REVIEW

Multiple mutations responsible for frequent genetic diseases in isolated populations

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The relatively frequent existence of an autosomal recessive disease in an isolated population suggests a founder effect. However, in many cases the high frequency is due to more than one mutation in either one or several genes. Several possibilities have been raised to explain these findings: a chance phenomenon, migration of families with affected patients or digenic inheritance. Although each of these possibilities may be responsible for a few of the cases, in most they are very improbable explanations. A selective advantage may explain most of the observations even if it is difficult to prove.
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Introduction

The relatively frequent existence of an autosomal recessive disease in an isolated population suggests a founder effect. Whereas this is true in most instances, in the past decade there have been several reports in which molecular investigations revealed that the high frequency is due to more than one mutation in either one or several genes. A review of some examples of this phenomenon and of the possibilities that have been raised to explain these puzzling findings is presented.

Multiple mutations in isolated populations
Pendred syndrome in an inbred family[1]
Pendred syndrome (MIM 274600) is an autosomal recessive disorder with symptoms including neurosensory congenital deafness and sometimes associated with defective vestibular function. Patients are usually euthyroid but the thyroid is moderately enlarged from childhood.

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In a consanguineous kindred originating from Turkey and living in The Netherlands, the syndrome was found in several families to be due to a homozygous splice site mutation (1143-2A>G) in the *PDS* gene. In one of the families with patients who were compound heterozygotes, the mother originating from the family was heterozygote for the splice site mutation whereas the nonrelated father from the Netherlands carried a mutation (1558 T>G) that has been characterized in other families from the same origin.

These observations may be explained by the marriage of a carrier of a founder mutation to a spouse originating from a population in which a rare mutation exists. The probability for such an event to occur was *a priori* relatively high in this kindred equal to twice the product of the allele frequency in the family to the allele frequency in the population in The Netherlands (2pq).

Congenital hypothyroidism in large inbred Amish kindred[2]
Congenital hypothyroidism is one of the most common causes of mental retardation that can be prevented by the early institution of replacement thyroid hormone treatment. Data from routine neonatal screening programmes indicate the prevalence is one in 4000 newborns. Although most cases are due to faulty embryonic development of the

thyroid gland, some are caused by inborn errors of thyroid hormone synthesis.

A high incidence of severe hypothyroidism owing to a complete iodide organification defect in the youngest generation of five nuclear families belonging to inbred Amish kindred was reported.[2] Genealogical records revealed their origin to an ancestral couple seven to eight generations ago and to identify an autosomal recessive pattern of inheritance. Two missense mutations, (E799K) and (R648Q) in the *TPO* gene, were found in the family. Both mutations occurred in CpG hotspots and possibly arose recently. Many rare genetic diseases have been reported to be relatively frequent in the Amish community and it is possible that *TPO* gene was involved twice by chance.

Spondylocostal dysostosis in a small village in Switzerland[3]

Spondylocostal dysostosis (MIM 277300) is an autosomal recessive disorder with characteristics including short thorax, short neck with limited mobility, winged scapulae and scoliosis or kyphoscoliosis. Noteworthy are the vertebral anomalies, including hemivertebrae and vertebral fusions affecting the whole vertebral column and the rib abnormalities. A cluster of four families with spondylocostal dysostosis was described in a small village in Switzerland.[3] The disease is caused by mutations in *DLL3* and the Swiss study found three different mutations. In two families, the patients were homozygous for one of the mutations (1285–1301dup) that was also found in the third family together with a second mutation (c.615delC). In the last family, the patients were compound heterozygotes for (c.615delC) and a third mutation (R238X).

The observation of rare diseases owing to a founder mutation has often been made in villages that were geographically isolated for centuries. Indeed (1285–1301dup) in the *DLL3* gene is a founder mutation responsible for the appearance of spondylocostal dysostosis in several families. However, the existence of two additional mutations in the same gene in such a small community is challenging to explain.

Autosomal recessive deafness in a village from the Galilee[4]

In northern Israel, a high frequency of hereditary deafness was reported in a village of lower Galilee.[4] The 9000 inhabitant Muslim Arabs are descendants from a single family that immigrated to this region from Jordan approximately 200 years (eight generations) ago. Three sons from this immigrant family each founded a Hamula (kinship group). Most marriages are between individuals born in the village, with a preference for marriages within the Hamula – particularly first cousins. The gene responsible for almost all cases of profound deafness is *GJB2* coding for connexin 26 and three distinct mutations were

found in the village. The three mutations appeared in the village approximately 100–150 years ago. At the present time, the general carrier frequency is 7.8% for (35G del), 2.4% for (W77R) and 4.8% for (V37I).

In the village, many genetic diseases have been characterized[5] owing to a high carrier frequency for some mutations in the surrounding population (thalassemia/ sickle cell anemia or familial Mediterranean fever), with others representing new random mutations. Among the mutations in the *GJB2* gene, (35Gdel) is relatively frequent among Israeli Arabs, whereas the other two mutations have never been reported in the region and probably occurred *de novo* in the village. The presence of three mutations in the *GJB2* gene, similar to the presence of three mutations in the *DLL3* gene in a small village off Switzerland, is difficult to explain as a random phenomenon.

Limb Girdle dystrophy in La Reunion Island[6]

Limb-girdle muscular dystrophy type 2A (MIM 253600) is an autosomal recessive disorder characterized by symmetrical and selective atrophy of the pelvic, scapular and trunk muscles. In most cases, the symptoms arise in childhood and progress gradually, often leading to the inability to walk. The disease was identified at a high frequency in a genetic isolate from La Reunion Island[6] (an Island in the Indian Ocean east of Madagascar).

The genealogical studies suggested a founder effect as the explanation for the high frequency of limb-girdle muscular dystrophy type 2A on the island. However, after the localization of the gene by linkage, the haplotypes suggested the existence of several mutations in *CPN3*. The analysis of the inter-related pedigrees revealed the existence of nine different mutations in the gene encoding for calpain-3, *CPN3*. A digenic model was proposed to explain these observations in which the clinical features in the homozygotes for calpain-3 mutations are secondary to the existence of a mutation in another gene frequently found on the island because of a founder effect.

Primary congenital glaucoma in Saudi Arabia[7]

Primary congenital glaucoma (MIM 231300) is a devastating autosomal recessive disorder associated with developmental defects of the anterior chamber. The disease occurs when intraocular pressure increases, leading to an enlarged cornea and eventually to optic nerve damage. Although primary congenital glaucoma is the most common form of glaucoma in infancy, it is uncommon in the North American population (<1:30 000) compared with Saudi Arabia where the estimated incidence is one in 2500. The early inhabitants of the Arabian peninsula formed a genetic isolate that was established by relatively few founders. Their descendants established various tribes with a high degree of consanguinity and large families. In Saudi Arabia, eight recent distinct mutations in the *CYP1B1* gene were found to be responsible for congenital glaucoma, the three

most common representing 72, 12 and 7% of all the chromosomes of the affected propositi.[7]

The camptodactyly-arthropathy-coxa vara-pericarditis syndrome in Saudi Arabia[8]

The camptodactyly-arthropathy-coxa vara-pericarditis syndrome is an autosomal recessive disorder (MIM 208250) manifesting with congenital camptodactyly and childhood-onset noninflammatory arthropathy. Most patients develop bilateral coxa vara deformity and some symptoms or signs of pericarditis. The syndrome is rare but was reported in several families from Saudi Arabia.[8] The responsible gene is *PRG4*, and among Saudi patients, five novel mutations were uncovered.

As expected in a very inbred population, many rare genetic syndromes are found in Saudi Arabia. However, the presence of several different mutations in each of the genes responsible for two otherwise rare disorders is difficult to reconcile with just a founder affect.

Bardet-Biedl syndrome in Newfoundland[9] among the Negev Bedouins[10] and in Lebanon[11]

Bardet-Biedl syndrome (BBS, MIM 209900) is an autosomal recessive disease characterized by various degrees of mental retardation, retinal dystrophy, renal structural abnormalities, obesity, polydactyly and hypogenitalism in males. BBS is rare with an estimated prevalence of 1:160 000 in Switzerland whereas it is relatively frequent among the Negev Bedouins (Israel) and in Newfoundland (Canada).

Newfoundland is a very young founder population (of less than 20 generations) that was genetically isolated. This isolation is evidenced by an abundance of several monogenic disorders mainly as a result of founder effects. However, the high prevalence of BBS (1:18 000) is due to mutations in at least five of the BBS loci.[10] At least nine different mutations were characterized, four of which are in the *BBS6* gene.

Among the Bedouin of the Negev in Israel, BBS is prevalent and mutations in three different genes *BBS2*, *BBS3* and *BBS4* were demonstrated.[9] Each of the mutations was a founder mutation each present in homozygozity in the patients of a single Bedouin tribe from the Negev.

In an extended consanguineous family living in a small Lebanese village, several individuals were diagnosed with BBS.[11] In one sibship, a homozygous mutation in *BBS2*, (G139V), was identified. In three other sibships, a homozygous *BBS10* mutation (S311A) was characterized. In the last sibship, a patient was compound heterozygote for two mutations in the *BBS10* gene (S311A) and (V11G).

In the three communities, mutations in more than one gene were responsible for the increased frequency. In the Lebanese extended family, the occurrence of the three different mutations was attributed to chance owing to the high frequency of the prevalence of BBS and of carriers in the population. Another possible explanation for at least

some of the observations is that the inheritance of BBS is often complex involving mutations in more than one of the BBS genes: a 'trialleic inheritance model'.[12]

Multiple mutations in a small geographic area
Israeli Arabs

Non-Jewish Israeli citizens include mainly Arabs being either Muslim or Christian and Druzes. Among Arab and Druze families, 45% of the marriages are between related spouses: half of whom are first cousins. This population has been living in small, relatively isolated localities, which were recently settled by a small number of founders. After the creation of the state of Israel in 1948, there was a significant internal increase in the population size and several larger towns developed, but since the preference for consanguineous marriages remained, the isolation of most communities is still preserved.

Most of the genetic diseases frequent among Arabs and Druzes are due to founder effects; in each case, a single mutation is responsible for the high frequency.[13] However, several examples in which multiple mutations cause rare genetic disorders have been reported: two of which are described here.

Metachromatic leukodystrophy in the Galilee[14]

Late infantile metachromatic leukodystrophy (MIM 250100) is an autosomal recessive disorder caused by the deficiency of the lysosomal enzyme arylsulfatase A (*ARASA*). The disease was diagnosed in seven different villages in a small area of Galilee.[14] In this region, five different mutations were characterized: two among Christian Arab patients, one in Bedouin Muslim patients and two in other Muslim patients. In each case, only one mutation was found in a single village and each patient was homozygous for the respective mutation.

Hurler syndrome in the Galilee[15]

Hurler syndrome (MIM 607014) is an autosomal recessive disorder caused by deficiency of the lysosomal enzyme iduronidase. The clinical features of Hurler syndrome include coarse facial features, corneal clouding, mental retardation, hernias, dysostosis multiplex and hepatosplenomegaly. Children with Hurler syndrome appear normal at birth and develop the characteristic appearance during the first years of life. The syndrome was diagnosed in seven families, Muslim Arabs and Druzes, residing in a very small area of northern Israel.[15] Three mutations were identified as mutations underlying Hurler syndrome in Muslim Israeli Arabs and among Druzes. In all instances, the probands were homozygous for the mutant alleles, as anticipated from the consanguinity in each family.

When these observations were made, it was thought that the existence of multiple mutations responsible for recessive diseases may be a phenomenon particular to

lysosomal diseases. However, the same phenomenon was also later observed in several other diseases in the Galilee including hyperoxaluria, ataxia telegiectasia, biotinidase deficiency, metylmalonic acidemia or molybdenum cofactor deficiency.

Lysinuric protein intolerance in Italy[16]

Lysinuric protein intolerance (MIM 222700) is a rare autosomal recessive disease that is relatively common in Italy. Clinical features of lysinuric protein intolerance include vomiting, diarrhea, failure to thrive, episodes of coma, hepatosplenomegaly and osteoporosis. Life-threatening lung involvement and severe renal defect have also been reported. The disease is considered relatively benign when appropriately treated with citrulline supplementation and a low-protein diet, but this treatment may be insufficient in preventing severe complications. Lysinuric protein intolerance is caused by a defective transport of cationic amino acid in the intestine and kidney. A lysinuric protein intolerance gene has been identified as member 7 of the solute carrier family 7A, *SLC7A7*.

In Italy, lysinuric protein intolerance is caused by several independent mutational events; in particular three different mutations were found in families originating less than 50 km apart.[16] One mutation was found in four families and the other two mutations were found each in a single family. In each patient, the mutation was homozygous.

Congenital chloride diarrhea in Poland [17]

The clinical picture of congenital chloride diarrhea (MIM214700) includes a life-long watery diarrhea with a high concentration of chloride, electrolyte disturbances and metabolic alkalosis. Patients receiving adequate salt and fluid supplementation grow and develop normally and may have a normal lifespan. Mutations in the *DRA* gene have been shown to cause congenital chloride diarrhea.

Seventeen Polish families with no close relation between them were studied. The distribution of the birthplaces of all known congenital chloride diarrhea grandparents shows clustering in the middle and southern parts of the country.[17] Molecular studies found several mutations in the congenital chloride diarrhea families. There was one major and three more local founder effects in the Polish population that have made congenital chloride diarrhea a relatively common disease. The rest of the mutations were found only in a single chromosome.

In Italy as well as in Poland, consanguineous marriages are rare but rural populations were often isolated. In each of the examples, a rare disorder was relatively frequent because of the presence of several mutations. These observations are parallel to those made in Galilee; the relatively high frequency in the small area being due to the presence of multiple mutations, each present in a homozygous state.

Usher syndrome type 1 in the Bressuire region[18]

Usher syndrome type 1 (MIM 276903) is an autosomal recessive condition characterized by profound congenital hearing impairment with early retinitis pigmentosa and vestibular dysfunction. Several different loci have been mapped and five of them were identified. The *USH1A* locus was identified in several French families in which the grandparents of the propositi were from the Poitou-Charente region around the town of Bressuire.[18] Further study found that there is no *USH1A* locus and in seven of the ten families from the region, a mutation in the *myosin VIIA* gene was characterized (*USH1B*). A total of 11 different mutations were found among the patients, and in two of the patients, the mutation – different in each case – was homozygous.

Because of the existence of an institution for the deaf and blind in Bressuire, the authors proposed that *USH1* families moved to the region, which could explain the finding of several mutations in the region.

Discussion

Many possibilities have been proposed to explain the observation that multiple mutations are responsible for a single disease in an isolated population or area. Among the hypotheses that have been raised are a chance phenomenon, migration, high mutation rates, a digenic model or selective advantage to carriers.

A chance phenomenon

The possibility that the observation of several mutations in isolated populations is a random phenomenon has often been suggested. This may indeed be true in some of the cases, for instance the inbred family from Turkey with Pendred syndrome described in this review, in which a carrier of the mutation frequent in the kindred married an unrelated individual who was a carrier of a mutation known to exist in his population of origin.[2] The existence of a mutation in a population increases the possibility that a second mutation will be revealed in a patient as a compound heterozygote. The probability for the second mutation to appear in an affected individual is related to the frequency of the first mutation ($2\,pq$). Whereas in this case the rare mutation is found in compound heterozygosity, in most of the examples discussed in this review, the affected individuals are homozygous. Therefore, most of the observations presented in the review do not fit this model.

Another possibility was proposed to explain the finding of three different mutations in two genes in the extended family with BBS from Lebanon.[11] According to the prevalence of the disease and the number of genes involved, the authors calculated a cumulative carrier frequency for BBS of one in 50 in the general population. From these calculations, it was proposed that the finding of

more than one mutation in a large family is expected by chance only and the consanguinity may bring the mutations to homozygosity. Still, even if this possibility is correct, it is difficult to explain by chance the finding of a third mutation in compound heterozygosity in another member of the family.

A chance phenomenon may explain some of the examples in which more than one mutation is found; however, in most cases this is improbable, particularly because of the diversity of the mutations and their relative frequency in different diseases and populations.

Migration of the families of affected individuals in a particular region

Non-random migration of affected families has been proposed as a possible mechanism for the clustering of the families of the patients in a small geographic area. This may have been because the grouping of individuals affected with similar disorders leads to social exclusion, a possibility that has been proposed to try and explain the high frequency of autosomal recessive deafness in most populations.[19]

Migration was suggested to explain the high frequency of Hurler syndrome in Israel and recently of Usher syndrome in France.[15,18] In the case of Hurler syndrome, the possibility that families of patients with the disease migrated to the Galilee was raised in the first description of the phenomenon but without any supporting evidence.[15] Hurler syndrome is a severe disorder leading to early death and it is difficult to imagine any reason why families with affected children would have a preference for migrating to Galilee. Another observation that is against this possibility is the finding that all the affected patients in the region are homozygous for each of the mutations, pointing to a relatively recent appearance of the mutations in the population.

In the case of Usher syndrome, it was suggested that after the creation of the first institution for the deaf and blind in the town of Bressuire in the middle of the 19th century, several *USH1* families moved to the region.[18] However, as it was the grandparents of the affected children who originated in the region, marriages between the descendants of the Usher families who migrated to the region must have taken place to explain the actual observations. The relatively small size of the population may support this possibility but only a detailed study of the genealogy of the families can demonstrate if it was indeed the case. Even if migration is indeed the explanation for the finding of multiple mutations in *USH1* in the Bressuire region, it is an exception and in the other cases there is no basis for favoring the hypothesis of a preferential migration.

The Reunion paradox and the digenic model

Genealogical studies suggest that La Reunion Island is an isolate with multiple links to the first settlers of the island.

The presence on La Reunion Island of multiple mutations responsible for the high frequency of the rare limb girdle muscular dystrophy was referred to as the Reunion paradox.[6] The authors of that study believed that the condition has a complex inheritance and that its expression depends on the genetic background. In their proposed digenic model, only the presence of specific alleles at a second unlinked locus would allow for the expression of the calpain-3 mutations. As mutations at both loci are needed for the expression of the disease, its prevalence will remain low. Under this model, the La Reunion island community would, as a result of a genetic drift, have a frequent allele at the second locus, which will explain the complete penetrance of the calpain mutations. In this model, the frequency of calpain mutations in the overall population is expected to be much higher than calculated according to the rarity of the disease. Another expectation is that some of the individuals that are homozygous/ compound heterozygotes for calpain-3 mutations will be healthy. The model has not been validated; no second locus has been found and the mutations in other populations are rare, as expected by the frequency of the disease in the general population. In addition, whereas many mutations in the calpain-3 gene were reported in various populations, the penetrance has always been complete and therefore the digenic model cannot explain the 'Reunion paradox'. The model is not applicable to most of the other disorders discussed in this review because in most of the cases a single gene is responsible for the occurrence of the disease. However, in the case of BBS, a digenic triallelic inheritance model has been demonstrated.[12] Mutation analysis of BBS genes identified families in which three mutations from genes at two different BBS loci segregate with expression of the disease. An oligogenic type of inheritance was proposed to explain the observations in BBS, in which mutations in a small number of genes may interact genetically to manifest the phenotype. However, among the Bedouin in Israel in each of the tribes where the syndrome is frequent, the data fit to a classical Mendelian single-gene disorder. No healthy individual homozygous for any of the mutations has been described and in each of the tribes up to now, only one mutation in one of the BBS genes has been found. However, the interaction between the different genes responsible for the syndrome probably explains in part the observations from Newfoundland.[20,21]

BBS highlights the difficulties of drawing meaningful conclusions about a phenomenon when the complete component of a disease is not well understood.

High mutation rates

The mutation rate in humans is estimated to be on average 1×10^{-8} to 2×10^{-8} per nucleotide per generation and is probably an order of magnitude higher in males than in females.[22] New mutations are expected to be frequent

because of the large numbers of nucleotides per genome. *De novo* mutations are easily detected in dominant disorders and frequently diagnosed in genetics clinics in families in whom healthy parents have a child with a dominant disease. In X-linked recessive diseases, the existence of new mutations is more difficult to detect, particularly because of the higher mutation rate in males, it takes at least two generations for new mutations to manifest. However, because of the mode of inheritance in which the hemizygote only requires one abnormal allele for clinical expression, examples of new mutations on the X-chromosome may also be suspected clinically. The existence of new autosomal recessive mutations is much more difficult to detect, which gives the impression that recessive mutations are rare. There are two main reasons that explain the observation that *de novo* autosomal recessive mutations are rarely detected. Firstly, an individual who inherited a new mutation that occurred in one of his parents cannot be detected on a clinical basis because carriers of autosomal recessive diseases are healthy. Secondly, to be affected, a patient must have received a mutant allele from each of his parents, the spouse of an individual in whom a new mutation occurred in the germ cells must also be a carrier. If indeed mutations in autosomal recessive genes have a pattern of occurrence similar to the one observed in dominant or X-linked mutations and a similar mutation rate, one would expect that during a long period of time, many recessive mutations took place in the population. Even if many of these mutations are lost in each generation, they should exist in the population and be observed in occasional patients. Indeed, in a rare disease such as ataxia telangiectasia, among 60 indigenous families of patients from the United Kingdom, 51 different mutations were detected, most of which were probably of local origin.[23] Eleven mutations were detected in more than one family and haplotype analysis demonstrated that they were founder mutations. As may have been expected by the structure of the population, most patients with ataxia telangiectasia originating from the 60 families were compound heterozygote and only in three families the patients were homozygous. On the other hand, if as the result of genetic drift with a founder effect in an isolated population there is one frequent mutation, then most patients will be homozygous for this mutation. In such populations, from time to time new mutations in the gene arise *de novo* and are revealed as compound heterozygotes.

The observations described in this review are different from those two situations as whereas more than one mutation is frequent, in many cases the mutations are detected in homozygosity. Therefore, the mutations are detected not because of the presence of a single frequent mutation but because of either the structure of the population and its isolation or because of the presence of other mutations.

Selective advantage to carriers

Most of the new mutations are expected to be lost. However, a new mutation could reach homozygozity when the conditions are appropriate: for instance, as a result of a founder effect in isolated or/and inbred populations. The particularity of inbred populations is that after the appearance of new recessive mutations, there is a significant probability that it may reappear three to four generations later in a homozygote state. Another possibility for a mutation to become frequent in the general population is if it confers a selective advantage to its carrier. The existence of such an advantage may explain the observation of multiple mutations in a small geographic area.[24] Although the conferred advantage to the carrier gives a better chance for its transmission to the next generation, the founder effect in an isolated population will lead to its rapid expansion. If a selective advantage is indeed the explanation for most of the cases presented here, then questions which remain to be solved are about the nature of the advantage and why this phenomenon remains isolated and not seen in larger populations. One possibility is that the selective mechanism is an advantage conferred to survive in a particular environment. In the case of BBS, both the Newfoundland population as well as the Bedouins are living in severe conditions. In the case of the Newfoundland population, it was proposed that the advantage might be an increased ability to store fat that may have provided a survival benefit in an environment where there was uncertainty about availability of food.[25] Another explanation for the positive selection may be that the advantage was particular to the population by itself with little or no influence of environmental factors. In other words, an advantage that is conferred by the mutations according to the genetic background of the population. This may then explain why the advantage is limited to a small region and that it is not observed in other populations.

Conclusions

Because of the high frequency of new mutations in humans, there is no need to assume an increased mutation rate to explain the finding of multiple mutations responsible for the prevalence of a disease in an isolated population. The unusual phenomenon is not the mutation rate but the clustering of related mutations in specific populations. A chance phenomenon, the migration of families with affected patients or a digenic inheritance may be responsible for the phenomenon in a few of the cases, but in most they are very improbable explanations. On the other hand, selection is a mechanism that may explain most of the observations, even if it is difficult to prove. The advantage to the individual that carries the mutation is to reduce the probability that a new mutation in the population may be lost. The type of selection may be

different in each case: with selection being advantageous either to environmental factors or to the genetic background of the population.

References

1 Coucke PJ, Van Hauwe P, Everett LA *et al*: Identification of two different mutations in the PDS gene in an inbred family with Pendred syndrome. *J Med Genet* 1999; **36**: 475–477.

2 Pannain S, Weiss RE, Jackson CE *et al*: Two different mutations in the thyroid peroxidase gene of a large inbred Amish kindred: power and limits of homozygosity mapping. *J Clin Endocrinol Metab* 1999; **84**: 1061–1071.

3 Bonafe L, Giunta C, Gassner M, Steinmann B, Superti-Furga A: A cluster of autosomal recessive spondylocostal dysostosis caused by three newly identified DLL3 mutations segregating in a small village. *Clin Genet* 2003; **64**: 28–35.

4 Zlotogora J, Carasquillo M, Barges S, Shalev SA, Hujerat Y, Chakravarti A: High incidence of deafness in an isolated community from three frequent Connexin 26 mutations. *Genetic testing* 2006; **10**: 40–43.

5 Zlotogora J, Shalev S, Habiballah H, Barjes S: Genetic disorders among Palestinian Arabs: 3. Autosomal recessive disorders in a single village. *Am J Med Genet* 2000; **92**: 343–345.

6 Richard I, Broux O, Allamand V *et al*: Mutations in the proteolytic enzyme calpain 3 cause limb-girdle muscular dystrophy type 2A. *Cell* 1995; **81**: 27–40.

7 Bejjani BA, Stockton DW, Lewis RA *et al*: Multiple CYP1B1 mutations and incomplete penetrance in an inbred population segregating primary congenital glaucoma suggest frequent de novo events and a dominant modifier locus. *Hum Mol Genet* 2000; **9**: 367–374.

8 Alazami AM, Al-Mayout SM, Wyngarard C-A, Meyer B: Novel PRG4 mutations underline CACP in Saudi families. *Hum Mut* 2006; **27**: 213.

9 Moore SJ, Green JS, Fan Y *et al*: Clinical and genetic epidemiology of Bardet-Biedl syndrome in Newfoundland: a 22-year prospective, population-based, cohort study. *Am J Med Genet A* 2005; **132**: 352–360.

10 Sheffield VC, Stone EM, Carmi R: Use of isolated inbred human populations for identification of disease genes. *Trends Genet* 1998; **14**: 391–396.

11 Laurier V, Stoetzel C, Muller J *et al*: Pitfalls of homozygosity mapping: an extended consanguineous Bardet-Biedl syndrome family with two mutant genes (BBS2, BBS10), three mutations, but no triallelism. *Eur J Hum Genet* 2007; **80**: 1–11.

12 Katsanis N, Ansley SJ, Badano JL *et al*: Triallelic inheritance in Bardet-Biedl syndrome, a Mendelian recessive disorder. *Science* 2001; **293**: 2256–2259.

13 Zlotogora J: The molecular basis of autosomal recessive diseases among the Palestinian Arabs. *Am J Med Genet* 2002; **109**: 176–182.

14 Heinisch U, Zlotogora J, Kafert S, Gieselmann V: Multiple mutations are responsible for the high frequency of metachromatic leukodystrophy in a small geographic area. *Am J Hum Genet* 1995; **56**: 51–57.

15 Bach G, Moskowitz SM, Tieu PT, Matynia A, Neufeld EF: Molecular analysis of Hurler syndrome in Druze and Muslim Arab patients in Israel: multiple allelic mutations of the IDUA gene in a small geographic area. *Am J Hum Genet* 1993; **53**: 330–338.

16 Sperandeo MP, Bassi MT, Riboni M *et al*: Structure of the SLC7A7 gene and mutational analysis of patients affected by lysinuric protein intolerance. *Am J Hum Genet* 2000; **66**: 92–99.

17 Hoglund P, Auranen M, Socha J *et al*: Genetic background of congenital chloride diarrhea in high-incidence populations: Finland, Poland, and Saudi Arabia and Kuwait. *Am J Hum Genet* 1998; **63**: 760–768.

18 Gerber S, Bonneau D, Gilbert B *et al*: USH1A: Chronicle of a Slow Death. *Am J Hum Genet* 2006; **78**: 357–359.

19 Nance WE, Kearsey MJ: Relevance of connexin deafness (DFNB1) to human evolution. *Am J Hum Genet* 2004; **74**: 1081–1087.

20 Katsanis N: The oligogenic properties of Bardet-Biedl syndrome. *Hum Mol Genet* 2004; **13R**: 65–71.

21 Mykytyn K, Nishimura DY, Searby CC *et al*: Evaluation of complex inheritance involving the most common Bardet-Biedl syndrome locus (BBS1). *Am J Hum Genet* 2003; **72**: 429–437.

22 Crow JF: Spontaneous mutation as a risk factor. *Exp Clin Immunogenet* 1995; **12**: 121–128.

23 Stankovic T, Kidd AM, Sutcliffe A *et al*: ATM mutations and phenotypes in ataxia-telangiectasia families in the British Isles: expression of mutant ATM and the risk of leukemia, lymphoma, and breast cancer. *Am J Hum Genet* 1998; **62**: 334–345.

24 Zlotogora J: Selection for carriers of recessive diseases, a common phenomenon? *Am J Med Genet* 1998; **80**: 266–268.

25 Davidson WS, Fan Y, Parfrey PS, Dicks E, Moore S, Green JS: Genetics of Bardet-Biedl syndrome: obesity and the Newfoundland population. *Progress in Obesity Research* 2003; **9**: 323–326.