

CURRENT TOPIC

Behavioural phenotypes: what do they teach us?

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The study of behavioural phenotypes could be said to go back to the days of the syndrome first described by Down.¹ He believed that affected children (whose genetic anomaly was of course not known to him) had distinctive personality characteristics, including “a lively sense of the ridiculous”, among others. Subsequent studies have failed to confirm these stereotypes.² Nevertheless, there have since been numerous attempts to discover, among individuals with known chromosomal or genetic anomalies, mental features that are causally related to the underlying condition. Opinions are divided, between those who regard the study of behavioural phenotypes as being worthwhile³ and those who have their doubts.⁴ Before discussing the arguments, a definition is required.

What is a behavioural phenotype?

There is no consensus. Flint² suggested that it is: “a behaviour, including cognitive processes and social interaction style, that is consistently associated with, and specific to, a syndrome which has a chromosomal or a genetic aetiology”. He added “where there is little doubt that the phenotype is a consequence of the underlying anomaly”, but others feel that definition is too restrictive.^{5,6} In practice, most behavioural phenotypes are associated with chromosomal aneuploidies and microdeletions rather than point mutations in a single gene.

The study of behavioural phenotypes aims to identify bona fide links between genotype and phenotype. A few moments’ reflection only is necessary to realise that there are many problems with the concept. First, is the term to be restricted only to disorders in which a genetic anomaly has been identified? There are many conditions (such as Rett syndrome⁷) that appear to have a genetic origin and characteristic behavioural features, but the nature of the genetic deficit is not known. Second, is it likely that any cognitive or behavioural features will be found consistently, unless they are part of the syndrome’s definition? The genetic background of the individual will inevitably modify phenotypic expression.⁸ Third, because behaviour is to a greater or lesser extent modified by experience, the phenotype is likely to vary in intensity for that reason alone, independent of genotype. How should we then differentiate between the direct effects of the genetic anomaly and the indirect influence of the circumstances of upbringing, such as education? Fourth, how is it possible in studies of

humans, as opposed to model systems,⁸ to clarify the mechanisms that link genetic anomalies to behaviour, and to show that they are causal?

The use of the term “behavioural phenotype” to describe the features of a disorder in which the genetic anomaly is only suspected, but where it has not been demonstrated, is liable to cause confusion. The danger is a widening of the concept to include the cognitive and behavioural features of all conditions that are highly heritable (such as autism or attention deficit hyperactivity disorder), irrespective of whether any chromosomal or genetic anomaly has been detected. Therefore, I shall restrict usage of the term to conditions in which a genetic deletion/mutation or at least a chromosomal anomaly has been identified.

Why should we be interested at all in the concept of behavioural phenotypes? What can they teach us? In the first place, they have clinical value. For many parents it is perplexing to be faced with a child suffering from a genetic disorder whose disturbed behaviour is “inexplicably” starkly different from that of their siblings. It is reassuring for parents to know that certain features of behaviour, such as the hyperphagia of Prader-Willi syndrome,⁹ or the self injurious behaviour of Lesch-Nyhan syndrome,⁴ can be as characteristic of the disorder as learning difficulties or facial dysmorphism. We are at risk of forgetting the guilt that is associated with having a child who is biologically “different”. Parents need to be reassured that behavioural manifestations of the condition are neither their fault, nor caused by wilfulness on the part of the child, even when they are manageable.⁶

In addition, one of the main reasons for the interest in behavioural phenotypes is the hope that they could guide us towards genes that contribute to the biology of specific human behavioural patterns.² Much behavioural genetic research to date has been “top-down” in its approach to quantitative genetic analysis of complex traits. We often use linkage or association strategies to examine naturally occurring alleles of candidate genes in a “wild-type” population. These alleles are likely to be functional polymorphisms rather than mutations and, if they are quantitative trait loci, might account for individual differences in the trait in question.¹⁰ Tully¹¹ argues that such genes are likely to have only a minor influence upon the phenotype of the individual, simply because

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alleles with a more dramatic effect could reduce fitness and so would be selected against during evolution. On the other hand, chromosomal deletions that have cognitive and behavioural consequences might be associated with monosomy, hence the loss of one copy of genes that are dosage sensitive.¹² Such genes might play a fundamental role in the developmental or functional organisation of the brain,⁴ although they are not necessarily important for individual differences in the general population.

Linking genotype to phenotype

To discover how the genotype of an individual with a naturally occurring genetic anomaly contributes to the behavioural manifestations of that condition caution is necessary. Conceptual flaws in study design and interpretation are almost certainly why such successes as have been reported to date¹³ have subsequently been countered by alternative claims.¹⁴ I shall illustrate the pitfalls awaiting the unwary with the example of Turner's syndrome, the subject of our own research programme in this area.^{10 15} We used a particular subset of patients with Turner's syndrome, those with X monosomy (45,X), to find evidence that the parental origin of that single X chromosome has a substantial influence upon social communication skills.¹⁶ Our data indicate the existence on the X chromosome of an imprinted locus that affects social cognitive development.

ASCERTAINMENT BIAS

Patients with a condition that has a distinctive behavioural characteristic are more likely to be detected if that characteristic is present. Thus, in an identified clinical population the key behavioural features are likely to be more common than in unidentified patients with the genetic anomaly from the general population. An example might be hyperphagia in Prader-Willi syndrome,⁹ or autistic features in association with fragile X syndrome.¹⁷ On the other hand, if it can be shown that the reason for ascertainment is never, or hardly ever, the cognitive or behavioural features, such bias would not be applicable and the ascertained sample would be representative of all potential cases. In our series of patients with Turner's syndrome, drawn from a national register, 22% of a series of 288 individuals were diagnosed at or before birth. A further 41% were ascertained as a consequence of short stature in middle childhood. Only 2% were ascertained as a consequence of learning difficulties or behavioural maladjustment, suggesting that biased recruitment is an unlikely explanation for our findings.

COMPARISON SUBJECTS

There are two main reasons for recruiting a comparison sample. First, it is necessary to find out the degree to which the behavioural feature of interest is linked directly to the genetic anomaly. To what extent is it associated with some other non-specific factor, such as low intelligence quotient (IQ)? For example, what proportion of children with IQs as low as those found in Lesch-Nyhan syndrome also show the

self injurious behaviours characteristic of that condition?² Second, it is necessary to control for the possibility that the behavioural feature of interest is linked to some other specific characteristic of that syndrome, which confounds the association of interest. For example, in Turner's syndrome poor social adjustment is common,¹⁸ but short stature is almost invariable.¹⁹ Could the social adjustment problems be primarily the result of living with the stigma of exceptionally short stature? In a survey of 110 female patients with other short stature syndromes (such as primary hypopituitarism and Silver-Russell syndrome) we found the mean score on a scale of social communication impairment¹⁶ was 4.1 (SD, 5.4). This was remarkably similar to that of the general population of age matched children and adolescents (n = 344; mean, 3.8; SD, 4.1). However, in a population of female patients with 45,X Turner's syndrome (n = 78), with a single maternal X chromosome, the mean was 9.0 (SD, 7.3), indicating some influence other than stature alone was relevant to their impaired social adjustment.

CHOICE OF PHENOTYPIC MARKERS

The choice of phenotype is crucial to a study of this nature. The aim is to find a measure of dysfunction that is closely and causally linked to the genetic anomaly. Realistically, no cognitive or behavioural feature is likely to be unique to a single genetic disorder and neither is that feature likely to be found in all instances of the disorder. By what means, then, is the choice of marker made? In my opinion, it is usually a waste of time using behavioural measures such as hyperactivity, self injury, impulsiveness, clumsiness, or personality characteristics, such as aloofness or anxiousness. These features are found in association with many genetic disorders³ and have low specificity. Cognitive measures such as IQ (whether verbal and/or non-verbal) are little better. Ideally, one wishes to find a marker that is unlikely to have been modified substantially by experience, and which is linked aetiologically to the genetic anomaly. In choosing such an indicator one must bear in mind its frequency in the general population. For example, poor visuospatial skills are known to be closely associated with Turner's syndrome.²⁰ A non-verbal IQ more than 1 SD below verbal IQ was found in 52% of our patients with 45,X Turner's syndrome (n = 88), but a difference of at least this magnitude occurs in 12% of the general population. Therefore, this characteristic falls far short of a strongly discriminating cognitive phenotype, and attempts to improve on it have not been especially successful.²¹

Another area of interest to those studying behavioural phenotypes is the association of genetic anomalies with what superficially resembles a psychiatric or neurodevelopmental disorder. Examples include autism, which is found in (loose) association with fragile X syndrome,¹⁷ and in perhaps 50% of cases of tuberous sclerosis. Its associations with Turner's syndrome,^{22 23} and with maternally derived small marker chromosomes on 15q,²⁴ are of particular theoretical

interest. Another co-morbidity, which might be worth following up, is that of schizo-affective disorder with velocardiofacial syndrome.²⁵ Further work is needed on the phenotypic similarities and differences of these syndromes from the idiopathic variants.

MEASURES

The choice of measures in the study of behavioural phenotypes is by no means easy. Although the use of standardised instruments has merit, there is no a priori reason why the phenotypic features of interest should be reflected by scores on such measures. We found there were few differences between patients with 45,X Turner's syndrome whose single X was maternally, rather than paternally, derived on a widely used behavioural questionnaire.^{18–26} Pilot studies indicated a different range of behavioural characteristics was present in most patients. Therefore, we devised a measure that summarised the main features of social maladjustment. Clear group differences emerged according to genotype.¹⁶ We have subsequently shown that this instrument has excellent discriminant and concurrent validity, especially for clinical syndromes characterised by social communication deficits, such as pervasive developmental disorders.²⁷ The fact that these were more common among female patients with Turner's syndrome and a single maternal, rather than a paternal X chromosome, has far reaching theoretical implications.²⁷

There seems little merit in comparing behavioural phenotypes with one another, so a generic measuring instrument is inappropriate. The closer the assessment gets to those aspects of the phenotype that are linked directly to variations in the genotype the better. In our study, poor social adjustment might reflect a fundamental dysfunction in the recognition of social cues, or in the pragmatics of language usage in social conversation. We may not yet have valid and reliable ways of measuring these skills in the general population, let alone a genetically deviant one. Thought needs to be given to the purpose of the investigation of behavioural phenotypes at a theoretical level, rather than reaching instinctively for that handy test battery or behavioural questionnaire.

DELETION MAPPING

Behavioural phenotypes are of greatest interest when it is possible to use the phenotypic features to locate a gene of interest by deletion mapping. This technique requires the molecular characterisation of the deletion's extent in a series of individuals with chromosomal deletions of varying size, all in approximately the same location. Deletion mapping of "cognitive" genes has been attempted in Williams syndrome, with inconsistent results.^{13–14} In contrast, it appears to have worked well in the identification of the SHOX gene, which influences growth in stature,²⁸ probably because deletion of this locus in the pseudoautosomal region of the X chromosome is invariably associated with impaired growth (a solid phenotypic marker). The pseudoautosomal region is about 2.5 megabases in size, and lies

at the tip of the short arm. Unlike most of the X chromosome this region is not subject to X inactivation, and genes expressed from this region are required in two copies for normal development.

Deletion mapping of cognitive or behavioural traits is fine in theory, but difficult in practice, as is illustrated by this cautionary tale. First, it is essential to know how variable the phenotype is in the genetic disorder. Where the deletion is itself variable in extent, that question can be hard to answer conclusively. About 50% of patients with Turner's syndrome have a single X chromosome.¹⁵ Therefore, the phenotypic features that distinguish them from normal girls or women must result from the haploinsufficiency of "Turner genes" (directly or indirectly),²⁹ assuming the absence of cellular mosaicism. A genetic locus is technically haploinsufficient when the protein produced by a single copy of an otherwise normal gene is not sufficient to ensure normal function. Despite the consistency of the genetic anomaly, the cognitive and behavioural phenotype within 45,X Turner syndrome is utterly inconsistent, in the presence of demonstrable structural brain deficits (Price CJ, *et al*, unpublished data, 1999). This might in part reflect the (unmeasurable) variability in the genetic background of the individuals concerned, but could reflect epigenetic stochastic events too.

Suppose we wish to deletion map genes for cognitive deficits on the X chromosome by taking a series of individuals with successively larger deletions of—for example, the short arm of the second X chromosome.³⁰ We hypothesise that a "Turner gene", which is expressed in the brain, is situated about half way down the short arm and is dosage sensitive. It escapes X inactivation. Thus, a small distal deletion will be associated with the retention of the skill. A substantial deletion, extending to the centromere, will be associated with its loss. If we have a large enough series, with differing extents of chromosomal loss, we should be able to map molecularly the position of the "cognitive" gene in relation to the cognitive deficits observed in our sample. However, to interpret these results we will need at the very least to know the proportion of monosomic individuals with a loss of that skill. Assuming no confounding mosaicism is present, they must have deleted one copy of the gene of interest. We have found no phenotypic marker of this nature that affects 100% of monosomic subjects, and most of those that we have studied affect a considerably smaller proportion than this. Accordingly, there is such phenotypic variability in a series of individuals with successively larger deletions³⁰ of the short arm of the X chromosome that the results of deletion mapping for any cognitive or behavioural marker we have identified to date are uninterpretable.

The investigation of behavioural phenotypes by means of any instrument, whether questionnaire or neuropsychological test procedure, will effectively constitute a screening strategy. The "true" incidence of the phenotype in the presence of a deletion of the gene of interest is

usually not known (although Turner's syndrome constitutes a probable exception). Any measure that is used will have limited sensitivity and specificity. Thus, there will be false positives, because of the prevalence of the phenotype in the general population, and the possibility that the phenotype is present even though the gene has not been deleted. There will also be false negatives, because of the variability of the phenotype within the syndrome. The phenotype might not be present, despite monosomy for the crucial region. Nevertheless, it should be possible, with due care, to devise a screening strategy that does minimise bias (the extent to which the strategy consistently overestimates or underestimates the behavioural phenotype in relation to the genotype). It should also maximise accuracy (the extent to which the screening strategy identifies phenotypes in which the gene of interest has indeed been deleted). To date, these issues have hardly been discussed, let alone dealt with.

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