4-4-2017

Finding Rare, Disease-Associated Variants in Isolated Groups: Potential Advantages of Mennonite Populations

Fabiana L. Lopes
Human Genetics Branch, National Institute of Mental Health, Intramural Research Program, National Institutes of Health, US Department of Health and Human Services, Bethesda, MD

Liping Hou
Human Genetics Branch, National Institute of Mental Health, Intramural Research Program, National Institutes of Health, US Department of Health and Human Services, Bethesda, MD

Angelica B. W. Boldt
Laboratório de Genética Molecular Humana, Departamento de Genética, Universidade Federal do Paraná, Curitiba, Brazil

Layla Kassem
Human Genetics Branch, National Institute of Mental Health, Intramural Research Program, National Institutes of Health, US Department of Health and Human Services, Bethesda, MD

Veronica M. Alves
Laboratory of Panic and Respiration, Institute of Psychiatry, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

See next page for additional authors

Title: Finding Rare, Disease-Associated Variants in Isolated Groups: Potential Advantages of Mennonite Populations

Authors: Fabiana L. Lopes, Liping Hou, Angelica B.W. Boldt, Layla Kassem, Veronica M. Alves, Antonio E. Nardi, [1]Francis J. McMahon
[1]*National Institutes of Health, Human Genetics Branch*

Corresponding author: Francis J McMahon, M.D., Chief, Human Genetics Branch, 35 Convent Drive, Bethesda, MD. 20892-3719. Phone:301-451-4453, mcmahonf@mail.nih.gov

Keywords: Amish, Anabaptist, bipolar, bottleneck, drift, isolate, Mennonite, migration
Header: Rare Variants in Mennonite Populations
Issue: 88.2

Abstract: Large-scale genotyping and next generation sequencing techniques have allowed great advances in the field of molecular genetics. Numerous common variants of low impact have been associated with many complex human traits and diseases, such as bipolar disorder and schizophrenia. Although they may exert a greater impact on risk, few rare disease variants have been found, owing to the greatly increased sample sizes that are typically necessary to demonstrate association with rarer variants. One alternative strategy is to study isolated populations, where historical bottlenecks reduce genetic diversity and some otherwise rare variants may drift to higher frequencies. Here we describe the Mennonite population settlements, considering their history of multiple bottlenecks followed by demographic expansion and a currently widespread geographical distribution. We argue that Mennonite populations are valuable partners for studies seeking to find genetic variants that exert a high impact on risk for a variety of common disorders, including mental illnesses.

Introduction

The past decade has seen a technological explosion in the field of molecular genetics, with the aim of elucidating the etiologic basis of complex diseases. Genome-wide association studies (GWAS) have made great strides in the identification of common single nucleotide polymorphisms (SNPs)– those with minor allele frequencies (MAF) generally over 5% --associated with complex diseases (Hindorff et al 2015). Despite this success, associated effects are small and a substantial heritability gap still remains to be elucidated (Zeggini 2011). Variants that are not well tagged by common SNPs and genetic heterogeneity of

clinical disease entities are now considered leading contributors to the "still missing heritability" of complex disorders (Wray & Maier 2014).

Thanks to next generation sequencing techniques, we are beginning to accumulate a large catalog of low-frequency and rare variants (1000 Genomes Project Consortium 2015; UK10K Consortium 2015; Genome of the Netherlands Consortium, 2014), a few of which have already been implicated in complex traits (Cohen et al 2005; Shuldiner et al 2009; Cruchaga et al 2014; Flannick et al 2014; Hoffman et al 2014; Lange et al 2014; Santos-Cortez et al 2015; Krumm et al 2015; Surakka et al 2015). However, current study designs are not optimal for detecting association with rare variants. The initial idea that the search for rare variants in small samples could reveal mutations with outsized effects on disease risk is becoming obsolete. It is now increasingly accepted that, as with common variants, rare variant association studies require very large samples (in the tens of thousands) to achieve adequate statistical power (Zuk et al 2014).

Some strategies have been proposed to boost the power of association studies. These include statistical methods to aggregate rare variants in chromosomal regions or in functional units (Li &Leal 2008), detection of *de novo* mutations that may exert large effects (Cooper & Shendure 2011), and the use of isolated populations (Hatzikotoulas et al 2014). We will emphasize the characteristics of isolated populations and how they may help to identify variants that contribute to the heritability of complex disorders. We will focus on the Mennonite population settlements. We consider their migration history, multiple genetic bottlenecks, recent demographic expansion, and current widespread geographical distribution.

Characteristics of Isolated Populations

Isolated populations are currently defined as sub-populations deriving from a relatively small number of individuals ("founders") who became isolated from their ancestral group (e.g., through the settlement of a new territory) and/or had experienced a significant reduction in population size ("bottleneck") and remained isolated for several generations afterward (Hartl & Clark 2007). As a consequence of isolation, there is often an increased rate of endogamy and a diminished gene flow between neighboring populations. Therefore, isolated populations have a small effective population size (N_e, or the effective number of individuals required to explain the observed genetic diversity) (Hartl & Clark 2007; Charlesworth & Willis 2009; Colonna et al 2013).

In the context of a small, reproductively isolated population, alleles fluctuate randomly toward higher and lower frequencies ("genetic drift"). The magnitude of genetic drift correlates inversely with effective population size. This may increase the frequency of ancestrally rare alleles, but more often these alleles

disappear entirely, leading to a rapid and important reduction in genetic diversity. Genetic drift also contributes to increased homozygosity, which is further promoted by continued endogamy (Hartl & Clark 2007), since related parents are more likely to carry the same allele at a locus. A population bottleneck coupled with random genetic drift can create local concentrations of otherwise rare traits and diseases, known as the founder effect.

Another important characteristic of isolated populations – and especially those with a recent history of bottlenecks – is a large increase in linkage disequilibrium (LD), leading to long stretches of shared chromosomal regions or haplotypes. Because an isolated population is younger than its ancestral population and was founded by a smaller number of individuals, linkage can be observed for loci that are quite distant from one another in the genome. When associated with a small Ne, increased LD promotes recombination between identical haplotypes, thus preserving long haplotypes that would otherwise be broken down through recombination. Consequently, distantly related members of the same isolated population may share identical chromosomal segments that descended from a distant common ancestor, known as identity by descent (IBD). This facilitates techniques such as long-range haplotyping, genotype imputation, and construction of population-specific reference panels (Colonna et al 2013; Carmi et al 2014; Gudbjartsson et al 2015). Shared homozygous blocks may be as large as several megabases and contain dozens or hundreds of genes (Puffenberger et al 2012). This may aid the identification of chromosomal segments that harbor disease alleles, but also complicates attempts to fine-map specific disease alleles.

Recent reports have highlighted how studies of isolated populations can help illuminate the genetic architecture of complex diseases. Studies in Finland (Lim et al 2014), Iceland (Gudbjartsson et al 2015), Greenland (Moltke et al 2014), Sardinia (Scott et al 2007), and among the Old Order Amish (Pollin et al 2008; Hoffman et al 2014) have all demonstrated how populations with a history of recent bottlenecks display large variations in allele frequencies.

Recently, Zuk et al (2014) introduced the concept of the combined allele frequency (CAF) - the combined frequency of minor alleles present at a given locus – to estimate power to detect genetic association in various populations. They showed that the expected value of CAF is essentially identical across large and diverse populations, but can be much more variable in those – like the Mennonites -- with a history of bottlenecks. Zuk et al (2014) modeled the bottleneck event thought to originate the Finnish population, allowing only approximately 100 chromosomes to pass, compared with 1000 chromosomes for the Icelandic population. As a consequence, the proportion of genes with intermediate standard deviations of CAF (e.g., 5- to 10-fold) was higher in the simulated Icelandic population, but the proportion of genes with high standard

deviations (e.g., 30-fold or more) was higher in the simulated Finns. These results support the idea that populations that have passed through bottlenecks should be good starting points for the discovery of otherwise rare, disease-associated gene variants, although the results also show that particular variants may be more or less frequent.

International efforts are underway that are aimed at identifying rare variants involved in several complex diseases. deCODE is studying rare variation at the population level in Iceland, using whole genome sequencing in a subset, augmented with genotype imputation in a larger sample (Gudbjartsson et al 2015). Analogous efforts are underway in Ashkenazi (Carmi et al 2014), Sardinian (Sidore et al 2015), Dutch (Genome of the Netherlands Consortium 2014), and Amish population samples (Crawford et al 2014). A consortium of researchers led by the NIMH is currently developing a whole genome reference panel for use in Anabaptist populations, which include Amish and Mennonites (Hou et al 2015).

While the Amish have been extensively studied, the Mennonites – especially those residing in South America -- have so far been little investigated. Multicenter efforts to pursue genetic studies in the developing countries are a big challenge (Forero et al 2014), but the potential payoffs for medical science and the participating populations could also be big.

Historical Isolation of the Mennonites

Mennonites are the largest and most complex group in the Anabaptist community, which also includes the Amish, Hutterites, and other groups. We will present a brief overview of the historical evolution of these groups, giving particular emphasis to South American Mennonites, who are the least studied. See Nolt (2003) for a complete history of the Anabaptists, and Crawford (2000) for an extensive treatment of Mennonite population history and migration. The major Mennonite migrations are shown in Figure 1.

Anabaptists trace their roots to 16th century Switzerland (Nolt 2003). The Anabaptist movement was triggered by the diffusion of books, including German translations of the Bible, and by the social disparities that persisted through the Protestant Reformation. “Anabaptism” means baptizing again. The newly converted agreed to be baptized again as adults and to refuse baptism for their newborns until they were old enough to consent themselves. This act threatened the power of the state, which relied on church baptism lists for taxpayer roles and other politically sensitive population counts. For many common people, Anabaptism presented an attractive opportunity for political change and religious freedom. For the Church and the state, however, the movement was a symbol of rebellion. This led to widespread persecution by both religious and civil authorities, progressing to civil war in some areas, especially in Germany.

Most of those Anabaptists who survived the strife and remained in Switzerland, southern Germany, and Alsace left Europe in the 18th century and headed for North America. Their history has been well documented (Nolt 2003). Their descendants now live mainly in the eastern United States (especially Pennsylvania and the Midwestern states of Ohio and Indiana) where they are now known as Amish and (Swiss) Mennonites.

Many other Anabaptists fled to the Netherlands in the 1530s, where they gradually coalesced around Menno Simmons, a former Dutch Catholic priest, and began to call themselves Mennonites. Here we will refer to these groups with the commonly-used designation "Dutch-German Mennonites," reflecting their predominant Dutch and northern German ancestry (Crawford 2000). Mennonites were soon widespread in both the Netherlands and western Germany. Shortly after Simmons's death in 1566, an important ecclesiastic split divided the Mennonites in two groups: "Frisians" and "Flemish." Despite these names, both groups were ethnically similar and also included some families who fled from South Germany. Although they lived in the same towns and settlements, they remained strictly separated for more than two centuries, with almost no gene flow between them or with outsiders (Neff & van der Zijpp, 1956). This population subdivision may have contributed to increased rates of homozygosity within each Mennonite subgroup, due to the Wahlund effect (Hartl & Clark, 2007). Increased homozygosity contributes, in turn, to increased rates of distinct, recessively-inherited disorders within the Frisian and Flemish subgroups (see below).

The Dutch-German Mennonite population suffered three major bottlenecks (Figures 2 and 3). The first was driven by plague, war, and persecution, which intensified under the Spanish dominion of the Netherlands, claiming the lives of at least 1,500 Mennonites. As a result of these misfortunes, in the year 1561 several families began to leave the Netherlands for Danzig, Prussia (now Gdánsk, Poland), where they settled in the delta of the Vistula river (Dyck, 1967). There they initiated a cooperative leasing system and built a solid reputation as workers and taxpayers. Although perhaps 80% of the early settlers died of disease (Stevenson & Everson 2000), the population expanded rapidly. By the end of 1780s, the population was estimated to be around 15,000, including least 369 families, many of Dutch origin (Weigle, 2006).

The second bottleneck, after about 200 years of reproductive isolation in Danzig, was brought about by a second large migration associated with political changes and insufficient land for the growing population. Between 1787 and 1796, at least 423 Danzig Mennonite families (about 2,000 individuals) were invited by Catherine the Great to farm lands recently seized from the Ottoman Empire in Ukraine (Stevenson & Everson 2000). During the following century, an additional 2,300 families (about 6,700 individuals) moved to Ukraine, leaving only about 8,300 individuals behind in Danzig.

In Ukraine, the population expanded greatly over about a century, from the initial settlements of Chortitza and Molotschna, to several others stretching out towards Siberia. Two of the largest settlements, Am Trakt and Alexandertal were settled in the 1850s, later spawning several daughter settlements. After World War I, the population reached around 120,000 individuals, of which 75,000 were within and 45,000 outside of Ukraine (Dyck, 1967) (Figure 3). Each family had an average of 6.2 children (Weigle, 2006; Bergmann & Krahn, 1995; Krahn & Sawatsky, 2011).

The move to Ukraine represents the second most important bottleneck for this population, but it was not the last (Figure 3). There were several migratory waves out of Russia in different directions. In 1874, political changes regarding exemption from military service again led to a mass migration of Mennonites, this time to North America. About 7,000 (mostly from Chortitza) emigrated to Manitoba, Canada and another 10,000 (mostly from Molotschna) went to the American western plains, where proprietors of the rapidly expanding steam railroads competed to lure new immigrants to their growing western settlements. The majority of Mennonites remained in Russia, however, where it was still possible to exchange agricultural or paramedical work for military service (Krahn, 1957; Krahn & Sawatsky, 2011).

After at least a century of isolation and prosperity in Russia, the remaining Mennonite settlements were driven out by the social and political changes caused by World War I and the Russian Revolution. This was accompanied by a big loss of population, initially due to raiding gangs, famine and typhus epidemics, and later to severe religious persecution, with more than 7,000 Mennonites sent to Gulags (Dyck 1967; Kroeker & Ward 2012). This is the third major bottleneck. From 1923 to 1926, about 20,000 Mennonites left Russia; most went to Canada. An additional 3,000 Mennonites fled to Germany, Mongolia, China, Japan and France, helped by Mennonite non-profit organizations in Europe and Canada. While precise figures are lacking, many undoubtedly perished (Bergmann & Krahn, 1995).

No longer allowed to immigrate to Canada after 1929, Mennonites moved from the refugee camps in Germany and Harbin (China) to South America. A total of 240 families (about 2,300 individuals) arrived in Brazil between 1930 and1934, increasing to an estimated 9,000 individuals today (Bender et al 2013). They founded three major Mennonite communities. The largest is in Curitiba, the capital city of Paraná state (ca. 4000 individuals, divided into the initial settlements of Boqueirão, Xaxim, and Água Verde). Another major settlement is Witmarsum, which lies 60 km away from Curitiba and was founded by 60 families in 1951 (currently, there are 342 families with approximately 1200 individuals). Colônia Nova, in Rio Grande do Sul state was founded in 1948 by 86 families with approximately 900 individuals. Although intermarriage with the

local Brazilian population is now accepted, especially in Curitiba, Mennonite cultural and religious practices are still retained (Bender et al. 2013).

Starting as early as the 1920s, an even larger number of Mennonites moved to the western regions of Paraguay. Discontent with Canadian educational politics, about 1,280 conservative Canadian Mennonites who left Russia in 1874 founded the first Paraguayan settlement, called Menno, in 1927 (currently about 9000 individuals) (Dyck, Friesen & Friesen, 2009). Slightly more than 2,000 Russian Mennonite refugees, including 50 Polish Mennonites and 367 who crossed the Chinese border, founded Fernheim in 1930 (now with 4,000 individuals). Of these, 748 emigrated and founded Friesland, in 1937 (Dyck, Klassen & Niebuhr, 2009; Fast & Ratzlaff, 2012). Russian Mennonites who fled the 1941-43 invasion of South Russia by the German army founded two other settlements in 1948: Neuland and Volendam, with an initial population of 2,389 and 1,172 individuals, respectively. There are now at least 33,000 Mennonites living in Paraguay, distributed in these and other more minor settlements, as well as in Asunción (Smith & Klassen, 2013).

Bolivia is also home to several settlements. In 1955, some 50 conservative families moved from the Menno colony in Paraguay to Bolivia (near Santa Cruz), joining 11 families from Fernheim, who settled there one year earlier (Chapman 1973). In fact, descendants of the Russian emigrants of 1874 founded most of the Bolivian Mennonite settlements, which today are probably the most isolated among all the Mennonite settlements in South America. By 1986, there were about 2,500 Mennonites distributed in seven Bergthaler-Sommerfelder colonies, founded after 1956 by Mennonites coming from Paraguay, Canada, and Mexico (where Canadian Mennonites had immigrated some years earlier). In addition, nearly15,000 individuals from Canada and Mexico founded nine Old-Colony Mennonite settlements. Currently, at least 60,000 Mennonites are estimated to inhabit this remote Andean region, maintaining almost complete social (and presumably reproductive) isolation (Bender et al 2013).

Mennonites also live in other South American countries. Some families from the 1948 Russian emigration wave (including many who stayed in Danzig in the eighteenth century) can now be found near Buenos Aires, Argentina. Another 750 individuals founded a settlement in Uruguay known as El Ombu (Gering & Bender, 1955).

Most of the South American communities (except the “Old Mennonites”) have incorporated aspects of the surrounding, modern society, while maintaining many traditions, such as the Low German dialect (Plattdeutsch). Nevertheless, endogamy is still common, with clear implications for genetic studies. Several unusual genetic disorders have been described in Mennonites of Dutch-North German ancestry, as we will discuss below. However, Mennonites in South

America have so far been little studied and not much is known about their genetic disease burden (Ferreira 2014).

Mendelian Diseases

Previous reviews have already described the profile of inherited (mainly Mendelian) diseases frequently (or exclusively) found among the Old Order Mennonites (OOM) (Strauss & Puffenberger, 2009), Dutch-German Mennonites (Orton et al. 2008), and related groups. Table 1 shows some of the inherited diseases that have been described among Dutch-German Mennonites in Canada. Mennonites with similar origins can also be found in the western regions of the USA (especially Kansas and Nebraska).

Diseases recently added to the Dutch-German Mennonite disease catalog (Sidore et al, 2006) include Fanconi Anemia - complementation group c (OMIM #227645; de Vries Y et al, 2012), progressive myoclonus epilepsy with ataxia (OMIM #611726; Farhan et al 2014), and severe combined immunodeficiency due to CD3-delta deficiency (OMIM #615617; Kavadas et al 2014). Hereditary non-polyposis colon cancer (HNPCC) is reported in several families in Manitoba (Orton et al 2008; Sidore et al, 2006) who share the same mutation, consistent with a founder mutation. Interestingly, the Brazilian National Census of Isolates (CENISO) (http://www.inagemp.bio.br/ceniso.php) reports an increased incidence of HNPCC in Rio Grande do Sul, where there are several Dutch-German Mennonite settlements. Other diseases reported in CENISO in geographically dispersed areas in the states of Parana and Rio Grande do Sul (where Mennonites are abundant) include: Breast-Ovarian Cancer, Familial (OMIM #604370), Adrenocortical Carcinoma, Hereditary (OMIM #202300), and Spinocerebellar Ataxia, Type 3 (OMIM#202300). Cases of bipolar disorder have been reported in the Colonia Nova and Witmarsum colonies. Since there is a long history of research on bipolar disorder among the Amish in the USA, we consider this topic in more detail in the next section.

Psychiatric Disorders

The high heritability and complex inheritance patterns of psychiatric disorders such as schizophrenia, major depression, and bipolar disorder have spurred a variety of studies aimed at identifying risk genes. While genome-wide association studies of large case-control samples have been the most successful (Neale & Sklar 2015), the identified alleles confer very low risk and collectively account for a minority of the heritability (Shinozaki & Potash 2014). Alleles that confer higher risk implicate genes that might be better targets for biological studies aimed at developing new treatments. If such high risk alleles exist, they are not

common in the general population. This idea leads naturally to isolated populations like those that are the focus of this review.

As noted above, there is a long history of bipolar disorder research among the Amish in Lancaster County, PA. This work was pioneered by Egeland and colleagues in the late 20th century (Egeland et al 1989; Egeland1983). Ongoing studies are now being pursued by several groups (Yang et al 2009; Egeland et al 2012; Hou et al 2013; Ginns et al 2014; Strauss et al 2014; Georgi et al 2014; Kember et al 2015; Byrne et al 2015).

Several characteristics make Anabaptist populations especially valuable for studies of psychiatric disorders (Hou et al 2013). For instance, they seem to have reduced rates of substance use disorders that can complicate psychiatric diagnosis. They usually live (and often receive their psychiatric care) in the same geographic area for several generations, easing ascertainment and access to clinical records. Reduced environmental variation in education, socioeconomic status, and stressful life experiences may tend to magnify inherited (genetic) sources of variation in behavioral traits. Furthermore, extended families provide ample family informants, improving diagnosis and reducing undetected cases.

Overall, the advantages of Anabaptist communities for studies of mental health and disease are strong enough that additional insights into the genetic architecture of mental health conditions seem likely to follow soon. But challenges remain. Many individuals live in remote areas with limited access to communications. The establishment of a trusting relationship with researchers who often stand at a distant point on the spectrum of modernity can be a major barrier. Basic data for designing experiments is lacking. For instance, no epidemiologic study of the prevalence of major mental illness in any of these groups has been published. Additional challenges arise from limited sample sizes and strong local population structures. For example, a recent study identified a missense mutation in the *KCNH7* gene among Old Order Amish in Pennsylvania, some of whom suffered from bipolar or other mood disorders (Strauss et al 2014). In vitro functional studies seemed to demonstrate a clear impact of the variant on HERG3 channels, but small sample size and lack of matched controls hampered the establishment of a significant association with the disorder.

The larger size and greater genetic diversity of Mennonite populations offer a solution to the sample size problem, but increase the risk of genetic heterogeneity (Zuk et al 2014), which can be problematic for studies of complex, multigenic disorders like bipolar disorder. “Gene-first” studies that seek to define the range of phenotypes that occur among carriers of a particular allele (Schulze & McMahon 2004; Stessman et al 2014), may prove to be an effective alternative strategy that could be pursued within groups where a large proportion of individuals are well-characterized genetically and phenotypically.

Conclusions

The unique history of the Mennonite populations, characterized by founder effects and multiple bottlenecks, presents some unique opportunities and challenges for genetic studies. There are clear differences between Mennonites of Swiss and southern German ancestry and those of Dutch and northern German ancestry, who spent centuries in Russia before settling in the Americas and experienced several major population bottlenecks along the way. Studies that exploit large-scale sequencing technology to assess the full range of allelic variation in Mennonite populations may lead to new insights into the genetic architecture of a variety of common disorders, including common mental illnesses.

Acknowledgements: Supported by the Intramural Research Program of the National Institute of Mental Health, Bethesda, MD (USA), the Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, and the Federal University of Paraná, Curitiba, Brazil.

Received 30 November, 2015; accepted 26 April 2016.

References

1000 Genomes Project Consortium, Auton A, Brooks LD, Durbin RM, et al. 2015. A global reference for human genetic variation. *Nature*. 526:68-74.

Bender, H. S., H. Ens, P. Pauls. 2013. Brasil. In: *Global Anabaptist Mennonite Encyclopedia Online*. http://gameo.org/index.php?title=Brazil&oldid=128404 Accessed 6 Oct 2015.

Bergmann, C. and C. Krahn. 1995. Chortitza Mennonite Settlement (Zaporizhia Oblast, Ukraine). In: Global Anabaptist Mennonite Encyclopedia Online. http://gameo.org/index.php?title=Chortitza_Mennonite_Settlement_(Zaporizhia_Oblast,_Ukraine)&oldid=127181. Accessed 3 Oct 2015.

Byrne, E. M., Psychiatric Genetics Consortium Major Depressive Disorder Working Group, U. K. Raheja, et al. 2015. Seasonality shows evidence for polygenic architecture and genetic correlation with schizophrenia and bipolar disorder. *J. Clin. Psychiat*. 76:128-34.

Carmi, S., K. Y. Hui, E. Kochav, et al. 2014. Sequencing an Ashkenazi reference panel supports population-targeted personal genomics and illuminates Jewish and European origins. *Nat. Commun*. 9;5:4835.

Charlesworth, D. and J. H. Willis. 2009. The genetics of inbreeding depression. *Nat. Rev. Genet*. 10: 783-796.

Cohen, J., A. Pertsemlidis, I. K. Kotowski, et al. 2005. Low LDL cholesterol in individuals of African descent resulting from frequent nonsense mutations in PCSK9. *Nat. Genet*. 37:161-5.

Colonna, V., G. Pistis, L. Bomba, et al. 2013. Small effective population size and genetic homogeneity in the Val Borbera isolate. *Eur. J. Hum. Genet. 21*: 89-94.

Cooper, G. M. and J. Shendure. 2011. Needles in stacks of needles: finding disease-causal variants in a wealth of genomic data. *Nat. Rev. Genet*. 12:628-40.

Crawford, D. C., L. Dumitrescu, R. Goodloe, et al. 2014. Rare variant APOC3 R19X is associated with cardio-protective profiles in a diverse population-based survey as part of the Epidemiologic Architecture for Genes Linked to Environment Study. *Circ. Cardiovasc. Genet*. 7:848-53.

Crawford, M. H. (ed). 2000. Different Seasons: Biological Aging among the Mennonites of the Midwestern United States. Publications in Anthropology. Lawrence, KS: University of Kansas.

Cruchaga, C., C. M. Karch, S. C. Jin, et al. 2014. Rare coding variants in the phospholipase D3 gene confer risk for Alzheimer's disease. *Nature*. 505:550-4.

Cummings, A. C., L. Jiang, D. R. Velez Edwards, et al. 2012. Genome-wide association and linkage study in the Amish detects a novel candidate late-onset Alzheimer disease gene. *Ann. Hum. Genet.* 76:342-51.

de Vries, Y., N. Lwiwski, M. Levitus, et al. 2012. A Dutch Fanconi Anemia FANCC Founder Mutation in Canadian Manitoba Mennonites. *Anemia*. 2012:865170.

Dyck, C. J. 1967. An introduction to Mennonite history. Scottdale, Pa.: Herald Press, 1967.

Dyck, C. J., M. W. Friesen, U. S. Friesen. 2009. Menno Colony (Boquerón Department, Paraguay). *Global Anabaptist Mennonite Encyclopedia Online*.

http://gameo.org/index.php?title=Menno_Colony_(Boquer%C3%B3n_Department,_Paraguay)&oldid=121240. Accessed 6 Oct 2015.

Dyck, C. J., P. P. Klassen, G. Niebuhr. 2009. Fernheim Colony (Boquerón Department, Paraguay). *Global Anabaptist Mennonite Encyclopedia Online*. http://gameo.org/index.php?title=Fernheim_Colony_(Boquer%C3%B3n_Department,_Paraguay)&oldid=121050.Accessed 6 Oct 2015.

Egeland, J. A., J. Endicott, A. M. Hostetter, et al. 2012. A 16-year prospective study of prodromal features prior to BPI onset in well Amish children. *J. Affect. Disorders*. 142:186-92.

Egeland, J. A., D. S. Gerhard, D. L. Pauls. 1989. Description of Amish Study data set. *Genet. Epidemiol*. 6: 195-199.

Egeland, J. A. and A. M. Hostetter. 1983. Amish Study I: Affective disorders among the Amish, 1976-1980. *Am. J. Psychiat*. 140:56-61.

Fast, A. and G. Ratzlaff. 2012. Friesland Colony (San Pedro Department, Paraguay). *Global Anabaptist Mennonite Encyclopedia Online*. http://gameo.org/index.php?title=Friesland_Colony_(San_Pedro_Department,_Paraguay)&oldid=91825. Accessed 6 Oct 2015.

Ferreira, C. R., M. Beatriz de Herreros. 2014. Medical genetics in Paraguay. *Mol. Genet. Genomic Med.* 2, 458-466.

Flannick, J., G. Thorleifsson, N. L. Beer, et al. 2014. Loss-of-function mutations in SLC30A8 protect against type2 diabetes. *Nat. Genet*. 46:357-63.

Forero, D. A., A. Velez-van-Meerbeke, S. N. Deshpande, et al. 2014. Neuropsychiatric genetics in developing countries: Current challenges. *World J. Psychiatry.* 4: 69-71.

Genome of the Netherlands Consortium 2014. Whole-genome sequence variation, population structure and demographic history of the Dutch population. *Nat. Genet*. 46:818-25.

Georgi, B., D. Craig, R. L. Kember, et al. 2014. Genomic view of bipolar disorder revealed by whole genome sequencing in a genetic isolate. *PloS Genet*. 10:e1004229.

Gering, W. and H. S. Bender. 1955. Danzig Refugees. Global Anabaptist Mennonite Encyclopedia Online.

http://gameo.org/index.php?title=Danzig_Refugees&oldid=120100. Retrieved 24 November 2015.

Ginns, E. I., M. Galdzicka, R. C. Elston, et al. 2015. Disruption of sonic hedgehog signaling in Ellis-van Creveld dwarfism confers protection against bipolar affective disorder. *Mol. Psychiatr*. 20: 1212-8.

Gudbjartsson, D. F., H. Helgason, S. A. Gudjonsson, et al. 2015. Large-scale whole-genome sequencing of the Icelandic population. *Nat. Genet*. 47:435-44.

Hartl, D. L. and G. Clark. 2007. Principles of Population Genetics. 4th Edition. Sinauer.

Hatzikotoulas, K., A. Gilly, E. Zeggini. 2014. Using population isolates in genetic association studies. *Brief Funct. Genomics* 13:371-7.

Hindorff, L. A. and J. MacArthur. (European Bioinformatics Institute), Morales J (European Bioinformatics Institute) et al. A Catalog of Published Genome-Wide Association Studies. Available at: www.genome.gov/gwastudies. Accessed Sept 2015.

Hoffman, J. D., J. N. Cooke Bailey, L. D'Aoust, et al. 2014. Rare complement factor H variant associated with age-related macular degeneration in the Amish. *Invest. Ophth. Vis. Sci*. 55:4455-60.

Hou, L., R. Kember, J. C. Roach, et al. 2015. A population-specific reference panel empowers genetic studies of Anabaptist participants through improved imputation and variant filtering. Abstract presented at the annual meeting of the American Society of Human Genetics, Baltimore MD, PgmNr 1436.

Hou, L., G. Faraci, D. T. W. Chen et al. 2013. Amish revisited: next-generation sequencing studies of psychiatric disorders among the Plain people. *Trends Genet.* 29: 412-418.

Kember, R. L., B. Georgi, J. E. Bailey-Wilson et al. 2015. Copy number variants encompassing Mendelian disease genes in a large multi-generational family segregating bipolar disorder. *BMC Genet*. 16:27.

Krahn, C. and W. W. Sawatsky. 2011. Russia *Global Anabaptist Mennonite Encyclopedia Online*.http://gameo.org/index.php?title=Russia&oldid=131482. Accessed 6 Oct 2015.

Krahn, C. 1957. Molotschna Mennonite Settlement (ZaporizhiaOblast, Ukraine) *Global Anabaptist Mennonite Encyclopedia Online.* http://gameo.org/index.php?title=Molotschna_Mennonite_Settlement_(Zaporizhia_Oblast,_Ukraine)&oldid=131469. Accessed 6 Oct 2015.

Kroeker, P. T. and B. K. Ward. 2012. Gulag Ethics: Russian and Mennonite Prison Memoirs from Siberia. *J. Mennonite Studies*. 30, 251-268.

Krumm, N., T. N. Turner, C. Baker, et al. 2015. Excess of rare, inherited truncating mutations in autism. *Nat. Genet*. 47:582-8.

Lange, L. A., Y. Hu, H. Zhang, et al. 2014. Whole-exome sequencing identifies rare and low-frequency coding variants associated with LDL cholesterol. *Am. J. Hum. Genet*. 94:233-45.

Li, B. and S. M. Leal. 2008. Methods for detecting associations with rare variants for common diseases: application to analysis of sequence data. *Am. J. Hum. Genet*. 83:311-21.

Lim, E. T., P. Wurtz, A. S. Havulinna, et al. 2014. Distribution and medical impact of loss-of-function variants in the Finnish founder population. *Plos Genet.* 10(7).

McKusick, V. A. 1965. Some computer applications to problems in human genetics. *Method. Inform. Med*. 9:4, 183-189.

Moltke, I., N. Grarup, M. E. Jorgensen, et al. 2014. A common Greenlandic TBC1D4 variant confers muscle insulin resistance and type 2 diabetes. *Nature*. 512:190-3.

Neale, B. M. and P. Sklar. 2015. Genetic analysis of schizophrenia and bipolar disorder reveals polygenicity but also suggests new directions for molecular interrogation. *Curr. Opin. Neurobiol*. 30:131-8.

Neff, C. and N. van der Zijpp. 1956. Flemish Mennonites. Global Anabaptist Mennonite Encyclopedia Online. http://gameo.org/index.php?title=Flemish_Mennonites&oldid=107374. Retrieved 24 November 2015.

Nolt, S. M. A History of the Amish, rev. ed. 2003. Intercourse, PA: Good Books, 2003.

Orton, N. C., A. M. Innes, A. E. Chudley, et al. 2008. Unique disease heritage of the Dutch-German Mennonite population. *Am. J. Med. Genet. A*. 146: 1072-1087.

Pollin, T. I., D. J. McBride, R. Agarwala. 2008. Investigations of the Y chromosome, male founder structure and YSTR mutation rates in the Old Order Amish. *Hum. Hered*. 65:91-104.

Puffenberger, E. G., R. N. Jinks, C. Sougnez, et al. 2012. Genetic mapping and exome sequencing identify variants associated with five novel diseases. *PLoS One, 7*: e28936.

Santos-Cortez, R. L., C. M. Chiong, M. R. Reyes-Quintos, et al. 2015. Rare A2ML1 variants confer susceptibility to otitis media. *Nat. Genet*. 47:917-20.

Schulze, T. G. and F. J. McMahon. 2004. Defining the phenotype in human genetic studies: forward genetics and reverse phenotyping. *Hum. Hered*. 58:131–138.

Scott, L. J., K. L. Mohlke, L. L. Bonnycastle, et al. 2007. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science* 316:1341-1345.

Shinozaki, G. J. B. and Potash. 2014. New developments in the genetics of bipolar disorder. *Curr. Psychiatry Rep*. 16:493.

Shuldiner, A. R., J. R. O'Connell, K. P. Bliden, et al. 2009. Association of cytochrome P450 2C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy. *JAMA*. 2009. 302:849-57.

Sidore, C., F. Busonero, A. Maschio, et al. 2015. Genome sequencing elucidates Sardinian genetic architecture and augments association analyses for lipid and blood inflammatory markers. *Nat. Genet*. 47:1272-81.

Sidore, C., F. Busonero, A. Maschio, et al. 2006. Amish, Mennonite, and Hutterite Genetic Disorders Database. http://www.biochemgenetics.ca/plainpeople/index.php. Retrieved April 20th 2015.

Smith, W. H. and P. P. Klassen. 2013. "Paraguay." *Global Anabaptist Mennonite Encyclopedia Online*. Web. 6 Oct 2015. http://gameo.org/index.php?title=Paraguay&oldid=131882.

Stessman, H. A., R. Bernier, E. E. Eichler. 2014. A genotype-first approach to defining the subtypes of a complex disease. *Cell*. 156:872-7.

Stevenson, J. and P. Everson. 2000. Historical demography of Mennonite populations. In: Different Seasons: Biological Aging among the Mennonites of the Midwestern United States, ed. Crawford MH, pp. 19-30. Lawrence, KS: University of Kansas Press.

Strauss, K. A., S. Marxx, B. Georgi, et al. 2014. A population-based study of KCNH7 p.Arg394His and bipolar spectrum disorder. *Hum. Mol. Genet.* 23: 6395-6406.

Strauss, K. A. and E. G. Puffenberger. 2009. Genetics, medicine, and the plain people. *Annu. Rev. Genom. Hum. G.* 10: 513-536.

Surakka, I., M. Horikoshi, R. Mägi, et al. 2015. The impact of low-frequency and rare variants on lipid levels. *Nat. Genet*. 47:589-97.

UK10K Consortium, K. Walter, J. L. Min, et al. 2015. The UK10K project identifies rare variants in health and disease. *Nature*. 526:82-90.

Weigle, J. Über Mennoniten. G-Gruppe - grenzenlose Genealogie über die Ostsee 2006. http://www.g-gruppen.net/mennot.htm.

Wray, N. R. and R. Maier. 2014. Genetic basis of complex genetic disease: The contribution of disease heterogeneity to missing heritability. *Curr. Epidemiol. Rep.* 1: 220-227.

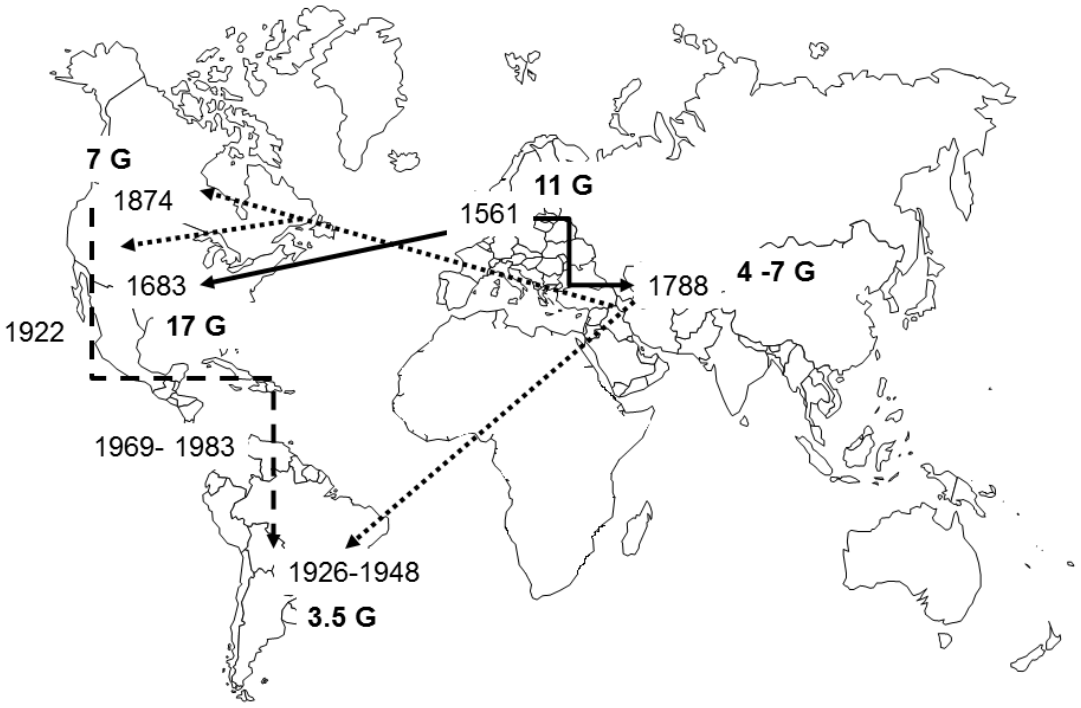
Yang, S., H. P. Van Dongen, K. Wang, et al. 2009. Assessment of circadian function in fibroblasts of patients with bipolar disorder. *Mol. Psychiatr*. 14:143-55.

Zeggini, E. 2011. Next-generation association studies for complex traits. *Nat. Genet. 43*: 287-288.

Zuk, O., S. F. Schaffner, K. Samocha, et al. 2014. Searching for missing heritability: Designing rare variant association studies. *Proc. Natl. Acad. Sci. USA.* 111, E455-E464.

Figure 1. Migration dates and routes of the Dutch-German Mennonites

Mennonites settled in Danzig in 1561 and eastern North America in 1683. From Danzig, consecutive migration waves went to Ukraine (1788) and spread within



Russia. This was followed by additional waves of migration to western North America (1874) and South America (after 1926), leading to the extinction of the original settlements. Additional population movements within the Americas peaked from 1969 – 83.

——— First waves of migration from Danzig to North America (1680s) and Ukraine (1780s)
····· Migrations from Russia to Canada & US (1870's) and to South America (1926-1948)
– – – Migrations from Canada to Mexico (1922), Belize, and Bolivia (1969-83)

G = Generations each settlement persisted since founding, assuming 20 years per generation.

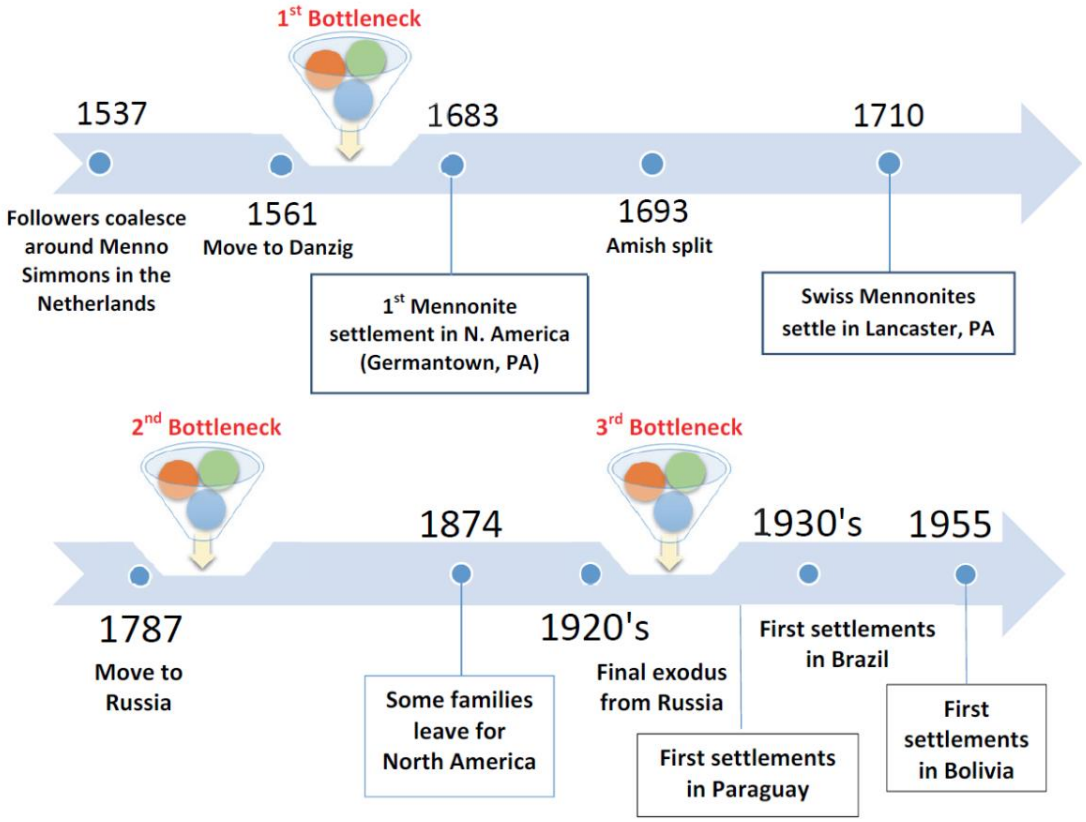


Figure 2. Brief population timeline of the Dutch-German Mennonites

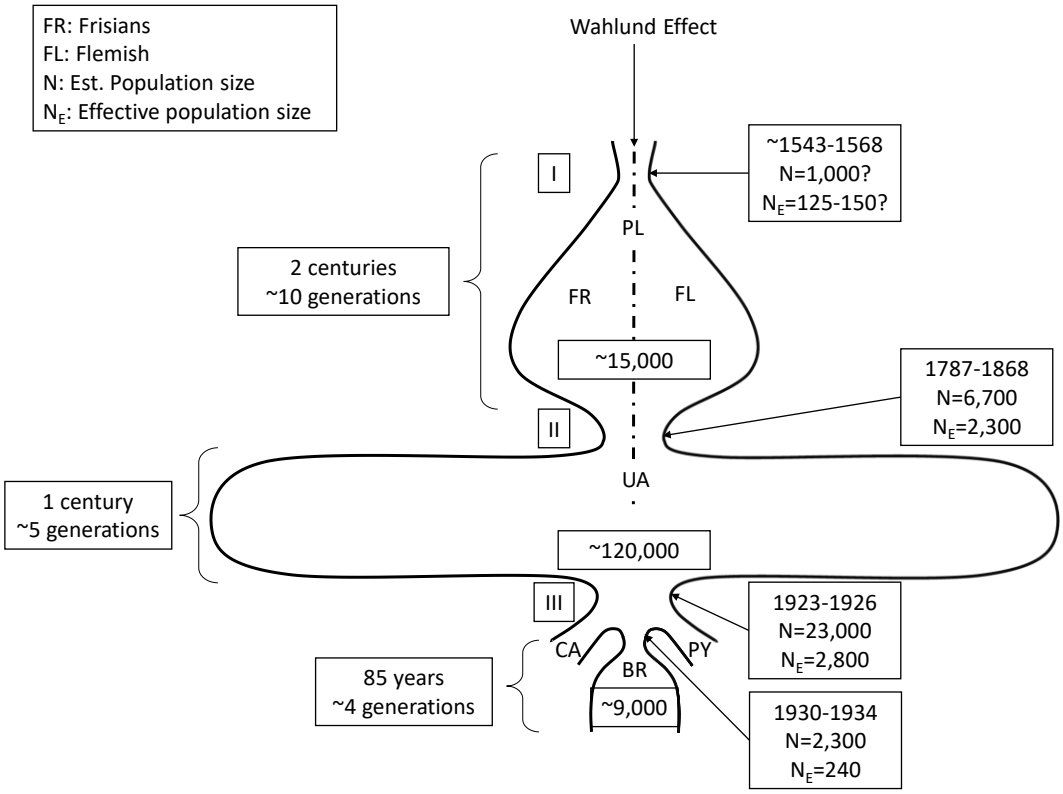


Figure 3. Major Population Bottlenecks in Dutch-North German Mennonites

The Mennonite population experienced a bottleneck during the move to Danzig (PL). Soon thereafter, Mennonites divided into two subgroups, called "Frisian" and "Flemish." This led to increased homozygosity and exposure of recessive alleles (Wahlund Effect). Another bottleneck coincided with the move to Ukraine (UA), followed by an unprecedented and rapid population expansion and gradual loss of the "Frisian"/"Flemish" population structure. Another severe bottleneck occurred a century later, when only about 20% of the population escaped to the Americas. Thereafter, an expanding population has split geographically among several regions in the US, Canada (CA), Brazil (BR), Paraguay (PY), and other countries in South America.

Table 1. Genetic Disorders Reported in Mennonites of Dutch and North German Ancestry

Disorder	Gene and Mutations				
	Gene symbol	Locus	Genotype	Amino Acid change	Refererences
Alstrom Syndrome	*ALMS1*	2p13	n.d	n.d	Collin et al. 2002, Orton et al. 2008
Apparent mineralocorticoid excess	*HSD11B2*	16q22	c.680C>T	p.Pro227Leu	Orton et al. 2008
Ataxia teleangiectasia	*ATM*	11q22.3	c.5932G>T	p.Glu1978X	Orton et al. 2008, Campbell et al. 2003
Biotinidase deficiency	*BTD*	3p25.1	c.380C>T	Pro127→leu	Sidore et al. 2006
Chudley-McCullough Syndrome	*n.d*	n.d.	n.d.	n.d.	Orton et al. 2008
Cleft palate with ankyloglossia	*TBX22*	Xq21.1	c.359G>T	p.Gly118Cys	Sidore et al. 2006
Cleidocranial dysplasia	*CBFA1*	6p21	n.d.	p.Thr200Ala	Orton et al. 2008, Sidore et al. 2006
CODAS syndrome	*n.d*	n.d	n.d	n.d.	Orton et al. 2008, Sidore et al. 2006
Congenital adrenal hyperplasia due to 17 α-hydroxylase deficiency	*CYP17*	10q24.3	c.1434-1437dupCATC	p.Pro480Hisfs Ter27	Orton et al. 2008, Sidore et al. 2006
Congenital stationary night blindness, incomplete	*CACNA1F*	Xp11.23	c.3166DupC	p.Leu1056 Profs	Orton et al. 2008
Cystic fibrosis	*CFTR*	7q31.2	c.1521-1523delCTT	p.F508del	Orton et al. 2008, Sidore et al. 2006
Familial hypertrophic cardiomyopathy	*MYBPC3*	11p11.2	c.2405insG	Frameshift	Orton et al. 2008, Sidore et al. 2006
Fanconi Anemia, complementation group C	*FANCC*	9q22.3	c.67delG	p.Asp23llefs	Sidore et al. 2006, de Vries et al. 2012
Fragile X Syndrome	*FMR1*	Xq27.3	(CGG)n expansion	n.d.	Orton et al. 2008, Sidore et al. 2006
Froese blood group	*SLC4A1*	17q21-q22	c.1438G>A	p.Glu480Lys	Orton et al. 2008

Gerodermia-Osteodysplastica	*SCYL1BP1*	1q24.7	c.367G>T	p.Glu123term	Sidore et al. 2006, Hennies et al. 2008
Tourette Syndrome	*n.d.*	n.d.	n.d.	n.d.	Orton et al. 2008, Sidore et al. 2006
Hereditary non-polyposis colon cancer	*MLH1*	3p21.3	n.d.	p.trp714term	Orton et al. 2008
Hypophosphatasia	*ALPL*	1p36.12	c.1117G>A	p.Gly317Asp	Orton et al. 2008, Sidore et al. 2006
Leigh Syndrome	*MT-RNR2*	mtDNA	m.3197T>C	-	Orton et al. 2008, Huntsman et al. 2005
Malignant hyperthermia susceptibility	*RYR1*	19q13.1	c.1840C>T	p.Arg6124Cys	Orton et al. 2008, Sidore et al. 2006
Mitochondrial DNA depletion, hepatocerebral form	*DGUOK*	mtDNA	m.763G>T	p.Asp255Tyr	Sidore et al. 2006
Mucopolysacchar-idosis, type VII	*GUSB*	7q21.11	c.526C>T	p.Leu176Phe	Sidore et al. 2006
Oculorenocerebellar Syndrome	*n.d.*	n.d.	n.d.	n.d.	Orton et al. 2008, Sidore et al. 2006
Progressive myoclonus epilepsy with ataxia	*KCTD7*	7q21.11	c.827A>G	p.Tyr276Cys	Sidore et al. 2006, Farhan et al. 2014
Renpenning Syndrome	*PQBP1, E5region*	Xp11.23	c.641insC	Frameshift, stop codon at 226	Orton et al. 2008, Sidore et al. 2006
Restrictive dermopathy, lethal	*ZMPSTE24*	1p34	c.1085dupT	Frameshift	Orton et al. 2008, Sidore et al. 2006, Loucks et al. 2012
Roberts / SC Phocomelia	*ESCO2*	8p21.1	unknown	Unknown	Orton et al. 2008
Severe Combined Immunodeficiency due to adenosine deaminase deficiency	*ADA*	20q13.1	c.424C>T	p.Arg142term	Sidore et al. 2006
Severe Combined Immunodeficiency due to CD3-delta deficiency	*CD3D*	11q23	c.202C>T	p.Arg68term	Sidore et al. 2006, Lam et al. 2014

Severe Combined Immunodeficiency due to ZAP70 deficiency	*ZAP70*	2q12	c.1624-11G>A	Splicing defect	Orton et al. 2008, Sidore et al. 2006, Arpaia et al. 1994
Spondylocostal-dysostosis with anal atresia and urogenital anomalies	*n.d*	n.d	n.d	n.d	Sidore et al. 2006, Casamassima et al. 1981
Tight Skin Contracture Syndrome, lethal	*ZMPSTE24,* E1 region	1p346	c.54insT	Frameshift	Sidore et al. 2006

n.d. = not determined