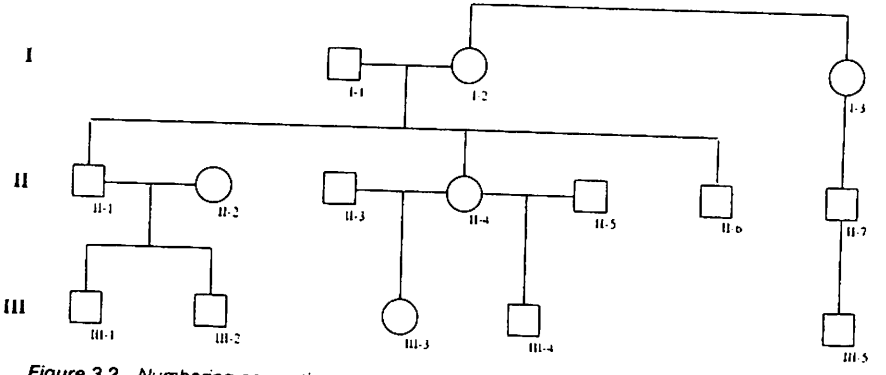


Figure 3.2 Numbering generations and individuals on a pedigree. This numbering system allows for easy reference to individuals on a pedigree when names are not recorded.

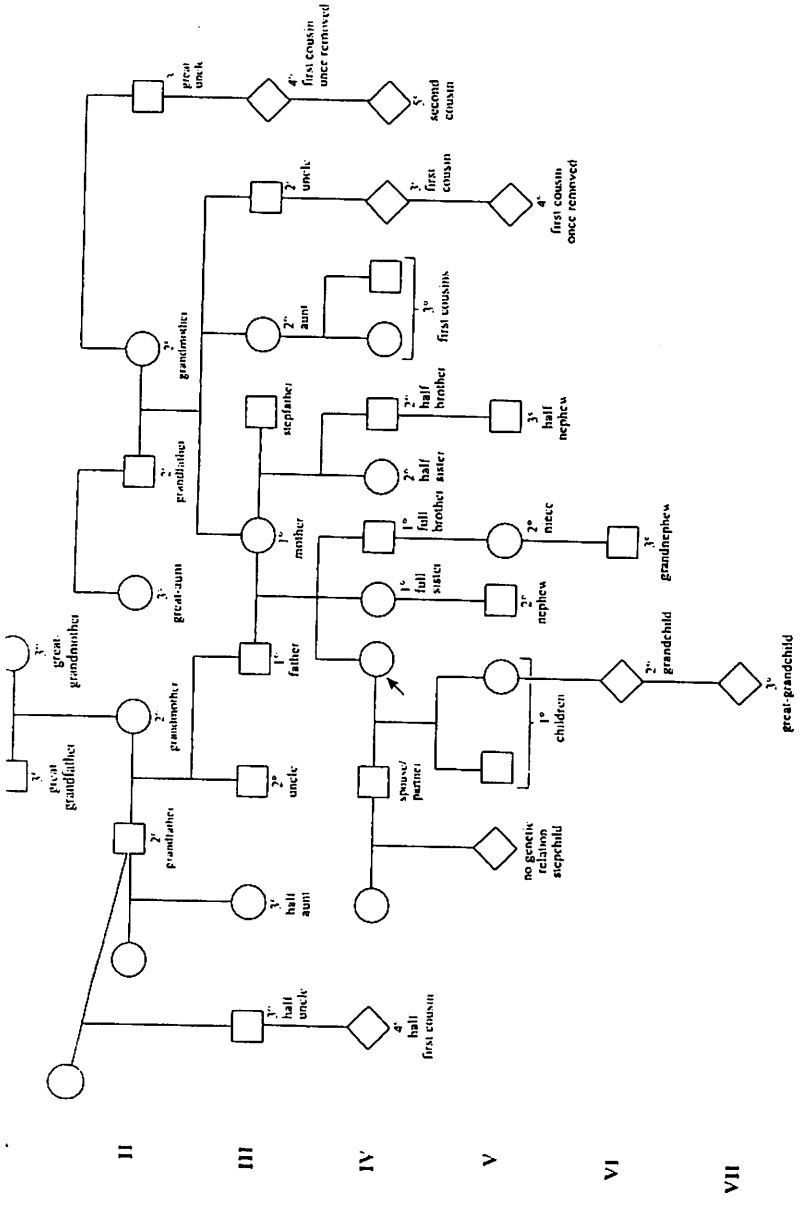


Figure 3.3 The pedigree framework for denoting a relative's relationship to the proband (i.e., first-degree, second-degree, and third-degree relatives).

ents if a positive family history were found. The pedigree for a genetic condition with a late age of symptom onset may be quite extensive.

THE BASIC PEDIGREE SYMBOLS

The most common pedigree symbols are shown in Figure 3.4. The gender of an individual is assigned by the outward phenotype. This is important when drawing a pedigree for a child with ambiguous genitalia. The age (or date of birth) is noted for each individual. Record the cause of death and age at death for all individuals on the pedigree. Noting the year of death is useful because this can provide you with clues as to the diagnostic tools available during that medical era. For example, DNA diagnostic testing did not exist before the mid-1980s. The identification of a structural brain anomaly may have been made with the aid of a pneumoencephalogram in the 1960s and 1970s, compared to the modern brain imaging techniques of computerized tomography (CT scans) or magnetic resonance imaging (MRI).

Relevant health information, such as height (h.) and weight (w.), is placed below the pedigree symbol. The recommended order of this information is as follows: (1) age, birth date, or year of birth; (2) age at death and cause; (3) relevant health information; and (4) pedigree number.

It is the rare historian who knows the precise details of such information as current ages, ages at death, and the heights of his or her extended family. A tilde (~) can be used when approximations are given.

Pedigree Symbols Related to Pregnancy and Reproduction

The various pedigree symbols related to pregnancy, spontaneous abortion, termination of pregnancy, stillbirth, and infertility are shown in Figure 3.5. Always include the gestational age, in weeks (wk), if known, below the symbol. An approximation of dates can be shown, such as "~12 wk." Usually the gestational age is stated as the date of the last menstrual period (LMP) or the estimated date of confinement (EDC). Some clinicians prefer using EDD (estimated date of delivery) because the description "confinement" seems archaic. You can note pregnancy dating by ultrasound (US) as "US 12 wk."

A stillbirth (SB) is defined as the "birth of a dead child with gestational age noted" (Bennett et al., 1995).

If the sex of the fetus is known, one can note male or female under the appropriate symbol. This is preferable to making the symbol a square or circle. If a chromosome study has confirmed the sex of the fetus, this can be noted under the symbol as "46,XX" or "46,XY."

Yours, Mine, and Ours—The Blended Family

Correct documentation of how individuals are biologically related to each other is essential for accurate pedigree assessment. It is almost inevitable when taking a

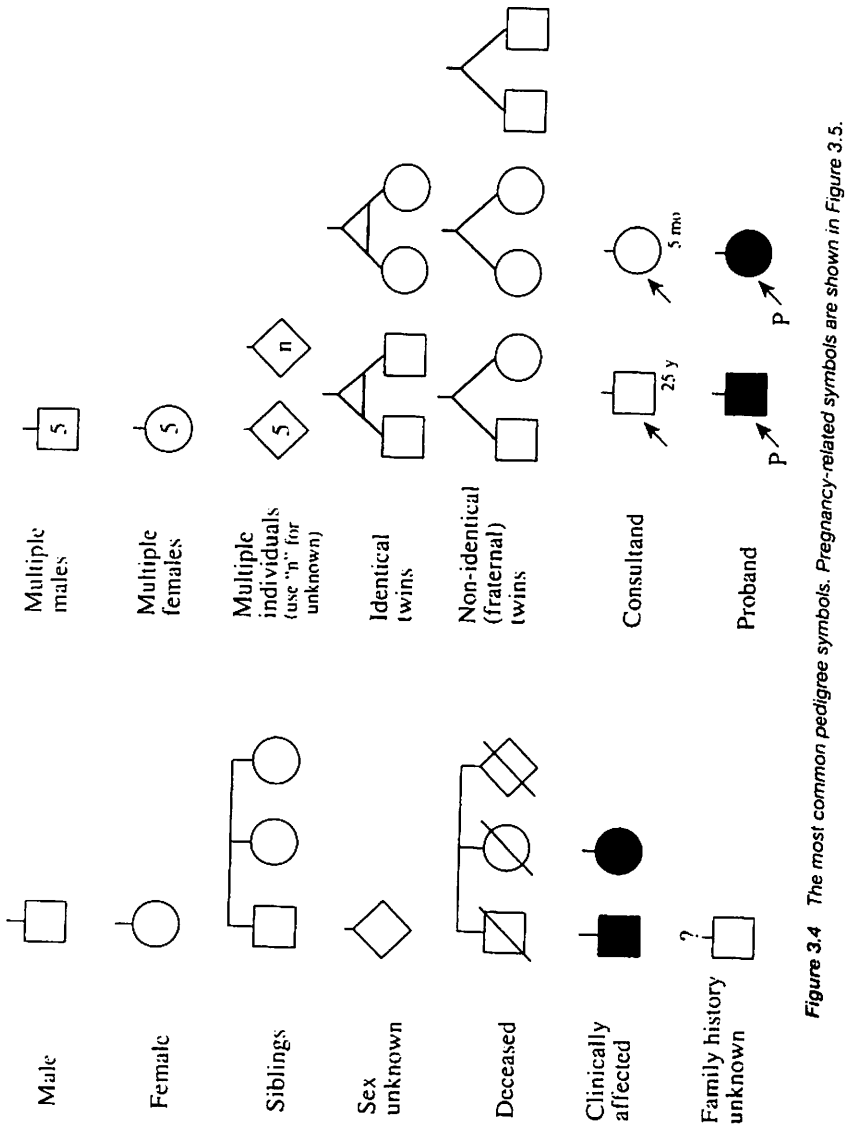


Figure 3.4 The most common pedigree symbols. Pregnancy-related symbols are shown in Figure 3.5.

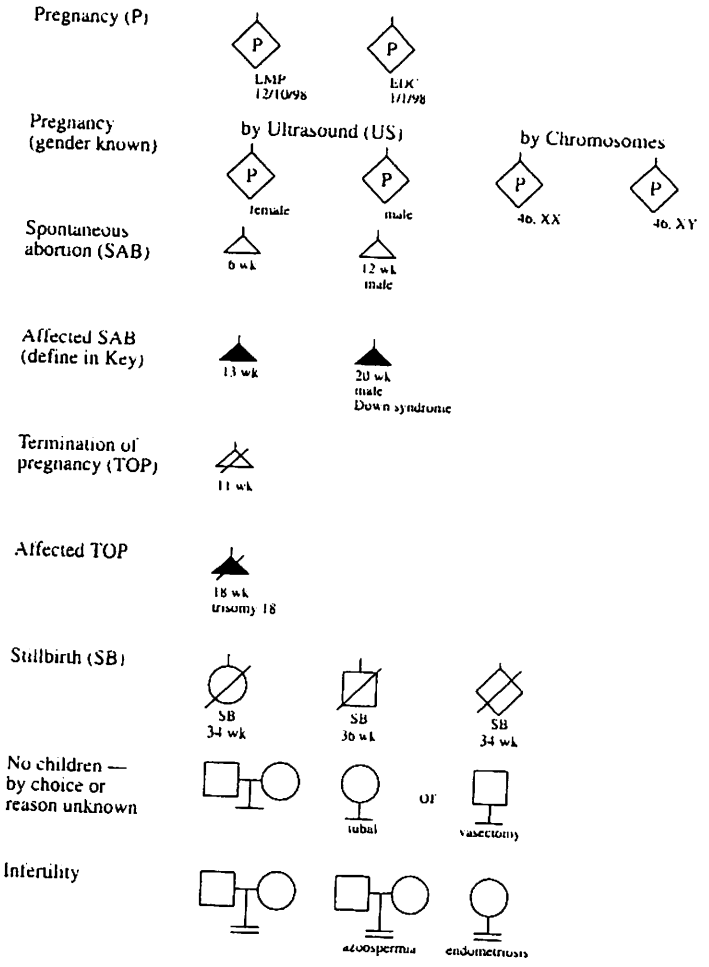


Figure 3.5 Pedigree symbols related to pregnancy.

family history that at least one person in the family will have more than one partner. For each sibling group in the pedigree, ask if they share the same mother and father. If the answer is "No," ask "Which of your brothers and sisters share the same mother, and which share the same father?"

If there is a big gap in ages between siblings, this is a clue that they may be half siblings. The gap may also be an indication of a period of infertility.

It is not necessary to include each partner of an individual on a pedigree, as is illustrated by actress Elizabeth Taylor's marriage history in Figure 3.6.

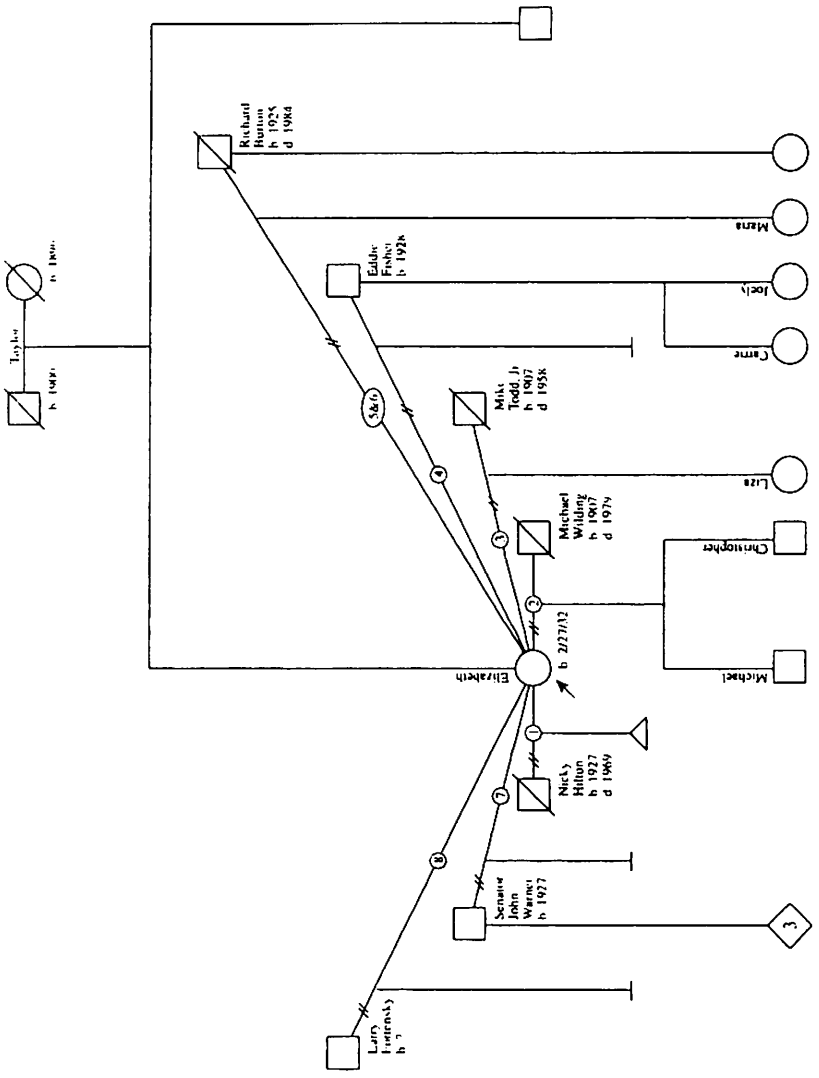


Figure 3.6 A pedigree of actress Elizabeth Taylor demonstrating how to illustrate multiple marriage partners, stepchildren, and half siblings. (Source: www.celebsite.com)

Adoption

It is important to distinguish a person who is adopted "in" to a family (nonbiological relative) from a person who is adopted "out" (a biological relative). For any adoption, brackets are placed on either side of the appropriate symbol for male, female, or sex-unknown (a diamond). If a person or couple adopts the person "in," the individual's line is dotted (indicating a nonbiological relationship). A person who is placed for adoption by birth parents has a solid individual's line.

It is not uncommon for a family member to adopt a relative (for example, a sibling adopting a niece or nephew, or grandparents adopting a grandchild). The method of recording this situation on a pedigree is shown in Figure 3.7.

When a person or couple adopts a child, it is useful to inquire if this was for a medical reason (such as an illness, a known genetic disease, or infertility in the adoptive parents). The reason can be noted below the line of descent.

When a child is placed for adoption, information about the child's medical her-

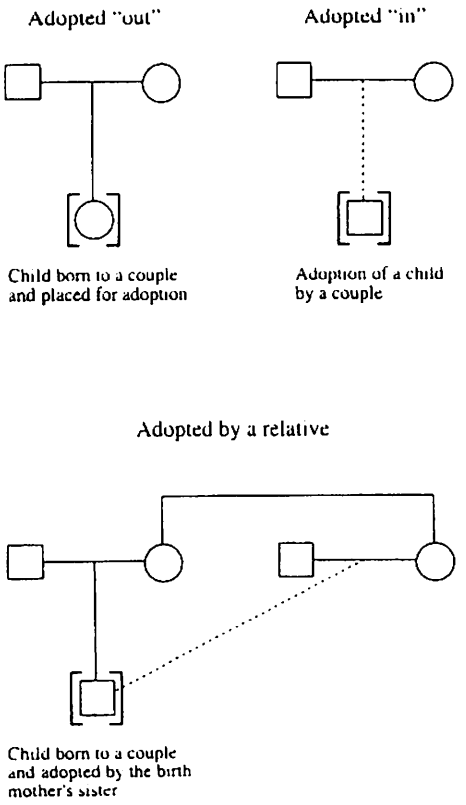


Figure 3.7 Pedigree symbolization of adoption.

itage is usually given to the new parents (See Chapter 7). A sample adoptive medical-family history form can be found in Appendix A.4.

Infertility Versus No Children By Choice

If a person (or couple) is of reproductive age and does not have children, inquire if this is by choice or for a biological reason. The cause of infertility should be noted below the individual's line (e.g., azoospermia, endometriosis) (Fig. 3.5).

Affected Status: Shading the Pedigree Symbols

Accurately documenting who is affected and unaffected on a pedigree is critical for pedigree analysis. A symbol should *only* be shaded in if the person (or pregnancy, miscarriage, etc.) is *clinically symptomatic* with the condition. Of course, this may be a function of the clinical "tool" that is used to define affected status. For example, the 2-year-old sister of a 5-year-old boy with classic symptoms of cystic fibrosis may have no detectable symptoms, yet have elevated sweat chloride levels.

Most families will have more than one genetic, or potentially heritable, medical condition. Different shading can be used to define the different diseases on the pedigree. For example, when documenting a family history of cancer, shading in the various quadrants of each square (male) or circle (female) is a way to symbolize multiple cancers (Fig. 3.8).

A&W

While seeing this abbreviation may cause you to salivate for your favorite root beer beverage, this is actually a simple method of showing on the pedigree that a family member is "alive and well." By noting "A&W" under each healthy person on the pedigree, you can be assured that you asked about the health of each member on the pedigree.

"He Died of A Broken Heart"—Family Hearsay

Family members often use nonmedical terms to describe illnesses in a family. If Uncle Billy "died of a broken heart," the clinician should not automatically assume that the person died of a heart attack. The family lore can be included on the pedigree in "quotes," using the words the historian used to describe the ailment. Asking questions such as, "Was this a long-standing illness or was the death unexpected?" might help to clarify the nature of the family member's illness.

Family History Unknown

Like the rogue in the 1970s Paul Simon tune, "Fifty Ways to Leave Your Lover," sometimes little information is known about a parent, grandparent, or other family member. Placing a question mark above the pedigree symbol shows that you in-

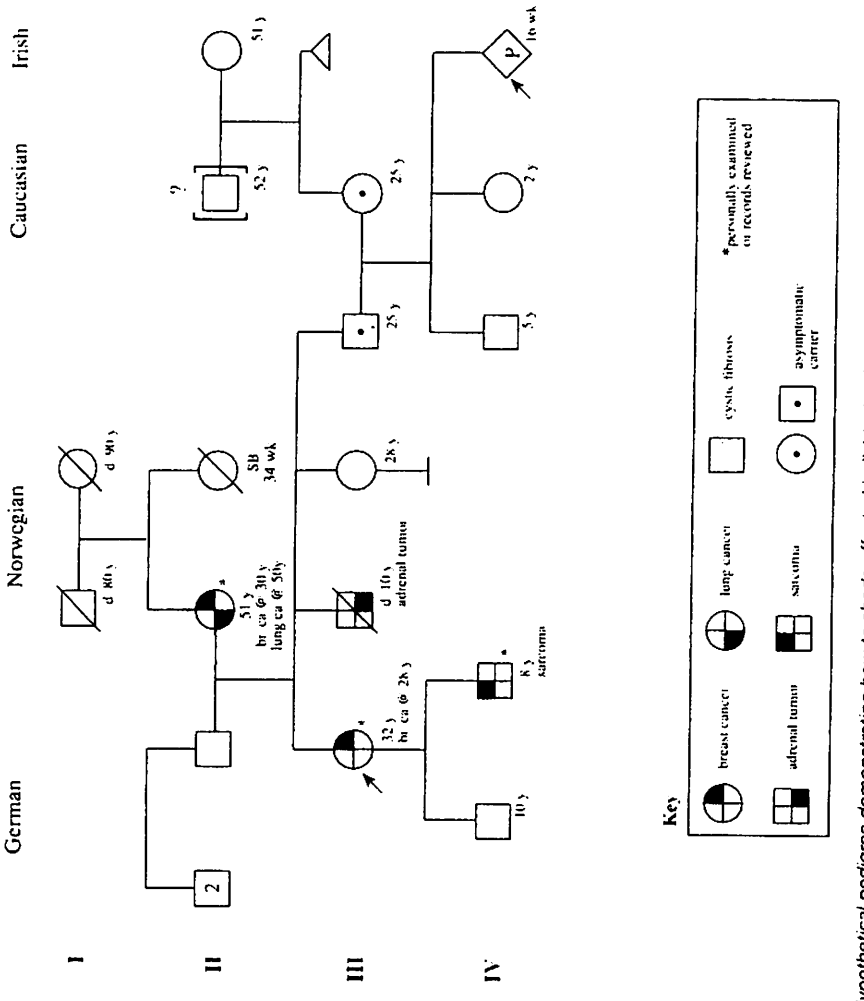


Figure 3.8 Hypothetical pedigree demonstrating how to shade affected individuals when more than one condition is segregating in a family.

quired about that individual's family history, but the information is unknown or unavailable (Fig. 3.4).

DOCUMENTING MEDICAL EXAMINATIONS AND EVALUATIONS

Genetic counseling requires accurate medical information. An asterisk (*) is placed near the lower right edge of the pedigree symbol for anyone who has been personally examined by you (or another team member), or if you have verified medical records (Fig. 3.9). See Chapter 6 for specific information on how to help families obtain medical records on family members.

Medical evaluations on family members can be recorded on the pedigree. Each clinical evaluation or test is represented by an "E" and defined in the key. An evaluation may represent information obtained by clinical examination, medical testing (e.g., brain imaging, nerve conduction studies), or molecular genetic test results. If a test or examination result is abnormal it is considered a "+" result, and a normal result is documented as "–." If the test is uninformative, a "u" follows the "E." For example, there are hundreds of mutations in the cystic fibrosis gene. A child with known cystic fibrosis may have only one mutation identified. This could be noted on the pedigree as ΔF508/u (Fig. 3.9).

A Note on Genetic Testing

Genetic testing confuses many patients. Some patients falsely assume any genetic test is a chromosome test. Others assume that all genetic tests involve direct analysis of the DNA (versus a linkage study or enzyme analysis). Patients also mistakenly think that when they have a DNA test, their DNA is screened for all genetic diseases. It is always important to obtain documentation of the patient's or family member's actual laboratory result.

The Healthy Person with An Abnormal Genetic Test Result: The Difference between a Presymptomatic or Asymptomatic Carrier and an Obligate Carrier

Advances in genetic testing now allow us to test a healthy person for a genetic condition that they may or will develop in the future. Many geneticists reserve the description *presymptomatic carrier* for a healthy person who is likely to develop a genetic disease in his or her lifetime (Bennett et al, 1995). For example, a healthy person at risk for Huntington disease who has a CAG expansion of more than 40 repeats is likely to have symptoms of HD if he or she reaches the age of 70 (Brinkman et al., 1997). In contrast, a woman who has a mutation in a breast cancer gene has a higher lifetime risk to develop breast and possibly other cancers, but the development of cancer is *not* inevitable. This is usually referred to as *predisposition* or *susceptibility genetic testing*. The description *asymptomatic carrier* is sometimes used for a person who carries a susceptibility or predisposition mutation. Many geneti-

Instructions:
— Evaluation (E) is used to represent clinical and/or test information on the pedigree.
  a. E is to be defined in key/legend.
  b. If more than one evaluation, use subscript (E_1, E_2, E_3) and define in key. May be written side by side or below each other depending on available space.
  c. Test results should be put in parentheses or defined in key/legend.
  d. If results of exam/family study/testing not documented or unavailable, may use a question mark (e.g., E?).
— Documented evaluation (*)
  a. Asterisk is placed next to lower right edge of symbol.
  b. Use ***only*** if examined/evaluated by **you** or ***your*** research/clinical team or if the outside evaluation has been personally reviewed and verified.
— A symbol is shaded only when an individual is clinically symptomatic.
— For linkage studies, haplotype information is written below the individual. The haplotype of interest should be on left and appropriately highlighted.
— Repetitive sequences, trinucleotides and expansion numbers are written with affected allele first and placed in parentheses.
— If mutation known, identify and place in parentheses.
— Recommended order of information:
  1) age/date of birth or age at death
  2) evaluation information
  3) pedigree number (e.g., I-1, I-2, I-3)

Definition	Symbol	Scenario	Example
1. Documented evaluation (*)	*	Woman with normal physical exam and negative fragile X chromosome study (normal phenotype and negative test result).	E- *
2. Obligate carrier (will **not** manifest disease).	*	Woman with normal physical exam and premutation for fragile X (normal phenotype and positive test result).	E+(100n/35n) *
3. Asymptomatic/presymptomatic carrier (clinically unaffected at this time but could later exhibit symptoms)		Man age 25 with normal physical exam and positive DNA test for Huntington disease (symbol filled in if/when symptoms develop).	25 * E+(45n/18n)
4. Uninformative study (u)	E	Man age 25 with normal physical exam and uninformative DNA test for Huntington disease (E_1) and negative brain MRI study (E_2).	25 * E_1u(36n/18n) E_2-
5. Affected individual with positive evaluation (E+)	E	Individual with cystic fibrosis and positive mutation study, although only one mutation has currently been identified.	E+(F508) E * E+(F508/u)
		18 week male fetus with abnormalities on ultrasound and a trisomy 18 karyotype.	P 18 wk * E+(tri 18)

Figure 3.9 *How to document results of medical evaluations and genetic testing on a pedigree (including presymptomatic testing and obligate carrier status) (reprinted with permission from Bennett et al., 1995, University of Chicago Press).*

cists take exception to the use of the terminology "predictive testing" because no genetic test provides an absolute predictive gaze into a person's medical future.

A person who "carries" a gene mutation but will *not* develop clinical symptoms is referred to as an *obligate carrier* (Bennett et al., 1995). For example, the parents of children affected with a classic autosomal recessive disorder are obligate carriers. A healthy mother of two boys with an X-linked recessive condition (such as Duchenne muscular dystrophy) is an obligate carrier.

Figure 3.9 demonstrates how to document genetic test results, and asymptomatic/presymptomatic and obligate carrier status. Individuals who are obligate carriers are represented on the pedigree with a dot in the middle of the male (square) or female (circle) symbol. Persons who are asymptomatic or presymptomatic carriers are represented with a line down the middle of the pedigree symbol. If the person later develops the disease, the symbol is shaded.

PEDIGREE ETIQUETTE

The Skeletons in the Closet

For many reasons, people tend to keep genetic information private. There is often a sense of stigmatization, even embarrassment, about the "bad blood" or "curse" in the family. An episode from the popular television series *Northern Exposure* described a person with a potential genetic disorder as a "genetic Chernobyl" (Nelkin, 1998). As Francis Galton observed, "Most men and women shrink from having their hereditary worth recorded. There may be family diseases of which they hardly dare to speak, except on rare occasions, and then in whispered hints, or obscure phrases, as though timidity of utterance could hush thoughts . . ." (Resta, 1995). People may also be reluctant to share information because of fear they will be "blamed" for the "family imperfections."

Choose Your Words Wisely

When you take a medical family history, you are inquiring about the very essence of an individual. You are asking not only about the individual's personal health, but also about their intimate relationships and the health of family members (with whom they may have little contact). Before you begin taking a genetic family history, it is helpful to warn the client: "I need to ask you some personal questions about the health of people in your family. Your answers to these questions are an important part of providing you with appropriate medical care."

The clinician should be careful not to perpetuate feelings of guilt and stigmatization. Use words such as "altered" or "changed" to describe genes, instead of "mutated" or "bad." Emphasize to the patient that people have no choice in the genetic conditions that are "passed" in a family; the disease is nobody's fault.

Be sensitive to terms like an "uneventful pregnancy." Although a healthy pregnancy may be uneventful to the clinician, it is very eventful to the proud parents! I often hear clinicians refer to a family history with no apparent genetic problems as a "negative" family history, as compared to a "positive" family history if a genetic condition exists. A "positive" family history is usually very "negative" for the patient (Fisher, 1996)! I usually describe health problems in the family history as being "contributory" or "noncontributory" to the medical problem in question.

Acknowledge Significant Life Events

Common courtesy should be the rule in taking a family history. If a woman tells you that she recently miscarried, or that her mother died of breast cancer a few months ago, it is appropriate to acknowledge this with "I am sorry to hear of your loss" or "This must be a difficult time for you." Conversely, the news of a recent birth, marriage, or desired pregnancy can be greeted with "Congratulations."

Beware the Leading Question

If you say, "So, your brothers and sisters are healthy, right? No problems in your parents?" you will most likely receive a reply of "Un-huh," regardless of whether or not this is a true statement. Instead, try to be specific with your questioning by asking, "Do your brothers and sisters have any health problems?"

Use Common Language

You are more likely to be successful in obtaining an accurate family history if you use terms that are familiar to people. Rather than asking about myopathies in the family, inquire if individuals have muscle weakness, or if anyone uses a cane or a wheelchair?

Be Sensitive to Cultural Issues

If your patient does not speak English, get an interpreter. Do not rely on a family member to provide interpretation. The family member may be tempted to interject his or her opinions, particularly about "family matters," as part of the translation!

Culture consists of shared patterns, knowledge, meaning, and behaviors of a social group (Fisher, 1996). Individuals have different customs and beliefs based on their race, socioeconomic status, sex, religious beliefs, sexual orientation, education, or health status. When taking a family history, it is important to acknowledge belief systems that are different from one's own. For example, a traditional Latino woman may believe that her child's cleft lip and palate are the result of supernatural forces during a lunar eclipse (Cohen et al, 1998). Individuals from a traditional Southeast Asian culture may have strong belief in karma and fate. Persons from certain religious and cultural groups may believe bad thoughts or sins cause a birth defect or genetic disorder (Cohen et al., 1998). References to "bad" things in genetic counseling may disturb the "good aura" of the family or ancestors (Fisher and Lew, 1996).

An individual's belief system is likely to influence the type of health information he or she shares with the health care provider. A vivid example of this is a Hopi woman with severely disabling congenital kyphoscoliosis who was described by her sister as being small and having pain in her legs and back that kept her from her normal activities. The woman was not portrayed as disabled because she had high status in the community due to her ability to make piki, a thin wafer bread (Hauck and Knoki-Wilson, 1996).

Two excellent references on providing health care for diverse population groups are *Cultural and Ethnic Diversity: A Guide for Genetics Professionals*, edited by Nancy Fisher, and *Developing Cross-Cultural Competence: A Guide For Working with Young Children and Their Families*, by Eleanor Lynch and Marci Hanson.

RECORDING A BASIC PEDIGREE: THE QUESTIONS TO ASK

Obtaining an extended medical-family history is really no different from obtaining the medical history from an individual. I usually inform the consultand, "I will now ask you questions about you and your extended family. I am interested in your family members who are both living and dead." Then I ask general questions, reviewing medical systems "from head to toe." If a "positive history" is found, I ask directed questions based on that "system," and the genetic diseases that are associated with it. For additional directed family history questions focused on a positive family history for several common medical conditions (e.g., mental retardation, hearing loss), see Chapter 4.

Medical-Family History Queries by Systems Review

Head, Face, and Neck

Begin by asking, "Does anyone have anything unusual about the way he or she looks?" If yes, have the historian describe the unusual facial features. In particular, inquire about unusual placement or shape of the eyes and ears.

Anyone with an unusually large or small head?

Are there problems with vision, blindness, cataracts, or glaucoma? If so, inquire as to the age the problems began, the severity, and any treatment.

Anyone with unusual eye coloring? For example, eyes that are different colors, or whites of the eyes that are blue?

Do any family members have cleft lip, with or without cleft palate?

Anyone with unusual problems with his or her teeth (e.g., missing, extra, misshapen, fragile, early teeth loss)?

Any problems with hearing or speech?

Anyone with a short or webbed neck?

Anything unusual about the hair (e.g., coarse, fine, early balding, white patch)?

Skeletal System

Is any family member unusually tall or short? (If so, record the heights of the individuals, the parents, and siblings.)

If someone is short, is he or she in proportion or not?

Anyone with curvature of the spine? If so, did this require surgery or bracing?

Anyone with multiple fractures? If yes, inquire as to how many fractures, how the breaks occurred, the bones that were broken, and the age the fractures occurred.

Anyone with an unusual shape to his or her chest?

Anyone with unusually formed bones?

Anyone with unusually long or short fingers or toes? Anyone with extra or missing fingers or toes, or an unusual shape to the hands or feet? Have the historian describe these anomalies.

Anyone with joint problems such that they are unusually stiff or flexible, or dislocate frequently?

Skin

Anyone with unusual lumps, bumps, or birthmarks? If so, have the patient describe them, their location, their coloration, and number. "Were these skin changes ever biopsied or treated?"

Any problems with excessive bruising, or problems with healing or scarring?

Anyone with unusual problems with fingernails or toenails, such as absent nails or growths under the nails?

Respiratory System

Any family members with any lung diseases? If so, were they smokers? Were they treated for the lung condition, and how?

Cardiac System

Anyone with heart disease? If so, at what age, and how were they treated?

Was anyone born with a heart defect? If so, did they have birth defects or mental retardation?

Anyone with heart murmurs?

Anyone with high blood pressure?

Were there any heart surgeries? If so, what was done?

Gastrointestinal System

Anyone with stomach or intestinal tract problems? If so, were they treated for the problem, and how?

Renal System

Anyone with kidney disease? If so, were they treated for the problem, and how? Any problems with alcohol?

Hematologic System

Anyone with bleeding, clotting, or healing problems?

Have any family members been told they are anemic?

Have there been family members who needed transfusions?

Endocrine

Anyone with thyroid problems? Anyone with diabetes? Anyone who is overly heavy or thin?

Immune System

Anyone with frequent infections or hospitalizations, or difficulties healing?

Reproduction

Have any relatives had miscarriages or babies who died, severe pregnancy complications, or infertility?

Neurological/Neuromuscular

Anyone with muscle weakness, or with problems with walking? Do any family members use a cane or wheelchair? If there are muscle problems, inquire as to the age at which the problems began and what type of testing was done, such as a muscle biopsy, nerve conduction velocity, or brain imaging.

Anyone with strokes or seizures? If so, at what age did they begin, and what medications were given?

Anyone with uncontrolled movements, tics, difficulties with coordination, or spasticity? If so, at what age did they begin? Were medications given?

Anyone with slurred speech?

Mental Functioning

Anyone in the family with mental retardation or severe learning problems? Did anyone attend special classes or school, or need help to finish school? If yes, describe the level of functioning and any dysmorphic features. Was the mother taking any drugs, alcohol or medications during the pregnancy; or was she ill?

Are there any family members with problems with thinking or judgment, mental illness, or severe depression? (If so, have the patient describe the relative's symptoms, the age symptoms began, and any known medications.)

General Interview Questions

Occupation Asking about a patient's occupation helps develop rapport. It also may be a clue to a potential environmental exposure that is contributing to a disease.

Birth Defects I also ask near the end of the interview if any family members had children with birth defects, particularly of the heart, spine, hands, or feet.

Drug and Alcohol Abuse Knowing about drug and alcohol abuse in the patient and other family members is important for many reasons. If there are abnormal ultrasound findings in a pregnancy, or a child has birth defects with or without mental retardation, there could be a maternal teratogenic etiology for the problems. The known and suspected human teratogens are list in Table 3.2. Neurological problems can also be related to, or exacerbated by, drug and alcohol use.

There are three specific areas to note when inquiring about possible maternal teratogens:

1. What is the drug or medicine? For medications, ask the patient to bring her prescription to the appointment.
2. When in pregnancy did you take the medication?
3. How much did you take?

When inquiring about a patient's drug and alcohol use, remember that people invariably *underestimate* usage. Do not ask, "Are you a heavy drinker?" Your patient will not want to be judged, and will probably reply "No." Instead ask, "How much alcohol do (did) you use?" or "What drugs do (did) you take?"

Cancer I usually specifically ask about cancers in the family, because this information may not be volunteered in the medical systems review. Inquire about any family members with cancer, the types of primary cancers, the age of onset, and treatments if known (such as a mastectomy, colectomy, or chemotherapy). Also ask about potential environmental exposures (such as smoking) or occupational exposures. The details of inquiring about specific familial cancer syndromes are outlined in Chapter 5.

TABLE 3.2 Human Teratogens: Proven, Possible, and Unlikely

Known Teratogens

Radiation
- Atomic weapons
- Radioiodine
- Therapeutic radiation

Maternal infections
- Cytomegalovirus (CMV)
- Herpes simplex virus I and II
- Parvovirus B-19
- Rubella virus
- Syphilis
- Toxoplasmosis
- Varicella virus
- Venezuelan equine encephalitis

Maternal and metabolic factors
- Alcoholism
- Early amniocentesis (before day 70 post-conception)
- Chorionic villus sampling (before day 60 post-conception)
- Cretinism, endemic
- Diabetes
- Folic acid deficiency
- Hyperthermia
- Phenylketonuria
- Rheumatic disease
- Sjogren's syndrome
- Virilizing tumors

Drugs and environmental chemicals
- Excessive alcohol
- Aminopterin
- Androgenic hormones
- Busulfan
- Captopril
- Chlorobiphenyls
- Cocaine
- Coumarin anticoagulants
- Cyclophosphamide
- Diethylstilbestrol
- Enalapril
- Etretinate
- Iodides
- Isotretinoin (Accutane®)
- Lithium
- Mercury, organic
- Methotrexate (methylaminopterin)
- Methylene blue (via intramniotic injection)
- Misoprostol
- Methimazole
- Penicillamine
- Phenytoin (Hydantoin)
- Tetracyclines
- Thalidomide
- Toluene (abuse)
- Trimethadione
- Valproic acid

Possible Teratogens

- Binge alcohol use
- Carbamazepine
- Cigarette smoking
- Colchicine
- Disulfiram
- Ergotamine
- Fluconazole (high doses)
- Lead
- Primidone
- Quinine (suicidal doses)
- Streptomycin
- Vitamin A (high doses)
- Zinc deficiency

Unlikely Teratogens

- Agent Orange
- Anesthetics
- Aspartame
- Aspirin[a]
- Bendectin
- Hydroxyprogesterone
- LSD
- Marijuana
- Medroxyprogesterone
- Metronidazole
- Oral contraceptives
- Progesterone
- Rubella vaccine
- Spermicides
- Video display terminals and electromagnetic waves
- Ultrasound

[a]Aspirin use may increase cerebral hemorrhage during delivery if used in the second half of pregnancy.
Source: Adapted from Cohen, 1997; Shepard, 1996.

Ethnicity Inquiring about ethnicity is one of the last questions to ask when taking a pedigree. Certain genetic conditions, particularly autosomal recessive disorders, are more common in certain ethnic groups (see Table 3.3 and Chapter 2). For some genetic disorders, the sensitivity of DNA testing depends on the person's ethnicity. The ethnicity should be recorded for all four of the grandparents of the consultand. Usually this information is placed at the top of the pedigree, at the head of each lineage. If ethnicity is unknown, you can draw a question mark or write "unknown" at the top of the lineage.

Patients may wonder why you are inquiring about their ethnic background. They may fear that you have singled out their ethnic group for genetic screening or testing. I usually say, "Information about the origins of your ancestors can help us offer you the most appropriate genetic information and testing. Do you know your country of origin, where your ancestors were originally from?" You may get a reply of "South Dakota or Nebraska", to which I reply, "Can you be more specific? For instance, were your ancestors Black, Spanish, Native American, or European?." If someone is Native American, it is helpful to ask the tribal background. Likewise, knowing the village name from small countries is useful.

Because more and more genetic tests are becoming available to people of Ashke-

Table 3.3 Genetic Disorders That Have a High Incidence in Certain Ethnic Groups

Population Group	*Condition*	*Inheritance Pattern*[a]	*Approximate Prevalence*
African (Central)	Sickle cell anemia	AR	1/50
Ashkenazi Jewish	Gaucher type I disease	AR	1/1000
	Cystic fibrosis	AR	1/3300
	Tay–Sachs disease	AR	1/3600
	Familial dysautonomia	AR	1/3700
	Canavan disease	AR	1/6400
Caucasian	Phenyketonuria (PKU)	AR	1/20,000
	Cystic fibrosis	AR	1/3300
French Canadian (Saguenay-Lac Saint-Jean)	Tyrosinemia	AR	5.4/10,000
South African (white)	Porphyria variegata	AD	3/1000
Yupik Eskimo	Congenital adrenal hyperplasia	AR	1/500
Turkish	PKU	AR	3.8/10,000
Yemenite Jewish	PKU	AR	1.9/10,000
Ojibway Indian (Canada)	Glutaric aciduria I	AR	1/2000
Hopi Native American	Oculoctaneous albinism II	AR	1/227
Pueblo Native American	Cystic fibrosis	AR	1/3970
Zuni Native American	Cystic fibrosis	AR	1/1580
Swedish	Glutaric aciduria I	AR	3.3/100,000
Mennonite (Pennsylvania)	Maple syrup urine disease	AR	56.8/10,000
Amish (Eastern Pennsylvania)	Ellis van Creveld	AR	1/700
	Glutaric aciduria I	AR	1/400
Finnish	Congenital nephrotic syndrome	AR	1/8000

[a]AR—autosomal recessive, AD—autosomal dominant.
Sources: Clarke, 1996; NIH Consensus Statement, 1997; OMIM, 1998; Scriver et al., 1995.

nazi Jewish heritage, I specifically ask, "Is anyone in your family of Jewish heritage?"

Never assume ethnicity by dress, skin color, or language. I learned this the hard way once when I requested a Spanish interpreter for a non-English speaking client. When a Japanese gentleman arrived from interpreter services, I said, "Oh, you must be in the wrong clinic." He politely informed me that I was the one who was misinformed.

It's All Relative: Consanguinity

One of the final pedigree history questions is: "Has anyone in your family ever had a child with a relative? For example, were your parents or grandparents related as cousins?" Often you are greeted with a nervous laugh, and an answer of "not that we know of." In the United States, people are very sensitive to questions about unions between relatives. Marriage between first cousins is legal in about half of the states in America. In some parts of the world, particularly the Middle East, close to half the population is married to a cousin or more distant relative.

People are often confused by the terms describing kinship. For example, a second cousin is commonly confused with a first cousin once removed (Fig. 3.10). It is important to work "back" through the family history to document exactly how the couple is related. For example, the clinician might say, "Let me see if I have this straight, your father and your wife's mother are brother and sister, therefore you are first cousins." If the degree of relationship is not implicit from the pedigree, it should be stated above the relationship line, as shown in the pedigree of the prestigious Darwin and Wedgwood families (Fig. 3.11).

Individuals from different branches of the family, with the same last name, may

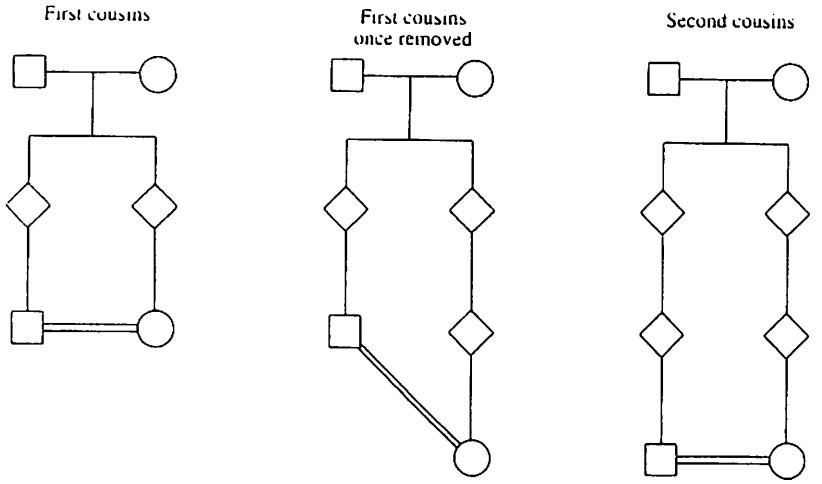


Figure 3.10 *Symbolization of first cousins, first cousins once removed, and second cousins.*

be distantly related. Ancestors from the same small tribes or villages may also be consanguineous.

Documenting that you have inquired about consanguinity should be noted on every pedigree. The information "consanguinity denied," or "consanguinity as shown" can be incorporated into the key, or written near the top of the pedigree.

The Closing Questions

Before I finish the interview, I always end with these questions: "Aside from the information that you have given me, is there anything that people say 'runs' in your family? Do you have any other questions about something that you are concerned may be genetic or inherited in your family? Is there anything that I have not asked you about, that you feel is important for me to know?" Patients always seem to save their burning questions for the last few minutes of their appointment! Perhaps it takes them some time to warm up to the interview. Maybe they are embarrassed about something in their family history. Or possibly nervousness just causes temporary memory loss. Regardless of the reason, always provide the consultand with a final opportunity to think about additional concerns in his or her medical-family history.

The Family Photo Album

If a person in the family is described as having unusual facial or other physical features, have your patient raid the family photo album. Pictures from a wedding or family reunion are an easy way to see if people resemble each other. Such pictures are also a way to determine if an individual is unusually tall or short for the family. Summer pictures of a beach outing show more "flesh," if you are trying to investigate unusual skeletal features or skin findings. Looking at chronological pictures of the same individual over a several year period can be useful; some syndromes may be difficult to identify in childhood, or may be less obvious in an adult than in a child.

WHAT'S REMARKABLE ABOUT AN UNREMARKABLE FAMILY HISTORY?

Many times when you take a family history, there are no obvious genetic diseases or familial aggregation of disease. Table 3.4 reviews some common reasons that a family history of a genetic condition is missed. Clinicians often note in the medical record that the family history is "negative" or the person has "no family history." I prefer stating the family history is unremarkable or noncontributory. The word "negative" may be misconstrued as a value judgment. All humans have a family history, though the medical–family history may not affect the current indication for seeing a health professional.

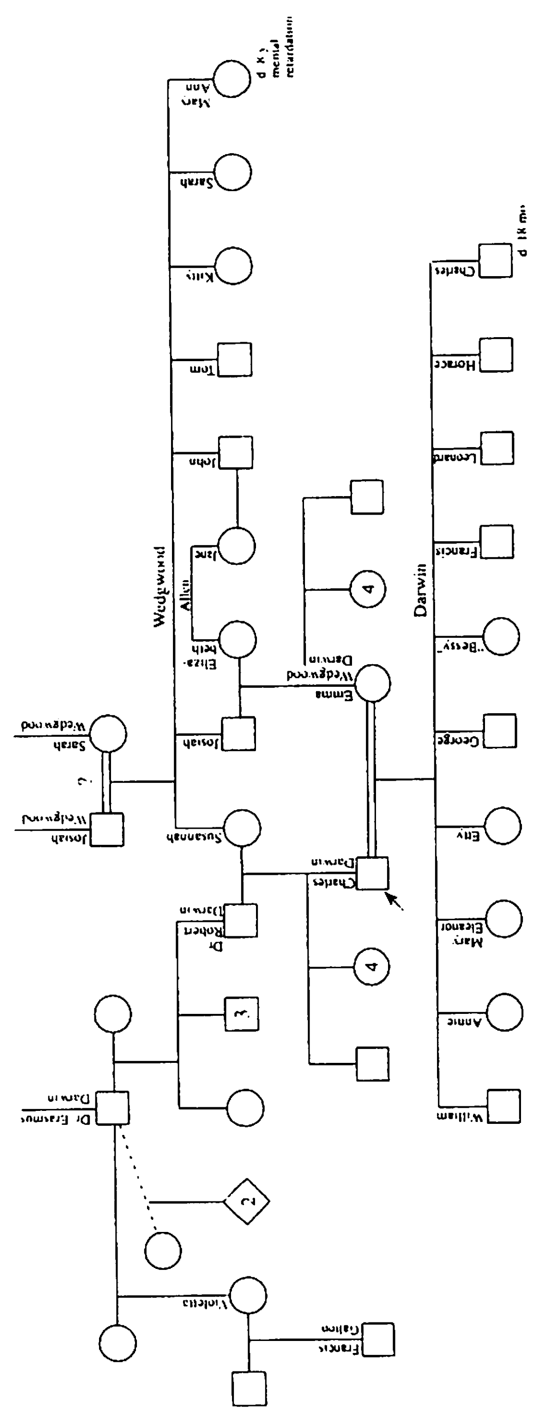


Figure 3.11 A pedigree of first cousins, Charles Darwin and his wife Emma Wedgwood Darwin. Their mutual grandparents, Josiah and Sarah Wedgwood, were also related as cousins.

TABLE 3.4 Possible Explanations for a Seemingly Unremarkable Family History

Individual has a new mutation (dominant, mitochondrial)
New X-linked mutation (in a male)
Affected person is not the natural child of parent(s) (e.g., nonpaternity, adoption, artificial insemination by donor semen)
Sex-limited inheritance
Seemingly unaffected parent actually has subtle expression of disease/syndrome (always examine the parents!)
Delayed age of onset of symptoms
Reduced penetrance
Small family size
Failure of the clinician to take a three-generation pedigree
Lack of knowledge of person giving history to clinician
Intentional withholding of information by historian (i.e., person may feel guilty or embarrassed, or fear discrimination)

WHEN IS A GENETIC FAMILY HISTORY SIGNIFICANT?

This is a tough question to answer. My feeling is that a significant family history is one that the patient is concerned about, or one that the patient *should* be concerned about. A 22-year-old woman with an abnormal ultrasound finding at 16 weeks may have a strong family history of breast and ovarian cancer, but a desire for information about inherited cancer susceptibility testing is probably the farthest thing from her mind at that moment. Instead, delving further into this information at a future visit is warranted, and might be communicated to the woman in a follow-up letter.

THE ULTIMATE PEDIGREE CHALLENGE

The following fictitious family history is provided for you to practice drawing each standardized pedigree symbol. This teaching scenario is republished with permission from the American Journal of Human Genetics (Bennett et al., 1995). The "answer" is in Appendix A.1.

The consultand, Mrs. Feene O'Type, presents to your office with a pregnancy at 16 wk gestation. She has questions about risks to her fetus because of her age. She is 35 years old and her husband, Gene O'Type, is 36 years old. Mrs. O'Type had one prior pregnancy, an elective termination (TOP) at 18 wk, of a female fetus with trisomy 21.

Mrs. O'Type's Family History

- Mrs. O'Type had three prior pregnancies with her first husband. The first pregnancy was a TOP, the second a spontaneous abortion (SAB) of a female fetus at 19 wk gestation, and the third a healthy 10-year-old son who was subsequently adopted by her 33-year-old sister, Stacy.

- Stacy had three pregnancies, two SABs (the second a male fetus at 20 wk with a neural tube defect and a karyotype of trisomy 18) and a stillborn female at 32 wk.
- Mrs. O'Type has a 31-year-old brother, Sam, who is affected with cystic fibrosis (CF) and is infertile.
- Her youngest brother, Donald, age 29 years, is healthy and married. By means of gametes from Donald and his wife, an unrelated surrogate mother has been successfully impregnated.
- Mrs. O'Type's father died at age 72 years and her mother at age 70 years, both from "natural causes." Mrs. O'Type's mother had five healthy full sibs, who themselves had many healthy children.

Mr. O'Type's Family History

- Mr. O'Type has two siblings, a monozygotic twin brother, Cary, whose wife is 6 wk pregnant by donor insemination (donor's history unknown), and a 32-year old sister, Sterrie.
- Sterrie is married to Proto, her first cousin (Sterrie's father's sister's son), who has red/green color blindness. She is carrying a pregnancy conceived from Proto's sperm, and ovum from an unknown donor. Sterrie and Proto also have an adopted son.
- The family history of Sterrie's mother, who has Huntington disease (HD), is unknown.
- Mr. Gene O'Type's father has a set of twin brothers, zygosity unknown, and another brother and sister (Proto's mother), who are also twins.

REFERENCES

Bennett RL, Steinhaus KA, Uhrich SB, et al. (1995). Recommendations for standardized human pedigree nomenclature. Am J Hum Genet 56:745–752.

Brinkman RR, Mezei MM, Theilmann J, Almqvuist E, Hayden MR (1997). The likelihood of being affected with Huntington disease by a particular age, for a specific CAG size. Am J Hum Genet 60:1202–1210.

Cohen MM (1997). *The Child with Multiple Birth Defects*, 2nd ed. New York: Oxford University Press.

Cohen LH, Fine BA, Pergament (1998). An assessment of ethnocultural beliefs regarding the causes of birth defects and genetic disorders. J Genet Couns 7:15–29.

Fisher NL (ed) (1996). *Cultural and Ethnic Diversity: A Guide for Genetics Professionals*. Baltimore: Johns Hopkins University Press.

Fisher NL, Lew L (1996). Culture of the countries of Southeast Asia. In Fisher NF (ed), *Cultural and Ethnic Diversity: A Guide for Genetics Professionals*. Baltimore: Johns Hopkins University Press, pp. 113–128.

Hauck L, Knoki-Wilson UM (1996). Culture of Native Americans of the Southwest. In Fisher NF (ed), *Cultural and Ethnic Diversity: A Guide for Genetics Professionals*. Baltimore: Johns Hopkins University Press, pp. 60–85.

Lynch EW, Hanson MJ (1992). *Developing Cross-Cultural Competence: A Guide for Working with Young Children and Their Families*. Baltimore: Paul H. Brookes Publishing Co.

Marazita ML (1995). Defining "proband." Am J Hum Genet 57:981–82.

Nelkin D (1998). Cancer genetics and public expectations. In Foulkes WD, Hodgson SV (eds), *Inherited Susceptibility to Cancer: Clinical, Predictive and Ethical Perspectives*. Cambridge: Cambridge University Press, p. 55.

NIH Consensus Statement (1997 Apr 14–16). Genetic Testing for Cystic Fibrosis 15(4):1–37.

Online Mendelian Inheritance in Man, OMIM™. Center for Medical Genetics, Johns Hopkins University (Baltimore, MD), 1997. Accessed June 19, 1998. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/

Resta RG (1993). The crane's foot: the rise of the pedigree in human genetics. J Genet Couns 2(4):235–260.

Resta RG (1995). Whispered hints. Am J Med Genet 59:131–133.

Scriver CR, Beaudet AL, Sly WS, Valle D (eds) (1995). *The Metabolic and Molecular Bases of Inherited Disease*, 7th ed. New York: McGraw-Hill.

Shepard TH (1996). *Catalog of Teratogenic Agents*, 8th ed. Baltimore: The Johns Hopkins University Press.

GENETICS GLOSSARY

GLOSSARY OF ABBREVIATIONS

aCGH array comparative genomic hybridization (microarray)

AFP alpha fetoprotein

AFAFP amniotic fluid alpha fetoprotein

AS Angelman syndrome

ASO allele specific oligonucleotide

BRCA Breast Cancer gene (1 & 2)

CAH congenital adrenal hyperplasia

CF cystic fibrosis

CPC choroid plexus cyst

CVS chorionic villus sampling

DNA deoxyribonucleic acid

DS Down syndrome

EDD (or EDC) expected date of delivery

EIF echogenic intracardiac focus

ERA early risk assessment

FISH fluorescence *in situ* hybridization

hCG human chorionic gonadotropin

HNPCC Hereditary Non-polyposis Colon Cancer (also called Lynch syndrome)

HD Huntington disease

LMP last menstrual period

MCA multiple congenital anomalies

MoM multiple of median

PHILTRUM The vertical groove in the median portion of the upper lip.

PHOCOMELIA Absence of the proximal portion of a limb or limbs; the hands or feet being attached to the trunk by a small, irregularly shaped bone.

PIGMENTARY SKIN ANOMALIES Cafe-au-lait spots (brown spots in neurofibromatosis), ash leaf spots (depigmented leaf shaped spots in tuberous sclerosis), axillary freckles (freckles under the arms in neurofibromatosis).

POLYDACTYLY The presence of extra digits (fingers and toes) on the hands and feet; called postaxial when on the ulnar side or preaxial when on the radial side.

PREAURICULAR PITS An indentation situated in front of the auricle of the ear.

PREAURICULAR TAGS An appendage situated in front of the auricle of the ear.

PROGNATHISM Marked protrusion of the jaw.

RETROFLEXED THUMBS Bent backward.

ROCKER-BOTTOM FEET The plantar surface of the foot is shaped like the rocker of a rocking chair, with an abnormally prominent heel.

SIMIAN LINE Single crease on the palm, common in Down syndrome. ***We prefer "single palmar crease"***

SKELETAL ANOMALIES - SPINE Lordosis (swayback), kyphosis (hunchback), scoliosis (s-shaped curve).

SYNDACTYLY Persistence of the webbing between adjacent digits (digits that are more or less completely attached) usually between the 3rd and 4th fingers and the 2nd and 3rd toes.

SYNOPHRYS The eyebrows meet or fuse in the midline.

TELECANTHUS Increased distance between the inner corners of the eyes.

TRIPHALANGEAL THUMB The presence of three phalanges in the thumb normally composed of only two.

from http://www.usd.edu/med/som/genetics/curriculum/

May 2010

For more terms, some with pictures, see: http://elementsofmorphology.nih.gov/index.cgi

BRUSHFIELD SPOTS A speckled ring about 2/3 of the distance from the pupil to the periphery of the iris with a relative lack of patterning beyond the ring. Found in about 80% of babies with Down syndrome.

CAMPTODACTYLY Permanent and irreducible flexion of one or more fingers.

COLOBOMA The absence of a portion of ocular tissue resulting in a notch or cleft-like defect in the structure, usually resulting from the failure to close part of a fetal fissure. May affect the iris, retina, or optic nerve.

CRANIOSYNOSTOSIS Premature closure of one or more sutures of the skull.

EPICANTHAL FOLD A vertical fold of skin on either side of the nose, covering the inner canthus (corner of the eye).

HIRSUTISM Excess body hair.

HYDROCEPHALUS A condition marked by dilatation of the ventricles of the brain.

HYPERTELORISM Increased distance between the eyes.

HYPOPLASTIC Poorly formed or small (e.g., hypoplastic toenails, fingernails, digits).

HYPOSPADIAS Urethral opening along the ventral surface (rather than the tip) of the penis.

HYPOTELORISM Decreased distance between the eyes.

MACROCEPHALY Large head size.

MACROSTOMIA Increased width of the mouth, resulting from failure of union of the maxillary and mandibular processes, with extension of the oral orifice toward the ear.

MICROCEPHALY Small head size, often associated with mental retardation.

MICROGNATHIA Small jaw.

MICROMELIA Abnormally small or short limb.

MICROPHTHALMIA Abnormally small eyes (one or both).

MICROSTOMIA Small mouth.

NEURAL TUBE DEFECT (NTD) A congenital abnormality of the spinal cord or brain resulting from the abnormal closure of the neural groove early in embryogenesis (e.g., spina bifida, anencephaly).

OLIGODACTYLY Missing fingers.

PALPEBRAL FISSURES Pertaining to the eye slits.

SEX CHROMOSOMES The X and Y chromosomes, which are responsible for sex determination.

SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs) DNA sequence variations that occur when a single nucleotide (A, T, C or G) in the genome differs between members of a species or within populations. Almost all common SNPs have only two alleles. See *dbSNP* (a SNP database hosted by NCBI).

SOMATIC CELLS All of the cells in the body except the eggs and sperm.

SYNDROME A pathogenetically related, recognizable pattern of structural anomalies, often with predictable natural history, that can be identified amongst several patients.

TERATOGEN An agent capable of causing congenital malformation(s) (e.g. environmental agents, recreational drugs, medications, infectious diseases, etc.).

TRANSLOCATION A rearrangement occurring when a piece of one chromosome is broken off and joined to another chromosome. An individual with a **balanced** translocation has the normal amount of chromosomal material. A person with an **unbalanced** translocation will have a loss or gain of chromosomal material.

TRIPLET REPEAT A unit of three DNA bases (e.g. CAG, CTG, CGG) that is present in multiple copies, scattered throughout the genome. A number of neurogenetic conditions have been ascribed to expansion of triplet repeat size (e.g. fragile X syndrome).

TRISOMY The presence of three homologous chromosomes rather than the normal two (e.g. trisomy 21).

UNIPARENTAL DISOMY A pair of homologous chromosomes inherited from one parent with no contribution from the other parent.

X-LINKED A gene located on the X chromosome (formerly known as sex-linked).

ZYGOTE A fertilized egg.

*A talking glossary may be found at http://www.genome.gov/Glossary/

GLOSSARY OF MALFORMATIONS

ANOPHTHALMIA A developmental defect characterized by absence of the eyes (rare).

ARACHNODACTYLY A condition characterized by abnormally long and slender fingers/toes.

BRACHYCEPHALY "Short" head due to a decreased anteroposterior distance.

BRACHYDACTYLY Short fingers, all digits or only one or two.

MEIOSIS A special type of cell division that occurs when mature eggs and sperm are formed. Through the process of meiosis the number of chromosomes present in a cell is decreased by half. In humans the diploid number of chromosomes is 46. This is decreased to 23 during meiosis.

MITOSIS The division process that occurs in somatic cells resulting in the formation of two cells, each with the same number of chromosomes that were present in the original parent cell.

MONOSOMY One chromosome of a pair is missing.

MOSAICISM The presence of two or more cell lines (cell populations) which differ from each other in genotype or chromosome number.

MULTIFACTORIAL A trait that is determined by the interaction between a number of genes and environmental factors.

MUTATION A permanent, heritable change in a gene or in chromosome structure.

NONDISJUNCTION Failure of paired chromosomes to separate (into each daughter cell) during cell division.

PEDIGREE A graphic representation of a person's family created using a standardized set of symbols.

PENETRANCE The frequency with which individuals carrying a given gene will show the clinical manifestations associated with the gene.

PHENOTYPE The clinical features or the observable characteristics of an individual determined by a pair of genes at a given locus (or genotype). The phenotype can vary following interaction with modifying genes or the environment.

PLEIOTROPIC A single gene or gene pair which produces multiple effects, usually because of clinical abnormalities in various organ systems.

RECESSIVE A gene (allele) which is only expressed clinically in the homozygous state. In a recessive disorder, both genes at a given locus must be abnormal to manifest the disorder.

RESTRICTION ENDONUCLEASES Naturally occurring enzymes which cut DNA at specific sites. Each enzyme recognizes a specific sequence of base pairs and will only cut the DNA at these sites.

RESTRICTION FRAGMENT LENGTH POLYMORPHISMS Variations in DNA nucleotide sequences which occur throughout the human genome and have no apparent phenotypic effects since they usually do not involve the (RFLPs) coding sequence of genes. Each is inherited codominantly. RFLPs are used as markers to track the inheritance of abnormal genes in families in linkage analysis.

SEQUENCE A pattern of anomalies derived from a single prior anomaly or factor (e.g. Pierre-Robin)

GAMETE An oocyte or sperm.

GENE A small segment of DNA that codes for the synthesis of a specific protein. Genes are located on the chromosomes. Examples: ABO blood group gene, Rh blood group gene.

GENETIC COUNSELING A communication process intended to help affected individuals and/or their families to comprehend the diagnosis, prognosis, recurrence risks and reproductive choices and to make the best possible adjustment to the condition.

GENOME All of the genes present on a set of chromosomes.

GENOTYPE The specific pair of alleles present at a single locus.

GERMLINE MOSAICISM Two or more genetic or cytogenetic cell lines confined to the precurser (germline) cells of the eggs or sperm; formerly called gonadal mosaicism.

HAPLOID The normal number of chromosomes present in an egg or sperm. In humans, the haploid number of chromosomes is 23.

HETEROGENEITY The phenomenon by which a certain phenotype (or clinical feature) can be produced by different genetic mechanisms.

HETEROZYGOTE An individual who has two different alleles at a given locus on a pair of homologous chromosomes.

HOMOLOGOUS CHROMOSOMES A pair of chromosomes, one from each parent, carrying genes for the same traits, in the same order. In a karyotype, the members of a homologous pair look alike (e.g., a pair of 1s, 2s, etc.).

HOMOZYGOTE An individual who has two identical alleles at a given locus on a pair of homologous chromosomes.

IMPRINTING Refers to the modification of a gene as it is transmitted through the father or the mother. One mechanism may be the methylation (inactivation) of a gene.

INDEX CASE The affected individual who brings the family to the attention of the geneticist, also known as the proband or propositus.

KARYOTYPE The chromosome complement of an individual arranged in a standard order from large to small.

LINKAGE Refers to genes (or DNA markers) that are located in close proximity to one another on the same chromosome. Closely linked genes tend to be inherited together as a unit.

LOCUS Position or location of a gene on a chromosome.

LYONIZATION The random inactivation of loci on one of a pair of X chromosomes. In each cell, only one X chromosome is active. This process of inactivation occurs early in fetal development and explains the variable expressivity of X-linked traits in females.

ANOMALY A structural variation present from birth. Considered minor if there are no significant health or cosmetic implications (i.e. would not require intervention).

ASSOCIATION A nonrandom occurence of multiple anomalies without known cause (e.g. VATER). Compare to syndrome.

AUTOSOME The chromosomes males and females have in common; chromosomes 1 to 22.

BIRTH DEFECT An abnormality of structure, function, or body metabolism which often results in a physical or mental handicap. It may be inherited (genetic) or environmental.

CARRIER A person who has one normal gene and a gene coding for a recessively inherited disease, or a person with a balanced chromosomal rearrangement; in either case a carrier usually has a normal phenotype. However, a carrier may manifest some or all features of the disease, particularly when female with an X-linked disorder.

CHORIONIC VILLUS SAMPLING (CVS) A procedure done between 10-12 weeks of gestation in which a few milligrams of fetal placenta are aspirated vaginally or abdominally. The aspirated tissue is then tested for various genetic diseases.

CODOMINANT Alleles are codominant if each is expressed independent of the presence of the other. Example: AB blood type.

CONGENITAL Present at birth; may or may not have a genetic cause.

CONSANGUINITY Refers to a mating between persons who share a common ancestor.

CONTIGUOUS GENE SYNDROME Conditions that occur secondary to microdeletions or microduplications that involve several adjacent genes.

DIPLOID The number of chromosomes normally present in a somatic cell. In humans there are 46 chromosomes in a somatic cell. This is twice the number of chromosomes present in a haploid (23 chromosomes) gamete.

DOMINANT A gene (allele) which is expressed clinically in the heterozygous state. In a dominant disorder only one mutant allele need be present as it covers up, or masks, the normal allele.

EPIGENETIC Heritable changes in gene expression caused by mechanisms *other* than changes in the underlying DNA sequence; often occurs in embryonic development (e.g. DNA methylation, chromatin remodeling, X-inactivation).

EXPRESSIVITY The extent to which a gene is clinically evident (expressed) in an individual. Variable expressivity refers to the variation in severity of symptoms produced by the same gene in different individuals.

FAMILIAL Any trait that occurs more often in the relatives of an affected person than in the general population; traits that tend to "run in families" (e.g., diabetes, neural tube defects).

MSAFP maternal serum alpha fetoprotein

NF neurofibromatosis

NTD neural tube defect

OMIM Online Mendelian Inheritance in Man

OTIS Organization of Teratogen Information Services

PAH phenylalanine hydroxylase

PCB polychlorinated biphenyl

PCR polymerase chain reaction

PKU phenylketonuria

PWS Prader-Willi syndrome

Rb retinoblastoma

RNA ribonucleic acid

TIS Teratogen Information Service

UPD uniparental disomy

U/S ultrasound

VNTR variable number of tandem repeats

GLOSSARY OF GENETIC TERMS

ALLELE Refers to the different forms of a gene at one locus. Example: A, B and O are alleles for a blood group gene. A person with blood type AB has the A allele on one chromosome and the B allele at the same locus on the other homologous chromosome.

AMNIOCENTESIS A clinical procedure in which a few milliliters of amniotic fluid is withdrawn from the pregnant uterus. This fluid and the fetal cells that are present in the fluid may be tested to detect various genetic conditions.

ANEUPLOID The total number of chromosomes in a cell if not an exact multiple of the haploid number of 23. The most common aneuploid numbers are 45 (Turner syndrome) and 47 (Down syndrome).

TRANSLATIONAL GENETICS

Molecular genetic testing and the future of clinical genomics

Sara Huston Katsanis[1] and Nicholas Katsanis[2]

Abstract | Genomic technologies are reaching the point of being able to detect genetic variation in patients at high accuracy and reduced cost, offering the promise of fundamentally altering medicine. Still, although scientists and policy advisers grapple with how to interpret and how to handle the onslaught and ambiguity of genome-wide data, established and well-validated molecular technologies continue to have an important role, especially in regions of the world that have more limited access to next-generation sequencing capabilities. Here we review the range of methods currently available in a clinical setting as well as emerging approaches in clinical molecular diagnostics. In parallel, we outline implementation challenges that will be necessary to address to ensure the future of genetic medicine.

Large-insert clone
A large haplotype fragment that is inserted into, for example, a bacterial artificial chromosome.

Oligonucleotide arrays
Hybridization of a nucleic acid sample to a very large set of oligonucleotide probes, which are attached to a solid support, to determine sequence, to detect variations or to carry out gene expression or mapping.

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doi:10.1038/nrg3493

Molecular diagnostic testing in a symptomatic individual has become increasingly sophisticated. Until recently, such testing was carried out on, at most, one or a few loci. The advent of large-insert clone arrays and, later, oligonucleotide arrays changed this landscape by allowing a patient's entire genome to be queried at improved resolution, thereby allowing the detection of medium to large genomic lesions. Today, this can be done at single-nucleotide resolution thanks to cheaper, faster and increasingly accurate whole-exome sequencing (WES) and whole-genome sequencing (WGS)[1]. Although genome sequencing is expected to transform diagnostics, non-sequencing molecular technologies remain crucial for efficiently and precisely screening and defining variation. Patients and families rely on molecular diagnoses for health-care management, disease prognosis and family planning, and they personally benefit when an answer is provided for the afflicting condition.

Amid the euphoria surrounding these advances, major analytical and interpretative challenges have emerged, ranging from the validation of large numbers of genomic changes in a patient, to the economic feasibility of this approach and its deployment in standard care, to managing the terabytes of data that accompany a single sequenced genome[2]. Deciphering the information that is locked in a patient's genome is not trivial. However, the effort invested towards development of informatic and molecular tools that are immediately applicable to both common and rare genetic disease has the potential to inform a broad range of clinical phenotypes[3–11].

Here we review a range of methods available for molecular diagnosis, their relative value for detecting genomic variation and some key challenges for each technology. With the rapidly changing technological platforms, we direct the reader to other articles for comparisons of next-generation sequencing (NGS) and other genomic technology reviews[12–14]. Further, we discuss the challenges of implementing these technologies into clinical practice, including policy development and ethical considerations. Although we concentrate our Review on laboratory testing in the United States, as it is a focal point for policy discussion and technological development, we present approaches to be considered in other countries and regions with more limited resources. We also focus on genetic testing for heritable genotypes or karyotypes as opposed to somatic mutations in cancer or viral load genetic testing. We do not cover newborn screening technologies, ancestry testing or identity DNA testing; for a scholarly discussion of prenatal genetic testing and ethical considerations, see REFS 15,16. We start with a discussion of the scope of genetic services and applications and current relevant technologies. We then focus on the challenging interpretation of genome variation, particularly in the nascent use of WGS and WES. Finally, we discuss the breadth of considerations and social implications of clinical genome sequencing, including access, ethics, genetics education and the regulatory landscape. At the conclusion of this Review, we discuss the upcoming challenges to integrating the next wave of genome sequencing into clinical practice.

Exome
The collection of protein-coding regions (exons) in the genome. As exons comprise only 1% of the genome and contain the most easily understood and functionally relevant information, sequencing of only the exome is an efficient method of identifying many variants that are likely to affect a trait.

Next-generation sequencing (NGS). NGS platforms sequence as many as billions of DNA strands in parallel, yielding substantially more throughput than Sanger sequencing and minimizing the need for the fragment-cloning methods that are often used in Sanger sequencing of genomes.

The scope of clinical genetic testing

Genetic testing has grown from a niche speciality for rare disorders to a broad scope of applications for complex disease and personal use[17,18]. Not surprisingly, the definition of a genetic test has changed as the applications have evolved. Applications of clinical genetic testing span medical disciplines, including: newborn screening for highly penetrant disorders; diagnostic and carrier testing for inherited disorders; predictive and pre-symptomatic testing for adult-onset and complex disorders; and pharmacogenetic testing to guide individual drug dosage, selection and response (TABLE 1). Currently, genetic tests may be indicated in different clinical contexts and ordered by multiple health-care providers (see Further information for resources of available genetic tests). The circumstances of the individual genetic test — including the acute nature of the phenotype, the age of the patient, family history and specimen availability — guide the selection of tests and test platforms. For example, prenatal WGS can detect carrier status for a host of rare genetic disorders[19] but might be considered to be impractical for routine screening. Genetic tests in under-funded regions may continue to be driven by the candidate gene approach on the basis of the phenotype of a patient, as has been the paradigm in the United States for two decades. Still, these approaches hold a valuable role in certain classic monogenic syndromes and in families with a previously attributed molecular cause. However, in naive cases for genetic work-up, an argument could be made that (not accounting for cost) whole-genome analysis may be valuable in determining mutation load and identifying other genetic factors relevant to health planning.

Post-millennium genetic technologies

For the most part, clinical molecular diagnostic technologies remain focused on identifying patients' underlying pathogenic mechanisms. TABLE 2 summarizes the methodologies that are applicable to heritable genotypes and karyotypes. With direct genetic testing, the laboratory looks for the particular genetic variant (or variants) that contributes to a condition, whereas

Table 1 | **Factors considered in selecting a genetic test**

Test	Description	Example	Embryo or blastocyst (pre-implantation genetic diagnosis)	Fetus (prenatal testing)	Child	Adult
Newborn screening	Targeted tests for recessive genetic disorders	Phenylketonuria, cystic fibrosis, sickle-cell anaemia	Not applicable	Not applicable	Tests provided at birth vary by country and state or region	Not applicable
Diagnostic testing	Confirmatory test or differential diagnosis testing for a symptomatic individual	Skeletal dysplasias, thalassaemias, craniosynostoses	Specimen type and limited available amount for sampling may restrict platform selection (for example, WES or WGS versus SNP or STR typing) Turnaround time necessary may restrict platform selection		Where treatment is desired, turnaround time may restrict platform selection	
Carrier testing	Targeted testing for asymptomatic individuals potentially carrying one or more recessive mutation	Cystic fibrosis, thalassaemias, Tay–Sachs disease	Applied typically for rare disease but applicable for other familial mutations		Carrier testing of minors is considered in the context of individual paediatric cases[164,165]	According to standard of care
Predictive testing	Tests for variants causing or associated with diseases or disorders with a hereditary component, usually with adult-onset symptoms	Most cancers, cardiovascular disease, diabetes	Some have discouraged genetic testing of asymptomatic minors for adult-onset conditions			According to standard of care
Pre-symptomatic testing	Tests for variants causing or associated with diseases or disorders known to be inherited in the family, often with adult-onset symptoms	Huntington's disease, haemochromatosis, Alzheimer's disease	Some have discouraged genetic testing of asymptomatic minors for adult-onset conditions[152,153] Interpretation of VUSs will depend on presenting phenotypes in the family			According to standard of care
Pharmacogenetics	Targeted tests for variants associated with pharmaceutical dosage choice or adverse reactions	DNA tests for abacavir, warfarin, carbamazepine	Application not currently conducted but theoretically feasible	Application not currently conducted, but conceivably applicable for screening treatment approaches *in utero*	Pharmacogenetic testing is considered in context of individual paediatric cases[166]	According to standard of care

SNP, single-nucleotide polymorphism; STR, short tandem repeat; WES, whole-exome sequencing; WGS, whole-genome sequencing; VUS, variant of unknown significance.

Direct genetic testing
Testing that looks at the presence or absence of known genetic variants that contribute to pathogenicity

Indirect genetic testing
Testing that compares the genetic regions of multiple affected persons to unaffected persons. Indirect genetic tests may evaluate patterns of inheritance in multiple family members with a known trait and look at the segregation of the trait with genetic markers

Linkage analysis
A statistical method for identifying a region of the genome that is implicated in a trait by observing which region is inherited from the parental strain carrying the trait in offspring that carry the trait.

indirect genetic testing relies on the comparison of DNA markers that are linked to a trait of interest but that do not cause the genetic condition.

Every shift in technology is accompanied by the need to assess the quality and feasibility of the new platform for diagnosis. (TABLE 3 defines the terms that are useful for evaluating diagnostic tests.) Analytical validity is a measure of the ability of a molecular test to detect a genetic or genomic variant, both in terms of the analytical sensitivity of the assay (false-negative rate) and the analytical specificity of an assay (false-positive rate). By contrast, the clinical validity refers to the ability of the test to predict the presence or absence of a clinical condition.

Indirect testing. Despite the surge of new technologies to interrogate disease-causing variants in a patient in well-funded laboratories, indirect methodologies continue to have a prominent role in diagnostics in regions of the world with more limited resources (and thus a substantial fraction of the human population); in particular, linkage analysis using single-nucleotide polymorphisms (SNPs) and short tandem repeats (STRs) can be applied[20]. Classical indirect approaches (for example, single-strand conformation polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE) and heteroduplex analysis) have mostly been phased out in the United States, but these techniques are still highly used in developing regions with limited resources[21–23]. In some cases, indirect tests could inform whole-genome data (see discussion below) by narrowing in on regions of interest; this is an approach that is commonly used in research to save costs of WGS[20]. Further, for some specialized applications, such as non-invasive prenatal testing (NIPT) and pre-implantation genetic diagnosis (PGD), the ability to amplify and to differentiate STRs from trace samples or even single cells makes microsatellite linkage analysis an attractive approach[24–26].

Targeted allele-specific mutation detection. Amplification combined with restriction digest, hybridization or another means of detecting a mutation remains among the cheapest and most robust methods in clinical molecular diagnostics. The simplicity of PCR mutation detection makes throughput of multiple samples feasible and offers high confidence to detect variants. For example, common disease-causing repeat

Table 2 | **Clinical genetic testing methodologies**

Method	Common point mutations	Rare point mutations	Copy number variants	Uniparental disomy*	Balanced inversions or translocations	Repeat expansions	Analytical sensitivity‡§	Analytical specificity‡‖	Turnaround time‡¶	Cost#	Examples
Linkage analysis (commonly STRs)	X		X**				Low	Low	Low	Low	Historical familial mutation
FISH			X		X		Low	Low	Low	Low	Angelman's syndrome
Array CGH or virtual karyotyping			X	X			Average	Average	Average	Average	A new referral or challenging diagnostic case
Genome-wide SNP microarrays	X		X				Low	Low	Low	Low	Cardiovascular disease risk assessment
Target PCR	X	X**				X	High	High	Low	Low	Cystic fibrosis carrier testing
Sanger gene sequencing	X	X					High	High	Average–high	Average	Treacher Collins syndrome diagnosis
Southern blot or MLPA			X			X	High	High	High	Low	Fragile X syndrome
Panel or pathway sequencing	X	X					Average	Low	Average	Average	Long QT syndrome
WES or WGS	X	X	X‡‡				Low	Low	High	High	A new referral or challenging case to diagnose

CGH, comparative genomic hybridization; FISH, fluorescent *in situ* hybridization; MLPA, multiplex ligation-dependent probe amplification; SNP, single-nucleotide polymorphism; STR, short tandem repeat; WES, whole-exome sequencing; WGS, whole-genome sequencing. *Familial mutations or genomic rearrangements can be assayed. ‡Categorical assignments in these columns are subjective and vary according to context of the tests being ordered and the laboratory conducting the tests. The 'low', 'average' and 'high' are presented to simplify and to compare platforms generally. §Low, <80%; average, 80–98%; high, >98%. ‖Low, <80%; average, 80–98%; high, >98%. ¶Low, <1 week; average, 1 week–1 month; high, >1 month. #Costs of the testing will widely vary from one laboratory to the next; however, these estimates are based on the charge of the test from a sampling of laboratories, not on the costs of consumables or the reimbursed amount. Low, less than US$400; average, $400–$2,000; high, >$2,000. **Uniparental disomy can be detected by any method if both parents are genotyped. However, only the indicated approaches will detect uniparental disomy in absence of the parental genetic samples. ‡‡Copy number variant detections are improving in next-generation sequencing applications but are more efficient in WGS than WES, although they are of limited reliability for clinical diagnostics.

Table 3 | **Evaluating the validity of genetic tests**

Term	Definition	Complications in molecular tests	Calculation
Analytical sensitivity	Refers to the proportion of assays with the genotype that have a positive test result (false-negative rate of the assay)	Allele drop out; preferential amplification; mosaicism	True positives / (true positives + false negatives)
Analytical specificity	Refers to the proportion of assays without the genotype that have a negative test result (false-positive rate of the assay)		True negatives / (true negatives + false positives)
Clinical sensitivity	Refers to the proportion of people with a disease who have a positive test result (false-negative rate of diagnosis)	Variable penetrance; variable expressivity	True positives / (true positives + false negatives)
Clinical specificity	Refers to the proportion of people without a disease who have a negative test result (false-positive rate of diagnosis)		True negatives / (true negatives + false positives)
Positive predictive value (PPV)	Refers to the likelihood that a patient has the disease given that the test result is positive		True positives / (true positives + false positives)
Negative predictive value (NPV)	Refers to the likelihood that a patient does not have the disease given that the test result is negative		True negatives / (true negatives + false negatives)
Clinical utility	Refers to the value of the test for determining treatment, patient management and family planning	Depends on health-care system and environment	Subjectively determined on the basis of reports supporting use and economic benefits
Personal utility	Refers to the value of the test for personal and family choices	Depends on personal vantage	Subjectively determined from an individual's perspective

expansions, such as those in fragile X syndrome, are frequently tested for by direct amplification of the repeated fragment[27]. This approach is ideal for carrying out simple assays on common variants, such as a Taqman® assay for genotyping a pharmacogenetic variant or factor V Leiden mutation. The disadvantage of allele-specific PCR is, of course, the inability to detect any relevant variants that have not been assayed. Nonetheless, these approaches retain a high value, especially in laboratories with limited resources and/or access to advanced instrumentation and are likely to remain core clinical assays.

Gene-specific Sanger sequencing. For detection of point mutations and small variants, bidirectional Sanger sequencing has been considered the 'gold standard' in clinical genetic testing for the past decade[28]. This direct approach has high analytical validity (TABLE 3), although long reads can deteriorate quality for base calling, and minute specimens can produce PCR artefacts[29,30]. The fundamental value in directly sequencing one or more entire genes is the ability to combine a clinical indication for a candidate gene with the high sensitivity and specificity of the assay (TABLE 3). For instance, focused sequencing of a single gene (namely, *FGFR2*) can confirm or rule out a diagnosis of Apert's syndrome at fairly low cost[31], sequencing *TCOF1* will to detect up to 90% of mutations in patients with Treacher Collins syndrome[32], whereas testing six genes known to cause Noonan's syndrome (namely, *PTPN11*, *SOS1*, *RAF1*, *NRAS*, *CBL* and *KRAS*) detected mutations in 30% of individuals with clinical features suggestive of Noonan's syndrome[33]. As the analytical validity of whole-genome technologies improves (TABLE 3), genome sequencing will probably become the first-pass instrument of genetic analysis to inform candidate gene Sanger sequencing (see below). It is important to note that although Sanger sequencing is of high analytical validity, the clinical validity of the approach is dependent on the genetic drivers of a condition. Sanger sequencing does not detect most structural changes, so it alone is not sufficient for diagnosis for many genetic disorders.

Genome-wide SNP microarrays. Microarray-based genotyping can be divided into three main applications: array comparative genomic hybridization (array CGH) to detect structural anomalies (see discussion below), phenotype-specific SNP panels, and genome-wide SNP panels. Efforts in academic and commercial laboratories have produced phenotype-specific panels containing alleles that are known to drive specific phenotypes, such as panels for retinal degeneration[34,35]. The utility of this approach is that a low-cost, expeditious experiment interrogating multiple genes can offer high-quality molecular diagnoses. However, the continuous discovery of novel causal alleles and genes, as well as variable penetrance and expressivity of known mutations[36] limits the clinical validity of this approach (TABLE 3).

By contrast, large-scale genome-wide SNP genotyping offers a single, cost-efficient platform to assess risk of multiple common genetic disorders with variably documented associations in one test[36–38]. Predictive and presymptomatic testing is available as a multiplex platform for a host of conditions, including certain cancers and pharmacogenetic tests, as well as for ophthalmologic, cardiac, renal and neurological disorders (among others). Several personal genome companies now provide versions of commercial clinical genotyping services to consumers, such as the Personal Genome Service from 23andMe, Pathway Genomics and Navigenics, to name but a few[39]. Although the tests are designed for and available to consumers, because the analytical tests are conducted in clinical (that is, CLIA-certified) laboratories, such genome-wide SNP tests may also be ordered by clinicians. With genome-wide SNP tests, particular loci

Single-nucleotide polymorphisms (SNPs) Differences in the nucleotide composition at single positions in the DNA sequence

Short tandem repeats (STRs) DNA sequences containing a variable number of highly polymorphic, tandemly repeated short (2–6 bp) sequences

Non-invasive prenatal testing (NIPT) A method of obtaining a prenatal diagnosis by detecting fetal cells circulating in maternal blood

Pre-implantation genetic diagnosis (PGD) An *in vitro* method of identifying genetic defects in *in vitro* fertilization embryos before maternal transfer and implant

Sanger sequencing A method used to determine the nucleotides present in a fragment of DNA. It is based on the chain terminator method developed by Frederick Sanger but currently uses labelling of the chain terminator dideoxynucleotides, allowing sequencing in a single reaction.

may be evaluated with high analytical validity (TABLE 5), but the limited scope of variant detection confines analysis to pre-selected points in the genome. Further, most SNP-based diagnostics are probabilistic, not deterministic, with variable degrees of clinical validity[40], as arrays identify a limited range of variants. For instance, homozygosity of common alleles at the two major loci for age-related macular degeneration (AMD; namely, *CFH* and *HTRA1*) have a high probabilistic value for disease onset[41–45] and might induce behavioural modification in patient management owing to the documented high association of the homozygosity of some SNPs and smoking[46], but the test has limited ability to predict AMD per se. Newer hybrid platforms, such as exome chips that contain all known coding variants reported both in patients and in control individuals might offer improved efficiency in identifying the mutational load of patients for both rare and common alleles that are relevant to disease status, although they too might have limited clinical validity[39].

Detection of structural and chromosomal variation. Recent improvements in chemistry and microscopy have substantially augmented the resolution of cytogenetics, most notably through the development of multi-probe fluorescent *in situ* hybridization (FISH; for a detailed review, see REF. 47) and chromosomal CGH. Economic factors aside, cytogenetic methods are gradually being phased out in the clinic in favour of a combined SNP–array CGH approach that uses probes to detect chromosomal and genomic rearrangements as well as deletions with greater precision and smaller genomic variations than FISH (for a thorough review of structural variation and medical genomics, see REF. 48). Depending on design and probe density, array CGH can offer resolution from whole chromosomes to deletions and duplications of a few kilobases in size[49]. Array CGH imparts improved sensitivity (TABLE 2) of rearrangement detection (with the important exception of balanced inversions and translocations) and the ability to detect readily uniparental disomy (UPD), which is not detectable through chromosomal CGH. At the same time, improved resolution has been accompanied by a massive increase in detection of submicroscopic genomic rearrangements of unclear importance to the clinical phenotype of tested patients. Resources such as Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources (DECIPHER) and International Standards for Cytogenomic Arrays Consortium (ISCA Consortium) are cataloguing submicroscopic deletions and duplications that may affect the copy number of dosage-sensitive genes or disrupt normal gene expression, leading to disease. These databases provide a common depot for aiding interpretation of the often novel and often *de novo* structural changes found in diagnostics[50,51]. Even so, non-uniform deposition of phenotypic data deposited represents a substantial limitation to the utility of such databases.

In parallel, other molecular techniques have exponentially improved in their ability to detect subchromosomal rearrangements of varying sizes and complexity. Southern blotting, which used to be widely used in combination with restriction fragment length polymorphism (RFLP) for molecular diagnoses, continues to be used to detect small genetic changes as well as large repeat variants that are not amenable to PCR amplification (for example, *FMR1* expansions)[27]. However, more recently, multiplex ligation-dependent probe amplification (MLPA) assays have replaced Southern blotting for some applications. In addition to standard copy number variants (CNVs), MLPA can detect mosaic mutations, as well as methylation status[52]. Further, MLPA can be used to confirm structural anomalies detected by FISH or CGH[53]. However, in most cases MLPA does not detect balanced genomic rearrangements, such as translocations or inversions[52], which is a substantial limitation, given the emergent realization of these types of events in human genetic disease[54]. We anticipate that for some types of genetic lesions, such as large trinucleotide expansions, classical molecular methods, including Southern blotting and MLPA, will remain assays of choice.

Whole-genome and whole-exome sequencing. NGS uses powerful massively parallel sequencing assays to sequence many genes of interest, the whole exome or the whole genome for variants in a broad range of rare and complex disorders. Targeted exon capture before genome sequencing (that is, WES) facilitates efficient analysis of most of the coding regions of the genome, whereas WGS evaluates almost all of the euchromatic human genome (it is important to note that heterochromatic regions will remain off limits for some time until read lengths become long enough to resolve repeat-dense regions). WES has proven to be a fast and accurate discovery approach for some mutations causing Mendelian disorders[3,5–10,55–59]. The plummeting cost of genome sequencing is reducing reagent costs below those of Sanger sequencing for some candidate genes (this is especially true for focused gene panels), making application of WES and WGS economically feasible[60,61]. At this time, WGS is not clinically available, but WES is available from select clinical laboratories (for a scholarly discussion of WES and WGS in clinical diagnostics, see REF. 62). Interpretation of clinical WES is limited, with few reported results clinically actionable. However, medical geneticists at major academic centres now routinely counsel for and order WES for unexplained genetic disorders. Although the choice between the two technologies is primarily driven by cost, after WGS has been offered as a clinical service, WGS is expected to supersede WES in the coming years, at least in well-funded arenas. Naturally, as with every disruptive technology, WGS data will introduce a new challenge over WES of interpreting non-coding variants that may contribute to the genetic load of a patient's phenotype.

In some cases, a targeted NGS approach based on a suspected syndrome may be taken to minimize costs and to maximize variant identification (for a review of disease-targeted sequencing, see REF. 63).

In addition to its use in WES and WGS for diagnostics and discovery, NGS can be used to detect methylation status, alternative splicing, small RNAs, allele-specific expression and even haplotypes and rearrangements[64–67].

Array comparative genomic hybridization
(Array CGH) A microarray-based method of identifying differences in DNA copy number by comparing a sampled genome to a reference genome.

Penetrance
The proportion of individuals with a given genotype who display a particular phenotype

Fluorescent *in situ* hybridization
(FISH) A molecular and cytogenetic method using a fluorescently labelled DNA probe to detect a particular chromosome or gene using fluorescence microscopy

Uniparental disomy
(UPD) An occurrence of an individual inheriting both copies of her chromosome from one parent

Restriction fragment length polymorphisms
(RFLP) Variations between individuals in the lengths of DNA regions that are cut by a particular endonuclease.

Multiplex ligation-dependent probe amplification
(MLPA) A molecular technique involving the ligation of two adjacent annealing oligonucleotides followed by quantitative PCR amplification of the ligated products, allowing the characterization of chromosomal aberrations in copy number or sequence and single-nucleotide polymorphism or mutation detection

Copy number variants
(CNVs) Structural genomic variants that result in copy number changes in specific chromosomal regions. Usually, there are two copies of each locus, but if, for example, duplications or triplications occur then the number of copies will increase

Although it has not yet been fully vetted for applications other than WES and WGS, NGS could potentially become a robust platform for a range of other 'omics' applications.

Cost considerations notwithstanding, the primary practical barrier to the use of WES and WGS in clinical settings is the limited ability of the technology to detect reliably the absence or presence of mutations. Different sequencing platforms have been shown to deliver results of variable quality, with some instruments more accurate at individual base calls and others covering a broader range of the genome[68–70]. Targeted approaches, including specific gene panels and whole exomes may be of greater analytical sensitivity (that is, they have a better coverage of the target to detect heterozygous changes) but restrict the clinical sensitivity (TABLE 3) in comparison to WGS, which might limit the interpretive scope to coding lesions. Even then, it is not possible at present to obtain high-quality sequence from the entire human genome, or even the euchromatic genome, that is sufficient for exhaustive clinical interpretation.

Further, to parse sequencing data efficiently, WES and WGS efforts for diagnosis often include sequencing of the proband and both unaffected parents in a trio to ascertain efficiently *de novo* and inherited mutations under limited information with regard to the mode of inheritance. With interpretation limited in current WES clinical tests to *de novo* and previously reported variants, some clinical WES laboratories sequence only the proband and confirm variants of interest in the parents. Nonetheless, access to the biological relatives remains valuable in interpretation of genetic variation. To refine further the vast amounts of data, confirmation testing by Sanger sequencing in probands and family members is typical. We are optimistic that the technical challenges of WES and WGS will be solved as market forces and clinical needs drive the field forwards. However, there remain acute interpretive problems that are dependent on the scope of the initial genome analysis (see the discussion of interpretive challenges below).

Taken together, the economical and analytical constraints of these technologies will limit WES and WGS to being an attractive first step in differential diagnosis, requiring secondary confirmations and possibly parallel testing by other methods for some time to come[71].

Evolving results

The success cases in rare diseases of WES are promising[72–74]; however, routine clinical genomic sequencing is waft with complications, resulting from both its unprecedented scale and interpretive challenges. Reliable interpretation of the multiple and *de novo* variants found through NGS will require additional experience and validation before it reaches the clinic on a large scale, particularly for diagnosis of complex traits[75,76]. Nonetheless, the clinical implementation of WES and WGS will probably transform clinical genetic testing, especially after genome-wide data become integrated into electronic medical records (EMRs)[77–79]. After this transition has occurred, specialized, phenotype-driven tests will probably wane and eventually disappear, and molecular diagnostics will focus instead on the interpretation of existing data[80].

Until recently, genetic data did not drive diagnosis but had a primarily confirmatory role. Moreover, the knowledge of pathogenic lesions typically leads to population-based arguments about possible patient outcomes. A major challenge is to convert pathogenic genetic data into a primary diagnostic tool that, in combination with clinical observation and biometric data, can shape clinical decisions and long-term management in a proactive way. Most emerging clinical genome-sequencing paradigms focus on a narrow phenotypic band in order to probe its genetic architecture in detail. A broader approach — namely, sequencing and parsing the total load of variants irrelevant to phenotype — will contribute meaningfully to what is a core question: should we sequence every patient admitted to a hospital and, if so, how do we interpret these data for clinical use?

Causal disease variants. In clinical diagnostic genetic testing, the American College of Medical Geneticists[81] recommends that variants be assigned to one of the following six categories:

- 'Disease causing': sequence variation has previously been reported and is a recognized cause of the disorder (for example, deletion of F508 in *CFTR*);
- 'Likely disease causing': sequence variation has not previously been reported and is of a type expected to cause the disorder, usually in a known disease gene (for example, a nonsense mutation in a gene for which other mutations of this type, but at a different residue, have been reported);
- 'Possibly disease causing': sequence variation has not previously been reported and is of the type that may or may not be causative of the disorder;
- 'Likely not disease causing': sequence variation has not previously been reported and is probably not causative of disease;
- 'Not disease causing': sequence variation has previously been reported and is a recognized neutral variant;
- 'Variant of unknown clinical significance': sequence variation is not known or expected to be causative of disease but is found to be associated with a clinical presentation.

Most of these categories of variants are subject to additional interpretation on the basis of literature, population frequencies, clinical findings, mutation databases and possibly case-specific research data. In addition, a variant may be considered to be protective or related to drug response.

Interpreting sparsely documented genetic mutations lacking evidence in co-morbidity has been challenging since the outset of molecular diagnostics, relying historically on the segregation patterns of inheritance, statistical incidence of a variant and the conservation of the altered amino acid in non-human species. To complicate this problem further, some laboratories responsible for assigning the importance of a molecular finding make decisions largely on the basis of experience of that laboratory in the analyte of interest. The current onslaught of

Box 1 | Variant interpretation: a case study

Consider an individual with a family history of amyotrophic lateral sclerosis (ALS), which is a lethal disorder with no treatment options (the index has two brothers, both of whom died at the age of 50). Motivation for the test is both for family planning but also for personal life planning. Whole-exome sequencing (WES) identifies a known mutation in superoxide dismutase 1, soluble (*SOD1*), one of the known ALS genes for which the confidence level for its pathogenic potential is high, given that the allele has been seen in other patients and confirmed in the deceased brothers from the index case. However, the test also detected a heterozygous nonsense mutation in ciliary neurotrophic factor (*CNTF*) that was deemed insufficient to drive disease as neither deceased brother carried it, and it was found in three control exomes. Under most simplistic models in effect today, alleles with such characteristics might not be reported to the index, as they are variants of unknown significance (VUSs) and might be interpreted as not being 'medically relevant'. However, studies in model organisms and humans have shown that haploinsufficiency at *CNTF* can have a potent effect on the age of onset of ALS, potentially reducing a patient's lifespan by two decades[145].

WES and WGS data requires a sophisticated and transparent exchange of variants associated with detailed phenotypes or clinical indications. Disease-centric mutation databases have morphed into human disease variant databases that are valuable for documenting clinical variation, such as the Human Gene Mutation Database (HGMD)[82] and the hand-curated databases ClinVar and MutaDatabase[83]. With concentrated effort, these latter pilots could expand into the broader, focused exchange necessary to facilitate interpretation of both rare and common variation across varying platforms and laboratories around the world.

Variants of unknown significance. We now recognize that with hundreds of loss-of-function variants and thousands of variants of unknown significance (VUSs) in each person's genome[84,85], prioritizing variants remains a primary challenge[56]. Genetic filters are of modest value, and with a shortened list of variants of interest, it is possible to enrich for specific variants[86], to analyse multiple family members, to examine concordance in computational algorithms[87–89] and to parse the morbid human and mouse genomes for variation[82,83]. Additional uncommon alleles may also be compared to human disease gene[90,91] and model organism databases[92,93]. These narrow approaches fail to take into consideration the potentially clinically useful trove of data from a WES or WGS experiment and are subject to a high false-negative rate for various reasons, including poor quality of sequence within a particular gene, mutational mechanisms not easily detectable by this technology and technical biases inherent to each instrument used[94].

Even in the context of a single gene or rare disorder, variant interpretation remains problematic, as a substantial fraction of alleles have poor predictive value, whereas modifier alleles are often excluded from consideration even though they can have profound phenotypic effects (BOX 1). This issue is amplified in genome-wide data. At present, for alleles that had not previously been associated with human pathology or for which there is limited biological insight (for example, model organisms and biochemical studies), *in silico* prediction algorithms (such as PolyPhen, VAAST and ESEfinder)[87,95–97] represent a common source of interpretation, and such analyses can be incorporated into clinical reports. This is problematic for two principal reasons. First, more commonly than not, such interpretations are taken at face value without an appreciation of the caveats and limitations of each algorithm. Second, the community has no metrics on the specificity or sensitivity (TABLE 3) of each of these programs to guide us with regard to possible false-positive and false-negative interpretations.

One solution that is currently in place exclusively in the research setting is the deployment of physiologically relevant functional assays that, in essence, 'functionalize' the morbid human genome. Such tools already exist for a small subset of disorders, most notably metabolic disorders, disorders of mitochondrial function and a handful of other conditions[98,99]. In addition, research studies ranging from protein stability studies to transcriptional activity and allele- and/or gene-specific animal models (for example, mice, fish, worms and flies) have all been used on multiple occasions to investigate the pathogenic potential of alleles relevant to clinical mutation findings[99–104]. However, no clinical laboratories can or do carry out such tests, and the challenge remains for functional annotation to be incorporated into clinical-grade interpretation of results. We do not envisage a time when such non-human studies will become bona fide clinical tests, as not only will they remain expensive, labour-intensive, difficult to automate and challenging to interpret in the context of human mutation, but they are outside the scope of existing regulatory guidelines in the United States (see the section below and BOX 2 on the regulation of genetic tests). Our hope is that clinical testing laboratories may collaborate with functional modelling laboratories to inform the variant findings. Consensus guidelines might be developed to annotate the hundreds of unique and/or rare alleles identified in patient genomes — and their functional consequences — in a fashion that will allow improved interpretation of genome variants and the introduction of such annotations into EMRs for patient use and health management.

Other considerations

Ethical considerations. The application of WES and WGS in the clinic has appropriately generated substantial debate in the community with regard to the delivery and impact of the information on physicians, patients and society in general[105–107]. Much consideration has been given to the ethical implications of genomic information provided to research participants (for example, see REFS 108–110), but less is known about the implications in a clinical setting[111,112]. BOX 2 discusses two of the key issues: how to handle secondary findings in whole-genome data and genetic privacy concerns.

Genetic education. Keeping pace with emerging clinical genetic technologies requires specialized genetic training as well as broad genetic literacy for patients and clinicians ordering and receiving genetics test results. In reality, genetics literacy in the United States is sorely lacking from elementary school through to medical

Variants of unknown significance (VUSs). Alterations in the sequence of a gene, the significance of which are unclear.

Box 2 | **Ethical considerations for genetic testing**

Secondary findings in genomic data

For most patients, whole-exome sequencing (WES) and whole-genome sequencing (WGS) will identify one or more novel (or rare) variants that are suspected to be disease-causing mutations but may also identify mutations that are relevant to adult medical care (for example, breast cancer and Alzheimer's disease)[146,147]. Although there is no consensus on whether and how to share this information with a patient, there is broad agreement that results must be confirmed by a clinical laboratory before returning to a patient[81]. Some advocate returning only results with certain findings of high medical importance, whereas others have proposed tiered return of results on the basis of relative risk[55]. Some clinical laboratories are exploring informed consent models to allow patients to elect what information to disclose. The Personal Genome Project, although not a clinical test, has taken the approach to return all secondary findings requested by the participant and to make WGS data on all participants publicly accessible[148,149]. Ultimately, the duty to inform patients of predictable risks could be influenced by the legal pressure and threat of malpractice[150].

Paediatric genetic testing raises the additional ethical challenge of deciding whether to test or to disclose results for adult-onset genetic conditions. There is no consensus on whether to withhold genomic information on a minor until he or she is of a consenting age to receive the data personally[151]; some policies discourage genetic testing of asymptomatic minors for adult-onset conditions such as Huntington's disease[152,153]. Longitudinal studies chronicle adverse events on minors receiving genetic test results[153], and predictive testing for Huntington's disease demonstrates minimal harm and a need to individualize to a patient's needs rather than to develop blanket policies[151–156].

Privacy and discrimination

A network of country-specific legislation protects Europeans from life and health insurance discrimination on the basis of genetics[157]. In the United States, clinical test results are subject to Health Insurance Portability and Accountability Act (HIPAA) protections; however, the HIPAA rule does not explicitly provide privacy protections for genetic information[158]. The US Genetic Information Nondiscrimination Act (GINA) of 2008 addresses this oversight to some extent[159]. GINA prohibits genetic discrimination in most health insurance and employment scenarios. However, the provisions do not apply to life insurance, disability insurance or long-term care insurance[158]. Despite the HIPAA and GINA protections, the public remains nervous about genetic information being used against them[160], and physicians are wary of genetic information being included in medical records[161]. As the applications and utility (both clinical and personal) of genetic testing expand, so too does the risk that discovered genetic information could be used against individuals. The protections of the existing US legal framework assuredly will be tested in courts. In the meantime, one key issue is how and where the delicate data resulting from WES and WGS clinical tests are hosted.

training[113,114,115]. By and large, the US public views genetics through the lens of genetic determinism. For the public to gain an understanding of polygenic inheritance and complex traits, primary and secondary genetics education must move beyond the mathematics of 'one gene, one phenotype' Mendelian inheritance[116] and embrace concepts of complex inheritance. Implementation of genomic sciences into clinical applications requires that clinicians be sufficiently versed in genetics and genomics to prevent that the result of these tests are misunderstood or misused[113,117]. The distinct role of the genetic counsellor in the genetics profession is extremely valuable in translating genetics and genomics concepts. However, the dearth of professionals trained for this role necessitates centralized telemedicine to provide broad access to genetics services[118]. Recent efforts to push genetics curricula into medical and nursing schools to attract professionals led to the successful development of core genetics competencies in nursing and medicine[113,117,119].

Genetic determinism
The idea that genes and genetic variants are the primary factor determining and shaping human traits.

Epigenomics
Describes a heritable effect on chromosome or gene function that is not accompanied by a change in DNA sequence but rather by modifications of chromatin or DNA.

Regulatory policy and standards. Regulating genetic tests continues to challenge authorities attempting to protect patients and consumers from misguided misuse of genomic technologies. This is not a new issue but one that continues to complicate existing models for regulating analytical and diagnostic tests in the United States (BOX 3) and around the world. With the emerging availability of WES and WGS in the clinic, the challenges are multiplied in some regards. For one, the analyte-specific model for regulating tests is no longer practicable when thousands or billions of analytes are assayed in a single clinical test. In addition, the burden on the regulator is evolving into one for regulating interpretation of rather than execution of results, authority for which is not clearly defined for genetic tests.

Mode of delivery. Until recently, physician and patient information exchange has been asymmetrical, if not paternalistic: patients are expected to adhere to regimens prescribed by a physician. However, it is clear that people with Internet access will seek medical information online[120,121], refuting the idea that patients want only a small amount of information or nothing more than a prescriptive regimen. We also know that the rise of 'crowdsourced' patient websites (see Further information for resources) fulfils a need that is not otherwise being met by the traditional health-care system[122–126]. We expect crowd-sourcing to raise funds for rare disease testing or to create online communities to be integral to genetic interpretation on a personal level. Current evidence indicates that most people want to know their genetic test results and want choice in whether and how to access this information[109,127–131]. With increasing public interest in and attention to genetic services and decreasing availability of genetic experts to filter the information, patients are likely to seek their own modes of information gathering.

As genome sequencing enters the clinical realm, we must develop ways to communicate relevant findings to best inform clinical practice while remaining alert to the dangers of genetic determinism. Genetic variants that appear to precipitate a phenotype may also depend on environmental factors, modifier genes, epigenomics and the additive and synergistic effects from multiple variants[57]. Even simple genetic test results can be misunderstood in clinical translation[132]. Thus, communicating complex genomic results with a range of interpretations is challenging to say the least.

Costs, coverage and implementation. The availability of clinical genetic diagnostics in the United States depends on the practicability of both development of laboratory tests and payment for laboratory services. Clinical diagnostic laboratory directors select tests for development that will fit into existing throughput platforms, maximize efficiency and costs, and be subject to minimal competition. Laboratories that hold gene patents or that have exclusive licences for genetic testing benefit from such intellectual property by restricting test development and offerings by competing laboratories[133,134]. Newer technologies carry the additional costs of validation of novel platforms for clinical use, whereas WES and WGS in particular carry

Box 3 | US regulatory policy and standards for genetic testing: a case study

The vast majority of genetic tests offered in the United States are laboratory-developed tests (LDTs; sometimes called 'home brew' tests). In the United States, the Center for Medicare/Medicaid Services (CMS) currently regulates the analytical validity of LDTs through oversight of clinical laboratories under the Clinical Laboratory Improvement Amendments (CLIA) of 1988. CLIA certification is determined and maintained through CMS or through an independent accrediting body to verify quality standards and proficiency testing (for example, the College of American Pathologists and The Joint Commission). Genetic testing is not a speciality under CLIA so is usually regulated as a high-complexity chemistry test[162]. The US Food and Drug Administration (FDA) holds discretionary power to enforce oversight of LDTs and reviews *in vitro* diagnostic (IVD) devices (or assays) marketed commercially. Several states provide additional state-specific oversight of LDTs, and New York State requires evaluation of clinical validity for state certification. Recent focus on regulation of genetic tests stem in part from the advent of direct-to-consumer marketing and offering of personal genetic and genomic tests[163]. False claims of validity or utility of genetic tests are subject to Federal Trade Commission (FTC) enforcement. In addition to regulatory authority, guidelines for testing may be developed by professional organizations, such as the American College of Medical Genetics, for both rare disease diagnostics and broader technological platforms designed for risk prediction.

The analytical validity of most genetic tests is fairly high in comparison to other chemical assays subject to CLIA certification. However, the clinical validity can vastly vary depending on the genotype and the corresponding phenotype. As such, the crux of regulation of genetic tests lies not with the evaluation of the analytical validity of the IVD device or laboratory-developed test (LDT) but with the interpretation of any discovered genomic variants in context of a particular patient and a particular phenotype. However, clinical validity is not evaluated under CLIA and only claims of an IVD device are reviewed by the FDA. Moreover, newer NGS technologies (for example, microarrays and whole-exome and whole-genome sequencing) interrogate tens of thousands of analytes rather than a single or a few analytes. This substantially complicates the review processes of laboratory tests conducted both by the FDA and CMS. It is unclear at this point how to develop sufficient evidence for test validation, what controls are appropriate for such tests and how to establish proficiency routinely within a laboratory.

substantial costs in long-term data storage and informatics for interpretation of genomic variation. Reimbursement of genetic testing services by payers depends on the level of evidence for clinical utility (or it should do), the impact of such services on clinical decision making and the cost-effectiveness of genetic testing for a diagnosis[135–138]. With these economic constraints, diagnostic tests for rare diseases are not as commercially profitable as the tests for common disorders, given the expense of validation and proficiency testing. Integration of clinical diagnostics into practice depends on the speciality that is being considered for testing, but clinical decision support tools are vital for introducing testing options into hospital and outpatient workflow, particularly within EMRs[139,140].

Conclusions

The continued erosion of sequencing costs, driven in part by increased capacity of existing technologies and improvements in chemistry, as well as the emergence of single-molecule third- and fourth-generation sequencing[141,142], such as nanopore sequencing[64], suggest that in the fullness of time, most patients entering the healthcare system will have had their genome sequenced before clinical evaluation. Therefore, the composition of genetic testing will be fundamentally altered to focus on interpretation of genomic data in the context of an individual, their immediate and long-term needs, their personal choices and their environment. This will not be an overnight revolution, not least because it will be some time before emergent genomic technologies are of a sufficient quality and of a low enough cost to be accessible to most of the world population that does not have access to high-quality health care. It is almost certain that technological problems relating to accuracy of sequencing information will shortly be solved; however, the same is not true for the challenges in interpretation.

Although a detailed discussion of interpretation paradigms deserves detailed scholarly study and robust discussion among basic sciences, clinicians and policy makers, it is important to highlight some key points. The scientific community has heavily focused on the sequencing of phenotypic extremes, derived models of genetic architecture and allelic causality from these extremes, and is now seeking to superimpose these models on the general population. Given that we have at present a poor understanding of the effect of individual alleles that are superimposed on the genetic context of the rest of the genome, these assumptions are premature. We now understand that each individual can carry dozens of non-sense mutations, some of which appear to lie in genes thought to be crucial to biological function[66]. However, discarding such alleles from clinical relevance could be fundamentally flawed in the context of other alleles, epialleles and environmental exposures. Likewise, we are troubled by the flaws in the approaches to sequencing for prenatal defects from maternal fetal blood as a guiding tool, as such efforts are still grounded on a narrow view of genetic causality. It is important to stress that, given our limited ability to predict phenotypic outcomes on the basis of the genotype, offering pre-emptive guidance might be catastrophic. From our own work, we understand that patients bearing the M390R allele in *BBS1* may have no phenotype, may develop isolated retinal degeneration or may experience the full spectrum of Bardet–Biedl syndrome. Finally, variable penetrance and variable expressivity remain acute problems in clinical management and interpretation, the genetic basis of which must be understood more fully to improve the clinical utility of WGS data[143,144].

We strongly encourage the systematic study of both patient and control populations wherein genomic data are systematically annotated with detailed clinical information and physiologically relevant biological assays. We propose that these activities will be necessary to gain a sufficient understanding of the genetic architecture of human pathology and to improve the validity of computational prediction algorithms to the point at which their implementation in the clinical setting can be executed with confidence.

Finally, amid the discussion of what information should be delivered and how, we must be diligent to avoid genetic exceptionalism and threatening paternalistic approaches. Rather, we should work on bilateral communication mechanisms and policies that facilitate the exchange of annotated genetic information, accompanied by lucid assessment of the shortcomings and risks of such data, between clinical laboratories and patients.

Epialleles
An epigenetic variant of an allele. The activity of an epiallele is dependent on epigenetic modifications such as histone deacetylation or cytosine methylation

Genetic exceptionalism
The view that genetic information, traits and properties are qualitatively different and deserving of exceptional consideration

1. Pasche, B. & Absher, D. Whole-genome sequencing: a step closer to personalized medicine. *JAMA* **305**, 1596–1597 (2011).
2. Green, E. D. & Guyer, M. S. Charting a course for genomic medicine from base pairs to bedside. *Nature* **470**, 204–213 (2011).
3. Bainbridge, M. N. *et al.* Whole-genome sequencing for optimized patient management. *Sci. Transl. Med.* **3**, 87re3 (2011).
4. Berg, J. S. *et al.* Next generation massively parallel sequencing of targeted exomes to identify genetic mutations in primary ciliary dyskinesia: implications for application to clinical testing. *Genet. Med.* **13**, 218–229 (2011).
5. Choi, M. *et al.* Genetic diagnosis by whole exome capture and massively parallel DNA sequencing. *Proc. Natl Acad. Sci. USA* **106**, 19096–19101 (2009).
6. Lupski, J. R. *et al.* Whole-genome sequencing in a patient with Charcot–Marie–Tooth neuropathy. *N. Engl. J. Med.* **362**, 1181–1191 (2010).
7. Maxmen, A. Exome sequencing deciphers rare diseases. *Cell* **144**, 635–637 (2011).
8. Ng, S. B. *et al.* Exome sequencing identifies the cause of a Mendelian disorder. *Nature Genet.* **42**, 30–35 (2010).
9. Rios, J., Stein, E., Shendure, J., Hobbs, H. H. & Cohen, J. C. Identification by whole-genome resequencing of gene defect responsible for severe hypercholesterolemia. *Hum. Mol. Genet.* **19**, 4313–4318 (2010).
10. Roach, J. C. *et al.* Analysis of genetic inheritance in a family quartet by whole-genome sequencing. *Science* **328**, 636–639 (2010).
11. Worthey, E. A. *et al.* Making a definitive diagnosis: successful clinical application of whole exome sequencing in a child with intractable inflammatory bowel disease. *Genet. Med.* **13**, 255–262 (2010).
12. Mestan, K. K., Ilkhanoff, L., Mouli, S. & Lin, S. Genomic sequencing in clinical trials. *J. Transl. Med.* **9**, 222 (2011).
13. Quail, M. A. *et al.* A tale of three next generation sequencing platforms: comparison of Ion Torrent, Pacific Biosciences and Illumina MiSeq sequencers. *BMC Genomics* **13**, 341 (2012).
14. Liu, L. *et al.* Comparison of next-generation sequencing systems. *J. Biomed. Biotechnol.* **2012**, 251364 (2012).
15. de Jong, A., Dondorp, W. J., Frints, S. G., de Die-Smulders, C. E. & de Wert, G. M. Advances in prenatal screening: the ethical dimension. *Nature Rev. Genet.* **12**, 657–663 (2011).
This Review covers prenatal screening strategies from ultrasound scans to genome-wide molecular tests and considers the important ethical questions concerning reproductive choice, autonomy rights of future children, equity of access and the proportionality of testing.
16. Phimister, E. G., Feero, W. G. & Guttmacher, A. E. Realizing genomic medicine. *N. Engl. J. Med.* **366**, 757–759 (2012).
17. Sequeiros, J. *et al.* The wide variation of definitions of genetic testing in international recommendations, guidelines and reports. *J. Commun. Genet.* **3**, 113–124 (2012).
18. Kiezun, A. *et al.* Exome sequencing and the genetic basis of complex traits. *Nature Genet.* **44**, 623–630 (2012).
19. Kitzman, J. O. *et al.* Noninvasive whole-genome sequencing of a human fetus. *Sci. Transl. Med.* **4**, 137ra76 (2012).
20. Lipman, P. J. *et al.* On the analysis of sequence data: testing for disease susceptibility loci using patterns of linkage disequilibrium. *Genet. Epidemiol.* **35**, 880–886 (2011).
21. Massaro, J. D. *et al.* Analysis of five polymorphic DNA markers for indirect genetic diagnosis of haemophilia A in the Brazilian population. *Haemophilia* **17**, e936–943 (2011).
22. Michaelides, M. *et al. ABCA4* mutations and discordant *ABCA4* alleles in patients and siblings with bull's-eye maculopathy. *Br. J. Ophthalmol.* **91**, 1650–1655 (2007).
23. Pereira Fdos, S. *et al.* Mutations, clinical findings and survival estimates in South American patients with X-linked adrenoleukodystrophy. *PLoS ONE* **7**, e34195 (2012).
24. Phylipsen, M. *et al.* Non-invasive prenatal diagnosis of β-thalassemia and sickle-cell disease using pyrophosphorolysis-activated polymerization and melting curve analysis. *Prenat. Diagn.* **32**, 578–587 (2012).
25. Kearns, W. G. *et al.* Preimplantation genetic diagnosis and screening. *Semin. Reprod. Med.* **23**, 336–347 (2005).
This paper reviews the scope of PGD to identify genetic abnormalities prior to embryo transfer and the techniques used for single cell detection of genetic variants.
26. Laurie, A. D. *et al.* Preimplantation genetic diagnosis for hemophilia A using indirect linkage analysis and direct genotyping approaches. *J. Thromb. Haemost.* **8**, 783–789 (2010).
27. Wallace, A. J. Detection of unstable trinucleotide repeats. *Methods Mol. Med.* **5**, 37–62 (1996).
28. Bakker, E. Is the DNA sequence the gold standard in genetic testing? Quality of molecular genetic tests assessed. *Clin. Chem.* **52**, 557–558 (2006).
29. Murphy, K. M., Berg, K. D. & Eshleman, J. R. Sequencing of genomic DNA by combined amplification and cycle sequencing reaction. *Clin. Chem.* **51**, 35–39 (2005).
30. SenGupta, D. J. & Cookson, B. T. SeqSharp: A general approach for improving cycle-sequencing that facilitates a robust one-step combined amplification and sequencing method. *J. Mol. Diagn.* **12**, 272–277 (2010).
31. Robin, N. H., Falk, M. J. & Haldeman-Englert, C. R. *FGFR*-related craniosynostosis syndromes. *GeneReviews* [online], http://www.ncbi.nlm.nih.gov/books/NBK1455 (updated 7 Jun 2011).
32. Katsanis, S. H. & Jabs, E. W. Treacher Collins syndrome. *GeneReviews* [online], http://www.ncbi.nlm.nih.gov/books/NBK1532 (updated 30 Aug 2012).
33. Tartaglia, M., Gelb, B. D. & Zenker, M. Noonan syndrome and clinically related disorders. *Best Pract. Res. Clin. Endocrinol. Metab.* **25**, 161–179 (2011).
34. Hageman, G. S. *et al.* Clinical validation of a genetic model to estimate the risk of developing choroidal neovascular age-related macular degeneration. *Hum. Genom.* **5**, 420–440 (2011).
35. Zanke, B., Hawken, S., Carter, R. & Chow, D. A genetic approach to stratification of risk for age-related macular degeneration. *Can. J. Ophthalmol.* **45**, 22–27 (2010).
36. Schaaf, C. P., Wiszniewska, J. & Beaudet, A. L. Copy number and SNP arrays in clinical diagnostics. *Annu. Rev. Genom. Hum. Genet.* **12**, 25–51 (2011).
37. Meschia, J. F. *et al.* Genomic risk profiling of ischemic stroke: results of an international genome-wide association meta-analysis. *PLoS ONE* **6**, e23161 (2011).
38. Tiu, R. V. *et al.* Prognostic impact of SNP array karyotyping in myelodysplastic syndromes and related myeloid malignancies. *Blood* **117**, 4552–4560 (2011).
39. Imai, K., Kricka, L. J. & Fortina, P. Concordance study of 3 direct-to-consumer genetic-testing services. *Clin. Chem.* **57**, 518–521 (2010).
40. Reid, R. J. *et al.* Association between health-service use and multiplex genetic testing. *Genet. Med.* **14**, 852–859 (2012).
41. Haines, J. L. *et al.* Complement factor H variant increases the risk of age-related macular degeneration. *Science* **308**, 419–421 (2005).
42. Klein, R. J. *et al.* Complement factor H polymorphism in age-related macular degeneration. *Science* **308**, 385–389 (2005).
43. Edwards, A. O. *et al.* Complement factor H polymorphism and age-related macular degeneration. *Science* **308**, 421–424 (2005).
44. Hageman, G. S. *et al.* A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. *Proc. Natl Acad. Sci. USA* **102**, 7227–7232 (2005).
45. Gold, B. *et al.* Variation in factor B (*BF*) and complement component 2 (*C2*) genes is associated with age-related macular degeneration. *Nature Genet.* **38**, 458–462 (2006).
46. Despriet, D. D. *et al.* Complement factor H polymorphism, complement activators, and risk of age-related macular degeneration. *JAMA* **296**, 301–309 (2006).
47. Tsuchiya, K. D. Fluorescence *in situ* hybridization. *Clin. Lab. Med.* **31**, 525–542 (2011).
48. Raphael, B. Chapter 6: structural variation and medical genomics. *PLoS Comput. Biol.* **8**, e1002821 (2012).
49. Fruhman, G. & Van den Veyver, I. B. Applications of array comparative genomic hybridization in obstetrics. *Obstet. Gynecol. Clin. North Am.* **37**, 71–85 (2010).
50. Swaminathan, G. J. *et al.* DECIPHER: Web-based, community resource for clinical interpretation of rare variants in developmental disorders. *Hum. Mol. Genet.* **21**, R37–R44 (2012).
51. Wapner, R. J. *et al.* Chromosomal microarray versus karyotyping for prenatal diagnosis. *N. Engl. J. Med.* **367**, 2175–2184 (2012).
This paper compares the accuracy, efficacy and yield of chromosomal microarray analysis to karyotyping as a primary diagnostic tool for the evaluation of developmental delay and structural malformations in children.
52. Kozlowski, P., Jasinska, A. J. & Kwiatkowski, D. J. New applications and developments in the use of multiplex ligation-dependent probe amplification. *Electrophoresis* **29**, 4627–4636 (2008).
This paper describes the MLPA technique and explores its utility in copy number variation diagnostics.
53. Hills, A. *et al.* MLPA for confirmation of array CGH results and determination of inheritance. *Mol. Cytogenet.* **3**, 19 (2010).
54. Talkowski, M. E. *et al.* Sequencing chromosomal abnormalities reveals neurodevelopmental loci that confer risk across diagnostic boundaries. *Cell* **149**, 525–537 (2012).
55. Berg, J. S., Khoury, M. J. & Evans, J. P. Deploying whole genome sequencing in clinical practice and public health: meeting the challenge one bin at a time. *Genet. Med.* (2011).
To address the challenge of interpreting WGS data for personal use, this paper describes a 'binning' approach to reporting genomic variants.
56. Cooper, G. M. & Shendure, J. Needles in stacks of needles: finding disease-causal variants in a wealth of genomic data. *Nature Rev. Genet.* **12**, 628–640 (2011).
A Review of approaches is presented here for determining pathogenicity of single-nucleotide variants using comparative and *in silico* functional genomics.
57. Majewski, J., Schwartzentruber, J., Lalonde, E., Montpetit, A. & Jabado, N. What can exome sequencing do for you? *J. Med. Genet.* **48**, 580–589 (2011).
58. Worthey, E. A. *et al.* Making a definitive diagnosis: successful clinical application of whole exome sequencing in a child with intractable inflammatory bowel disease. *Genet. Med.* **13**, 255–262 (2011).
59. Need, A. C. *et al.* Clinical application of exome sequencing in undiagnosed genetic conditions. *J. Med. Genet.* **49**, 353–361 (2012).
60. Mayer, A. N. *et al.* A timely arrival for genomic medicine. *Genet. Med.* **13**, 195–196 (2010).
61. Ormanac, R. The advent of personal genome sequencing. *Genet. Med.* **13**, 188–190 (2011).
62. Gonzaga-Jauregui, C., Lupski, J. R. & Gibbs, R. A. Human genome sequencing in health and disease. *Annu. Rev. Med.* **63**, 35–61 (2012).
63. Rehm, H. L. Disease-targeted sequencing: a cornerstone in the clinic. *Nature Rev. Genet.* **14**, 295–300 (2013).
64. Branton, D. *et al.* The potential and challenges of nanopore sequencing. *Nature Biotech.* **26**, 1146–1153 (2008).
65. Skovgaard, O., Bak, M., Lobner-Olesen, A. & Tommerup, N. Genome-wide detection of chromosomal rearrangements, indels, and mutations in circular chromosomes by short read sequencing. *Genome Res.* **21**, 1388–1393 (2011).
66. Smith, H. E. Identifying insertion mutations by whole-genome sequencing. *Biotechniques* **50**, 96–97 (2011).
67. Metzker, M. L. Sequencing technologies — the next generation. *Nature Rev. Genet.* **11**, 31–46 (2010).
This is a technical Review of NGS technologies and recent advances in commercially available NGS instruments.
68. Hedges, D. J. *et al.* Comparison of three targeted enrichment strategies on the SOLiD sequencing platform. *PLoS ONE* **6**, e18595 (2011).
69. Quail, M. *et al.* A tale of three next generation sequencing platforms: comparison of Ion Torrent, Pacific Biosciences and Illumina MiSeq sequencers. *BMC Genomics* **13**, 341 (2012).
70. Sulonen, A. M. *et al.* Comparison of solution-based exome capture methods for next generation sequencing. *Genome Biol.* **12**, R94 (2011).
71. Ashley, E. A. *et al.* Clinical assessment incorporating a personal genome. *Lancet* **375**, 1525–1535 (2010).
72. Saunders, C. J. *et al.* Rapid whole-genome sequencing for genetic disease diagnosis in neonatal intensive care units. *Sci. Transl. Med.* **4**, 154ra135 (2012).
73. Rauch, A. *et al.* Range of genetic mutations associated with severe non-syndromic sporadic intellectual disability: an exome sequencing study. *Lancet* **380**, 1674–1682 (2012).

74. de Ligt, J. *et al.* Diagnostic exome sequencing in persons with severe intellectual disability. *N. Engl. J. Med.* **367**, 1921–1929 (2012).
75. Roberts, N. J. *et al.* The predictive capacity of personal genome sequencing. *Sci. Transl. Med.* **4**, 133ra58 (2012).
76. Chen, R. *et al.* Personal omics profiling reveals dynamic molecular and medical phenotypes. *Cell* **148**, 1293–1307 (2012).
77. Zuvich, R. L. *et al.* Pitfalls of merging GWAS data: lessons learned in the eMERGE network and quality control procedures to maintain high data quality. *Genet. Epidemiol.* **35**, 887–898 (2011).
78. Pathak, J. *et al.* Evaluating phenotypic data elements for genetics and epidemiological research: experiences from the eMERGE and PhenX network projects. *AMIA Summits Transl. Sci. Proc.* **2011**, 41–45 (2011).
79. McCarty, C. A. *et al.* The eMERGE network: a consortium of biorepositories linked to electronic medical records data for conducting genomic studies. *BMC Med. Genom.* **4**, 13 (2011).
This paper describes the eMERGE (Electronic Medical Records and Genomics) network and how it is exploring the utility of DNA repositories coupled with EMR systems.
80. Belmont, J. & McGuire, A. L. The futility of genomic counseling: essential role of electronic health records. *Genome Med.* **1**, 48 (2009).
81. Richards, C. S. *et al.* ACMG recommendations for standards for interpretation and reporting of sequence variations: revisions 2007. *Genet. Med.* **10**, 294–300 (2008)
ACMG developed recommendations for standards for interpretation of sequence variations.
82. Stenson, P. D. *et al.* The Human Gene Mutation Database: 2008 update. *Genome Med.* **1**, 13 (2009).
83. Bale, S. *et al.* MutaDATABASE: a centralized and standardized DNA variation database. *Nature Biotech.* **29**, 117–118 (2011).
84. MacArthur, D. G. & Tyler-Smith, C. Loss-of-function variants in the genomes of healthy humans. *Hum. Mol. Genet.* **19**, R125–R130 (2010).
85. MacArthur, D. G. *et al.* A systematic survey of loss-of-function variants in human protein-coding genes. *Science* **335**, 823–828 (2012).
This paper determined how many genetic variants predicted to cause loss of function of protein-coding genes humans carry.
86. Ng, S. B., Nickerson, D. A., Bamshad, M. J. & Shendure, J. Massively parallel sequencing and rare disease. *Hum. Mol. Genet.* **19**, R119–R124 (2010).
87. Adzhubei, I. A. *et al.* A method and server for predicting damaging missense mutations. *Nature Methods* **7**, 248–249 (2010).
88. Kumar, P., Henikoff, S. & Ng, P. C. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nature Protoc.* **4**, 1073–1081 (2009).
89. Stone, E. A. & Sidow, A. Physicochemical constraint violation by missense substitutions mediates impairment of protein function and disease severity. *Genome Res.* **15**, 978–986 (2005).
90. Amberger, J., Bocchini, C. & Hamosh, A. A new face and new challenges for Online Mendelian Inheritance in Man (OMIM®). *Hum. Mutat.* **32**, 564–567 (2011).
91. Robinson, P. N. *et al.* The Human Phenotype Ontology: a tool for annotating and analyzing human hereditary disease. *Am. J. Hum. Genet.* **83**, 610–615 (2008).
92. Blake, J. A., Bult, C. J., Kadin, J. A., Richardson, J. E. & Eppig, J. T. The Mouse Genome Database (MGD): premier model organism resource for mammalian genomics and genetics. *Nucleic Acids Res.* **39**, D842–D848 (2011).
93. Sprague, J. *et al.* The Zebrafish Information Network: the zebrafish model organism database. *Nucleic Acids Res.* **34**, D581–D585 (2006).
94. Minoche, A. E., Dohm, J. C. & Himmelbauer, H. Evaluation of genomic high-throughput sequencing data generated on Illumina HiSeq and genome analyzer systems. *Genome Biol.* **12**, R112 (2011).
95. Houdayer, C. *et al.* Evaluation of *in silico* splice tools for decision-making in molecular diagnosis. *Hum. Mut.* **29**, 975–982 (2008).
96. Cartegni, L., Wang, J., Zhu, Z., Zhang, M. Q. & Krainer, A. R. ESEfinder: A web resource to identify exonic splicing enhancers. *Nucleic Acids Res.* **31**, 3568–3571 (2003).
97. Yandell, M. *et al.* A probabilistic disease-gene finder for personal genomes. *Genome Res.* **21**, 1529–1542 (2011).
98. Pelak, K. *et al.* The characterization of twenty sequenced human genomes. *PLoS Genet.* **6**, e1001111 (2010).
99. Zaghloul, N. A. *et al.* Functional analyses of variants reveal a significant role for dominant negative and common alleles in oligogenic Bardet–Biedl syndrome. *Proc. Natl Acad. Sci. USA* **107**, 10602–10607 (2010).
100. Chassaing, N. *et al. OTX2* mutations contribute to the otocephaly–dysgnathia complex. *J. Med. Genet.* **49**, 373–379 (2012).
101. Kato, S. *et al.* Understanding the function–structure and function–mutation relationships of p53 tumor suppressor protein by high-resolution missense mutation analysis. *Proc. Natl Acad. Sci. USA* **100**, 8424–8429 (2003).
102. Merveille, A. C. *et al.* CCDC39 is required for assembly of inner dynein arms and the dynein regulatory complex and for normal ciliary motility in humans and dogs. *Nature Genet.* **43**, 72–78 (2011).
103. Rosenthal, N. & Brown, S. The mouse ascending: perspectives for human-disease models. *Nature Cell Biol.* **9**, 993–999 (2007).
104. Siddiqui, S. S. *et al. C. elegans* as a model organism for *in vivo* screening in cancer: effects of human c-Met in lung cancer affect *C. elegans* vulva phenotypes. *Cancer Biol. Ther.* **7**, 856–863 (2008).
105. Bick, D. & Dimmock, D. Whole exome and whole genome sequencing. *Curr. Opin. Pediatr.* **23**, 594–600 (2011).
106. Drmanac, R. Medicine. The ultimate genetic test. *Science* **336**, 1110–1112 (2012).
107. Brunham, L. R. & Hayden, M. R. Medicine. Whole-genome sequencing: the new standard of care? *Science* **336**, 1112–1113 (2012).
108. Facio, F. M. *et al.* Intentions to receive individual results from whole-genome sequencing among participants in the ClinSeq study. *Eur. J. Hum. Genet.* **21**, 261–265 (2013).
109. Murphy, J. *et al.* Public expectations for return of results from large-cohort genetic research. *Am. J. Bioeth.* **8**, 36–43 (2008).
110. Fullerton, S. M. *et al.* Return of individual research results from genome-wide association studies: experience of the Electronic Medical Records and Genomics (eMERGE) network. *Genet. Med.* **14**, 424–431 (2012).
111. Jamal, S. M. *et al.* Practices and policies of clinical exome sequencing providers: analysis and implications. *Am. J. Med. Genet. A* 5 Apr 2013 (doi:10.1002/j.1552-4833.2013.35942.x).
This paper presents ACMG guidelines for clinical exome sequencing.
112. Tabor, H. K. *et al.* Informed consent for whole genome sequencing: a qualitative analysis of participant expectations and perceptions of risks, benefits, and harms. *Am. J. Med. Genet. A* **158**, 1310–1319 (2012).
113. Feero, W. G. & Green, E. D. Genomics education for health care professionals in the 21st century. *JAMA* **306**, 989–990 (2011).
114. Haga, S. *et al.* Survey of genetic counselors and clinical geneticists' use and attitudes toward pharmacogenetic testing. *Clin. Genet.* **82**, 115–120 (2012).
115. Dougherty, M. J., Pleasants, C., Solow, L., Wong, A. & Zhang, H. A comprehensive analysis of high school genetics standards: are States keeping pace with modern genetics? *CBE Life Sci. Educ.* **10**, 318–327 (2011).
116. Redfield, R. J. "Why do we have to learn this stuff?"—a new genetics for 21st century students. *PLoS Biol.* **10**, e1001356 (2012).
117. Guttmacher, A. E., Porteous, M. E. & McInerney, J. D. Educating health-care professionals about genetics and genomics. *Nature Rev. Genet.* **8**, 151–157 (2007).
118. O'Daniel, J. M. The prospect of genome-guided preventive medicine: a need and opportunity for genetic counselors. *J. Genet. Couns.* **19**, 315–327 (2010).
119. Jenkins, J. & Calzone, K. A. Establishing the essential nursing competencies for genetics and genomics. *J. Nurs. Scholarsh.* **39**, 10–16 (2007).
120. Atkinson, N. L., Saperstein, S. L. & Pleis, J. Using the internet for health-related activities: findings from a national probability sample. *J. Med. Internet Res.* **11**, e4 (2009).
121. van Uden-Kraan, C. F. *et al.* Health-related Internet use by patients with somatic diseases: frequency of use and characteristics of users. *Inform Health Soc. Care* **34**, 18–29 (2009).
122. Bell, R. A., Hu, X., Orrange, S. E. & Kravitz, R. L. Lingering questions and doubts: online information-seeking of support forum members following their medical visits. *Patient Educ. Couns.* **85**, 525–528 (2011).
123. Nambisan, P. Evaluating patient experience in online health communities: implications for health care organizations. *Health Care Manage. Rev.* **36**, 124–133 (2010).
124. Shute, N. Connecting and sharing on the Web. At 'crowd-sourced' disease sites, patients can swap stories—and data. *US News World Rep.* **146**, 82–83 (2009).
125. Swan, M. Crowdsourced health research studies: an important emerging complement to clinical trials in the public health research ecosystem. *J. Med. Internet Res.* **14**, e46 (2012).
126. Wicks, P. *et al.* Sharing health data for better outcomes on PatientsLikeMe. *J. Med. Internet Res.* **12**, e19 (2010).
127. Bollinger, J. M., Scott, J., Dvoskin, R. & Kaufman, D. Public preferences regarding the return of individual genetic research results: findings from a qualitative focus group study. *Genet. Med.* **14**, 451–457 (2012).
128. Kaufman, D., Murphy, J., Scott, J. & Hudson, K. Subjects matter: a survey of public opinions about a large genetic cohort study. *Genet. Med.* **10**, 831–839 (2008).
129. Lemke, A. A., Wolf, W. A., Hebert-Beirne, J. & Smith, M. E. Public and biobank participant attitudes toward genetic research participation and data sharing. *Publ. Health Genom.* **13**, 368–377 (2010).
130. Murphy, J. *et al.* Public perspectives on informed consent for biobanking. *Am. J. Publ. Health* **99**, 2128–2134 (2009).
131. Willison, D. J. *et al.* Consent for use of personal information for health research: do people with potentially stigmatizing health conditions and the general public differ in their opinions? *BMC Med. Eth.* **10**, 10 (2009).
132. Leighton, J. W., Valverde, K. & Bernhardt, B. A. The general public's understanding and perception of direct-to-consumer genetic test results. *Pub. Health Genom.* **15**, 11–21 (2011).
133. Carbone, J. *et al.* DNA patents and diagnostics: not a pretty picture. *Nature Biotech.* **28**, 784–791 (2010).
134. Cook-Deegan, R. *et al.* Impact of gene patents and licensing practices on access to genetic testing for inherited susceptibility to cancer: comparing breast and ovarian cancers with colon cancers. *Genet. Med.* **12**, S15–S38 (2010).
135. Grosse, S. D., Kalman, L. & Khoury, M. J. Evaluation of the validity and utility of genetic testing for rare diseases. *Adv. Exp. Med. Biol.* **686**, 115–131 (2010).
136. Eckermann, S. & Willan, A. R. Presenting evidence and summary measures to best inform societal decisions when comparing multiple strategies. *Pharmacoeconomics* **29**, 563–577 (2011).
137. Douglas, P. S. & Ginsburg, G. S. Clinical genomic testing: getting it right. *J. Cardiovasc. Transl. Res.* **1**, 17–20 (2008).
138. Dinan, M. A., Simmons, L. A. & Snyderman, R. Commentary: personalized health planning and the patient protection and affordable care act: an opportunity for academic medicine to lead health care reform. *Acad. Med.* **85**, 1665–1668 (2010).
139. Chan, I. S. & Ginsburg, G. S. Personalized medicine: progress and promise. *Annu. Rev. Genom. Hum. Genet.* **12**, 217–244 (2011).
140. Strauss, K. A., Puffenberger, E. G. & Morton, D. H. One community's effort to control genetic disease *Am. J. Publ. Health* **102**, 1300–1306 (2012).
141. Schadt, E. E., Turner, S. & Kasarskis, A. A window into third-generation sequencing. *Hum. Mol. Genet.* **19**, R227–R240 (2010).
142. Sanderson, K. Personal genomes: standard and pores. *Nature* **456**, 23–25 (2008).
143. Badano, J. L. & Katsanis, N. Beyond Mendel: an evolving view of human genetic disease transmission. *Nature Rev. Genet.* **3**, 779–789 (2002).
This Review describes muti-locus inheritance as seen in both rare and complex disorders.
144. Zaghloul, N. A. & Katsanis, N. Functional modules, mutational load and human genetic disease. *Trends Genet.* **26**, 168–176 (2010).
This is a review of approaches to model genomic variants *in vivo* to determine functionality.
145. Giess, R. *et al.* Early onset of severe familial amyotrophic lateral sclerosis with a SOD-1 mutation: potential impact of CNTF as a candidate modifier gene. *Am. J. Hum. Genet.* **70**, 1277–1286 (2002).

146. Lyon, G. J. *et al.* Exome sequencing and unrelated findings in the context of complex disease research: ethical and clinical implications. *Discov. Med.* **12**, 41–55 (2011).
147. Christenhusz, G. M., Devriendt, K. & Dierickx, K. To tell or not to tell? A systematic review of ethical reflections on incidental findings arising in genetics contexts. *Eur. J. Hum. Genet.* **21**, 248–255 (2012).
148. Lunshof, J. E., Chadwick, R., Vorhaus, D. B. & Church, G. M. From genetic privacy to open consent. *Nature Rev. Genet.* **9**, 406–411 (2008).
This paper describes the challenges of handling genome-wide data in a research setting.
149. Ball, M. P. *et al.* A public resource facilitating clinical use of genomes. *Proc. Natl Acad. Sci. USA* **109**, 11920–11927 (2012).
The Genome–Environment–Trait Evidence (GET-Evidence) tool is introduced here; it is used for processing personal whole-genome data.
150. Marchant, G. E., Milligan, R. J. & Wilhelmi, B. Legal pressures and incentives for personalized medicine. *Per. Med.* **3**, 391–397 (2006).
151. Bradbury, A. R. *et al.* Parent opinions regarding the genetic testing of minors for *BRCA1/2*. *J. Clin. Oncol.* **28**, 3498–3505 (2010).
152. Clarke, A. The genetic testing of children. Working Party of the Clinical Genetics Society (UK). *J. Med. Genet.* **31**, 785–797 (1994).
153. Duncan, R. E., Savulescu, J., Gillam, L., Williamson, R. & Delatycki, M. B. An international survey of predictive genetic testing in children for adult onset conditions. *Genet. Med.* **7**, 390–396 (2005).
154. Hawkins, A. K., Ho, A. & Hayden, M. R. Lessons from predictive testing for Huntington disease: 25 years on. *J. Med. Genet.* **48**, 649–650 (2011).
155. Cohen, C. B. Wrestling with the future: should we test children for adult-onset genetic conditions? *Kennedy Inst. Eth. J.* **8**, 111–130 (1998).
156. Robertson, S. & Savulescu, J. Is there a case in favour of predictive genetic testing in young children? *Bioethics* **15**, 26–49 (2001).
157. Van Hoyweghen, I. & Horstman, K. European practices of genetic information and insurance: lessons for the Genetic Information Nondiscrimination Act. *JAMA* **300**, 326–327 (2008).
158. Hudson, K. L. Genomics, health care, and society. *N. Engl. J. Med.* **365**, 1033–1041 (2011).
159. Department of Health and Human Services. HIPAA administrative simplification: standards for privacy of individually identifiable health information. *Federal Register* [online], https://www.federalregister.gov/articles/2009/10/07/E9-22492/hipaa-administrative-simplification-standards-for-privacy-of-individually-identifiable-health (2009).
160. Allain, D. C., Friedman, S. & Senter, L. Consumer awareness and attitudes about insurance discrimination post enactment of the Genetic Information Nondiscrimination Act. *Fam. Cancer* **11**, 637–644 (2012).
161. Haga, S. B. *et al.* Genomic risk profiling: attitudes and use in personal and clinical care of primary care physicians who offer risk profiling. *J. Gen. Intern. Med.* **26**, *834–840* (2011).
162. Hudson, K. L. *et al.* Oversight of US genetic testing laboratories. *Nature Biotech.* **24**, 1083–1090 (2006).
163. Horn, E. J. & Terry, S. F. Regulating genetic tests: issues that guide policy decisions. *Genet. Test. Mol. Biomarkers* **16**, 1–2 (2012).
164. Borry, P., Nys, H. & Dierickx, K. Carrier testing in minors: conflicting views. *Nature Rev. Genet.* **8**, 828 (2007).
165. Borry, P., Stultiens, L., Nys, H. & Dierickx, K. Attitudes towards predictive genetic testing in minors for familial breast cancer: a systematic review. *Crit. Rev. Oncol. Hematol.* **64**, 173–181 (2007).
166. Moran, C., Thornburg, C. D. & Barfield, R. C. Ethical considerations for pharmacogenomic testing in pediatric clinical care and research. *Pharmacogenomics* **12**, 889–895 (2011).

Acknowledgements
The authors thank E. Davis and M. Angrist for their thoughtful suggestions for this manuscript.

Competing interests statement
The authors declare no competing financial interests

FURTHER INFORMATION
23andMe: http://www.23andme.com
ClinVar: http://www.ncbi.nlm.nih.gov/clinvar
DECIPHER: http://decipher.sanger.ac.uk
Duke Center for Human Disease Modeling: http://www.cellbio.duke.edu/CHDM/Home.html
Duke Task force for Neonatal Genomics: https://www.dukegenes.org
Genetic Testing Registry (GTR): http://www.ncbi.nlm.nih.gov/gtr
GeneTests: http://www.ncbi.nlm.nih.gov/sites/GeneTests
GeneTests: Reviews: http://www.ncbi.nlm.nih.gov/sites/GeneTests/review
Human Gene Mutation Database (HGMD): http://www.hgmd.cf.ac.uk/ac/index.php
ISCA Consortium: http://www.iscaconsortium.org
MutaDatabase: http://www.mutadatabase.org
Online Mendelian Inheritance in Man (OMIM): http://www.ncbi.nlm.nih.gov/omim
PatientsLikeMe: http://www.patientslikeme.com
ALL LINKS ARE ACTIVE IN THE ONLINE PDF

REASONS FOR REFERRAL

While there are no screening guidelines you can use to detect all genetic disorders, the following is a list of common reasons for referral. If you are working with a family and are not sure whether a referral is appropriate, contact the Genetics center in your area.

PRECONCEPTION

1. A positive family history of a genetic disorder (e.g. fragile X syndrome, muscular dystrophy, cystic fibrosis) and concern about recurrence.

2. Members of a high-risk ethnic group (African Americans, Asians, Ashkenazi Jews).

3. Infertility or sterility problems.

4. Exposure to potential teratogenic or mutagenic agents (e.g. prior chemotherapy).

5. Maternal health (e.g. diabetes, PKU, epilepsy).

6. Consanguineous marriage (i.e. the prospective parents are related).

7. Two or more miscarriages/pregnancy losses.

8. A previous stillborn child.

9. A previous child with a genetic disorder or birth defect (e.g. neural tube defect, Down syndrome, PKU).

PRENATAL

1. Women who will be 35 years old or older at delivery.

2. A woman and/or her partner who is a carrier (or could be a carrier) for a genetic disorder.

3. A woman or her partner who carries a chromosome rearrangement or abnormality.

4. Couples with a family history of a neural tube defect or cleft lip/palate.

5. Couples with a previous child born with multiple congenital anomalies or a chromosome abnormality.

6. Women with an abnormal maternal serum screen.

7. Women exposed to an infectious disease, radiation, drugs or other environmental agents during pregnancy.

INFANTS AND CHILDREN

1. A history of intrauterine growth retardation or failure to thrive.

2. Abnormal growth patterns (e.g. short stature, obesity, excessive growth).

3. Ambiguous or abnormal genitalia; early onset of puberty.

4. Microcephaly, macrocephaly or craniosynostosis.

5. Psychomotor delay or mental retardation.

6. Hypotonia or hypertonia.

7. A family history of similar problems as seen in the patient.

8. Abnormal or unusual facial features, particularly when not familial.

9. Abnormal body and limb proportions, asymmetry between right and left or between paired structures.

10. Major - or multiple minor - congenital anomalies.

11. Metabolic disorder.

12. Muscular weakness.

13. Bleeding tendency.

14. Blindness or deafness.

15. A significant regression in developmental progress.

16. An unusual body odor.

17. Excessive or unexplained vomiting.

18. Unusual behaviors (hand biting, hand flapping, autistic symptoms, abnormal sleep patterns, etc.), especially when associated with minor malformations.

ADOLESCENTS

1. Abnormal sexual maturation.

2. Amenorrhea (failure to menstruate), delayed puberty.

3. Growth retardation.

4. Excessive tall stature.

ADULTS

1. A diagnosis of an adult onset genetic disease in the patient or a family member (e.g., Huntington disease, Marfan syndrome, myotonic dystrophy, Charcot-Marie-Tooth).

2. History of apparently familial disorders (e.g., colon cancer, breast/ovarian cancer, familial hypercholesterolemia, psychiatric or behavioral disorders).

3. Members of high-risk groups who want to pursue carrier testing for single gene disorders or chromosome abnormalities (e.g., Tay-Sachs disease, Duchenne muscular dystrophy, hemophilia, sickle cell anemia).

4. Questions about genetic diseases or birth defects in immediate or extended family members.

5. Paternity testing [*N.B. At UMass, we will refer to another provider.*]

From http://www.usd.edu/med/som/genetics/curriculum/

Updated May 2010