

Clinical Applications of Molecular Genetics: The Model of Congenital Adrenal Hyperplasia

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Abstract

Spectacular advances in molecular genetics have enabled the molecular characterisation of many genetic disorders. The clinical applications include: (i) identification of pre-symptomatic and symptomatic affected individuals (monogenic diseases), allowing for early treatment and prevention of complications, (ii) carrier testing for genetic counselling, (iii) pharmacogenetic testing to guide medical treatment, and (iv) susceptibility testing (in polygenic diseases) to determine the risk of developing future disease. Using the model of congenital adrenal hyperplasia (CAH), direct mutational analysis can be applied to: (i) confirm the diagnosis when hormone assays have been equivocal, which would allow for early treatment and prevention of adrenal crisis, (ii) prenatal diagnosis and prenatal treatment in affected females to prevent or reduce prenatal virilisation, (iii) heterozygote carrier identification for genetic counselling, (iv) novel therapeutic applications to optimise treatment, including adjusting the steroid dose based on consistent genotype-phenotype correlations, so as to reduce the incidence of growth-inhibiting effects of steroid excess. However, molecular analysis can occasionally be complicated by multiple mutations on one allele, which may potentially affect genotype-phenotype correlations. Hence, molecular genetic analysis of CAH may eventually be adopted as a second tier confirmation of the disease, but is unlikely to replace the current first tier screening assays of precursor steroid metabolites proximal to the enzyme deficiency.

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Introduction

Spectacular advances in genetics have enabled the molecular characterisation of many genetic disorders, and an improved understanding of disease pathogenesis has resulted in significant clinical applications. The early days before the 1960s, was the era of phenotype recognition, identification and classification. However, with the advent of new molecular technologies in the 1980s, the gene can now be amplified and examined, and this heralds the era of genotype identification.

After amplification by polymerase chain reaction (PCR), genotyping can be performed by a variety of techniques, including restriction digests, the use of DNA probes and sequencing. However, large scale association studies by genotyping many single nucleotide polymorphisms (SNPs) in individuals with well characterised phenotypes, are now promising methods of identifying the cause of many complex diseases. For diseases with established genetic causation, sequencing the entire gene will be time consuming and will

not be cost effective. In these diseases, a good strategy would be to screen for known common mutations of the gene in question, followed by sequencing of the entire gene if no mutations are detected.

Although genetic testing studies the genome directly, the sensitivity (or percentage of positive tests among subjects who will develop the disease) is not necessarily high.¹ Heterogeneity (the concept that more than one gene can cause a given disease) and the location of promoters or other gene-controlling elements outside the portion of the gene that is tested, are some reasons to explain why DNA tests may fail to identify affected individuals, thus accounting for false negatives.

With regards to specificity (the percentage of negative test results among subjects who will not develop the disease), a diagnosis is not always made by the presence of a DNA change.¹ Some gene changes are harmless variants, and mutations in a single gene can sometimes cause several different diseases. Hence, the interpretation of many genetic

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tests can be complex, because of several factors which include: (i) the effect of a given mutation which may be modified by other genes and the environment; (ii) different changes in a given gene may have different results; (iii) intermediate alleles may cause disease in only a fraction of cases; (iv) other genes, the environment and individual factors such as age and gender can affect penetrance so that 2 individuals with the exact same change may have different clinical presentations; and (v) a person with a 'disease causing mutation' may appear unaffected.¹

Nonetheless, the completion of the Human Genome Project has now provided a reference of the entire human genetic instruction book. It is clear that changes in genetic code can result in changes in the encoded amino acids. If these changes are functionally significant, they may affect the proteins, enzymes and receptors, with resultant clinical pathology and significant morbidity and mortality. Increasingly, the complexities of gene regulation, gene to gene interaction and gene expression are just being unravelled, and many clinical applications have arisen.

Clinical Applications of Molecular Genetics

Clinical applications of molecular genetics include the following:

1. Diagnostic testing: Molecular genetics can provide a genetic diagnosis and confirm an existing disorder.
2. Predictive testing: This determines the presence of a genetic condition for pre-symptomatic late onset disorders, for example in neurodegenerative disorders like X-linked adrenoleukodystrophy and Huntington's chorea. The tests predict the future, so that individuals can be better prepared. However, predictive testing raises its own ethical issues in relation to emotional trauma, depression and discrimination, as a consequence of knowing that disease will eventually develop.

Nonetheless, in both diagnostic and predictive testing, the identification of affected individuals may allow for early treatment, prognostication and the prevention of complications.

3. Carrier testing: In carrier testing, heterozygote carriers for X-linked and autosomal recessive disorders are identified so as to determine the probability of the birth of a normal or affected child. This would aid in reproductive choices. If the risk is unacceptable, couples may decide not to marry or to have children, or plan to terminate the pregnancy, if the foetus is affected. Other couples who are at risk of having a child with a serious abnormality may seek in-vitro fertilisation. However, the caveat is that carrier testing by genetic studies is only informative if the mutations have been fully identified in the propositus.

4. Prenatal testing: In prenatal testing, DNA is extracted from the chorionic villous sample between 10 to 13 weeks gestation, with a risk to the foetus of 1% to 2%. The foetus can be genotyped to predict risk of disease, and genetic counselling can be provided.
5. Pharmacogenetic testing: Pharmacogenetics is a study of how genetic polymorphisms can influence drug metabolism. The genotype can then be used to predict an individual's response to a specific drug, and the dosage can be modified accordingly.
6. Susceptibility testing: In susceptibility testing, the individual susceptibility to develop common polygenic disorders such as heart disease, diabetes and cancer is determined. At-risk individuals can be identified for primary prevention (diet/exercise) and secondary prevention (pharmacologic intervention).

The Model of Congenital Adrenal Hyperplasia

Congenital adrenal hyperplasia refers to any of several autosomal recessive diseases resulting from mutations of genes for enzymes mediating the biochemical steps of cortisol production from cholesterol by the adrenal glands. The most common form, 21-hydroxylase deficiency, can present with 3 phenotypes, based on the mutation which determines the degree of enzyme deficiency: (i) The Classical Salt Waster will present with a salt-losing adrenal crisis, and affected females present with ambiguous genitalia. (ii) In the Classical Simple Viriliser, affected females will have prenatal virilisation without salt loss, while affected males will present with pseudoprecocious puberty. (iii) The Non-classical CAH presents later with hirsutism, acne and irregular menstruation.

Likewise in the second most common form of CAH, 11- β hydroxylase deficiency, there is prenatal virilisation in affected females, but the hallmark is hypertension and hypokalemic alkalosis as a result of accumulation of precursor metabolites with mineralocorticoid activity, such as 11-deoxycortisol and deoxycorticosterone.² However, it is well recognised that hypertension may be absent or intermittent in the first few years, resulting in misdiagnosis.³

Clinical Applications of Genotyping for Congenital Adrenal Hyperplasia

Although hormonal screening can diagnose CAH effectively, genotyping can be useful in these circumstances:

1. Hormonal assays can diagnose CAH, but there may be false positives with mildly elevated serum 17-hydroxyprogesterone (17-OHP) levels associated with low birth weight, prematurity or the presence of stress-inducing illness which stimulates the adrenal production of steroids, including serum 17OHP.⁴ In contrast, direct

mutational analysis is not affected by these factors, and can also be applied to confirm the diagnosis when hormone results are equivocal.

2. If steroid treatment has been started in a child suspected of CAH before adequate diagnostic blood tests have been performed, genotyping can confirm the diagnosis without stopping treatment.
3. Genotyping may also help to differentiate 11- β hydroxylase deficiency from the simple virilising form of 21-hydroxylase deficiency. In both these conditions, there is no salt loss. Unfortunately, the hallmark hypertension in 11- β hydroxylase is variable, and the hormonal profile can be identical to 21-hydroxylase deficiency (low serum cortisol levels, elevated plasma ACTH, serum androstenedione, serum testosterone levels, with modest increases in serum 17-hydroxyprogesterone), making them indistinguishable. Hence genotyping provides an alternative, fast and accurate diagnosis of both 21-hydroxylase and 11- β hydroxylase deficiency. It is also important to make a diagnosis of 11- β hydroxylase deficiency so that fludrocortisone is not used for treatment. Whereas the hypertension in 21-hydroxylase deficiency will respond to a reduction in glucocorticoid dose (hypertension arising from presumed overdose of steroids), the treatment of hypertension in 11- β hydroxylase deficiency, conversely, necessitates an increase in glucocorticoid dose with the addition of spironolactone or amiloride. Even more importantly, undetected hypertension is potentially life-threatening, and may lead to fatal vascular accidents observed even in mildly virilised patients.
4. Generally, consistent genotype-phenotype correlations in CAH allow for prognostication, which can aid management in the field of pharmacogenetics. Conventional treatment for CAH with the replacement of glucocorticoid and mineralocorticoid is never perfect, as it is impossible to exactly mimic physiological secretion of cortisol and aldosterone.

With pharmacogenetics, it is potentially possible to adjust the glucocorticoid dose based on the genotype-phenotype correlations. The more severe genotypes may require a higher dose of glucocorticoid, and the milder genotypes may require lower doses, so as to avoid steroid toxicity and yet avoid hyperandrogenism.⁵

In addition, there are new treatment modalities which may improve growth potential in CAH due to severe mutations, which are currently being researched:

- a. Peripheral blockade of androgen action and estrogen production, which can be achieved with an androgen

receptor antagonist (flutamide) and an aromatase inhibitor (testolactone), which blocks the conversion of androgens to estrogens and allows for the use of a lower glucocorticoid dose.⁶

- b. Prophylactic bilateral adrenalectomy has been proposed for those with severe CAH with non-functional *CYP21* genes. In the null mutation of CAH, the adrenal gland is not functional and over-produces androgens. Hence, a bilateral adrenalectomy will eliminate excess androgens and avoid the risk of overtreatment with glucocorticoids.⁷
5. In carrier detection, if the index case is genotyped, the heterozygote carrier status of parents and siblings can be accurately determined. Genotyping has the advantage in carrier detection because hormonal assays for carriers are not always accurate, and there is considerable overlap in serum 17OHP for normals and carriers, even after a synacthen stimulation test. Knowing the parental carrier status enables accurate prediction of recurrence risks for future pregnancies.
6. In prenatal diagnosis, genetic studies of the DNA from chorionic villous cells are generally preferred over hormonal studies of the amniotic fluid. Following prenatal diagnosis, CAH is an example of an inborn error of metabolism which can be treated prenatally. The aims of prenatal diagnosis and prenatal treatment are to effectively prevent virilisation of an affected female foetus, to avoid gender confusion and sex mis-assignment, to avoid genital surgery and psychological trauma and the burden of genital ambiguity to the family. More significantly, with prenatal diagnosis, early treatment can be commenced so as to prevent a potentially life threatening adrenal crisis in the neonate.

From our studies on the *CYP21* gene in Singapore, the relatively low carrier frequency of 1.7% (1 in 60) does not warrant screening by genetic analysis.⁸ In addition, molecular analysis can occasionally be complicated by multiple mutations in one allele, which may potentially affect genotype-phenotype correlations. Hence, molecular genetic analysis of CAH may eventually be adopted as a second tier confirmation of the diagnosis, but is unlikely to replace the current first tier screening assays to quantify precursor steroid metabolites.

In conclusion, while neonatal screening of metabolites can diagnose disease, molecular genetics can confirm the diagnosis of some diseases, allow for specific genetic counseling with regard to heterozygote detection, prenatal diagnosis and treatment. Having screened and detected disease, genotyping may then help in optimising therapy to minimise morbidity, and to improve the quality of life, as illustrated in the model of CAH.

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