Curren advances in genetic knowledge and analysis have facilitated the shift in emphasis from single gene disorders to complex traits such as cancer and atopic disorders where changes in more than one gene usually contribute to the disease phenotype. A challenging area of complex trait research is the determination of the genetic basis of behavioural phenotypes. Early studies in this area focused on classical linkage analysis in pedigrees which segregated the trait of interest and led to the identification of genes for intellectual disability, such as fraX(A). In this disorder, mutations in a single gene are sufficient to generate the disease phenotype.

In contrast, with other neurodevelopmental disorders, results to date have been much more complex. With ADHD, for example, a large number of genes affecting neurotransmitter function, particularly dopamine, have been implicated. However, results from linkage or association studies have not been reliably replicated. Even the most promising gene candidates segregated the trait of interest and led to the identification of genes for intellectual disability, such as fraX(A). Identification of the genes involved is a challenging task. Firstly, accurate phenotype definition is crucial to successful outcomes in these pooled studies. Secondly, neurodevelopmental disorders such as ADHD are multifactorial, with a number of different genes, likely to be of varying effect and interacting with environmental influences, presumably contributing to the development of the observed phenotype.

We have identified an extended family where the proband, a child with moderate intellectual disability as well as severe conduct disturbance, was found to have a pericentric inversion of chromosome 3. The inversion was found to cosegregate with developmental-behavioural problems in other members of the family. In order to describe the phenotype, a developmental-behavioural paediatrician performed a clinical standardised clinical evaluation of eight of the juvenile members of the extended family. The findings are presented in this paper.

METHODS
Participants
The parents/guardians of eight juvenile family members were approached by the geneticist and invited to participate in the research. Children were chosen for this evaluation because the measures for the study of behavioural symptoms in children are better established than those for adults. In addition, one other child (no inversion) and six adults in the family (four with the inversion and two without) also underwent IQ testing. This child was unavailable for detailed assessment, and the adults were not assessed other than IQ testing, as discussed above. All agreed to be involved and gave informed consent. The project was approved by the Royal Children's Hospital Ethics in Human Research Committee (EHRC 98037C) and that of the University of Tasmania (approval No H4077).

Key points
- A child with a major neurodevelopmental impairment and severe impulse control problems was found to have a pericentric inversion of chromosome 3 (46N inv(3)(p14q21)). As several relatives also had developmental-behavioural symptoms, chromosomal analysis was undertaken on a number of family members. This showed 10 other subjects who carried the inversion.
- In order to define which phenotypic characteristics were attributable to the inversion, as opposed to familial or individual traits, standardised comprehensive assessments of eight subjects (four with the inversion, four without) were undertaken by a developmental-behavioural paediatrician blinded as to the subjects’ inversion status. In addition, intelligence testing was performed on 15 family members (eight with the inversion, seven without).
- Subjects with the inversion were found to have more significant developmental disabilities than those with normal karyotypes. The clinical phenotypic features associated with this inversion were intellectual impairment and an impulsive behaviour style. This is the first report of a behavioural phenotype associated with these loci and identifies a novel resource for defining the gene(s) involved.
- We hypothesise that one or more genes are disrupted by this inversion, resulting in the observed phenotype. Further molecular analysis of this family should provide an important contribution to the identification and characterisation of genes involved in this behavioural phenotype, which presents with some of the features commonly seen in attention deficit hyperactivity disorder (ADHD).

Cytogenetic analysis
Chromosome preparations were prepared using standard procedures and G banded chromosomes were assembled and described according to the recommendations of the International Standing Committee on Human Cytogenetic Nomenclature (ISCN).

Clinical assessment
A standardised assessment procedure was undertaken, involving the completion of behaviour rating scales by the children’s parents/guardians and teachers. The developmental-behavioural paediatrician performed a clinical
assessments. The clinical evaluation consisted of a semi-structured interview (pregnancy/perinatal period, early development, temperament/behaviour, learning, general health) and a neurodevelopmental assessment.

Psychological instruments

Wechsler Intelligence Scale for Children-III (WISC-III) and Wechsler Adult Intelligence Scale-Revised (WAIS-R) were undertaken by Amy Langbein. The Child Behaviour Checklist (CBCL) and complimentary Teacher’s Report Form (TRF) are broad band standardised behaviour rating scales. These 113 item checklists provide a multidimensional profile of empirically derived problem behaviour syndromes. Conners’ Parent Rating Scale - Revised and Conners’ Teacher Rating Scale - Revised have been the most frequently used behaviour rating scales in ADHD research. The revised 48 item version of the Conners’ Parent Rating Scale (CPRS-R) and the 28 item version of the Conners’ Teacher Rating Scale (CTRS-R) were used in this study. DSM-IV ADHD Parent and Teacher Rating Scales consists of the 18 items in The American Psychiatric Association’s Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) diagnostic criteria for ADHD, listed such that the Inattention items alternate with the Hyperactive/Impulsive items.

Blinding

The paediatrician knew that some of the children assessed had the inversion, but did not know which ones. An attempt was made to remain blinded to the inversion status of all subjects. This was successful in all cases except the proband (subject 1) whose carer inadvertantly revealed that he had the inversion. As this subject clearly had the most severe developmental disability of all subjects assessed (and hence was very likely to be affected), this revelation did not constitute a major departure from the study protocol.
Statistical methodology
Analysis of the difference in IQ between those with and those without the inversion was undertaken using a one tailed t test (Microsoft Excel for Windows).

RESULTS
The proband (subject 1), a 10 year old male, had been managed jointly by a child psychiatrist and paediatrician. He had moderate intellectual disability, as well as severe impulse control problems with features of ADHD and conduct disorder. His symptoms responded to high doses of stimulants. Cytogenetic analysis was performed as part of the investigative process. The child was found to have a pericentric inversion of chromosome 3 (46N inv(3)(p14q21) (fig 1A, B). A family member suggested that several other relatives had a similar behavioural phenotype and predicted that they too would carry the inversion. The family was then referred to the genetics clinic. Twenty other family members supplied blood samples for karyotyping. Their inversion status was correctly predicted by the family member in 19 of the 20 cases. Of the 21 who had cytogenetic analysis, 11 had the inversion and 10 did not (fig 2). Most of the subjects with this inversion had clinically significant developmental-behavioural problems, and some had been diagnosed with attention deficit hyperactivity disorder (ADHD) and were being treated with psychostimulant medication (table 1).

Behavioural problems were present in six children. The results of the behavioural rating scales are shown in table 2. In subject 7 (no inversion), onset was at around the age of 8 years, suggesting environmental factors were a more important cause than constitutional factors. Two other subjects without the inversion (3 and 8) had primarily learning difficulties. These children were different from one another

| Table 1  Clinical assessment findings |
|---|---|---|---|---|---|
| Subject | Sex | Age (y.m) | Positive findings from assessment | IQ testing | Summary formulation |
| | | | | Verbal | Perf | FS | |
| 1 | M | 13.9 | Developmental concerns from 18/12; severe behavioural disturbance and learning difficulties; multiple neuro-developmental delays; conduct disturbance; features of anxiety and depression. | 56 | 48 | 48 | Moderate intellectual disability; marked impulsivity, conduct disturbance |
| 2 | F | 9.3 | Behavioural problems from pre-school age; delayed short term auditory memory; poor fine motor skills | 69 | 77 | 70 | Consistent with treated ADHD (combined type)† |
| 3 | M | 12.6 | Learning difficulties; poor impulse control | 67 | 66 | 64 | Learning difficulties |
| 4 | F | 12.2 | Learning difficulties; impulsive, uncooperative; delayed short term auditory memory. | 74 | 78 | 74 | Mild intellectual disability; impulsivity, behavioural disturbance |
| 5 | F | 10.4 | Behavioural disturbance, learning difficulties | 104 | 100 | 101 | Normal child |
| 6 | M | 15.6 | | 83 | 83 | 83 | Borderline intellectual function with behavioural disturbance |
| 7 | M | 12.8 | Aggression, withdrawn, low self-esteem; onset 8 y; delayed short term auditory memory | 90 | 104 | 96 | Emotional disturbance |
| 8 | M | 11.4 | Learning difficulties | 73 | 91 | 80 | Learning difficulties |

| Table 2  Results of the various rating scales used in evaluating the eight subjects. The results presented are those where the factors/scales with scores were >2 SD above the mean for CPRS-R, CTRS-R, CBCL, and TRF. For DSM-IV scales, results are given where six or more items were present |
|---|---|---|---|---|---|---|
| Subject | DSM-IV Parent | DSM-IV Teacher | CPRS-R | CTRS-R | CBCL | TRF |
| | Inattent, hyp/imp | Inattent, hyp/imp | Conduct problem, learning problem, impulsive-hyperactive, anxiety | Conduct problem, hyperactivity, inattentive-passive | Anxious-depressed, social problems, thought problems, attention problems, delinquent behaviour, aggressive behaviour | Anxious-depressed, social problems, delinquent behaviour, aggressive behaviour |
| 2 | – | – | Conduct problem | – | Delinquent behaviour | – |
| 3 | – | – | – | – | – | – |
| 4 | – | – | Conduct problem, learning problem, psychosomatic, impulsive-hyperactive, | – | Withdrawn, thought problems, attention problems, delinquent behaviour, aggressive behaviour | – |
| 5 | – | – | – | – | – | – |
| 6 | Inattent | – | Learning problem, psychosomatic, impulsive-hyperactive | – | Withdrawn, somatic complaints, anxious-depressed, social problems, thought problems, attention problems, delinquent behaviour, aggressive behaviour | – |
| 7 | – | – | Conduct problem, impulsive-hyperactive | – | Withdrawn, anxious-depressed, social problems | Somatic complaints, anxious-depressed, thought problems |
| 8 | – | – | – | – | – | – |

CPRS-R = Conners’ Parent Rating Scale - Revised; CTRS-R = Conners’ Teacher Rating Scale - Revised; CBCL = Child Behaviour Checklist; TRF = Teacher’s Report Form.
temperamentally. Subject 3 had problems with impulsivity and aggression.

Of the eight children assessed, the clinical presentation in three (subjects 1, 4, and 6, all with inversion) strongly suggested a predominantly constitutional pathogenesis (early developmental difficulties, persistent, pervasive, and severe symptomatology with poor impulse control). Subject 2 had been taking stimulant medication for two years; however, historically she also had a constitutional problem, ADHD (table 1).

In addition, one other child (no inversion) and six adults in the family (four with the inversion and two without) also underwent IQ testing. Overall, the mean IQ of eight subjects with the inversion (four adults, four children) was 76.6, compared to 93.7 for the seven subjects (two adults, five children) without the inversion (p=0.03).

DISCUSSION

On clinical assessment at one point in time, those children who carried a pericentric inversion of chromosome 3 were found to have more significant developmental disabilities than those without the inversion. The clinical phenotypic features in common were intellectual disabilities and an impulsive behaviour style. The difference in IQ scores between subjects with the inversion and those without was statistically significant. Three of the four control family members had less severe developmental-behavioural problems. These tended to be qualitatively different from those with the inversion (one had learning difficulties, one reactive emotional disturbance), though the phenotype of subject 3 (learning difficulties with poor impulse control) did have similarities to that of subjects with the inversion.

These findings suggest that the inversion is causally related to the phenotype described. A number of disease causing genes have been localised by the cosegregation of the disease with an apparently balanced chromosomal rearrangement. An example of this is the localisation of the neurofibromatosis type 1 gene to chromosome 17q11.2 by mapping a translocation breakpoint cosegregating with the disease. More recently, chromosomal translocations have been identified in some patients with behavioural disorders such as autism. Breakpoints have been mapped to chromosome 7q31 and 22 and candidate genes are being examined.

The chromosomal abnormality observed in this family may mediate the development of the observed phenotype by physically disrupting or altering a gene or genes involved in intelligence and/or behaviour. Alternatively, the disruption or alteration of a suppressor or an enhancer element at one or both of the breakpoints may change the level of expression of the target gene(s). Similarly, the physical rearrangement of the chromosome may alter the chromatin structure or reposition the gene of interest such that gene transcription is enhanced or suppressed. More remotely, it is possible that a genetic element is transferred to the insertion breakpoint cosegregating with the disease.

If the impact this gene has on the aetiology of ADHD is confirmed, mutations or polymorphisms at this locus will determine the diagnostic profile of two of the three subtypes of patients with ADHD. Analysis of a cohort of such patients for mutations or polymorphisms at this locus will determine the impact this gene has on the aetiology of ADHD. If the impact is significant, better diagnostic testing based on genetic analysis may be possible for some children presenting with ADHD-like symptoms. This family affords the opportunity firmly to implicate a known biological pathway in the development of this behavioural trait or, alternatively, implicate a new gene family.

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Authors' affiliations

D Efron, Centre for Community Child Health, Royal Children's Hospital, Parkville, Victoria, Australia
D Efron, M B Delatycki, M G de Silva, H-M M Dahl, S M Forrest, Murdoch Children's Research Institute, Royal Children's Hospital, Parkville, Victoria, Australia
D Efron, M B Delatycki, H-H M Dahl, S M Forrest, Department of Paediatrics, University of Melbourne, Parkville, Victoria, Australia
M B Delatycki, Genetic Health Services Victoria, Royal Children's Hospital, Parkville, Victoria, Australia
M B Delatycki, M G de Silva, H-H M Dahl, S M Forrest, Cooperative Research Centre for Discovery of Genes for Common Human Diseases, Parkville, Victoria, Australia
M B Delatycki, Department of Paediatrics, Monash University, Clayton, Victoria, Australia
A Longbein, W Slaghaui, Department of Psychology, University of Tasmania, Sandy Bay, Tasmania, Australia
A Larson, Calvary Rehabilitation Service, New Town, Tasmania, Australia
Correspondence to: Dr M B Delatycki, Genetic Health Services Victoria, Royal Children's Hospital, Flemington Road, Parkville, Victoria 3052, Australia; delatymc@cryptic.rch.unimelb.edu.au

REFERENCES

3 Madras BK, Miller GM, Fischman AJ. The dopamine transporter: relevance to attention deficit hyperactivity disorder (ADHD). Behav Brain Res 2002; 130:57-63.
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D Efron, M B Delatycki, M G de Silva, A Langbein, W Slaghuis, A Larson, H-H M Dahl and S M Forrest

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