

Modifier Genes and Oligogenic Disease

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Abstract

It is now increasingly apparent that modifier genes have a considerable role to play in phenotypic variations of single-gene disorders. Intrafamilial variations, altered penetrance, and altered severity are now common features of single gene disorders because of the involvement of several genes in the expression of the disease phenotype. Oligogenic disorders occur because of a second gene modifying the action of a dominant gene. It is now certain that cancer occurs due to the action of the environment acting in combination with several genes. Although modifier genes make it impossible to predict phenotype from the genotype and cause considerable difficulties in genetic counseling, they have their uses. In the future, it is hoped that modifier genes will allow us to understand cell and protein interactions and thus allow us to understand the pathogenesis of disease.

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Key words: modifier genes, oligogenic diseases, genotype phenotype correlation

Introduction

The rapid identification of genes that are associated with human disease has revolutionized the field of medical genetics. Medical genetics is now capable of providing more accurate diagnostic, prognostic, and, potentially, therapeutic tools. The classical model that is used in medical genetics for the discovery of several single-gene disorders is founded on the assumption that the spread of a trait in a family is synonymous with a single molecular defect. However, in recent times it has been seen that the number of diseases that can be explained with the classical Mendelian genetics model is gradually diminishing.

It has been suggested that the division of diseases into monogenic and multifactorial may be an

oversimplification¹. Several diseases are caused primarily by a mutation of one gene but are influenced by several other genes. Some diseases, such as Becker type muscular dystrophy, which were initially believed to be caused by a single gene defect also fall into this category². Thus, the concept of oligogenic disease and modifier genes has been developed.

The Appearance of Oligogenic Disease

Oligogenic disorders are based on the concept that inheritance patterns occur somewhere between the dichotomies of monogenic inheritance and polygenic disease. Oligogenic disorders remain primarily genetic in origin, in contrast to polygenic traits, which are believed to occur because of a complex interaction between the genes and the

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environment³. These disorders require the synergistic action of a small number of mutant alleles at a small number of loci. The position along this continuum depends on three main variables. It depends on whether a major locus is involved, the number of loci involved, and the extent of environmental participation³.

Modifier genes form the basis of oligogenic disease. Modifier genes act to vary the expression of traits that are inherited from one gene. Modifier genes are defined as inherited genetic variations that lead to a qualitative or quantitative difference in any aspect of the disease phenotype⁴. Strictly, modifiers do not determine whether a disease develops, but this definition is relaxed in case, certain such as breast cancer and the BRCA gene⁵. Modifier genes are almost certainly common polymorphisms; otherwise, their effects would go unnoticed⁶.

The following paragraphs will describe a few of the oligogenic disorders and the genetic modifiers in each of them. The diseases will also explain how oligogenicity was established for the disease and how the various genes influence the phenotype.

Familial Amyotrophic Lateral Sclerosis: Intrafamilial Variation

Familial amyotrophic lateral sclerosis (FALS) is a neurological disorder that is transmitted as an autosomal dominant trait⁷. Mutations in the copper/zinc superoxide dismutase 1 (SOD-1) gene are found in approximately 20% of patients with FALS, or amyotrophic lateral sclerosis 1. In addition to this mutation, analysis of a family with FALS has revealed that a second mutation of the ciliary neurotrophic factor (CNTF) gene is responsible for the variation in the phenotype⁸. It has also been noted that patients with sporadic amyotrophic lateral sclerosis who have a homozygous CNTF gene defect show significantly earlier disease onset but do not show a significant difference in disease duration. The same CNTF mutation is also seen in Japanese patients with neurological disease, but because no correlations could be drawn, the significance of this mutation is unknown⁹. In this

case, the variation between the genotype and the phenotype was the factor responsible for the identification of the modifier gene.

Familial Adenomatous Polyposis

Familial adenomatous polyposis (FAP) is an autosomal dominant disorder with a population frequency of approximately 1 in 8,000 and a penetrance of almost 100%⁶. The disease is typified by florid adenomatous polyposis of the colon and the rectum, although a less severe attenuated form, attenuated adenomatous polyposis Coli (AAPC), also exists. The minimum number of polyps for a diagnosis of FAP is 100.

Individuals with FAP have many clinicopathological features that are subject to variation both within and between families. Variations due to different APC gene mutations are more dramatic among families rather than within families^{10,11}. Variation in the severity of colonic disease may result either from a greater number of tumours being initiated or from faster tumour progression. The variation in the phenotype has brought into question the role of modifier genes.

It was initially believed that the differences in the phenotype could be explained on the basis of the types of mutations in the APC genes¹²⁻¹⁵. However, it was later shown that the phenotypic differences were mainly because of the influence of modifier genes^{16,17}. Important evidence for the existence of modifier genes in FAP comes from the work using the Min (multiple intestinal neoplasia) mouse model of polyposis. This work has demonstrated the existence of a modifier locus (Mom 1; modifier of Min) on mouse chromosome 4 in the region syntenic with human chromosome 1p35~36¹⁸. The Mom1 mutation explains about 50% of the genetic variance in polyp number, indicating that other modifier genes with weaker effects probably exist. The secretory phospholipase A2 (Pla2s) gene was then identified as a strong candidate for the Mom1 gene¹⁹. Linkage studies have detected some evidence of a human FAP modifier on chromosome 1p²⁰. However, no functional variants of PLA2S have been identified in humans and the existence of an FAP modifier on

1p remains unproved¹⁷.

Other candidate genes with a possible modifier effect upon phenotype are APC11307K, APC E1317Q, carcinogen-metabolizing genes such as those of the N acetyl transferase family, SMAD3, COX2, and Ha-ras VNTR²¹. Power calculations using discordant relative pairs (DRPs) will be required to identify the FAP modifier genes by linkage analysis. It is likely that using about 150 DRPs will identify most of the other genes having a modifier effect in FAP.

Retinitis Pigmentosa and Digenic Inheritance

Retinitis pigmentosa (RP) is a genetically and clinically heterogenous disease that can be inherited as an autosomal dominant, autosomal recessive, or an X-linked trait. The genes implicated in the disease are the retinal outer segment protein 1 (Rom 1) gene and the peripherin/retinal degeneration slow (RDS) genes. These are related-membrane proteins of the photoreceptor outer segments. Both proteins are located at the rims of the photoreceptor disks, where they may act jointly in disk biogenesis. Mutations in the gene (RDS) encoding peripherin cause autosomal dominant retinitis pigmentosa²². Three families have been identified with mutations in these unlinked photoreceptor-specific genes in which only double heterozygotes developed retinitis pigmentosa²³. No cases of RP caused by ROM1 mutations alone have been discovered thus far²⁴. This shows that mutations of the RDS gene cause dominant or digenic RP and that mutations of the ROM1 gene cause digenic RP²⁵. The ROM1 gene acts as a modifier gene for the RDS gene in modifying the phenotype of RP.

Hirschsprung's Disease and Altered Penetrance

Hirschsprung's disease (HSCR), or congenital intestinal aganglionosis, is a relatively common disorder of neural crest migration. Two variants of HSCR are known to exist, the short segment HSCR (S-HSCR) and the long segment HSCR (L-HSCR). It has a strong genetic basis, although simple Mendelian inheritance is rarely observed²⁶.

Mutations in the RET gene are responsible for approximately half of familial cases and a smaller fraction of sporadic cases. Mutations of genes that encode RET ligands (GDNF and NTN)²⁷, components of another signaling pathway (EDNRB, EDN3, ECE-1)^{28,29}, and a transcription factor, SOX10³⁰, have been identified in patients with HSCR. For almost every HSCR gene, incomplete penetrance of the HSCR phenotype has been observed, probably due to genetic modifier loci. The nature of the RET mutation in patients with Hirschsprung's disease is also a strong predictor for the phenotype. For the L-HSCR phenotype, a strong modifier is the gene on 9q13 which produces a severe phenotype even if there is a potentially weak RET mutation³¹. The mode of inheritance of S-HSCR is considerably more complex than that of the L-HSCR, with several other genes other than RET modifying the phenotype. Modifier genes in this disease are capable of altering the phenotype even when the predominant gene involved (RET) shows weak penetrance.

Spinal Muscular Atrophy: Major Phenotypic Variations

Results of the genotype-phenotype studies in spinal muscular atrophy (SMA) suggest that an SMA-modifying locus distinct from the survival motor neuron 1 (SMN 1) locus lies in the 5q13 interval. Nearly all patients with SMA have some alteration in the SMN 1 locus, although the phenotypic diversity is considerable. Mild SMA can go unnoticed for decades, whereas severe SMA or Werdnig-Hoffmann disease presents in the first year of life. The phenotypic modification of SMA could be due to an increase in the number of copies of the SMN 2 genes or because of the absence or presence of the deletion of additional microsatellite markers, C272 and C212^{32,33}. Neuronal apoptosis inhibitory protein (NAIP), a gene that lies 16.5 kb downstream of SMN, has also been proposed as a modifier gene due to its proximity to SMN and its homology to apoptosis inhibitory proteins³⁴.

Another gene that has been postulated to play a role in SMA is the H4F5 gene, which lies closer to SMN1 than any previously identified gene in this

region³⁵. The gene is believed to function in snRNP biosynthesis and is a possible treatment target.

Multiple Modifier Genes in Alzheimers Disease

Alzheimer's disease (AD) is a devastating neurodegenerative disease. Three early-onset AD genes have been identified: presenelin 1 (PS1), presenelin 2 (PS2), and β APP (amyloid precursor peptide). Modifier genes identified in AD are the E4 allele of APOE, which is a major risk factor for AD regardless of age of onset or family history. However, it has been observed that the APO E4 allele is neither necessary nor sufficient for the expression of AD. This suggests the involvement of other genetic elements. One of the candidate genes is the gene coding for alpha 1 antichymotrypsin (ACT)³⁶. Like APO E, ACT binds to beta amyloid peptide with a high affinity in the filamentous deposits found in the AD brain and serves as a strong stimulatory factor in the polymerization of beta amyloid peptide into amyloid filaments. The APO E4 gene dosage effect associated with the AD risk is also significantly modified by the ACT polymorphism³⁷. Multiple genes perform modifier functions and significantly alter the phenotype of sporadic late-onset AD.

Malignant Melanoma

Malignant melanoma is a highly malignant tumour of melanocytes. Although several environmental factors have been implicated in the pathogenesis of melanomas, it is now increasingly evident that genetic factors play an important role in the pathogenesis of malignant melanoma. Predisposition to melanoma is genetically heterogenous. Mutations in the exons of the cyclin-dependent kinase inhibitor gene CDKN2A are melanoma-predisposition alleles that have high penetrance³⁸. Another high penetrance susceptibility gene, CDK4, has also been identified³⁹. A pigmentation gene, the melanocortin-1 receptor gene (MC1R) gene, has not only been found to be a low penetrance melanoma gene but has also been shown to act as a genetic modifier of melanoma risk in individuals carrying CDKN2A

mutations. Variants of the MC1R confer much lower melanoma risk but are common in European populations³⁸. Recently, ultraviolet radiation, has also been established as a modifier of melanoma risk in carriers of CDKN2A mutations. An environmental agent has been seen to function as a modifier of melanoma risk in patients with a mutation of a modifier gene. Hence, melanoma is turning out to be an excellent paradigm for studying gene-gene and gene-environment interactions.

Familial Hypercholesterolemia: Disease Severity Modifier

Familial hypercholesterolemia (FH) is a co-dominant disorder caused by to a variety of mutations of the low-density lipoprotein (LDL) receptor (LDLR) gene that result in an elevation of plasma levels of LDL-cholesterol (LDL-C). Plasma levels of LDL-C show large interindividual variation even in persons carrying the same mutation of the LDL receptor gene. Variants of FH clearly illustrate the evidence of modifier genes in the pathology of the disease. Two variants of FH, hyperlipoproteinemia IIa (HLP IIa) with elevated plasma cholesterol and hyperlipoproteinemia IIb (HLP IIb) with elevated plasma cholesterol and triglycerides, show the same mutation in the LDLR gene. However, there is a significant difference in the associated mutations of soluble epoxide hydrolase (EPHX2)⁴⁰. The EPHX 2-287 Arg allele, when co-inherited with the defective LDLR allele, predisposes to hyperlipoproteinemia IIb. The absence of the EPHX 2-287 Arg allele along with a defective LDLR allele predisposes to hyperlipoproteinemia IIa. A similar study has noted the effect of the apoH 247Leu allele. Patients with a defective LDLR allele with the apoH 247Leu allele have HLP IIb. The absence of the apoH 247Leu allele co-inherited with the defective LDLR allele lead to HLP IIa.

Another disease that is believed to have a profound effect on FH is β thalassemia which has a profound LDL-lowering effect. Plasma LDL-C in FH heterozygotes carrying the β^0 thalassemia trait is 25% lower than in noncarriers, regardless of the

LDL receptor gene mutation. It is likely that this effect is due to either increased uptake of LDL by the bone marrow to provide cholesterol for the increased proliferation of erythroid progenitor cells or because of increased production of inflammatory cytokines that reduce the hepatic secretion and increase the catabolism of LDL. In view of its LDL-C-lowering effect, β -thalassemia trait may protect FH heterozygotes against premature coronary atherosclerosis⁴².

Thalassemia Intermedia: The Paradigm of Modifier Genes

There are three main clinical phenotypes of β thalassemia: thalassemia major (TM), thalassemia trait (TT), and thalassemia intermedia (TI)¹. TI is an ill defined clinical term use to describe patients with phenotypes that are more severe than the asymptomatic TT but are milder than the transfusion-dependant TM⁴³. The clinical severity of β thalassemia is influenced not only by the types of β thalassemia mutations but also by other factors, including those affecting α and γ globin gene expression⁴⁴.

Silent β thalassemias are seen in asymptomatic individuals in whom the β thalassemia may be missed by haematological indices. Compound heterozygotes for silent and severe β thalassemia mutations usually have mild TI⁴⁵⁻⁴⁷. A mild β thalassemia phenotype, when inherited in the heterozygous state with a severe phenotype, will also lead to moderately severe β TI^{48,49}. Again, considerable variation in phenotypic severity is seen from interactions of severe β^+ and β^0 alleles. Patients with mild β^+/β^0 , severe β^+/β^0 , or β^0/β^0 genotypes have phenotypes ranging from mild to severe^{43,50}.

Some homozygous or compound heterozygous states for β thalassemia can be sufficiently ameliorated by the co-inheritance of α thalassemia determinants. Heterozygous β thalassemia inherited along with a homozygosity for the α^+ or heterozygosity for α^0 thalassemia can result in a normal or an near-normal haematological picture⁵⁰⁻⁵³. The inheritance of one or more α thalassemia alleles is less effective in ameliorating the effects of β^0

thalassemias^{54,55}. However, a triplication of the α gene can produce TI in an otherwise mild phenotype of the β thalassemia trait⁵⁶⁻⁵⁹.

Homozygosity or heterozygosity for the Xmn1 polymorphism plays a role in modifying the phenotype of β thalassemias. Homozygotes for this mutation have a slightly milder disease when it is co-inherited with β^0 thalassemia^{60,61}. Besides the Xmn 1^G γ polymorphism, the other genes in the γ gene cluster that can modify the phenotype are the deletional and nondeletional variants of HPFH and occasional cases of γ gene triplication or quadruplication⁶².

Beta TI is one of the first diseases for which a systematic effort has been made to predict the phenotype based upon the primary mutation and the action of the modifier genes. In the future, it is hoped that more will be known about modifier genes and that the phenotype can be accurately predicted from the genotype on the basis of the modifier alleles.

Modifier Genes in Cystic Fibrosis

Cystic fibrosis (CF) is the most common lethal genetic disorder in whites. The disease is due to alterations of the CF transmembrane regulator (CFTR) protein, a-cAMP activated chloride channel located in the apical membrane of most secretory cells. The altered protein causes dense mucous epithelial secretions and a clinical phenotype that typically involves the gastrointestinal and respiratory tracts, pancreas, and liver⁶³.

Respiratory disorders account for about 95% of deaths in patients with CF and the severity of the pulmonary manifestations varies. The hypersecretion of dense mucus obstructs the small airways. The mucus obstruction leads to an alteration in defence mechanisms and consequent bacterial colonization.

The pulmonary phenotype is altered by several modifier genes including the gene, associated with the migration of the CFTR protein to the cell surface⁶⁴. Low levels of mannose-binding protein^{65,66} also influence the severity of the disease. This occurs because mannose-binding protein is a lectin

involved in the opsonization and phagocytosis of microorganisms. A decrease in the destruction of microorganisms would lead to an increase in infection and a consequent increase in the severity of symptoms.

Polymorphisms in cytokine genes^{67,68} are also important in influencing the severity of the disease, probably by modulating the severity of the inflammatory response. Different alleles of the $\alpha 1$ antitrypsin (A1AT) gene are known to influence the severity of the disease⁶⁹. Pulmonary function was better in patients with CF bearing the S and Z alleles of the A1AT gene.

The prevalence of hepatic involvement in patients with CF varies from 4.0% to 30%^{70,71}. The pathogenesis of hepatic involvement remains unclear. Factors that modify the hepatic phenotype are mutations of the mannose-binding lectin gene⁷², which is associated with a higher risk of severe liver disease. The Z and S mutations of the A1AT gene confer a three–seven-fold increased risk for severe liver disease⁷³. Mutations of the A1AT and the mannose-binding lectin genes act as independent risk factors for CF liver disease. Variants causing overexpression of transforming growth factor beta interacts with the A1AT gene mutations, thereby increasing the adverse effects of CF liver expression⁷³.

Conclusion

Oligogenic disease has modified the concept of a 'single gene, single disease' considerably. It has modified our approach to studying genetic disease, particularly with reference to familial inheritance. It has also modified treatment and management protocols. It has made the prediction of the phenotype on the basis of the genotype more difficult. Considerable problems in genetic counseling are now encountered because of modifier genes. However, it is hoped that the study of modifier genes will allow us to better understand protein function and cellular pathways and allow better therapeutic intervention in the future with better technology.

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