Molecular basis of virulence

Advances in molecular biology, cell biology, and several other areas of science have changed the way we understand the mechanisms in which microbial pathogens interact with their hosts. This trend is set to continue with the advent of microbial genome sequencing, in vivo gene expression analysis, and other related techniques. The availability of these techniques and advances in other areas such as protein expression and crystallography has allowed the understanding of host pathogen interaction at the molecular and even atomic level. However, despite these powerful approaches the basic concept advanced many years ago by Smith that pathogenicity or virulence is a multifactorial property that consists of five basic steps is still valid today. The molecular basis of virulence can still be considered under these five headings: (1) attachment to the host (via mucous membranes); (2) entry into the host (usually); (3) multiplication within the host; (4) interference with host defence systems; (5) damage to the host.

These five stages are not mutually exclusive. The factors produced by pathogens that mediate these steps are termed the determinants of microbial pathogenicity. The molecular basis of these steps will be considered and specific examples will be given to demonstrate the basic principles. Finally it should be emphasised that expression of determinants of pathogenicity is usually regulated and systems exist for environmental sensing and quorum sensing to allow appropriate expression of virulence factors.

(1) Attachment to the host
Attachment of bacteria to host cells is mediated by adhesins, which have been identified for many bacterial species. Most adhesins are proteins which usually bind to carbohydrate receptors on the host cell surface. Perhaps the most studied adhesins are the fimbrial adhesins of Escherichia coli. These can be divided into two families. The K88, K99, CFA/I, and CFA/II adhesins mediate attachment to gut epithelium while type 1, P, and S fimbriae are associated with infections of the urinary tract. Fimbriae tend to be composed of a helical structure of major protein subunits which act as a support for the minor protein subunits. The minor protein subunits determine the receptor specificity for type 1, P, and S fimbriae whereas receptor specificity is determined by the major protein subunits in the attachment to gut epithelium. Fimbriae genes have been cloned and sequenced and the individual amino acids responsible for the interaction with the host cell receptors have in some cases been determined, for example lysine and arginine residues at positions 116 and 118 appear to be important for the Sfa minor subunit of S fimbriae. Host cell receptors for attachment of bacteria have also been characterised at the molecular level, for example β-D-Gal groups are the ligand for K88 fimbriae. It should be pointed out that interaction between a pathogen and host is not mediated by a single adhesin/receptor interaction. A good example of this fact is the interaction of the respiratory pathogen Streptococcus pneumoniae (the pneumococcus) with host cells. At least four types of receptor have been proposed for the pneumococcus and the interaction with these receptors depends on the phenotype of the organism. Pneumococci present at least three interchangeable variants which can be distinguished by their colony morphology into opaque, semitransparent, and transparent. The molecular basis of the phase variation is unknown but these phenotypes differ in their ability to colonise the nasopharynx. Nasopharyngeal cells bear a receptor, GlcNAcβ1-3Gal, which is recognised by transparent phase variants. Lung cells in the resting state bear two types of receptor, GalNcβ1-4Gal and GalNcβ1-3Gal, which are recognised by both opaque and transparent types. Cytokine activated lung cells also express the resting receptors but also present platelet activating factor (PAF) receptors. Transparent phase variants are able to adhere to PAF receptors whereas opaque variants are not. Thus, adhesion to epithelial cells should be considered as a dynamic situation in which both bacteria and host cell receptors differ according to site of isolation or activation state.

(2) Entry into the host
Entry involves either direct invasion of the epithelial cells of a mucus membrane or passage between them followed by invasion of the deeper tissues. Not all pathogens invade; Vibrio cholerae causes disease by toxin production from within the intestinal lumen.

Invasion of epithelial cells by the gut pathogen Shigella flexneri has been investigated in some detailed and elegant studies. Entry of shigella into cells is triggered by three gene products IpaB (invasion plasmid antigen B), IpaC and IpaD. IpaB is a haemolysin responsible for the release of shigella from the vacuole into the cytoplasm where the organisms then move by polymerisation of host actin under the influence of the intracellular spread gene icsA. This...
motility results in the generation of protrusions from one cell to the next. The protrusion is ‘clipped-off’ to form a vacuole within the next cell. The double membranes of this vacuole are then lysed by the product of the icsB gene, the organisms are released into the cytoplasm and the cycle begins again. A note of caution is warranted here with regard to the use of tissue culture cells. Although the mechanism described above applies to HeLa cells, shigella cannot invade across a brush border. Further studies using animal models indicated that invasion was more complex. Shigella enter the colonic mucosa through M-cells and then infect macrophages causing apoptosis programmed cell death). Apoptosis is mediated by the product of the ipaB gene. Inflammation resulting from the release of cytokines results in infiltration of phagocytes which damage the basal membrane and disrupt the epithelium. The brush border is disrupted and shigella invade the cells. This example highlights the need to use several approaches to understand the events occurring during pathogenesis.

(3) Multiplication in the host
Once in its specific niche the pathogen must multiply. The ability to multiply is a characteristic of all living organisms and the success of a pathogen depends on the degree to which it can multiply upon reaching its specific niche and secure its potential transmission to a new host. The speed of multiplication will also affect the type of disease caused. Rapid multiplication leads to acute disease whereas slow multiplication may be advantageous in chronic disease. Little is known of the molecular basis of multiplication within the host. The environmental factors and nutrients that determine growth rate in tissues remain largely unknown. Most detailed studies on factors controlling growth in vivo have concerned molecular mechanisms for overcoming iron restriction. Pathogens attempt to obtain iron by one of several mechanisms including (a) from haemoglobin as hemin or heme, for example _S pneumoniae_, (b) directly from ferrated transferrin or lactoferrin, for example _Neisseria gonorrhoeae_, (c) indirectly from iron binding proteins by the production of siderophores, for example _E coli_, (d) from intracellular iron stores, for example _Mycobacterium tuberculosis_.

(4) Interference with host defence systems
To survive within the host the pathogen must either prevent the immune response or circumvent its action. There are many virulence factors associated with interference of host defence, including polysaccharide capsules, protein toxins and lipopolysaccharides. Polysaccharide capsules interfere with the processes of phagocytosis and complement mediated bacterial killing. The importance of the capsule in the virulence of some organisms has been demonstrated at the molecular level. A non-capsular mutant of the pneumococcus generated by transposon mutagenesis was one million times less virulent than its capsular parent. The molecular mechanisms of activity of capsular polysaccharide still remain to be defined. It is known that siaIy groups on capsular polysaccharides of _E coli_ K1 and group B streptococci may prevent the activation of the complement pathway by these organisms. Antibodies to capsular polysaccharide usually confer protective immunity through opsonisation of bacteria promoting phagocytosis. Encapsulated pathogens are still major causes of human disease due to the production of diverse capsular types (for example pneumococcus) or to the capsule being non-immunogenic (for example _E coli_ K1 and _Neisseria meningitidis_ group B). The non-immunogenicity of these organisms may be due to the molecular structure of the polysaccharide subunits being similar to sugars found on host cells.

Pathogens may also produce toxins that interfere with the immune response. The pneumococcus, for example, produces pneumolysin. At high concentrations this membrane damaging toxin lyses all eukaryotic cells. At sublytic concentrations it has a range of effects on the cells and soluble molecules of the immune system. Pneumolysin inhibits the respiratory burst of phagocytes and also inhibits random migration and chemotaxis by these cells. The toxin inhibits antibody production by lymphocytes and blocks mitogen induced proliferation of B-cells. In vivo the toxin induces a large inflammatory response and this may be due to its ability to stimulate the production of inflammatory cytokines (interleukin-1 and tumour necrosis factor-a) and to activate the classical complement pathway. Activation of the classical complement pathway is due, at least in part, to the ability of the toxin to bind to the Fc portion of IgG. Molecular analysis has allowed the regions of the toxin responsible for these activities to be identified and modified. The role of the activities of the toxin have been investigated in the context of the whole bacterium by using gene replacement techniques to construct versions of the pneumococcus expressing altered versions of the toxin. These studies demonstrate the power of molecular analysis when used in combination with other techniques such as animal models in allowing the role of individual proteins, protein domains or even amino acids in the pathogenic process to be investigated.

Studies on the molecular basis of the activity of lipopolysaccharides (endotoxin) are not as advanced as studies with protein virulence factors. It is known that long O side chains are required for serum resistance in _E coli_ and salmonella.

(5) Damage to the host
Damage to the host can be mediated either directly by production of toxins or indirectly by the induction of gross inflammation and immunopathologic reactions.

There is a wealth of information in the literature concerning the molecular action of bacterial protein toxins (reviewed in Alouf and Freer ). In some cases the contribution of the toxin to disease process is obvious (for example in cholera or tetanus) and the action of the toxins concerned is understood at the molecular level. Cholera toxin, for example, is an example of an ADP ribosylating toxin. The enzymatic action of the toxin results in the ADP ribosylation of a regulatory G protein of the adenylate cyclase complex in enterocytes. This results in increased levels of cAMP which in turn alters ion transport across the epithelium and leads to the diarrhoea which is the key feature of the disease. The heat labile toxin of _E coli_ works in a similar manner to cholera toxin. Other examples of ADP ribosylating toxins include pertussis toxin, diphtheria toxin and toxin A of _Pseudomonas aeruginosa_. The effects of these toxins vary according to the cellular target of ADP ribosylation. Diphtheria and toxin A inhibit protein synthesis while pertussis toxin uncouples signal transduction. Other toxins are proteases. Tetanus toxin is a zinc protease that cleaves synaptobrevin, a protein involved in neurotransmitter release. Another large group of toxins are known as the membrane damaging toxins. These include pore forming proteins such as _Staphylococcus aureus_ alpha toxin and thiol-activated toxins including pneumolysin. The molecular mechanisms of action of some activities of these toxins have been elucidated, but others and contribution to disease of the process of pore formation is still unclear.

Stimulation of inflammation may occur due to the inappropriate or excessive production of cytokines or activation of the complement pathway. Inappropriate cytokine production or activation of the complement cascade may...
be triggered by bacterial toxins such as pneumolysin. Bacterial cell wall components also stimulate cytokine production. Stimulation of inflammatory cytokine production by lipopolysaccharide of Gram negative bacterial cell walls mediates endotoxin shock. Release of inflammatory cell wall fragments from the pneumococcus during autolysis mediates inflammation and data suggest that this inflammation is the major contributing factor to pathology in pneumococcal meningitis. Treatment of pneumococcal meningitis with cell wall active antibiotics such as penicillin may have the short term effect of promoting inflammation and it has been suggested that these antibiotics should be used in conjunction with anti-inflammatory agents.24

The molecular mechanisms of induction of cytokines by lipopolysaccharide and Gram positive cell walls are now beginning to be understood at the molecular level and have been reviewed.24

Immunopathologic reactions may also lead to damage to the host. These reactions occur due to bacterial antigens including the production of antibodies that are cross reactive to human structures as is seen for example in endocarditis after infection with group A streptococci. Glomerulonephritis can also occur after streptococcal infection and is due to the deposition of immune complexes in the kidney. The molecular determinants of some of these reactions have been established. The M-protein of streptococci, for example, has been shown to share epitopes with antigens expressed in heart tissue.25 Ankylosing spondylitis may be caused by antibodies to klebsiella reacting with antigens expressed by lymphocytes from individuals with the HLA B27.26

Regulation of virulence

When it encounters a host, a pathogen must adapt to changing environments and express appropriate virulence factors. Virulence genes may be regulated in response to a range of environmental stimuli including pH, temperature, oxygen tension, and inorganic metal ion concentration. Knowledge about the molecular basis of virulence gene regulation is rapidly increasing. The commonest mechanisms involve two component regulatory systems in which one component (the sensor) detects the environmental stimulus while the other (the repressor, usually a DNA binding protein) is responsible for altering gene expression. The sensor protein is usually a membrane spanning kinase which autophosphorylates on stimulation. The phosphate is then transferred to the response regulator proteins which then affect gene expression. Examples of this type of system are regulation of permeability in *E. coli* in response to osmotic stimuli (EnvZ/OmpR) and regulation of motor control in *E. coli* chemotaxis (CheA/CheY, CheB). These types of system, which have been comprehensively reviewed,27 have common features but differ in their exact mechanisms.

Bacterial pathogens produce a range of molecules that allow them to cause disease via the five stages described above. It should be emphasised that these five stages are not discrete steps and the interaction of the pathogen with its host is dynamic. The pathogen continually monitors its environment and produces virulence factors according to the signals it receives. There is also a host contribution to the process such that genetic differences between individuals will make one host environment different to another and affect the interactions and signals that occur between the pathogen and its environment. An understanding of the events that occur at the molecular level both in the action of individual virulence factors and in the coordinate regulation of virulence as a whole is a continuing aim that will be aided by the new trend of total genome sequencing of pathogens. An understanding of the molecular events involved in the disease process will allow us to generate new weapons to use in the continuing battle against infectious diseases.

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Commentary

The issues discussed by Professor Mitchell raise some important questions about the characteristics of the many human diseases that occur as a consequence of infection. Micro-organisms have evolved alongside humans and, indeed, are able to evolve much more rapidly due to their short generation times. The nature of their relationships with us vary from, at one extreme, imperceptible parasitism (for example commensal colonisation of the skin and mouth) or even full blown symbiosis (for example the mitochondria in all our cells—thought to derive from exogenous microbes) to overwhelming illness and death at
Growing interest in overgrowth

Congenital malformations or complex malformation syndromes are frequently associated with growth failure and have been the subject of much research and discussion in the paediatric literature. The less common overgrowth syndromes (OGSs) have until recently received little attention. The disordered growth in OGSs is, however, a primary anomaly and, unlike growth failure, is not explained away as a secondary phenomenon as is the case with many other complex syndromes. OGSs may therefore provide a fascinating window into the mechanisms of growth and the consequences of the failure of this regulation.

Ancient literature has many references to giants such as Goliath, Polyphemus, Gargantuа, or the Patagonian giants. Whether real or fictional, these reports show that such patterns have been present throughout history and serve to highlight two of the central issues—what is a “true overgrowth syndrome and how many overgrowth syndromes exist?”

Previously, overgrowth patterns were often categorised as primary or secondary. In primary disorders, the growth would be an intrinsic (unexplained) feature of the condition secondary to cellular hyperplasia, whereas in secondary disorders an identifiable cause, often endocrinological, would be expected to result in growth excess.1 The limitations of this rather simplistic differentiation have been illustrated by the identification of “novel” growth factors in a number of OGSs, such as Beckwith-Wiedemann syndrome (BWS) and Simpson-Golabi-Behmel syndrome (SGBS). In these disorders, abnormalities of insulin-like growth factor II (IGF II) and glypican 3 have been implicated.2 3 It would seem that if the term secondary growth factor II (IGF II) and glypican 3 have been implicated.2 3 It would seem that if the term secondary growth excess is still relevant, it should be limited to situations dependent on extrinsic growth promoters, such as fetal macrosomia secondary to maternal diabetes and hyperglycaemia and subsequent fetal hyperinsulinaemia. For the foreseeable future most “primary” OGSs will be classified by a process of clinical assessment and/or laboratory exclusion with the possible exceptions of BWS and SGBS, where clinical application of molecular tests might be feasible within a few years.

The constraints of this review preclude the discussion of another group of overgrowth patterns: those syndromes exhibiting regional/tissue specific overgrowth (table 1). These may provide further clues to specific growth promoters, and will have to be considered when developing a model of overall growth control.
Table 1 Examples of regional or tissue specific overgrowth

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<th>Condition</th>
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<td>Autosomal dominant macrocephaly</td>
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<td>Macrocephaly cutis marmorata telangiectasia</td>
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<tr>
<td>Neurofibromatosis</td>
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<tr>
<td>Proteus syndrome</td>
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<tr>
<td>Encephalocrianiocutaneous lipomatosis</td>
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<tr>
<td>Hemihypertrophy</td>
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<tr>
<td>Klippel-Trenaunay-Weber syndrome</td>
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<tr>
<td>Maffucci's syndrome</td>
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<tr>
<td>Olliers syndrome</td>
</tr>
<tr>
<td>Prader-Willi syndrome</td>
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<tr>
<td>Cohen's syndrome</td>
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<tr>
<td>Carbohydrate glycoprotein deficiency</td>
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<tr>
<td>Fragile X syndrome</td>
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<td>Trisomy 8 mosaicism</td>
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Jamaica, I would, however, be concerned of the influence of the high frequency of umbilical hernias (also known to be associated with an increased familial frequency of exomphalos) in this population. This might have led to over diagnosis, or might indicate that the population as a whole had a higher than average frequency of some of the predisposing mechanisms of BWS.

In BWS, the three main components are abdominal wall defects (exomphalos—E), macrocoglossia (M), and increased birth weight or growth (G) present in 80%, 88%, and 97% respectively, resulting in the alternative name EMG. The paediatric relevance of BWS is maintained because approximately 4–7.5% of patients develop childhood tumours, most commonly Wilms' tumours. The haemihypertrophy, present in 13–24% of patients with BWS, is reported in 40% of cases with tumours, therefore the presence of hemihypertrophy appears to be associated with an increased risk of tumourigenesis.

Endocrinological and metabolic disorders with an overgrowth component

Abnormalities of the hypothalamic pituitary adrenal axis may result in early overgrowth. A number of these are autosomal recessive and the most common is congenital adrenal hyperplasia due to 21-hydroxylase deficiency. In untreated cases, the production of excess 7-hydroxyprogesterone and androgenic steroids results in increased growth from birth and precocious puberty secondary to the anabolic effects of the steroids. Secretory tumours within this axis may also result in similar overgrowth patterns. A much rarer recessive adrenal disorder is adrenocorticotropic hormone receptor deficiency. This may present with symptoms suggestive of adrenal failure or overgrowth, or both, and prompt treatment of the adrenal insufficiency is of obvious importance.

The aetiology of the overgrowth and endocrinological confirmation of each of the above is clear. This has not always been the case for disorders with proved endocrinological abnormalities, for example, Seip-Barardinelli lipo-dystrophy syndrome. This autosomal recessive condition appears to show insulin resistance, but normal pituitary and adrenal function on formal testing. The growth excess can be striking (considerably more than 97th centile), and appears to be more than would be expected from the postulated anabolic component of the syndrome. After some 30 years of research, the mechanism of overgrowth and its link to insulin resistance is only now becoming clearer.

One further condition that may rarely present with overgrowth, which is important not to miss because of the neurological and genetic implications, is the recessive condition, mucopolysaccharidoses type III (Sanfilippo's syndrome). Early literature indicates that the excess growth is only apparent in the first two to three years of life, and that the regression and mental handicap is obvious by this stage. This is in fact frequently incorrect, and I have personal experience of a 6 year old patient over the 90th centile for all growth measurements, who was then only starting to show a decline of intellectual abilities into the range of mild mental handicap.

Congenital malformation syndromes with overgrowth as the major intrinsic component

The majority of conditions with “primary” overgrowth are poorly understood but are part of an ever increasing list of possible diagnoses. The current London dysmorphology database lists 283 conditions with either macrocephaly, obesity, increased birth weight, or excess stature. Personal experience in excess of 300 children, is that as many as 50% do not easily sit within the diagnostic categories currently recognised.

Any diagnostic assessment must include taking an adequate family history and measurements of the nuclear family, as the largest single factor is without doubt familial large stature or early maturation. The presence of some additional sign or symptom within an individual does not necessarily require a syndrome diagnosis because single malformations, or some degree of learning difficulty, is present in up to 10% of the population.

All possible diagnostic considerations will not be covered within this annotation, however the reader is referred to the excellent review of Cohen. Tables 1 and 2 (modified from Cole and Hughes) also list some of the range of regional and generalised overgrowth disorders.

BWS and SGBS—two OGSs but a common aetiological pathway

BWS, first described in 1963, has provided much of the recent impetus for research into overgrowth patterns. It has a reported frequency of one in 13 700 from a study in

Table 2 Generalised overgrowth conditions

<table>
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<tr>
<td>Familial/sporadic constitutional gigantism</td>
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<tr>
<td>Familial/sporadic precocious maturatation</td>
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<td>Banayan-Riley-Ruvalcaba syndrome</td>
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<td>Marfan’s syndrome</td>
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<tr>
<td>Beckwith-Wiedemann syndrome</td>
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<tr>
<td>Marshall-Smith syndrome</td>
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<tr>
<td>MOMO* syndrome</td>
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<tr>
<td>Nevo’s syndrome</td>
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<tr>
<td>Petitman’s syndrome</td>
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<tr>
<td>Seip-Barardinelli syndrome</td>
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<td>SGBS</td>
</tr>
<tr>
<td>SS</td>
</tr>
<tr>
<td>WSS</td>
</tr>
<tr>
<td>Congenital adrenal hyperplasia</td>
</tr>
<tr>
<td>Pituatry/adrenaland gonadal secreting tumours</td>
</tr>
<tr>
<td>Sanfilippo's syndrome</td>
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<tr>
<td>Klinefelter's syndrome</td>
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*MOMO = macrosomia-obesity-macrocephaly-ocular anomalies.
Mannens et al., showed that paternal duplications, maternal translocations, and paternal uniparental disomy (the presence of only paternal chromosome material) involving 11p15.5 result in BWS. There is evidence that the phenotype results from overexpression of IGF II, a gene usually only expressed on chromosomes of paternal origin. Recent papers have shown that mutations within p57KIP2, or disruption of the gene KVLQT1, which both map proximal to IGF II at 11p15.5, will result in the BWS phenotype. In both instances the maternally inherited gene is affected. At least one family with a translocation disrupting KVLQT1 had biallelic IGF II expression, while p57KIP2 may be involved in a common pathway with IGF II. A p57KIP2 gene “knock-out” mouse, and subsequent mutation studies of KIP2 in humans, also result in a BWS phenotype, but once again only if the maternal allele is affected. There also appears to be some early evidence for genotype/phenotype correlations with p57KIP2, mutation carriers having a high frequency of abdominal wall defects (five out of seven had exomphalos, one out of seven had an umbilical hernia) but no embryonal tumours, whereas uniparental disomy 11p15.5 may be associated with a slightly greater risk of tumourigenesis. This phenomenon of imprinting (the regulation of gene expression dependent on parent of origin) is believed to be important in numerous different conditions with disordered growth including Prader-Willi syndrome, Angelman’s syndrome, Russell-Silver syndrome, and certain tumours, and has therefore been the subject of intense investigation.

During research into BWS, clinicians noted the striking phenotypic overlap with SGBS, an X linked overgrowth disorder (table 3). In the report of Thorburn et al the diagnosis of SGBS in some, or all, of the three boys (all of whom died in infancy and had additional malformations recognised in SGBS) remains a possibility, and is one further source of error which could result in overestimation of the incidence in BWS in their paper.

After the identification of glypican 3 mutations as the cause of SGBS, subsequent antibody studies showed there was cross reaction of binding between the ligands for glypican and IGF II receptors. This in turn might explain the striking phenotypic overlap and also raised the possibility of further diagnostic tests in the literature or clinical setting. The latter has significant implications for genetic counselling. An additional consideration is the existence of an undiagnosed group of patients, as reported in the paper by Morrison et al. They described “non”-BWS patients with overexpression of IGF II. I would suggest that the presence of overgrowth and nephromegaly in these patients might represent the mild end of the spectrum of BWS. An alternative explanation is that many macrosomic babies have some underlying mechanism which results in abnormalities of IGF II expression, the BWS phenotype being just one of the resultant outcomes. If the outcome is not the BWS phenotype, it raises the fascinating possibility that overexpression of IGF II might explain the excess of neuroblastoma and Wilms’ tumours in babies weighing over 4000 g. As we start to unravel the mechanisms behind BWS and SGBS it appears that a more intriguing set of questions are raised.

While molecular studies for glypican 3 and IGF II are still at an early stage, it seems likely that within the next 2–3 years these will become part of the routine investigation for BWS and SGBS in an effort to confirm the diagnosis and the recurrence risk, and perhaps even help predict the individual natural histories.
particularly true of girls whose growth often stops earlier than their peers after a relatively early puberty. This pattern appears more variable in boys, but still very few exceed or even approach +3 SD (Agwu et al in preparation). It is the authors experience that while careful monitoring of growth, bone age, and pubertal status is advisable, therapeutic intervention is almost never necessary to obtain an “acceptable” final height.

SS was originally incorrectly reported to have a high frequency of mental handicap (88%). It is now clear that the figure is lower, and Finegan et al reported that 78% of children have an IQ above 70. This still remains one of the highest figures for OGSs and is often associated with significant behavioural problems. Other significant medical complications are individually uncommon.

Despite the similarities with WSS and MSS, these two conditions appear to exhibit higher frequencies of severe medical complications, including skeletal anomalies and infant death. Surprisingly, personal experience of the three older cases of WSS (two adults and one adolescent) and the two oldest literature cases of MSS is that intellectual abilities have been within, or near to, the normal range in all these individuals. This might be fortuitous, as significant intellectual impairment has been documented in both disorders.

The range of final heights in WSS remains unclear, however three literature reports of adults with WSS, and my own experience of two adult patients, may suggest that heights, significantly in excess of +2 SD, are more common than in SS. It should be stressed that ascertainment bias could be very relevant in four out of five of these cases.

**The genetics of OGSs**

The genetics of BWS is that the majority are sporadic, but approximately 15% follow autosomal dominant inheritance. In my experience of approximately 200 cases of SS, three cases, and one of 15 cases of WSS segregated in an autosomal dominant fashion, the remainder are sporadic. Unlike BWS, to date there has been no consistent region of chromosomal alteration in the other OGSs, and a study of uniparental disomy in SS was negative within the limitations of the study. The genetics therefore remain obscure in the majority of OGSs.

The clinical similarities among the OGSs should intrigue, yet alert, the researcher to the issue of whether the OGSs should be “lumped” or “split”. It seems certain that only as the molecular basis unfolds will it become clear if the disorders are differing ends of the same spectrum or separate conditions. Could it be that they will be allelic variations in the same gene, such as in the RET oncogene which results in the different multiple endocrine neoplasia II syndromes and Hirschsprung’s disease, or mutations in the same fibroblast growth factor gene associated with the different craniosynostosis syndromes such as Apert’s, Pfeiffer’s, Crouzon’s, and Jackson-Weiss? Or perhaps the different OGSs will be due to mutations within a group of genes like the fibroblast growth factor I, II, and III genes, each associated with syndromes including the feature of craniosynostosis, or the different fibrillin genes which are the cause of Marfan’s syndrome (fib 1), congenital contractual arachnodactyly (fib 2), and Shprintzen-Goldberg (fib 1). While these are possible mechanisms, the only precedent in OGSs is in BWS and SGBS. In these two conditions, none of the above mechanisms apply, but rather two different genes interact on the same pathway.
Diagnosing Friedreich’s ataxia

The condition that now bears his name was first described by Nicolaus Friedrich in a series of papers between 1865 and 1877. He noted the onset at around puberty of ataxia and dystarthisy; sensory loss and weakness developed later. The skeletal deformities of pes cavus and scoliosis were also reported. Over the following generations there was a tendency to lump the inherited ataxias together, and the essential features of Friedreich’s ataxia became diluted. Clinical studies in the 1970s and 1980s and subsequent genetic studies have helped clarify these features, and Friedreich’s ataxia is now known to be the commonest of the inherited ataxias, accounting for at least 50% in most large series and affecting approximately one in 50,000 individuals. Although at present it is an incurable and progressive disease, recent identification of the affected gene has not only provided a highly sensitive and specific diagnostic test, but has also given useful insight into the cellular pathology which may lead to the development of effective treatment.

Clinical diagnosis

Despite the relatively homogeneous clinical picture of an early onset of progressive ataxia involving the trunk and the limbs, it was necessary to formulate strict clinical criteria in order to perform genetic linkage analyses, and two notable studies1 provided these. Harding’s criteria (table 1) were widely adopted and are still useful today, although of course we are now able to reinterpret “atypical” cases in the light of available genetic data.

In addition to ataxia, there are several variable features, including pyramidal tract involvement. Initially this may be mild, with only extensor plantar responses, but after five or more years a pyramidal type of weakness in the legs invariably occurs and this can eventually lead to paralysis. The association of extensor plantar responses, absence of ankle reflexes, and a progressive course provide the core features. Skeletal abnormalities are also commonly found. These include scoliosis (85%), and foot deformities; although pes cavus is the best known of these, pes planus and equinovarus are also often found. Amyotrophy of the lower leg and rarely of the hands may also be found. When all these features are present in a case of early onset (before 20 years of age) autosomal recessive ataxia, genetic analysis will prove that Friedreich’s ataxia is the correct diagnosis in the vast majority. Additional clinical support for a suspected diagnosis includes optic atrophy, which occurs in 25% of cases; however, it is rare for there to be major visual impairment in Friedreich’s ataxia (less than 5%). Deafness is found in less than 10% of cases.
The most important non-neurological feature of Friedreich's ataxia is cardiomyopathy. The exact proportion of patients with cardiomyopathy is still debated. However, in a study where hearts were examined in detail, over 90% were found to have abnormalities, though the clinical significance of some of the lesser changes is unclear. About 65% of patients have an abnormal electrocardiogram (ECG), with widespread T wave inversion in the inferolateral chest leads. The most frequent echocardiographic abnormality is concentric ventricular hypertrophy. Although heart failure is a late event, referral to a cardiologist may be necessary as arrhythmias are an important cause of premature death. Review of the patient should therefore always include an ECG. It should also include an estimation of blood sugar, since diabetes is seen in approximately 10% of cases.

Before identification of the gene, additional investigations were done to screen for the associations listed above and to rule out other diseases with a similar presentation. Nerve conduction studies reveal a predominantly sensory neuronopathy with absent sensory action potentials. This differentiates Friedreich's from the Roussy-Levy variant of hereditary motor sensory neuropathy type 1, which was at one time thought to be a "forme-fruste" of Friedreich's ataxia as it produces a sensory ataxia with absent tendon reflexes. The neurophysiological findings in this condition are those of a severe demyelinating process rather than an axonopathy.

It was noted in the early 1980s that patients with vitamin E malabsorption associated with various disorders including abetalipoproteinaemia, chronic liver disease, and cystic fibrosis could develop a spinocerebellar syndrome which resembled Friedreich's ataxia but could be distinguished by those additional features. In 1985 Harding et al described a patient in whom vitamin E deficiency was seen in the absence of malabsorption or other identified problem. Although this disorder has undergone various name changes, including several acronyms, most people now refer to AVED (ataxia with isolated vitamin E deficiency). The gene responsible for this illness was linked to 8q14 and identified by Ouahchi et al. The gene encodes a protein called a tocopherol transporter protein, and abnormalities which ensue from mutations of this gene result in impaired incorporation of vitamin E into very low density lipoprotein. Therefore, although vitamin E is absorbed adequately, it is soon lost from the system as the circulating reservoir is dysfunctional. Although clinically there are similarities with Friedreich's ataxia, the neuronopathy is more central and therefore conduction studies are often normal. The clinical clue to the presence of this disease is a characteristic titubation which is rarely seen in classical Friedreich's ataxia. However, despite the rarity of this illness, supplementation with vitamin E can result in either modest improvement of the clinical syndrome, or at least in cessation of its progression, and therefore it is always worthwhile measuring vitamin E in such patients. Since absorption is normal, a direct inquiry should be made about the use of supplementary vitamins—it is possible to be misled by a normal vitamin E level if vitamin E supplements are being taken.

If the spinocerebellar syndrome is complicated by other neurological problems, for example dementia, then other illnesses should be considered, including hexosaminidase A deficiency, abetalipoproteinaemia, adrenoleucodystrophy, and related conditions.

The natural history of this disease unfortunately remains one of relentless progression, with dysarthria and pyramidal weakness presenting within a few years of onset, followed by jerky eye movements giving way to nystagmus. The patient usually becomes wheelchair bound within 10 to 15 years of onset. It is worth noting that patients may present to cardiologists with cardiomyopathy as the sole initial feature. We have recently seen two cases who presented with a choreiform movement disorder with no signs of ataxia. The clue that Friedreich's ataxia was the underlying condition was given by the absence of reflexes in both, scoliosis in one, and cardiomyopathy in the other.

### Genetic diagnosis

The gene for Friedreich's ataxia was mapped to chromosome 9q13 in 1988 by Chamberlain and colleagues. The gene responsible for the disease was predicted to be a "novel gene" and named Friedreich's ataxia locus (FRDA). In 1998, two research groups, working in the USA and France, independently identified the gene and sequenced it. Harding et al identified a novel gene with strong homology to the human frataxin gene. Several groups, working in France and elsewhere, subsequently confirmed the report of Harding et al. It was shown that the gene is not expressed solely in the spinal cord—mirrorsthatofthepathologyofthedisease. Further work showed that the predominant mutation was a trinucleotide repeat (GAA) in intron 1 of this gene. Expansion of both alleles was found in over 70 patients. In three patients point mutations were found on one allele and an expansion on the other. This is clear evidence that X25 is directly involved in Friedreich’s ataxia, although there is still some debate as to the exact construction of the gene itself. Moreover, initial mRNA studies have reported a decrease in frataxin protein. Interestingly, the tissue specific distribution seen in this disease—including expression in the pancreas, the heart, and the dorsal spinal cord—mirrors that of the pathology of the disease.

This was the first autosomal recessive condition found to be due to a dynamic repeat, and it permitted the introduction of a specific and sensitive diagnostic test as it is a relatively simple matter to measure the repeat size. On normal chromosomes the number of GAA repeats varies from seven to 22 units, whereas on disease chromosomes the range varies from around 100 to 2000 repeats. This is in sharp contrast to the modest exonic repeat expansions seen in the dominant genetic ataxias (SCA 1, 2, and 3), where an expansion of somewhere over 40 repeats is sufficient to cause a degenerative ataxia.

The polymerase chain reaction (PCR) with nucleotide primers spanning the repeated region is used to amplify the DNA in intron 1, and the products are then fractionated on an agarose or polyacrylamide gel (fig 1). The rarity of point mutations means that it is extremely unlikely that a case of Friedreich’s will have two point mutations, and therefore a normal sized repeat length on both chromosomes is strongly against a diagnosis of Friedreich’s ataxia. To put this into some sort of perspective, there is approximately a

### Table 1  Strict diagnostic criteria (after Harding, 1981)

<table>
<thead>
<tr>
<th>Essential</th>
<th>Age of onset before 25 years</th>
<th>Progressive ataxia of gait and limbs</th>
<th>Absent knee and ankle jerks</th>
<th>Axonal picture on neurophysiology</th>
<th>Dysarthria (if after five years from onset)</th>
<th>Failed tendon reflexes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Additional (present in over 66%)</td>
<td>Scoliosis</td>
<td>Pyramidal weakness in lower limbs</td>
<td>Absent reflexes in arms</td>
<td>Large fibre sensory loss on examination</td>
<td>Abnormal ECG</td>
<td></td>
</tr>
<tr>
<td>Others (less than 50%)</td>
<td>Nystagmus</td>
<td>Optic atrophy</td>
<td>Deafness</td>
<td>Distal amyotrophy</td>
<td>Pes cavus</td>
<td>Diabetes</td>
</tr>
</tbody>
</table>

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Annotations

Table 1  Strict diagnostic criteria (after Harding, 1981)
one in 2500 that a patient with typical Friedreich’s ataxia will have a double point mutation. However, the phenotype of atypical cases is still unresolved.

The exact mechanism of action of this repeat in intron 1 is not known, but it is likely that this huge expansion disrupts normal splicing because there is no splice acceptor site within the repeat. Because the repeat is encoded in both strands of DNA, it is possible that the abnormality in the spliceosomes could be due to the presence of both strands.

There has been progress in our understanding of the protein. A very recent report has investigated a yeast homolog of the protein and shown it to be a mitochondrial iron transporter. There is preliminary evidence in human studies that frataxin is also mitochondrial placed (M Pandolfo, personal communication). If this is indeed the case, this protein may be an iron transporter, which could be involved in oxidative phosphorylation. Clinically this fits—a syndrome of ataxia and neuropathy, in association with diabetes, cardiomyopathy, deafness and optic atrophy, has all the hallmarks of a mitochondrial disease, so perhaps Friedreich’s ataxia will turn out to be the commonest mitochondrial disease of all!

Genotype-phenotype correlations

The identification of a diagnostic test has allowed the clinical phenotype to be re-evaluated. It is now confirmed that retained tendon reflexes are present in a small proportion of patients with Friedreich’s ataxia. Although most patients present below the age of 25 onset, can be later than this and the oldest reported case was 51 years. Studies of large numbers of patients are in broad agreement that the length of repeat size is a determinant of the age of onset and therefore to some degree influences the severity of the disease, in that cases with early onset tend to progress more rapidly. However, this correlation applies to populations of patients and is not useful in guiding an individual patient or family. The presence of cardiomyopathy is also linked to the length of the repeat, but further studies are needed to disentangle the exact relation between the two.

We have also identified three families in whom inheritance initially appeared to be dominant, as in all three cases the parent had an ataxic syndrome. In one of these families, pseudodominance has been proved by identification of two abnormal alleles in the parental generation as well as two in the affected offspring, that is, it was a case of a patient marrying a carrier. In the other two families, the children have both clinical and genetic Friedreich’s ataxia. However, the fathers have a more complicated ataxia and a later onset. They are heterozygotes for the expansion, and the possibility arises that either they both have point mutations which are modifying the phenotype, or they have ataxia from some other cause.

Summary

Clinical diagnosis is still of the utmost importance and following our review of cases diagnosed using the strict criteria, 100% were homozygous for the expansion. However, now that there is a relatively simple direct genetic test, the diagnosis can be considered in more unusual cases. Genetic testing has been shown to be of value in establishing the correct diagnosis and in directing the appropriate screening tests, including cardiac evaluation and blood sugar estimation.

Perhaps the most interesting development following identification of the gene is the rapid progress in our understanding of the protein. If, as seems likely, it turns out to be a mitochondrial protein involved in iron transport, it gives cause for hope of effective treatment.

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Key messages

- Friedreich’s ataxia is the commonest inherited ataxia (1:50 000).
- Over 97% will have a homozygous GAA unstable repeat in intron 1 of the frataxin gene on chromosome 9q; a direct test is now available.
- There is a correlation between repeat length and age at onset.
- The frataxin protein may be an iron transporter within the mitochondria.

7 Elias E, Muller DP, Scott J. Association of spinocerebellar disorders with cystic fibrosis or chronic childhood cataract and very low serum vitamin E. Lancet 1981;i:319–21.
Graves’ disease is rare in childhood but occurs with increasing frequency into adolescence and young adult life. There is a strong familial predisposition but the precipitating cause is not known. Stimulation of the thyroid stimulating hormone (TSH) receptor by autoantibodies causes excessive thyroid hormone production and secretion, and diffuse enlargement of the thyroid. Other systems may be involved, notably the eyes with proptosis. In children the early symptoms of hyperthyroidism are non-specific and may be of gradual onset; unless there is an obvious goitre, a psychological or behavioural disorder is often suspected. Once considered, the diagnosis is readily confirmed biochemically by raised concentrations of circulating thyroid hormones and suppression of TSH.

The aim of treatment is to restore and maintain permanent euthyroidism as safely, quickly, and conveniently as possible. Especially in young people, there is no consensus on how this is best achieved and in this annotation we shall consider the options.

Initial management

The symptoms of hyperthyroidism may be distressing and can be relieved promptly to a great extent by blocking the peripheral effects of the excess thyroid hormones. A β blocking agent such as propranolol, 1 mg/kg/day in divided doses, is effective and useful to tide the patient over until the disease is controlled.

An antithyroid drug can be started at the same time. The thionamide drugs block the synthesis of thyroid hormones and also have ill understood immunosuppressive effects in Graves’ disease. Carbimazole is more widely used in Europe and propylthiouracil in the United States; although they are interchangeable, Carbimazole 0.5 to 1.0 mg/kg/day or propylthiouracil 5 to 10 mg/kg/day in divided doses brings the hyperthyroidism of Graves’ disease under control in four to eight weeks.

If there is urgent need to cure the disease as soon as possible, it is then safe to proceed to thyroidectomy but most clinicians favour a trial of medical treatment in the hope that a long term remission will occur. This entails treatment with an antithyroid drug for 18 months to two years under regular supervision. When the circulating thyroid hormones are restored to the normal range, treatment can be reduced to a single daily dose and titrated against regular thyroid function tests to maintain euthyroidism. A more convenient approach is to continue the antithyroid drug unchanged and add thyroxine 100 µg/m²/day in a “block and replace” regimen. Although there is continued exposure to the antithyroid drug in full dosage, less monitoring is needed and euthyroidism is more reliably maintained.

As with any long term treatment, many patients and their families find it remarkably difficult to remember to take the drugs regularly. Some 2–5% of patients develop a rash or other minor side effects with an antithyroid drug (usually nausea, headache, or arthralgia). If mild, the symptoms generally prove transient but if they persist the patient can be changed to the other major agent, as cross sensitivity is unusual. Serious side effects are rare, especially in young patients, but include neutropenia, agranulocytosis (which is nearly always reversible), and hepatotoxicity. If such effects occur they usually do so in the first few months of treatment. They cannot be predicted by frequent monitoring, so patients and parents must be warned to report promptly any episode of significant fever, sore throat, or other symptoms. It is our practice to request a blood count whenever checking on thyroid function but not to order tests otherwise.

At the end of the course of medical treatment the drugs are withdrawn and the child is observed for recurrent hyperthyroidism. Relapse is more likely in children than in adults, and in those with a low body mass index and a large goitre at presentation. The remission rate of children in the USA is reported to be 25% after each of repeated two year courses of medical treatment, with remission rates around 50–65% reported in adult studies. Of the wide variety of biochemical markers and dynamic tests that have been investigated in the hope of finding a method of predicting relapse, none has proved reliable. Hashizume and colleagues reported that giving thyroxine during and after treatment with antithyroid drugs greatly improved the remission rate, perhaps by reducing antigen presentation, but recent studies have not confirmed their findings.

Relapse generally occurs within the first few months after treatment is withdrawn but can be long delayed. Patients in remission from Graves’ disease are also at increased risk of becoming hypothyroid in the future, so a case can be made for continued infrequent checks on thyroid function.

If relapse occurs, there are three options: (1) to resume antithyroid drug treatment; (2) to proceed to thyroidectomy; (3) to treat the child with radioiodine. Here there is a transatlantic divide in approach. In Europe subtotal or total thyroidectomy has in general been the only option offered for definitive treatment. Some American units continue to report excellent surgical results but in many centres radioiodine treatment is preferred in all age groups.

Further medical treatment

If medical treatment was well tolerated and regular supervision with thyroid function testing did not prove a burden, some patients and their families will prefer to resume antithyroid drug treatment. This could be for a further full course with the hope of remission at the end—which may be expected in up to 25% of those who have relapsed after the first course of treatment—or for a shorter time, with definitive treatment planned at a convenient moment.

Annotations

Thyroidectomy

Surgery provides one definitive therapeutic option. In the past, partial thyroidectomy was favoured with the hope of leaving sufficient thyroid tissue for the child to remain euthyroid. Too conservative an operation resulted in a high rate of recurrent hyperthyroidism and too radical a thyroidectomy in hypothyroidism; either of these problems could occur unpredictably many years later. Most surgeons now aim to render the child hypothyroid at the first operation by removing essentially all the gland. As soon as hypothyroidism is confirmed, thyroxine replacement can be started. Although compliance with replacement treatment does remain a concern, thyroxine dose adjustment through the years of growth and development is straightforward and no further changes are necessary once the patient is fully grown. Many areas in the United Kingdom now keep thyroid registers, and patients can be followed up by their general practitioners without the need for continuing hospital review.

A short hospital stay is needed and potential problems include the discomfort of the operation, the scar (which may form keloid), the small but appreciable risk of general anaesthetic and surgical complications, the specific risks of damage to the recurrent laryngeal nerves, and transient or permanent hypoparathyroidism. All these risks are reduced in the hands of expert thyroid surgeons, but such expertise is rare now that hyperthyroidism in adults is generally treated with radioiodine.

Radioiodine

This elegant form of treatment was first used 50 years ago and it has earned a remarkable safety record. Radioiodine can be given with the aim of leaving the patient euthyroid, or in a higher dose with the specific intention of ablating the gland and accepting permanent hypothyroidism. Adjustment of the dose in relation to the size and uptake of the gland may result in the temporary restoration of euthyroidism but eventual progression to hypothyroidism is likely at some stage. Since a primary objective of definitive treatment is to relieve the patient of the need for long term monitoring, many centres have now accepted the policy of ablating the thyroid and accepting the need for thyroxine replacement; this is particularly appropriate in children.

There has been concern that radioiodine treatment may aggravate Graves’ ophthalmopathy, and steroid cover has been recommended to prevent this complication. In our own experience and that of others of this effect has not proved a problem; eye disease need not be considered a contraindication to radioiodine treatment.

In the past in many centres the use of radioiodine was restricted to patients beyond their reproductive age. With increasing confidence in its safety it is now used in the hands of expert thyroid surgeons, but such expertise is rare now that hyperthyroidism in adults is generally treated with radioiodine.

Some precautions are necessary after radioiodine treatment. Close and prolonged physical contact with others should be avoided for three days and we recommend that children stay away from school for two weeks. For older girls the importance of avoiding pregnancy for at least six months should be stressed.

Conclusions

Once diagnosed, Graves’ disease can readily be controlled, but there is no ideal form of treatment for young people. Detailed discussion of the advantages, disadvantages, and risks of each of the different forms of treatment is important so that a therapeutic plan can take into account the needs and wishes of the patient and family.

Unless there are compelling reasons to seek a rapid cure, an initial trial of medical treatment using a “block and replace” regimen for 18 to 24 months is recommended. For the majority who relapse when antithyroid drug treatment is withdrawn, medical treatment can be resumed but definitive treatment should be considered. If excellent surgical services are available, total thyroidectomy with subsequent thyroxine replacement provides a definitive solution with low risk. Treatment with radioiodine offers a simple alternative without short term risks and with an excellent long term safety record which justifies its use in young patients. Given the choice, many will opt for this form of treatment and our experience suggests that a dose of 400 mBq of will reduce the need for patients requiring a second dose. We recommend an approach in which the gland is ablated and the need for thyroxine replacement is accepted.

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Annotations

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