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Genetics, Medicine, and the Plain People

Kevin A. Strauss[1,2,3,*] and Erik G. Puffenberger[1,2,*]

[1]Clinic for Special Children, Strasburg, Pennsylvania 17579;
email: kstrauss@clinicforspecialchildren.org, epuffenberger@clinicforspecialchildren.org

[2]Department of Biology, Franklin and Marshall College, Lancaster, Pennsylvania 17603

[3]Lancaster General Hospital, Lancaster, Pennsylvania 17604

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*Equal contributors.

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Anabaptist, deme, haplotype, identity-by-descent, Mendelian, single-nucleotide polymorphism

Abstract

The Old Order Amish and Old Order Mennonite populations of Pennsylvania are descended from Swiss Anabaptist immigrants who came to the New World in the early eighteenth century. Today they live in many small endogamous demes across North America. Genetically, these demes have dissimilar allele frequencies and disease spectra owing to unique founders. Biological and social aspects of Old Order communities make them ideal for studies in population genetics and genomic medicine, and over the last 40 years, advances in genomic science coincided with investigational studies in Plain populations. Newer molecular genetic technologies are sufficiently informative, rapid, and flexible to use in a clinical setting, and we have successfully integrated these tools into a rural pediatric practice. Our studies with the Pennsylvania Plain communities show that population-specific genetic knowledge provides a powerful framework in which to prevent disease, reduce medical costs, and create new insights into human biology.

Knowledge of genetic anatomy is having a comparably strong and pervasive influence on all of medicine. . . . Genomic anatomy permits medicine to become more predictive and preventive. At the same time, diagnosis and treatment are rendered more sensitive, specific, effective, and safe.

–Victor A. McKusick, 2001 (50)

Be not conformed to this world, but be ye transformed by the renewing of your mind.

Romans 12:2

INTRODUCTION

The Old Order Amish and Old Order Mennonite (Plain) people of North America have contributed much to our understanding of human genetics. Although wary of technology, they have cooperated with clinical investigators for more than 40 years, believing the knowledge gained could help alleviate suffering in their own communities and elsewhere. The history of genetic discovery among Plain sects has broad relevance: It charts the transformation of human genetics from an esoteric discipline into a foundation of clinical medicine (50).

Beginning in 1962, inherited disorders among Old Order communities were the subject of exceptional observational studies, collected in the 1978 landmark *Medical Genetic Studies in the Amish* (1). Between 1975 and 1988, the advent of Southern blotting (80), genetic markers (17), linkage analysis, DNA sequencing (48, 76), and the polymerase chain reaction (PCR) (12) enabled investigators to describe these disorders in more precise molecular terms (31, 43, 66, 71, 74, 75, 81). Recently, single-nucleotide polymorphism (SNP) microarray technology has accelerated this process (67, 68, 89, 90) and provided fascinating details about the genetic structure of inbred populations.

Over the past two decades, the focus has shifted to treatment. Genetic studies within Old Order populations have revealed how personal genetic information can be integrated into everyday clinical practice (23, 37, 50). Here, we present original as well as previously published data that inform the larger field of genomics and fulfill a central promise of the Human Genome Project—to harness genetic knowledge to heal the sick (88), prevent disability (87), and reduce medical costs (59, 87–89).

THE PLAIN POPULATIONS

Contemporary Old Order groups fall under the collective term Plain people, which refers to Christian groups that live simply, dress plainly, and live in the modern world but remain separate from it. They include the Amish, Old Order and Conservative Mennonites, Old Order Brethren, and Hutterites (D. Kraybill, personal communication). Our clinical practice serves Old Order Amish and Mennonite people of Pennsylvania and Maryland, who are the focus of this review.

The Anabaptist movement began in 1525 amid the Protestant Reformation. It was based on the idea that baptism should be an intentional act of adults who wanted to join an austere Christian community (41, 65, 69). For this and other beliefs, early Anabaptists were tortured and killed as heretics for more than 200 years as they moved throughout Europe in exile. By the 1670s, most had settled in Switzerland (65). Jakob Amman (1656–1730), a Mennonite bishop living in the canton of Berne, believed that many Anabaptists had compromised their religious discipline to avoid persecution (41). He insisted upon the practice of shunning (*Meidung*), the social exclusion of noncompliant church members, and in 1693 forced a division among Mennonite preachers that marked the formation of the Amish church (41).

William Penn offered Anabaptists asylum in the New World, and several thousand arrived in the port of Philadelphia between 1683 and 1770. The Mennonites generally appeared earlier in the records, whereas the Amish arrived in the latter half of this migration (1727–1770). Several hundred Amish and Mennonites

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eventually settled in Lancaster County (27, 65, 69). The present-day Lancaster County Amish population of approximately 50,000 individuals is derived from perhaps 50 to 200 of these original founders (54). Most early Anabaptist settlements in Pennsylvania failed, and residual members joined later Anabaptist immigrants who moved west to Ohio, Indiana, and Illinois (54), where they remain concentrated. About 70% of contemporary Amish occupy just four counties: Lancaster County, Pennylvania; Holmes and Geauga Counties, Ohio; and LaGrange County, Indiana (54). The Old Order Amish in America have doubled in the last 16 years to approximately 226,395 individuals (D. Kraybill, personal communication).

Following the migration to North America, Amish culture became extinct in Europe (41), while forces of assimilation and change reached Anabaptist immigrants. Some regional Amish and Mennonite churches were further divided by ideological conflicts (65), and by the 1860s groups that held fast to tradition were designated the Old Order by their more progressive counterparts (41, 65). Philosophical differences, reinforced by geographical separation resulted in many endogamous subdivisions called demes.

Figure 1

This Amish boy with cartilage-hair hypoplasia has the characteristic physical features of short-limbed dwarfism, sparse hair, and small nails. He suffered from neuronal intestinal dysplasia and combined immune deficiency, rare complications of the disorder, and had a bone marrow transplant at 28 months of age. He died of intractable autoimmune hemolytic anemia one year later (photo used with parent permission).

MEDICAL GENETIC STUDIES

In 1962, Dr. Victor A. McKusick reviewed John Hostetler's *Amish Society* for Johns Hopkins University Press and recognized the potential for using the Amish to study medical genetics (**Table 1**) (54). That same year, he read about David Krusen, a Lancaster County doctor who reported a high prevalence of achondroplasia among the Amish. These events sparked a productive collaboration between McKusick and Hostetler (54), and McKusick made his first field trips to Lancaster County with Dr. Krusen (31). He soon recognized that dwarfism among the Amish was not achondroplasia, but rather two distinct recessive conditions: Ellis-van Creveld syndrome and cartilage-hair hypoplasia (**Figure 1**) (52, 53).

Over the next 16 years, the Amish were subjects of diverse genetic studies. Many of these were detailed clinical descriptions of recessive disorders, but there were also studies about blood groups (16), HLA antigens (42), immunoglobulin levels (56), chromosomal variation (35), and the heritability of complex traits (e.g., blood pressure, glucose tolerance, and cancer risk) (26, 44, 72). Seminal works from this era are collected in the 1978 publication *Medical Genetic Studies in the Amish* (1), edited by McKusick, which describes 18 previously recognized and 16 newly diagnosed Mendelian disorders among the Amish of Pennsylvania, Ohio, and Midwestern states. *Medical Genetic Studies in the Amish* established many known principles of population genetics that remain pertinent today (**Table 1**).

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Table 1 Characteristics of Plain populations conducive to genetic studies

McKusick et al. 1964 (53)
• A self-defined, closed population (little gene inflow)
• Western European origin
• Small number of founding members
• Concentrated population settlements (relative immobility)
• Extensive geneological records
• High standards of living (i.e., nutrition, hygiene) and physical health
• Strong interest in inherited illness and the healing arts
• Relatively uniform social, economic, and occupational circumstances
• Large sibships (e.g., average 5–8 per family)
• Low illegitimacy rate
• Children with birth defects or genetic disorders cared for at home
• Multiple discrete subpopulations (demes) allow comparative analysis
Molecular update, 2008
• Large haplotype blocks (small population of recent origin) from identity-by-descent around disease loci
• Mutation homogeneity (founder effect)
• Population-specific allele frequencies

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Before the advent of molecular genetic technology, McKusick used genealogical records to infer identity-by-descent for pathogenic alleles. He traced the mutation for pyruvate kinase deficiency to "Strong Jacob" Yoder and his wife, who immigrated in 1742, and traced the Ellis-van Creveld allele through 40 sibships, 73 affected individuals, and 80 parents to Samuel King and his wife, who also immigrated in the 1740s (49). Although complex pedigree analysis is seldom necessary today, these studies highlighted the advantages of genetic linkage analysis and disease gene mapping in Plain populations.

MOLECULAR GENETIC STUDIES

Population Genetics

Early investigators recognized the distinct nature of Amish demes based on their immigration history and surname distribution (49). Surnames are inherited through the paternal line, largely independent of selection or inbreeding. Deviations in their frequency within a population over time simulate random genetic drift. Presently, the name Stoltzfus accounts for 26% of Amish households in Lancaster County. Three other surnames (King, Fisher, and Beiler) each account for 10%–12% of the total. Similarly, among Weaverland and Groffdale Mennonites, the surname Martin accounts for 20% of households. Mitochondrial DNA haplotypes, inherited through the maternal line, demonstrate similar drift over the last 12–14 generations (**Table 2**). Within each Anabaptist subpopulation, pathogenic alleles may also increase (or decrease) in frequency by random chance. When the founding population is small, random allele frequency shifts from one generation to the next can be profound; some rare mutations become quite common, while others become extinct.

This underlying genetic structure made Plain populations ideal for early genetic mapping studies, which relied on large collections of patients and traditional linkage analysis. Formal linkage analysis was necessary because DNA markers were sparse and genotyping was time-consuming and expensive. Typically, 300–400 microsatellite markers were genotyped in affected individuals, their parents, and siblings in an attempt to link a phenotype to a genomic region. Many mapping studies in Plain populations have identified disease genes in this way (6, 13, 19, 20, 47, 62, 63, 66, 74, 75, 81, 92, 95).

It has long been known that isolated populations have a high incidence of rare genetic

Table 2 A frequency distribution of surnames and mitochondrial hypervariable region (HVR1) haplotypes among four Pennsylvania Amish and Mennonite demes[1]

	Groffdale Mennonites	Weaverland Mennonites	Lancaster Amish	Juniata and Mifflin Amish
Surname				
Martin	18%	21%	0%	0%
Zimmerman	18%	9%	0%	0%
Hoover	8%	6%	0%	0%
Nolt	7%	5%	0%	0%
Weaver	5%	7%	0%	0%
Stoltzfus/Stoltzfoos	0%	0%	26%	0%
King	0%	0%	12%	0%
Fisher	0%	0%	11%	0%
Beiler/Byler	0%	0%	11%	7%
Peachey	0%	0%	0%	29%
Swarey	0%	0%	0%	13%
Kanagy	0%	0%	0%	11%
Yoder	0%	0%	1%	11%
mtDNA haplotype				
16298C_16311C	20%	5%	0%	0%
1669T_16126C_16261T	13%	19%	0%	0%
Cambridge reference	8%	0%	0%	38%
16304C	7%	5%	0%	8%
1693C_16304C	6%	11%	0%	0%
16294T_16304C	2%	11%	3%	4%
16126C_16294T_16296T	4%	8%	0%	0%
16129A_16311C_16316G	2%	8%	0%	0%
16209C_16304C	1%	8%	0%	0%
16260T_16356C	0%	0%	36%	0%
16051G_16092C_16129C_16183C_16189C_16362C	4%	0%	26%	0%
16129A_16264T_16311C	0%	0%	15%	4%
16124C_16189C_16356A	0%	0%	5%	15%
16189C	2%	0%	0%	12%

[1]These data demonstrate significant genetic drift and distinct paternal and maternal lineages within Plain demes. In our local Amish population, the 16260T_16356C haplotype accounts for 36% of HVR1 haplotypes. By genealogical analysis, it can be shown that all copies of this haplotype were derived from a single ancestral female who migrated to Pennsylvania in the eighteenth century. In the Groffdale Mennonites, HVR1 16298C_16311C predominates (20%), whereas 1669T_16126C_16261T is the most common haplotype among the Weaverland Mennonites (19%). Thirty-eight percent of the Juniata and Mifflin County Amish harbor the HVR1 Cambridge reference sequence.

disorders. This, along with inbreeding and a restricted founder pool, has been taken as evidence of low genetic diversity. However, microsatellite marker (50) and SNP data challenge this view. Using Affymetrix 10K GeneChip arrays, we found that calculated heterozygosity across 9833 autosomal SNPs was 33.7% and 34.8% for Amish (N = 80) and Mennonite (N = 80) individuals, respectively. These values are similar to the 35.5% measured in an European control population (these data refer to the Affymetrix European control set for the 10K arrays; from Affymetrix, Santa Clara, CA). The comparisons may be affected by drift,

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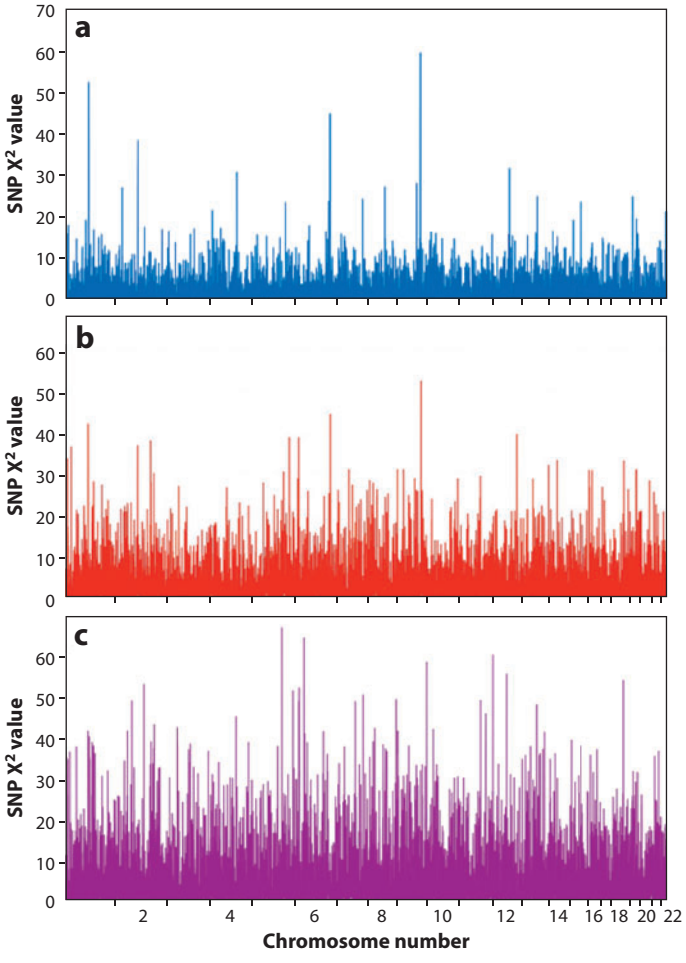
which can lead to allele fixation. Among Mennonites, 351 SNPs are monoallelic; 233 of these are also monoallelic in the European control population. Thus, 118 (i.e., 351–233) SNP alleles have become fixed in the Mennonite population, whereas 204 different SNP alleles have reached fixation in the Amish.

Further inspection of SNP allele frequencies shows that Amish and Mennonite populations are genetically dissimilar. We compared 10095 SNPs for significant allele frequency differences (chi-square > 10.828, alpha = 0.001) among Lancaster County Amish, Lancaster County Mennonites, and the Affymetrix European control set. Amish and Mennonite samples were more similar to European controls than to each other (**Figure 2**). This significant finding underscores the importance of choosing proper control groups for association and linkage mapping studies (**Figure 3**) (65). It is also relevant to clinical work; we have long appreciated that genetic diseases of Amish and Mennonite populations are quite distinct (**Table 3**) (59, 65). Of the 67 disorders we delineated among the Pennsylvania Amish and Menonnite people, only five are found in both populations and only two share the same molecular defect. Thus, for any particular child who presents with a medical problem, the genetic differential diagnosis depends critically on population of origin (59).

Random genetic drift is a compelling and parsimonious explanation for allele frequency



	Number of SNPs	Percentage
Mennonite vs. European	190	1.9
Amish vs. European	750	7.4
Amish vs. Mennonite	1493	14.8

Figure 2

Single-nucleotide polymorphism (SNP) allele frequencies are highly dissimilar between Old Order Amish and Mennonites. We performed a χ^2 analysis of allele frequencies for 10095 SNPs from the Affymetrix GeneChip Human Mapping 10K Arrays. We used 80 Amish and 80 Mennonite females and performed the analysis with high cut-off values ($\chi^2 > 10.828$, alpha = 0.001). We also compared the two Plain populations with Affymetrix European controls. Genome-wide SNP χ^2 values are plotted in panels (*a*), (*b*), and (*c*) to highlight overall differences. (*a*) The Mennonite population was moderately dissimilar to the European controls. One hundred ninety SNPs (1.9%) demonstrated statistically significant differences between these two groups. (*b*) The Amish demonstrated greater allele frequency differences; roughly 7.4% of SNPs exhibited significant differences with European controls. (*c*) The Amish and Mennonite comparison was most striking as nearly 1 in 7 SNPs showed significant allele frequency differences. We surmise that these large differences are due to genetic drift within each population. Since the Amish founding population was smaller, we expect larger shifts due to drift over time. This is evident by the comparison with European controls, where the Amish show a nearly fourfold increase in allele frequency differences as compared to the Mennonites.

shifts within a small, isolated population. However, allele frequencies might have been shaped by selection as well. For example, Anabaptist settlers were exposed to new pathogens that likely exerted significant selective pressures. Although difficult to measure, allele frequency shifts point to interesting gene candidates. From our GeneChip 10K genotype data, the SNP rs1986710 demonstrates the largest allele frequency differences between Plain populations and the Affymetrix European controls and HapMap data sets. The A allele frequency is 94% in Amish and 96% in Mennonites, compared to 54% in Affymetrix European controls. This SNP resides within an intron of *LPAR1*, a gene associated with immunity and inflammation.

When compared to control data (Americans of European descent), Amish and Mennonite samples are skewed toward larger blocks of homozygous SNPs (**Figure 4**). Within any isolated population, homozygous markers that occur in "runs" or blocks are likely autozygous (identical-by-descent); i.e., both the maternal and paternal alleles derive from a common ancestor. Now, we can use microarray data to quantify this—the genome-wide estimate of autozygosity is 4.1% and 2.5% in Amish and Mennonite individuals, respectively. Similar estimates based on homozygous megabases of genomic DNA yield comparable results (3.9% and 2.4%). This implies that the average Amish individual harbors ~120 Mb of autozygous DNA. The probability that any two random Amish individuals would be autozygous for the same genomic region and the same haplotype is very remote. Relatively high genetic diversity and low allele sharing help facilitate successful autozygosity mapping studies, even with very small sample sizes.

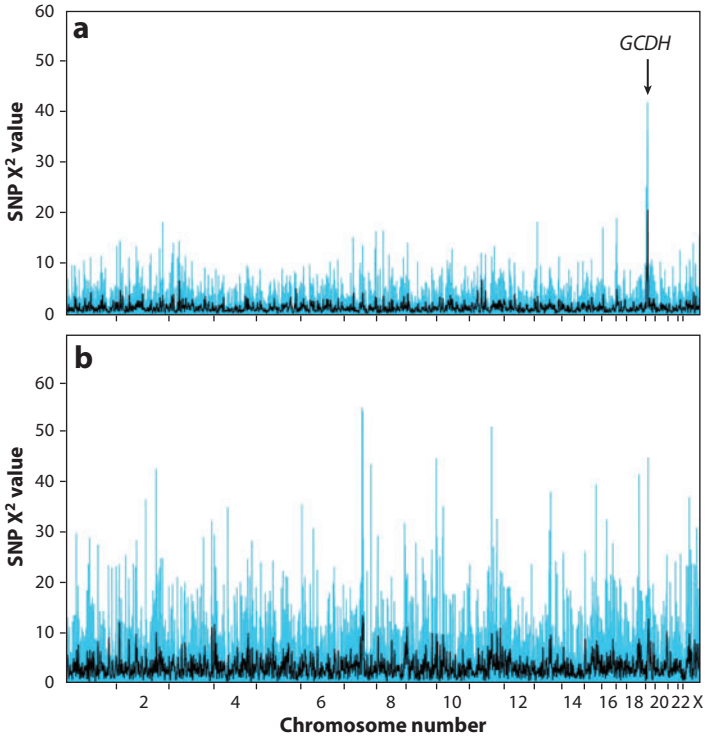


Figure 3

Population-specific SNP allele frequencies are critical for association mapping within Plain populations. As a test case, we analyzed SNP profiles of 30 Amish children with glutaric aciduria type 1 (GA1), who were all homozygous for *GCDH* c.1262C>T. The *GCDH* gene maps to a region of chromosome 19 that has poor SNP coverage on GeneChip 10K arrays, making homozygosity mapping impossible. As an alternative, we attempted to map the disease using X^2 analysis of SNP allele frequency differences between affected children and controls. This analysis depends on accurate control allele frequencies as well as extended linkage disequilibrium surrounding the disease gene. When we compared affected GA1 children to Old Order Amish controls, we easily identified the location of *GCDH*. However, when we used Old Order Mennonite allele frequency data, background noise increased and the mapping signal on chromosome 19 was obscured.

Modern Disease Gene Mapping

Large-scale SNP genotyping methodologies are a major technological breakthrough because they allow simultaneous and rapid genotyping of many markers randomly distributed across the entire genome. This reduces analytical time and cost, and allows autozygosity analysis to be done with fewer affected patients. With sufficiently dense SNP coverage, it is no longer necessary to infer genotype and haplotype sharing between distant markers; increased marker density provides enough information to reveal sharing directly. Higher marker density also simplifies analysis. Large shared blocks of homozygous SNPs can be detected with simple counting and graphing algorithms using standard spreadsheet software (67, 90). We have

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Table 3 Thirty-seven Amish and 29 Mennonite disorders understood at the molecular level among demes of Pennsylvania, Ohio, and Maryland

Disorder	Gene	DNA Variant	Protein Variant	Population	Deme*
3-methylcrotonylglycinuria	*MCCC2*	295G>C	Glu99Gln	Amish	A
3-ß-OH-steroid dehydrogenase deficiency	*HSD3B2*	35G>A	Gly11Glu	Amish	A
Aldosterone deficiency	*CYP11B2*	5 bp deletion		Amish	A
Amish microcephaly	*SLC25A19*	530G>C	Gly177Ala	Amish	A
Bardet-Biedl syndrome	*BBS1*	1169T>G	Met390Arg	Amish	A
Bartter syndrome	*CLCNKB*	gene deletion		Amish	A, B
Cardiomyopathy, dilated, with AV block	*LMNA*	568C>T	Arg190Trp	Amish	A
Cartilage-hair hypoplasia	*RMRP*	70A>G		Amish	A, O
Cortical dysplasia and focal epilepsy	*CNTNAP2*	3709delG		Amish	A, B, O
Crigler-Najjar syndrome	*UGT1A1*	222C>A	Tyr74Ter	Amish	A
Ellis-van Creveld syndrome	*EVC*	IVS13+5G>T		Amish	A
Familial hypercholanemia	*BAAT*	226A>G	Met76Val	Amish	A
Familial hypercholanemia	*TJP2*	143T>C	Val48Ala	Amish	A
Galactosemia	*GALT*	563A>G	Gln188Arg	Amish	A, E
Gittelman syndrome	*SLC12A3*	1924C>A	Arg642Gly	Amish	A, B
Gittelman syndrome	*SLC12A3*	8627 bp deletion		Amish	A, B
Gutaric aciduria, type 1	*GCDH*	1262C>T	Ala421Val	Amish	A
Gutaric aciduria, type 3	*C7orf10*	895C>T	Arg299Trp	Amish	A, B
McKusick-Kaufman syndrome	*MKKS*	[250C>T + 724G>T]	His84Tyr/Ala242Ser	Amish	A
Nemaline rod myopathy	*TNNT1*	505G>T	Glu180Ter	Amish	A
Nephrotic syndrome	*NPHS2*	413G>A	Arg138Gln	Amish	A
Osteogenesis imperfecta	*COL1A2*	2098G>T	Gly610Cys	Amish	A
Pelizaeus-Merzbacher-like syndrome	*GJA12*	203A>G	Tyr68Cys	Amish	A
Phenylketonuria	*PAH*	280-282delATC		Amish	A
Phenylketonuria	*PAH*	782G>A	Arg261Gln	Amish	A
Propionic acidemia	*PCCB*	1606A>G	Asn536Asp	Amish	A, B
Severe combined immune deficiency	*RAG1*	2974A>G	Lys992Glu	Amish	A
Sitosterolemia	*ABCG8*	1720G>A	Gly574Arg	Amish	A
Weil-Marchesani syndrome	*ADAMTS10*	15 exon del		Amish	A
Chronic granulomatous disease	*CYBB*	1335C>A	Cys445Ter	Amish	E
Troyer syndrome	*SPG20*	1110delA		Amish	O
11-hydroxylase deficiency	*CYP11B1*	1343G>A	Arg448His	Amish	B
Adenosine deaminase deficiency	*ADA*	646G>A	Gly216Arg	Amish	B
Byler disease	*ATP8B1*	923G>T	Gly308Val	Amish	B
Cockayne syndrome	*ERCC6*	IVS14+1G>T		Amish	B
GM3 synthase deficiency	*ST3GAL5*	694C>T	Arg232Ter	Amish	B, O
Primary ciliary dyskinesia	*DNAH5*	4348C>T	Gln1450Ter	Amish	B
Pyruvate kinase deficiency	*PKLR*	1436G>A	Arg479His	Amish	B
Sudden infant death with dysgenesis of the testes	*TSPYL1*	457_458insG		Amish	B
Homocystinuria	*MTHFR*	1129C>T	Arg377Cys	Amish	J

(*Continued*)

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Table 3 (*Continued*)

Disorder	Gene	DNA Variant	Protein Variant	Population	Deme*
3-methylcrotonylglycinuria	*MCCC2*	518insT		Mennonite	M, X
Alpha-1 antitrypsin deficiency	*SERPINA1*	1096G>A	Glu342Lys	Mennonite	M
Biotinidase deficiency	*BTD*	1459T>C	Trp487Arg	Mennonite	M
Congenital nephrotic syndrome	*NPHS1*	1481delC		Mennonite	M
Congenital nephrotic syndrome	*NPHS1*	3250delG		Mennonite	M
Crigler-Najjar syndrome	*UGT1A1*	222C>A	Tyr74Ter	Mennonite	M
Cystinuria	*SLC3A1*	IVS6+2T>C		Mennonite	M
Cystinuria	*SLC3A1*	1354C>T	Arg452Trp	Mennonite	M
Cystinuria	*SLC7A9*	201C>T	Ile67Ile	Mennonite	M
Cystinuria	*SLC7A9*	1166C>T	Thr389Met	Mennonite	M
Deafness, nonsyndromic	*GJB2*	35delG		Mennonite	M
Factor 11 deficiency	*F11*	1327C>T	Arg443Cys	Mennonite	M
Fragile X syndrome	*FMR1*	(CGG)n expansion		Mennonite	M
Glycogen storage disease, type 6	*PYGL*	IVS13+1G>A		Mennonite	M
Hirschsprung disease	*EDNRB*	828G>T	Trp276Cys	Mennonite	M, S
LYK5 deficiency	*LYK5*	7304 bp deletion		Mennonite	M
Maple syrup urine disease	*BCKDHA*	1312T>A	Tyr438Asn	Mennonite	M, S
Medium-chain acyl-CoA dehydrogenase deficiency	*ACADM*	985A>G	Lys329Glu	Mennonite	M
Medium-chain acyl-CoA dehydrogenase deficiency	*ACADM*	IVS4-30A>G		Mennonite	M
Mevalonate kinase deficiency	*MVK*	803T>C	Ile268Thr	Mennonite	M
Mevalonate kinase deficiency	*MVK*	1174G>A	Ala392Thr	Mennonite	M
Phenylketonuria	*PAH*	IVS12+1GA		Mennonite	M
Phenylketonuria	*PAH*	782G>A	Arg261Gln	Mennonite	M
Propionic acidemia	*PCCB*	1606A>G	Asn536Asp	Mennonite	M, S
Restrictive dermopathy	*ZMPSTE24*	54_55insT		Mennonite	M
Severe combined immune deficiency	*IL7R*	2T>G		Mennonite	M
Spinal muscular atrophy	*SMN1*	exon 7 deletion		Mennonite	M
Tyrosine hydroxylase deficiency	*TH*	698G>A	Arg233His	Mennonite	M
Tyrosinemia, type 3	*HPD*	85G>A	Ala29Thr	Mennonite	M
Vitamin B_{12} deficiency	*AMN*	44 bp deletion		Mennonite	M
Properdin deficiency	*PFC*	379T>G	Cys127Gly	Mennonite	X
Osteoporosis-pseudoglioma syndrome	*LRP5*	1225A>G	Thr409Ala	Mennonite	S
Osteoporosis-pseudoglioma syndrome	*LRP5*	1275G>A	Trp425Ter	Mennonite	S
Phenylketonuria	*PAH*	IVS10-11G>A		Mennonite	S
Cardiomyopathy, hypertrophic	*SLC25A4*	523delC		Mennonite	W
Periodic fever (TRAPS)	*TNFRSF1A*	362G>A	Arg121Gln	Mennonite	W
Salla disease	*SLC17A5*	115C>T	Arg39Cys	Mennonite	W
Tyrosinemia, type 3	*HPD*	479A>G	Tyr160Cys	Mennonite	W
Tyrosinemia, type 3	*HPD*	1005C>G	Ile335Met	Mennonite	W

*A, Lancaster County (PA) Amish; B, Juniata and Mifflin County (PA) Amish; O, Holmes and Geauga County (OH) Amish; J, Somerset County (PA) Amish; M, Weaverland and Groffdale Conference Mennonites (PA and elsewhere); S, Stauffer Mennonites (PA/MD); W, Western MD; X, other unspecified Mennonites; E, eastern shore of Maryland Amish.

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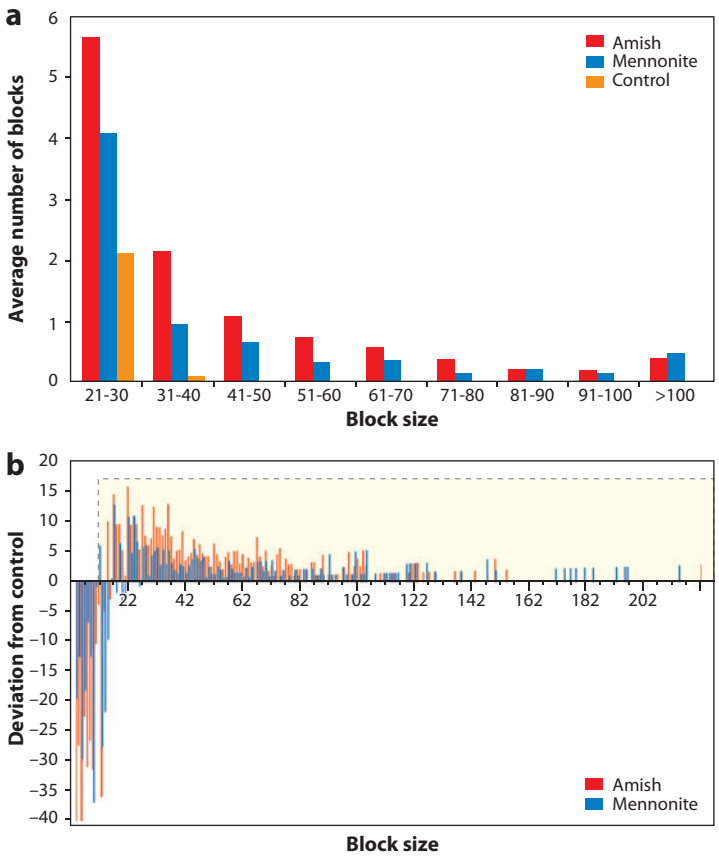

Figure 4

Plain populations demonstrate increases in homozygous block size and number compared to Americans of European descent. Blocks of homozygosity were quantified by their SNP content and physical size. (*a*) The average number of homozygous SNP blocks per person is shown. Amish and Mennonite individuals have more (and larger) runs of homozygosity than do controls. (*b*) The figure plots deviations from the average control block data by size (i.e., number of SNPs contained in each homozygous block). The Plain populations are relatively deficient in small homozygous runs, which are replaced by larger blocks. The excess runs of homozygosity range in size from about 20 to over 200 adjacent SNPs (*highlighted area*). For each Plain population, excess homozygosity relative to European controls is found in a relatively small number of large blocks, comprising roughly 116.7 and 72.5 Mb of homozygous DNA in Amish and Mennonite genomes, respectively.

used these techniques to map disorders with as few as two affected children (89).

We have applied various methods to identify 75 different mutations segregating in the Plain populations of southeastern Pennsylvania and elsewhere. The most straightforward method is candidate gene analysis—sequencing a candidate gene known to cause a phenotype. Phenylketonuria mutations in several unrelated Old Order Amish patients from Lancaster County were identified in this way, and the same strategy was applied to disorders such as 5,10-methylenetetrahydrofolate reductase (MTHFR) deficiency, factor XI deficiency, and Crigler-Najjar syndrome.

A related approach, candidate gene localization, uses SNP data to narrow a list of candidate genes that are associated with a particular phenotype and scattered across the genome, provided the child is autozygous surrounding the disease gene locus. By correlating homozygous blocks to the location of candidate genes, we can often identify a single gene for targeted sequencing. This method has been used successfully to map autosomal recessive deafness, Cockayne syndrome, megaloblastic anemia, and hereditary spastic paraplegia (**Figure 5**).

Finally, disease gene mapping uses genetic marker data to map unknown recessive phenotypes by comparing homozygous blocks among multiple affected individuals (67, 68, 89, 90). This strategy exploits genetic properties common to young populations with a small number of founders, and also assumes mutation homogeneity. We often delineate locus boundaries rapidly and efficiently, but the shared homozygous blocks tend to be quite large. The average shared block is 6.3 Mb (mean 3.7 patients per study) and contains dozens and sometimes hundreds of genes. Thus, although finding the relevant block can be simple, identifying the disease gene within it can be daunting.

We have initiated over 40 separate genetic mapping studies in Plain populations of Pennsylvania, Maryland, Indiana, and Ohio. For 14 of these, we unequivocally mapped the disease gene and identified the pathogenic sequence variant. For 10 others, we delineated a chromosomal region but have yet to identify the causative gene (**Table 4**). For the remainder, mapping results are inconclusive due to insufficient sample size or the presence of multiple shared homozygous blocks.

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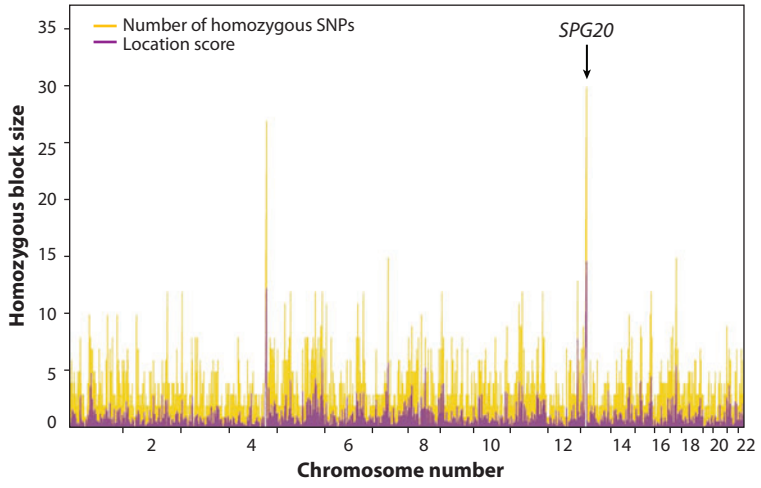

Locus	Location
SPG23	1q24
SPG30	2q37
SPG14	3q27
SPG25	6q23
SPG5A	8p12
SPG27	10q22
SPG20	13q13
SPG24	13q14
SPG32	14q
SPG28	14q21
SPG15	14q23
SPG11	15q14
SPG21	15q21
SPG7	16q24
SPG5B	
SPG35	

Figure 5

Two brothers from a Midwestern Amish deme presented to our clinic with developmental delay, short stature, and symmetric spastic diplegia. Recessive hereditary spastic paraplegia (SPG) was considered in the genetic diagnosis, but a review of *Online Mendelian Inheritance in Man* revealed multiple SPG loci scattered throughout the genome. This daunting gene list would likely preclude further genetic testing. However, assuming identity-by-descent for the disease gene, we used 10K SNP data to exclude the large majority of SPG loci and show that both boys were homozygous for a large block of DNA surrounding the locus for Troyer syndrome (SPG20); sequencing confirmed homozygosity for the known single-base-pair deletion (1110delA) in *SPG20*.

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MENDELIAN DISORDERS IN PLAIN POPULATIONS

The Evolution of Clinical Methods

Amish genetic studies between 1964 and 1988 predated the era of molecular mapping and did not deal explicitly with the problem of providing care. Many research subjects died or were crippled because they lacked appropriate medical services (57). In 1989, Holmes and Caroline Morton established the small nonprofit Clinic for Special Children to serve Amish and Mennonite children who suffered from genetic disorders (7, 8). The Clinic was sited in rural Lancaster County (**Figure 6**), which gave families nearby access to affordable care (60) and provided a framework for studying genetic disorders in a natural setting (41, 45). It is a paradigm of community genetics (46), designed to serve particular people living in a specific place and time (15, 57, 59, 65). The Old Order communities support the work as a way to care for their own (8, 59). Today, Clinic staff manage 1029 active patients representing over 105 different Mendelian disorders.

From the Clinic's inception, on-site laboratory studies were continually shaped by the needs of patients; research and clinical care were inseparable. Between 1989 and 1993, a gas chromatograph-mass spectrometer donated by Hewlett-Packard was used to screen 1232 Amish children for glutaric aciduria type 1 (GA1) (58). In 1990, two Mennonite churches donated a high-performance liquid chromatograph that has run more than 17,000 amino acid samples for the diagnosis and management of maple syrup urine disease (MSUD) (59). In the early years of the Clinic, operating these instruments on the front line of clinical practice meant that GA1 could be diagnosed within a few hours of life (i.e., from amniotic fluid obtained at delivery) (58), and MSUD could be detected in asymptomatic newborns less than 1 day old (60). These methods were a crucial

Table 4 Twenty-three genetic disorders mapped using SNP microarrays at the Clinic for Special Children since 2003. The causative gene was identified for 14 conditions, whereas 9 other disorders are mapped to a region but the gene remains unknown

Disease	Chromosome	Gene
Nephrotic syndrome, Amish	1	*NPHS2*
Pelizaeus-Merzbacher-like syndrome	1	*GJA12*
Bartter syndrome	1	*CLCNKB*
Hypertrophic cardiomyopathy	4	*SLC25A4*
Primary ciliary dyskinesia	5	*DNAH5*
Sudden infant death with dysgenesis of the testes	6	*TSPYL1*
Salla disease	6	*SLC17A5*
Cortical dysplasia and focal epilepsy	7	*CNTNAP2*
Glutaric aciduria, type 3	7	*C7orf10*
Severe combined immune deficiency	11	*RAG1*
Deafness, Mennonite	13	*GJB2*
Gitelman syndrome	16	*SLC12A3*
Polyhydramnios, megalencephaly, and symptomatic epilepsy	17	*STRADA*
Weil-Marchesani syndrome	19	*ADAMTS10*
Posterior column ataxia with retinitis pigmentosa	*1*	
Congenital nystagmus	*1*	
Usher-like syndrome	*5*	
Hydrocephalus	*6*	
Lethal seizure syndrome	*7*	
Mental retardation, nonsyndromic	*12*	
Junctional ectopic tachycardia	*15*	
Spastic paraplegia	*15*	
"Yoder" dystonia	*15*	
"Schwartz" syndrome	*20*	
Microcephaly	*22*	

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first step toward disease prevention, allowing affected newborns to start therapy days before state screening results were complete.

In 1999 we began direct DNA sequencing, which led quickly to mutation identification for numerous disorders and carrier testing for GA1, MSUD, and several other conditions (58, 60, 83, 91). Affymetrix donated a GeneChip scanner in 2005. It proved a powerful tool for mapping (67, 88, 89) and also revealed important details about allele dynamics within Plain populations. In 2007, we acquired a Roche real-time PCR system (LightCycler) through charitable contributions from the A.J. Stamps Foundation and CSL Behring. This instrument uses melting curve analysis to detect mutations in about an hour (94). It is ideal for community-based genetic testing [see sidebar 1, 5,10-Methylenetetrahydrofolate Reductase (MTHFR) Deficiency] and can be adapted to practical clinical problems such as low-resolution HLA matching (88).

Spectrum of Mendelian Disease

Table 3 lists 29 Mennonite and 37 Amish disorders understood at the molecular level, restricted for the purpose of this review to populations of Pennsylvania and Maryland. Although there is some crossover, demes of Ohio,

Indiana, and elsewhere suffer from different genetic disorders. Even within Pennsylvania, there are several genetically distinct groups, and these distinctions are clinically important (see sidebar 2, Genetic Epidemiology).

Ninety percent of the conditions listed in **Table 3** are recessive, and 90% of these result from a single allele restricted to a particular deme (54, 55). However, there are several exceptions. Among Old Order Mennonites, who stem from a larger founding population, we have identified multiple mutations for phenylketonuria, cystinuria, medium-chain acyl-CoA dehydrogenase deficiency, mevalonate kinase deficiency, osteoporosis-pseudoglioma syndrome, and tyrosinemia type 3. Among the Amish, biotinidase deficiency, galactosemia, Gitelman syndrome, and phenylketonuria demonstrate mutation heterogeneity, and familial hypercholanemia exhibits locus heterogeneity.

Although most of the disorders we manage are recessive, we have also identified several X-linked (e.g., fragile X syndrome, chronic granulomatous disease, and properdin deficiency) and dominant conditions (e.g., *LMNA* cardiomyopathy and polycystic kidney disease), and recognize others yet to be mapped (symphalangism, multiple exostoses, arteriovenous malformation). Although fewer in number, these disorders nevertheless exact a heavy toll when they occur in large extended families. We have also found de novo mutations in a child with lissencephaly (*PAFAH1B1* deletion), a child with Apert syndrome (*FGFR2)*, an adult with idiopathic torsion dystonia (*DYT1*), and several children with Prader-Willi or Angelman syndrome (chromosome 15 deletions). Finally, using 10K SNP arrays we have identified chromosomal abnormalities at the resolution of a standard karyotype in numerous children with syndromic developmental delay (**Figure 7**).

Although certain mutations are concentrated in Amish and Mennonite demes, they are seldom unique to these groups. About 20% of individuals leave the Old Order each generation, allowing mutations to flow into

Figure 6

The Clinic for Special Children is sited on an untillable corner of farmland in rural Lancaster County, Pennsylvania. The land was the gift of an Amishman who has two granddaughters with glutaric aciduria type 1. It was built with the donated labor of Amish and Mennonite workers. The Clinic is readily accessible to most of its patients and has on-site biochemical and molecular testing.

SIDEBAR 1. 5,10-METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR) DEFICIENCY

An Amish boy, born in Somerset County in 1998, had psychomotor delay and slow head growth by 3 months of age, and an MRI showed global cerebral atrophy and hypomyelination. He was seen by numerous pediatric subspecialists but remained without a diagnosis after more than $10,000 of diagnostic testing. We saw him in October 2003, and based on a biochemical workup identified a pathogenic 1129C>T change in *MTHFR* (87). Despite treatment with high-dose betaine, he remained severely retarded, autistic, and mute. We made several field trips to Somerset County where we identified 68 *MTHFR* c.1129C>T carriers among 230 healthy members of the deme (estimated population carrier frequency 30%). In collaboration with Dr. Naylor and his colleagues, we developed a LightCycler method for detecting *MTHFR* c.1129C>T in dried filter paper blood spots (28). Midwives offered Amish parents the option of getting a "fifth blood spot" to test for the *MTHFR* mutation. The first newborn diagnosed by LightCycler screening in 2004 was sister to the proband (87). She started therapy during her second week of life and has no signs of brain damage after four years of follow-up.

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SIDEBAR 2. GENETIC EPIDEMIOLOGY

Most Amish babies are delivered at home, breastfeed, and do not receive parenteral vitamin K. In Lancaster County, two recessive alleles in the Amish (*TJP2* c.143T>C and *BAAT* c.226A>G) disrupt bile salt metabolism and result in vitamin K malabsorption (20). Thus genetic factors interact with cultural practice to determine actual risk: over the past 10 years, three Amish infants with bile salt disorders presented with massive intracerebral and retinal hemorrhages from vitamin K deficiency. One child survived with dense spastic hemiparesis and unilateral blindness. The other two died of acute brain injuries. In one case, the parents were wrongfully accused of child abuse and temporarily lost custody of their other children. Screening for bile salt disorders and neonatal vitamin K administration are pressing public health concerns against this genetic background. In Juniata and Mifflin Counties, a mutation in *TSPYL* (457_458insG) among Amish children causes brain disease and a dysautonomia syndrome (SIDDT) that accounts for 20% of overall infant mortality and perhaps half of all sudden infant deaths in the region (67). To prevent sudden infant death in Mifflin County, efforts to understand and treat SIDDT syndrome are at least as important as the "back to sleep" campaign (**http://nichd.nih.gov/sids/**).

the outbred population (59). Moreover, most pathogenic alleles probably existed in Europe before the Anabaptist migration. For example, all four Plain mutations of the *PAH* gene were first identified in Europeans with phenylketonuria (**Table 3**), and the only *GCDH* variant known to cause glutaric aciduria type 1 in the Amish (c.1262C>T) is among the more common mutations found throughout Europe and the United States. Curiously, several founder mutations from Finland are also distributed in North American Plain sects (i.e., *RMRP* c.70A>G and *SLC17A5* c.115C>T). There are no known genealogical ties between Swiss Anabaptists and Finns, suggesting that these mutations are either ancient or recurrent (51).

For 21 (31%) conditions listed in **Table 3**, an experienced clinician can make a correct diagnosis based on the clinical presentation and a single confirmatory test. A few others are distinctive and easily recognized (e.g., Amish lethal microcephaly, Ellis-van Creveld syndrome, Weil-Marchesani syndrome, etc.). Only 8 (12%) are reliably detected by state newborn screening. Importantly, about 40% of the disorders cannot be diagnosed by routine clinical methods; they present to physicians as nonspecific problems such as failure to thrive, jaundice, bruising, itching, developmental delay, cerebral palsy, mental retardation, autism, seizures, short stature, or weakness. An understanding of genetic risks within a population makes it clear that these general terms apply to many discrete causes (**Table 5**) (see sidebar 3, Glutaryl-CoA Dehydrogenase Deficiency). The same is likely true in outbred populations, but it is much harder to investigate.

When we encounter a novel phenotype, investigational studies are needed to determine the molecular cause and then translate this information into useful clinical testing (67, 68, 90). Many core genetic laboratories offer DNA isolation and storage as well as biochemical genetics testing. However, few offer the flexible genotyping and sequencing services needed to map a new disease. Fewer still achieve these aims within a clinically relevant time frame or at an acceptable cost to self-pay families. Our laboratory functions as a specialized core facility with the sole purpose of solving patient- and community-centered problems quickly and economically (88). We have moved the tools of genetic research to the clinical laboratory and invested heavily in their continued use and efficacy. Consequently, when evaluating a new patient we are as likely to sequence a gene or generate SNP genotypes as we are to order an electrolyte profile.

TRANSLATIONAL MEDICINE AND THE TREATMENT OF GENETIC DISEASE

Treatment of Genetic Disease

Just over 20 years ago, half of known genetic disorders were considered untreatable and only about 10% could be treated in a highly effective

manner (93). We have seen extraordinary progress in Plain populations over the past two decades. For about 40% of the disorders we manage, simple dietary intervention has a decisive effect. These include conditions such as biotinidase deficiency, galactosemia, and vitamin B_{12} malabsorption. Protocols for the treatment of maple syrup urine disease (MSUD), Crigler-Najjar syndrome, and severe combined immune deficiency (SCID) are more complex but still quite effective (2, 57, 60, 91). These latter conditions can now be cured by tissue transplant, currently the only reliable form of gene therapy (85, 91). Problems such as pyruvate kinase deficiency, cystinuria, and deafness are treatable, but not perfectly so. Nevertheless, like diabetes, depression, and cancer, skillful medical care allows people to live longer, suffer less, and enjoy more independence.

For 13 (21%) genetic conditions listed in **Table 3**, eventual outcome is not affected by treatment; five of these disorders are lethal during infancy. They are among the most common genetic disorders we encounter; among the Amish, for example, we have a record of 85 children with lethal microcephaly born between 1960 and 2008 and 88 children born with nemaline rod myopathy (*TNNT1*) since 1989

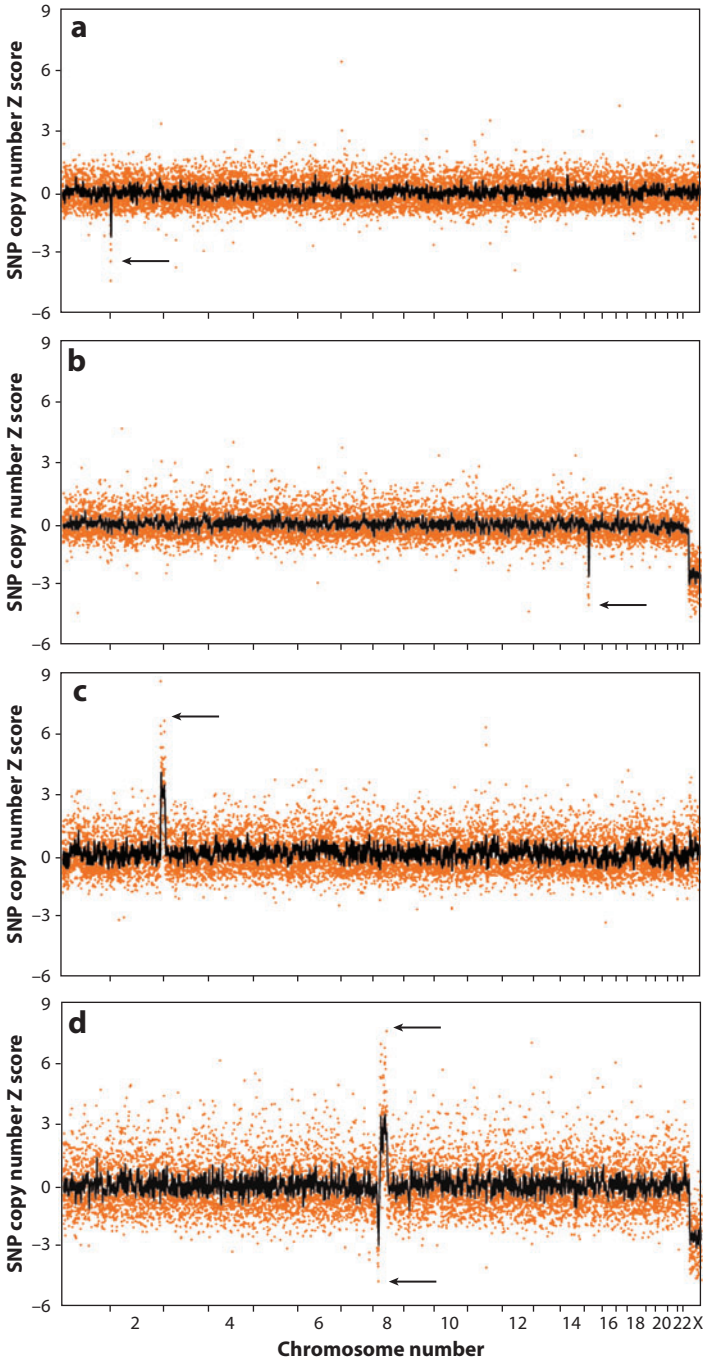

Figure 7

Affymetrix GeneChip Human Mapping 10K Arrays are useful for copy-number assessment. Although the 10K arrays lack fine resolution for this application, our data show that they perform at least as well as a standard karyotype. We readily identify copy-number abnormalities in the 2–4 Mb range, validated by standard metaphase karyotype (**http://www.mayomedicallaboratories.com/**). Our analyses use the copy-number data generated by the Affymetrix Genotype software. We calculate mean and standard deviations for the fluorescence at each SNP marker and then generate Z scores for the patient at each SNP marker. This partially corrects for polymorphic copy-number variation within the genome. Panels (*a*) and (*b*) show two small deletions of ~4.1 and ~4.2 Mb, respectively. The first patient had multiple congenital anomalies, and the second patient was diagnosed with Angelman syndrome after SNP analysis detected monosomy on chromosome 15. Panels (*c*) and (*d*) depict trisomy of chromosome 2q markers and a complex rearrangement involving partial deletion and duplication of chromosome 8. Data in panels (*b*) and (*d*) are from males, and hemizygosity for X chromosome markers is apparent.

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SIDEBAR 3. GLUTARYL-CoA DEHYDROGENASE DEFICIENCY

Before 1989, Amish children with GA1 were commonly misdiagnosed with cerebral palsy or acute viral encephalitis. The first genetic study at the Clinic for Special Children identified GA1 as the cause of "Amish cerebral palsy" in 16 disabled children who traced to a single founding couple (58). Beginning in 1989, the Clinic offered amniotic fluid and urine organic acid screening for newborns, comprehensive pediatric follow-up care, and inpatient management for illnesses. Dr. Naylor introduced statewide supplemental screening for GA1 in 1994. Between 1989 and the present, the rate of brain injury in GA1 decreased from 94% to 36% (83). Thus the efficacy of treatment in GA1, as with many genetic conditions, is relative. Although GA1 is the most common mimic of cerebral palsy in Lancaster County, other hereditary conditions can produce a similar phenotype and, equally importantly, not every child with dystonia has a Mendelian disorder. Acquired causes of brain injury are intermingled with, and difficult to distinguish from, genetic disease.

(43, 59). Even for such "untreatable" problems, medical care is important (57, 59). Before the Clinic was established, Amish children with nemaline rod myopathy were repeatedly subjected to invasive and costly interventions (e.g., muscle biopsies, nerve conduction testing, electromyography, magnetic resonance imaging, echocardiograms, etc.) that collectively cost the community millions of dollars. A thoughtful physician informed with the right genetic diagnosis can help a family determine appropriate limits of medical care while also protecting the child from futile interventions and the common miseries of hunger, thirst, dyspnea, and pain.

Presymptomatic Diagnosis and Disease Prevention

Children born today with conditions such as GA1, MTHFR deficiency, or MSUD—once thought untreatable (39)—now grow up healthy if diagnosed early in life (see sidebars 1, 3, and 4) (60, 83, 87). Presymptomatic diagnosis creates a window of opportunity (22, 33, 40, 83). This was the idea behind Guthrie & Susi's test for phenylketonuria in newborns, published in 1963 (36). Thirty years later, shortly after Dr. Morton began his work in Lancaster County, Edwin Naylor and colleagues were using tandem mass spectrometry to screen newborns for a much broader spectrum of genetic conditions (21).

Screening does not, however, assure good outcome. Early diagnosis is of no value without a pragmatic follow-up plan designed to deal with the dynamic interplay among genes, environment, and disease as it unfolds over time. Genes encode proteins embedded in complex biological networks. These networks are contained within cells, grouped into tissues, and able to interact within physiological systems. Understanding disturbances at all of these levels, as experienced by intact humans in their natural setting, is the basis for designing effective therapies (38, 70). This is vividly illustrated by the history of MSUD in Lancaster County (59).

Before 1989, one-third of Mennonite children with MSUD died during childhood. Most who survived were severely disabled [see sidebar 4, Changing the Natural History of Maple Syrup Urine Disease (MSUD)] (57, 59, 60). Since the Clinic started offering local services, outcomes for MSUD have improved (60). No child died while under our care for metabolic crisis, and all Mennonite children with MSUD born after 1988 attained normal developmental milestones. Knowledge of pathophysiology, not genetics, fueled this progress. Through careful study of many individuals in diverse clinical circumstances, we learned how deficiency of branched-chain ketoacid dehydrogenase disrupts critical biological processes such as metabolic adaptation to fasting and illness (86), cerebral amino acid transport (86, 87), and cell volume control (60). These processes change with age and are influenced by many nutritional and environmental variables. Our treatment protocol evolved in lockstep with our understanding of this complex physiology (60) and allowed us to prevent many serious neurological consequences of MSUD.

Table 5 Gene mutations result in specific medical conditions that are often described with nonspecific terms: mental retardation, cerebral palsy, autism, sudden infant death, sepsis, and epilepsy[1]

Clinical diagnosis	Disorder	Gene
Cerebral palsy	Glutaric aciduria type 1	*GCDH*
	Propionic acidemia	*PCCB*
	Gap junction deficiency	*GJA12*
	Crigler-Najjar syndrome	*UGT1A1*
	Hereditary spastic paraplegia	*SPG20*
	Dopamine-responsive dystonia	*TH*
	Idiopathic torsion dystonia	*DYT1*
Mental retardation	Phenylketonuria	*PAH*
	MTHFR deficiency	*MTHFR*
	Maple syrup urine disease	*BCKDHA*
	Salla disease	*SLC17A5*
	Bardet-Biedl syndrome	*BBS1*
	Fragile X syndrome	*FMR1*
Epilepsy	Biotinidase deficiency	*BTD*
	CDFE syndrome (CASPR2)	*CNTNAP2*
	GM3 synthase deficiency	*ST3GAL5*
	PMSE syndrome	*STRADA*
Stroke/hemorrhage	Hypercholanemia,TJP2 type	*TJP2*
	Hypercholanemia, BAAT type	*BAAT*
	Factor V Leiden	*F5*
	Sitosterolemia	*ABCG8*
	Alpha-1-antitrypsin deficiency	*SERPINA1*
Sudden death	SIDDT syndrome	*TSPYL*
	3-beta-HSD deficiency	*HSD3B2*
	Hypertrophic cardiomyopathy	*SLC25A4*
Lethal infection	Galactosemia	*GALT*
	Properdin deficiency	*PFC*
	SCID, IL7 receptor type	*IL7R*
	Adenosine deaminase deficiency	*ADA*
	RAG1 deficiency, Omenn	*RAG1*

[1]This table lists a selection of genetic syndromes that cause developmental disorders in Amish and Mennonite children. Recognizing genetic predispositions that underlie such disorders is a key step toward planning effective prevention strategies.

Disease prevention has substantial economic repercussions (57). Based on our experience with MSUD and several other treatable genetic conditions that affect the nervous system (**Table 3**), we have prevented severe motor disability or mental retardation in an estimated 150 children over a 20-year period. This saved the Plain communities about $180 million in direct and indirect costs (3). Thus annually, our operating budget of $1.4 million reduces the community economic burden by over $8 million.

To help uninsured patients, preventative therapies must be affordable. For example, early

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SIDEBAR 4. CHANGING THE NATURAL HISTORY OF MAPLE SYRUP URINE DISEASE (MSUD)

Between 1966 and 1988, 36 Mennonite children were born with MSUD in Lancaster County. They all became encephalopathic within the first week of life and required weeks or months of intensive hospital treatment that cost tens of thousands of dollars. Follow-up care was expensive, far away, and disorganized (59, 73). MSUD patients were often sick at home for days before arriving comatose in Philadelphia with critical cerebral edema. No aspect of therapy dealt specifically with this complication, and 14 (36%) of these children died of brain herniation within 24 h of hospitalization (59, 73).

Since 1989, we have managed 59 Mennonite infants with MSUD. Thirty-two (59%) were diagnosed between 12 and 24 h of life by on-site amino acid analysis or, more recently, direct mutation detection from umbilical cord blood using real-time PCR. Among infants diagnosed before 3 days of age, 94% were treated safely at home. To manage crises locally, we made MSUD hyperalimentation solution available 24 h a day at Lancaster General Hospital and had on-site amino acid analysis with a 30-min turnaround time. We have successfully managed over 200 metabolic crises at Lancaster General with an average hospital stay of just 4 days. By combining general pediatric care and specialized services, a much larger number of metabolic crises were averted out of hospital; the overall hospitalization rate for our MSUD cohort is currently only 0.4 hospital days per patient per year.

studies of vitamin B_{12} deficiency led to reliable and inexpensive forms of replacement therapy (61). Now, for Mennonite children with hereditary B_{12} malabsorption (*AMN* deletion), once daily sublingual methyl-B_{12} prevents life-threatening anemia and spinal cord injury for about $30 per year. However, if sublingual methyl-B_{12} were to be developed as a new therapeutic today, it would cost between $500 million and $2 billion to bring to the U.S. market, and its cost would be prohibitive for many self-pay families (4). Although we can expect exciting new advances in the treatment of genetic disease, attendant costs will put effective therapies beyond the reach of some families.

PLAIN PEOPLE AT THE FRONTIER OF GENOMIC MEDICINE

When McKusick and colleagues began their studies of the Amish in 1962 (31), medical genetics was just a fledging discipline and molecular studies were limited to descriptions of abnormal chromosome number and structure (9). Polymerase chain reaction was not introduced until 1986 (12), and polymorphic microsatellite markers were not widely used until the early 1990s (50). The Human Genome Project started in October 1990, and the complete draft of the human genome was published in February 2001 (25, 96). Thus, genetic discovery in the Plain communities developed in parallel with larger events taking place in the field of genomics.

Genetic science has become a cornerstone of medicine, but the practice of medicine has been equally vital to progress in genetic science. Bench scientists and clinicians have different interests and responsibilities. Basic scientists investigate physiologic systems, tissues, membranes, pathways, and molecules. They are primarily concerned with patterns and mechanisms. Physicians, in contrast, are principally concerned with the welfare of individuals, "for the patient is no mere collection of symptoms, signs, disordered functions, damaged organs, and disturbed emotions. [The patient] is human, fearful, and hopeful, seeking relief, help, and reassurance" (29).

These differing perspectives influence how one views progress in genomic science (82). Consider three decades of progress in cellular immunology. Over the past 30 years, immunogenetics research has expanded at an explosive pace; we now understand many cellular immune responses in exquisite molecular detail (18). Yet among 41 Amish and Mennonite children born with SCID during the same time period, only half received a bone marrow transplant and 63% died by 2 years of age (88). There was little funding for patient-centered research (34), and hospitals with expertise in SCID were far away and cost too much (57).

We came face to face with this problem in January 2007, when an Amish newborn presented to our Clinic with Omenn syndrome, a variant of SCID (**Figure 8**) (88). She was sick and uninsured, and needed timely, affordable care. We were able to use 10K SNP genotypes from the child and her siblings to delineate the *RAG1/RAG2* locus, and used these same data to quickly and inexpensively find an HLA-matched stem cell donor among her siblings. This entire process—from clinical presentation to genetic diagnosis to donor identification—took less than two weeks and saved the family between $20,000 and $80,000. Applied genomics enabled access to life-saving therapy.

In a medical-industrial research environment that views case reports and small cohorts as poor evidence (98), this one Amish child gave us a glimpse of McKusick's vision for genomic medicine (**Figure 9**) when he wrote: "[G]enomics is likely to render medicine more predictive and, therefore, more preventive... on the traditional turf of clinical medicine, diagnosis will become more specific and precise, and treatment also more specific and safer. Genomics-based *individualization* of medical care aims to achieve the right treatment for the right patient" (50) (italics added). Thus, the future of translational genomics is about *patient discovery*, not gene discovery (14, 34, 77). The idea has a modern emphasis but is not new; Hippocrates believed that diseases as such did not exist in nature. To him, what existed naturally were sick people, and no two people were sick in exactly the same way (77).

The ethical imperative to care for individuals with genetic disease is apparent, but the scientific value of such work should not be overlooked. The connection of a gene to a human phenotype is the only way to fully appreciate a gene's function in vivo (24, 49, 55, 59, 83). In this respect, mice, fish, and flies will always fall short of modeling human disease (see sidebar 5, New Disease Pathways in Human Brain Development). The astute physician, working daily to care for one or a few patients with a rare and complex disorder, can develop key insights that deepen our understanding (68, 90),

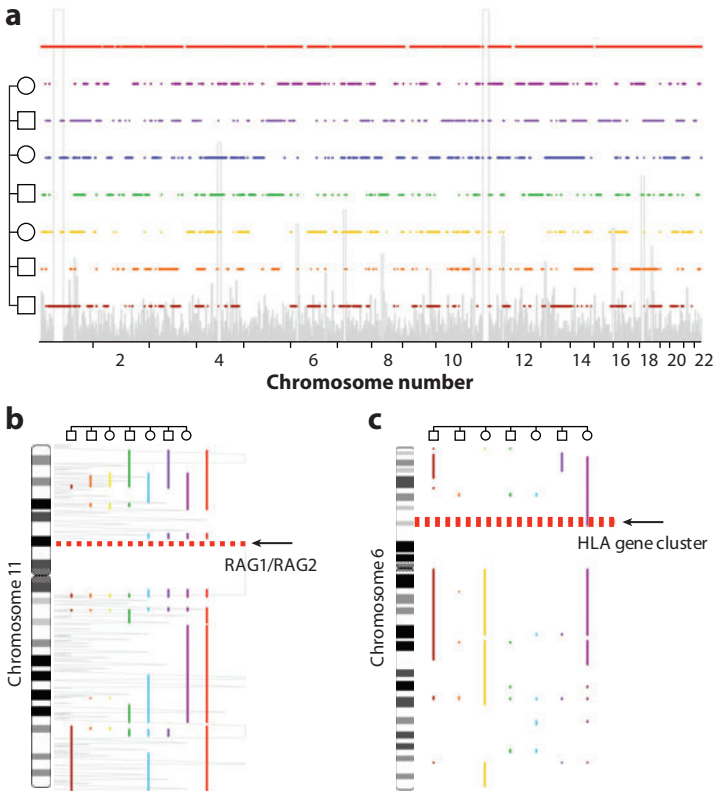

Figure 8

An Amish girl was born in 1993 with erythroderma, hepatosplenomegaly, hypereosinophilia, hypogammaglobulinemia, and B cell deficiency. She grew poorly and had recurrent infections. She was provisionally diagnosed with Omenn syndrome, a variant of severe combined immune deficiency (SCID), but died at 3 months of age before the diagnosis could be confirmed or a transplant attempted. In January 2007, her sister presented as a newborn with alopecia and erythroderma, developed a systemic *Staphylococcus aureus* infection at three weeks of age, and had immunologic studies consistent with SCID. (*a*) A homozygosity plot was generated for the proband (*gray peaks*); identity-by-state (IBS) plots for each pairwise comparison between the proband and her unaffected siblings were superimposed on this plot. Genotype-identical regions (*colored lines*) between the proband and her siblings (i.e., IBS = 2) denote regions where the disease gene cannot reside. The red line at the top is a summary of the exclusion mapping. This analysis demonstrated that only a single, homozygous genomic region on chromosome 11 was consistent with linkage within the pedigree. (*b*) The *RAG1* and *RAG2* loci mapped to the homozygous interval on chromosome 11. *RAG1* sequencing revealed homozygosity for a pathogenic c.2974A>G variant in the patient. (*c*) The homozygosity plot of chromosome 6 in the proband and her seven unaffected siblings. Compared to the proband, only one sibling is haplo-identical for the major HLA loci on chromosome 6. Subsequently, HLA typing of the patient and her potential sibling donor was performed using standard serotyping and molecular probes; these data corroborated the SNP matching. The patient had a successful unmodified bone marrow transplant at 62 days of age. She is now 2 years old, fully engrafted, and healthy. DNA extracted from paraffinized bone marrow showed that her sister, who died 14 years earlier, was also homozygous for *RAG1* c.2974A>G.

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SIDEBAR 5. NEW DISEASE PATHWAYS IN HUMAN BRAIN DEVELOPMENT

In 2006, we discovered a homozygous frameshift mutation of the *CNTNAP2* gene in a group of Amish children with cortical dysplasia, focal epilepsy, and developmental regression (90). Remarkably, mice with homozygous *Cntnap2* mutations have no central nervous system disease. Nevertheless, the findings prompted new studies about the role of *CNTNAP2* in human brain development. Within two years this gene was linked to idiopathic autism (5, 10, 11), schizophrenia (32), and mutism (97) in outbred populations across the country and throughout the world. Thus, the study of rare Mendelian disorders links specific gene families or protein networks—i.e., "interactomes"—to relevant processes in human biology. In the words of William Harvey (ca. 1657): "Nature is nowhere accustomed more openly to display her secret mysteries than in cases where she shows traces of her workings apart from the beaten path; nor is there any better way to advance the proper practice of medicine than to give our minds to the discovery of the usual law of nature by careful investigation of cases of rarer forms of disease."

Figure 9

Victor A. McKusick (*left*), D. Holmes Morton (*right*), and Bowie sitting on the front porch at the Clinic for Special Children, spring 2007.

expand our diagnostic capabilities (88, 89), and create new opportunities for prevention (34, 58, 60, 87). In the era of genomic medicine, physicians and molecular biologists who work closely together can translate these insights into real clinical benefits. Such collaborations will help us tackle big problems at the frontier of genomic medicine, including multigenic disease (e.g., atherosclerosis, hypertension, mental illness, etc.), modifier genes, and epigenetics (24, 30, 64, 78, 79).

Finally, it is important to remember that advances in molecular science only reveal what can be done with genetic information. Perhaps the Plain people can teach us something important about what should be done with it (24, 57). The Old Order communities measure the value of medical research not in grant awards, publications, or academic promotions, but in human terms: alleviation of pain, prevention of disability, equitable delivery, and fair cost. They understand that to translate genetic information into better public health, one must first commit to caring for individuals.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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deeply indebted to them for their extraordinary example and ongoing contributions to people of the Plain communities. Finally, we thank the children and families whom we serve; they continue to teach us much about science, medicine, and meaningful work (57).

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