A 3.2 Mb deletion on 18q12 in a patient with childhood autism and high-grade myopia.

Gilling, Mette; Lauritsen, Marlene Briciet; Møller, Morten; Henriksen, Karen Friis; Vicente, Astrid; Oliveira, Guiomar; Cintin, Christina; Eiberg, Hans; Andersen, Paal Skyt; Mors, Ole; Rosenberg, Thomas; Brøndum-Nielsen, Karen; Cotterill, Rodney M J; Lundsteen, Claes; Ropers, Hans-Hilger; Ullmann, Reinhard; Bache, Iben; Tümer, Zeynep; Tommerup, Niels

ARTICLE

A 3.2 Mb deletion on 18q12 in a patient with childhood autism and high-grade myopia

Mette Gilling*[,1], Marlene Briciet Lauritsen[2,3], Morten Møller[4], Karen Friis Henriksen[1], Astrid Vicente[5,6], Guiomar Oliveira[7], Christina Cintin[8], Hans Eiberg[9], Paal Skyt Andersen[10], Ole Mors[2], Thomas Rosenberg[11], Karen Brøndum-Nielsen[11], Rodney MJ Cotterill[12], Claes Lundsteen[13,15], Hans-Hilger Ropers[14], Reinhard Ullmann[14], Iben Bache[1], Zeynep Tümer[1] and Niels Tommerup[1]

[1]Department of Medical Genetics, Wilhelm Johannsen Centre for Functional Genome Research, Institute of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen N, Denmark; [2]Centre for Psychiatric Research, Aarhus University Hospital, Riskov, Denmark; [3]NANEA, Department of Epidemiology, Institute of Public Health, University of Aarhus, Aarhus, Denmark; [4]Department of Medical Anatomy, University of Copenhagen, Copenhagen N, Denmark; [5]Instituto Gulbenkian de Ciência, Oeiras, Portugal; [6]Instituto Nacional de Saúde Dr Ricardo Jorge, Lisbon, Portugal; [7]Hospital Pediátrico de Coimbra, Coimbra, Portugal; [8]Psychiatric Hospital, Hillerød, Denmark; [9]Department of Medical Genetics, Institute of Molecular and Cellular Medicine, University of Copenhagen, Copenhagen N, Denmark; [10]Department of Clinical Biochemistry, Statens Serum Institut, Copenhagen S, Denmark; [11]Kennedy Institute – National Eye Clinic, Glostrup, Denmark; [12]Biophysics Group, Department of physics, Danish Technical University, Lyngby, Denmark; [13]Department of Clinical Genetics, Rigshospitalet, Copenhagen, Denmark; [14]Max-Planck Institute for Molecular Genetics, Berlin, Germany

Autism spectrum disorders (ASDs) are a heterogeneous group of disorders with unknown aetiology. Even though ASDs are suggested to be among the most heritable complex disorders, only a few reproducible mutations leading to susceptibility for ASD have been identified. In an attempt to identify ASD susceptibility genes through chromosome rearrangements, we investigated a female patient with childhood autism and high-grade myopia, and an apparently balanced *de novo* translocation, t(5;18)(q34;q12.2). Further analyses revealed a 3.2 Mb deletion encompassing 17 genes at the 18q break point and an additional deletion of 1.27 Mb containing two genes on chromosome 4q35. Q-PCR analysis of 14 of the 17 genes deleted on chromosome 18 showed that 11 of these genes were expressed in the brain, suggesting that haploinsufficiency of one or more genes may have contributed to the childhood autism phenotype of the patient. Identification of multiple genetic changes in this patient with childhood autism agrees with the most frequently suggested genetic model of ASDs as complex, polygenic disorders.
European Journal of Human Genetics (2008) **16**, 312–319; doi:10.1038/sj.ejhg.5201985; published online 9 January 2008

Introduction

Childhood autism is a neurodevelopmental disorder with onset in early childhood. It is characterized by impairment of social interaction and communication accompanied by stereotypic behaviour or interests with onset of symptoms before the age of 3. The prevalence of childhood autism is

estimated to be between 10 and 60 in $10\,000^{1-3}$ with a male to female ratio of four to one.[4] Cumulative evidence from family and twin studies suggests that childhood autism is among the most heritable complex disorders with a concordance rate of 60–90% in monozygotic twins and a recurrence rate of 2–3% in siblings of affected probands.[4,5] The mode of inheritance is unknown, but the broad phenotypic variation together with the limited overlap in the numerous genome scans performed in autistic cohorts suggests genetic heterogeneity.[6]

Characterizing chromosomal rearrangements at the molecular level is an approach to identify disease or susceptibility genes for complex disorders. This approach makes no assumptions on the mode of inheritance or aetiological overlap with other patients and has successfully revealed disease genes for monogenic disorders as well as susceptibility genes for multifactorial disorders.[7-9] By combining this method with whole-genome screening methods, like array-based comparative genome hybridization (array CGH), it is possible to identify combinations of gene alterations that may confer susceptibility to complex disorders. We used this combined approach to identify potential susceptibility genes for childhood autism in a patient with a *de novo* translocation, t(5;18)(q34;q12).

Methods
Participants
The translocation patient The patient is a 38-year-old Danish woman with a *de novo* translocation t(5;18)(q34;q12). She is the first of two children of unrelated and healthy parents. Her younger sister is phenotypically normal. At birth, her mother was 21 and her father was 24 years old. She was born at term after a pregnancy with reduced intrauterine movement as described by the mother. Delivery was prolonged and asphyxia was noted at birth. Birth weight was 2500 g and birth length was 50 cm. Later in life, mild cerebral palsy, hyper flexible joints, excessive myopia (−12 dioptres, right eye; −11 dioptres, left eye), and hypersensitivity to sounds were observed. She did not have any dysmorphic features. She sat at 9 months of age, walked alone when 17 months old, and said her first words and sentences at 42 months of age. At 3 years of age, she was diagnosed with childhood autism. She attended a school for autistic children until the age of 18 years and afterwards she moved to an institution for autistic adults. At the age of 34 years, she was tested with Autism Diagnostic Observation Schedule (ADOS)[10] module 4 for adults with fluent speech, and her mother was interviewed with Autism Diagnostic Interview-Revised (ADI-R).[11] Both tests clearly showed that the patient fulfilled the criteria for a childhood autism diagnosis as defined in the International Classification of Diseases, tenth revision (ICD-10). In the ADOS test, the patient scored 7 points in both the 'communication' area (autism

cut-off 3) and 'qualitative impairment in reciprocal social interaction' area (autism cut-off 6), and thus, the total score was 14 points (autism cut-off 10). The results from the ADI-R gave equivalently a score of 27 in the 'qualitative impairment in reciprocal social interaction' area (autism cut-off 10); a score of 18 in the 'communication' area (autism cut-off 8); and a score of 9 in the 'restricted, repetitive behaviour' area (autism cut-off 3). At the same time, the Wechsler Adult Intelligence Scale-Revised (WAIS-R) showed a verbal IQ of 78, a performance IQ of 105, and a full IQ of 88. Today, she lives in a small sheltered house for adult autistic patients.

The National Ethics Committees and the Danish Data Protection Agency approved the study, and informed consent was obtained.

Patients used for sequencing analysis For mutation screening, DNA from a total of 32 high-grade myopia patients and 157 autistic patients was collected. The high-grade myopia patients were collected at The Kennedy Institute – National Eye Clinic (Glostrup, Denmark).

One hundred autistic patients were recruited at the Hospital Pediátrico de Coimbra, originating from mainland Portugal and the Azorean islands. The male–female ratio was 4.8:1, and the ages ranged between 2 and 18 years (mean age 6.8 years). Idiopathic subjects were included after clinical assessment and screening for known medical and genetic conditions associated with autism, including testing for Fragile X mutations (FRAXA and FRAXE), chromosomal abnormalities, neurocutaneous syndromes, endocrine (thyroid function screening), and metabolic disorders. Another 35 children diagnosed with childhood autism were recruited at child psychiatric hospitals in the western part of Denmark (Jutland) (age range 3–30 years, with mean age of 10 years and male–female ratio of 3:1). Part of the sample has been described elsewhere.[12] Thirteen autistic patients were ascertained at Kennedy Institute – National Eye Clinic (Glostrup, Denmark). These patients were all unrelated and were part of the IMGSAC group. Assessment methods and inclusion criteria have previously been described.[13] Eleven of the thirteen patients had siblings and some even additional relatives with a pervasive developmental disorder diagnosis. Four patients diagnosed within the autism spectrum were collected at Psychiatric Hospital (Frederiksborg Amt, Denmark). In all of these 152 patients, childhood autism was diagnosed in accordance with DSM-IV[14] or ICD-10[15] criteria using ADI-R in addition to ADOS or the Childhood Autism Rating Scale.[10,16] In addition, five DNA samples from autism spectrum disorder (ASD) patients with chromosomal rearrangements were included. Two of these DNA samples were collected at the Wilhelm Johannsen Centre for Functional Genome Research, University of Copenhagen (Denmark) and were from Danish men diagnosed with childhood autism in accordance with ICD-10. Two DNA

samples from a Swedish, male twin couple were collected at the Department of Clinical Genetics, University Hospital Lund (Sweden) and one male DNA sample was collected by James Lespinasse at Laboratoire de Genetique Chromosomique, Centre hospitalier Chambery (Chambery, France). These three patients have an ASD diagnosis but have not been diagnosed according to ICD-10 or DSM-IV.

Whole-genome DNA amplification
When necessary DNA samples were genome amplified using GenomiPhi™ DNA Amplification Kit (GE Healthcare, Buckinghamshire, UK).

The Phi29 WGA kit (Amplicon, Brighton, UK) was used to amplify DNA from blood spots.

Fluorescence *in situ* hybridization
Metaphase chromosomes were prepared from peripheral blood lymphocytes, and the karyotype of the translocation patient was determined by G-banding. Fluorescence *in situ* hybridisation (FISH) was performed using 250 ng biotin-14-dATP-labelled bacterial artificial chromosome (BAC) clones from the RPCI-11 library according to standard protocols.

Array CGH
Array CGH with a whole-genome 32K BAC array was performed for the translocation patient as described previously.[17]

Real-time quantitative PCR analysis (RT Q-PCR)
cDNA synthesis of mRNA or total RNA (tissues used listed in Supplementary Table S1A and S1B) (Clontech, CA, USA) was performed using SuperScript II reverse transcriptase (Invitrogen) according to manufacturer. cDNA was investigated for DNA contamination by PCR using three primer pairs located in a region with no known genes (Supplementary Table S2). Real-time quantitative PCR (RT Q-PCR) analysis was carried out on a DNA Engine Opticon 2 (Bio-Rad, Göteborg, Sweden) using LightCycler FastStart DNA Master[PLUS] SYBR GreenI (Roche, Hvidovre, Denmark). From 12 analysed housekeeping genes, 6 were selected for normalization by using the BestKeeper software.[18] Primers used are listed in Supplementary Table S3.

In situ hybridization
Coronal cryostat sections (12 μm thick) of the mouse brain were prepared and mounted on Superfrost Plus® slides. The sections were hybridized as previously described[19] with three 38-mer ^{35}S-labelled oligonucleotide probes complementary to *Brunol4* mRNA. An oligonucleotide probe was used as sense control (Supplementary Table S4).

Images of the sections on X-ray film were transferred to a computer using a light box, a COHU 4912 high-performance CCD camera, and Image 1.42 software (Wayne Rasband, NIH, Bethesda, MD, USA). The pictures were visualized with Adobe Photoshop 7.0.

Sequencing
Mutation analysis of the *BRUNOL4* gene (NM_020180) was carried out by direct sequencing of all the 12 coding exons and exon–intron boundaries in 157 ASD patients and 32 high-grade myopia patients. The sequencing reactions were carried out by Macrogen Inc. in Korea (http://www.macrogen.com/), and ChromasPro version 1.33 (Technelysium Pty Ltd, Australia) was used to analyse the data. Primers and conditions are listed in Supplementary Table S5.

Results
The chromosome break points of the translocation patient were characterized by FISH. On chromosome 5, the BAC clone RP11-541P9 was spanning the break point, while RP11-256N5 was proximal and RP11-2A20 was distal. No known genes were located within this break point region. On chromosome 18, an approximately 3.2 Mb microdeletion containing 17 annotated RefSeq genes and two ultraconserved sequences (UCSs)[20] was identified (Supplementary Table S6; Figure 1). At the proximal deletion break point, BAC clone RP11-812d8 (chr18:30 184–30 378 Mb; NCBI35; HG17) was present and RP11-108g18 (chr18: 30 197–30 378 Mb; NCBI35; HG17) was deleted. At the distal break point, the fosmid clone G248P85590D6 (chr18:33 355–33 392 Mb; NCBI35; HG17) was deleted and the BAC clone RP11-1147p1 (chr18:33 276– 33 467 Mb; NCBI35; HG17) was present. FISH analyses of the parents showed that the deletion occurred *de novo*.

Presence of microdeletions/duplications elsewhere in the genome was investigated by array CGH. Apart from the deletion at the 18q break point, a deletion of approximately 1.2 Mb was identified at 4q35 (RP11-215A19 to RP11-746B09; chr4:187 648–188 915 Mb; NCBI35; HG17). This deletion comprised two RefSeq genes: *MTNR1A* (Melatonin receptor 1A) and *FAT* (Human homologue of the Drosophila *fat* tumour suppressor gene). Presence of the deletion was confirmed by FISH analyses, which showed that the deletion was inherited from the father.

As the published information on most of the genes deleted at 18q12 was sparse, we determined the tissue expression profile of 14 of the 17 deleted genes by RT Q-PCR. Dystrobrevin alpha (*DTNA*) and polypeptide *N*-acetylgalactosaminyltransferase (*GALNT1*) were already well described and were therefore not included in this study, and *KIAA1328* was not annotated at the time of investigations. Eleven of the fourteen genes were expressed in fetal and/or adult brain (normalized expression pattern of the 14 genes in human brain tissue is shown in Figure 2). *BRUNOL4* expression was much higher in both fetal and adult brain than any of the other genes (Supplementary Table S1A), and we therefore investigated the tissue expression pattern of this gene further with mRNA *in situ*
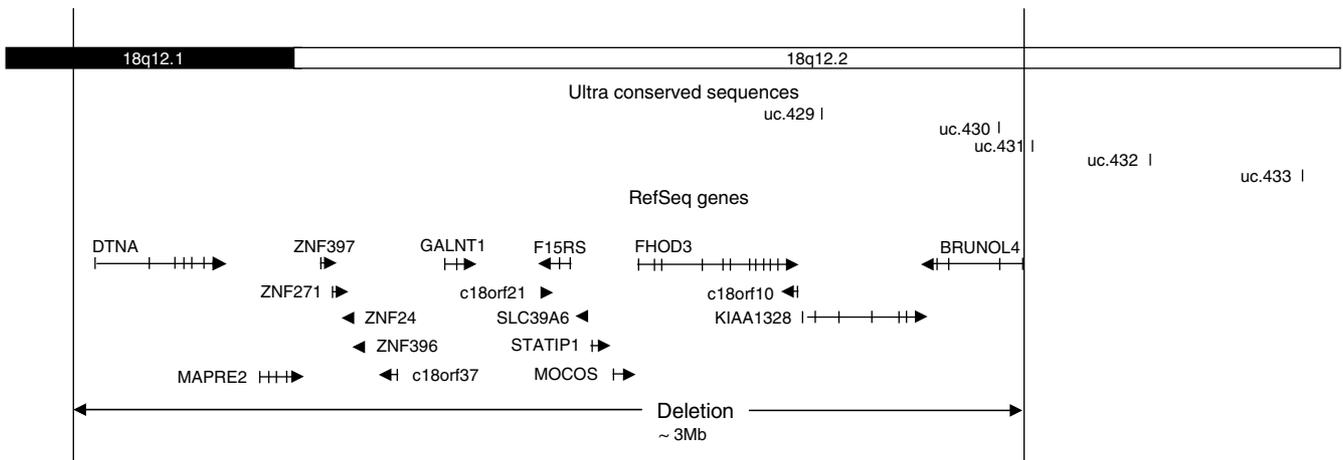
Figure 1 The 3.2 Mb deletion involving 17 genes on chromosome 18q12 in the translocation patient. Five ultraconserved non-coding sequences (UCSs) are located close to *BRUNOL4* within a region spanning from 343 kb downstream within intron 5 of *KIAA1328* to 918 kb upstream within the gene dessert. RefSeq genes and UCSs (uc.429–uc.433) are shown (in accordance with UCSC Genome Browser, March 2006 assembly). UCSs are numbered in accordance with Bejerano *et al.*[20]
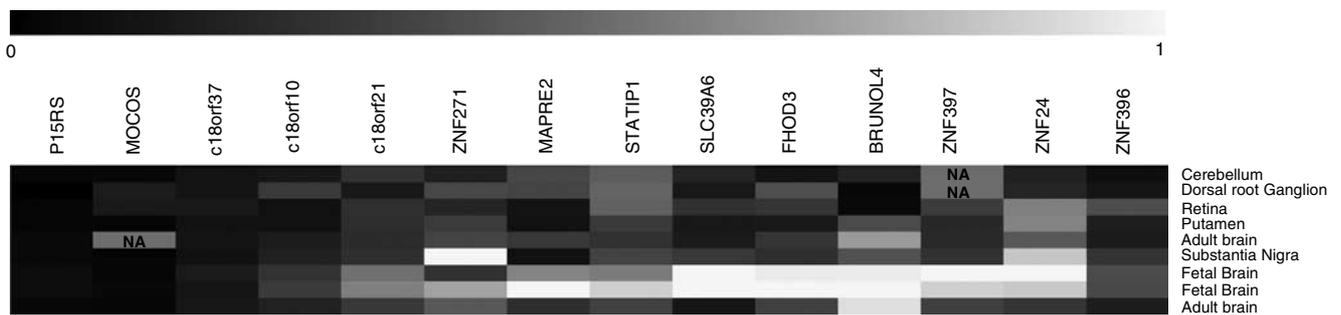

Figure 2 Cluster analysis of brain-specific, normalized Q-PCR data for 14 of 17 genes within the deletion of our translocation patient. Yellow is high expression, black is low expression, and NA (not a number) refers to expression outside of the standard curve. Multiple Array Viewer has been used to produce this figure. The Q-PCR data are listed in Supplementary Table S1A and S1B (see online version for colour figure).

hybridization on mouse brain sections. A strong hybridization signal for *Brunol4* was detected in the mouse neocortex, striatum, cerebellum, amygdala, hippocampus, piriform cortex, and hypothalamus (Figure 3). We further investigated involvement of *BRUNOL4* in ASD and myopia, by sequencing 157 ASD patients and 32 high-grade myopia patients.[21,22] Three new silent nucleotide changes were identified within the coding region of *BRUNOL4* in three unrelated ASD patients and submitted to the NCBI SNP database: ss67005831, ss67005837, and ss67005840. Furthermore, two new silent nucleotide changes within the coding region were identified in two unrelated high-grade myopia patients and were also submitted to the NCBI SNP database: ss67005834 and ss67005843.

Discussion

In a female patient diagnosed with childhood autism and a *de novo* translocation t(5;18)(q34;q12), we identified a 3.2 Mb deletion encompassing 17 genes (Figure 1) at the 18q12 translocation break point and a 1.27 Mb deletion on chromosome 4q35. Since chromosomal imbalances are known causes of mental retardation and other congenital anomalies,[23] and comorbidity of mental retardation with autism is a frequent finding, it is likely that deletion of one or more genes in this patient may lead to the observed childhood autism phenotype due to haploinsufficiency.

On chromosome 4, two known genes, *MTNR1A* (Melatonin receptor 1A) and *FAT* (homologue of Drosophila tumour suppressor fat), and an approximately 900 kb gene desert located 5′ to these genes were deleted (chr4:187 648–188 915 Mb, NCBI35; HG17). These deletions were inherited from the father, who had ADHD-like features. Previously, five copy number variations have been identified in this area (Database of Genomic Variants; http://projects.tcag.ca/variation/). Three normal control subjects have duplications of a large area of chromosome 4q35 encompassing *MTNR1A* and *FAT* (chr4:188 251–188 282 Mb, NCBI35; HG17).[24] Another normal control
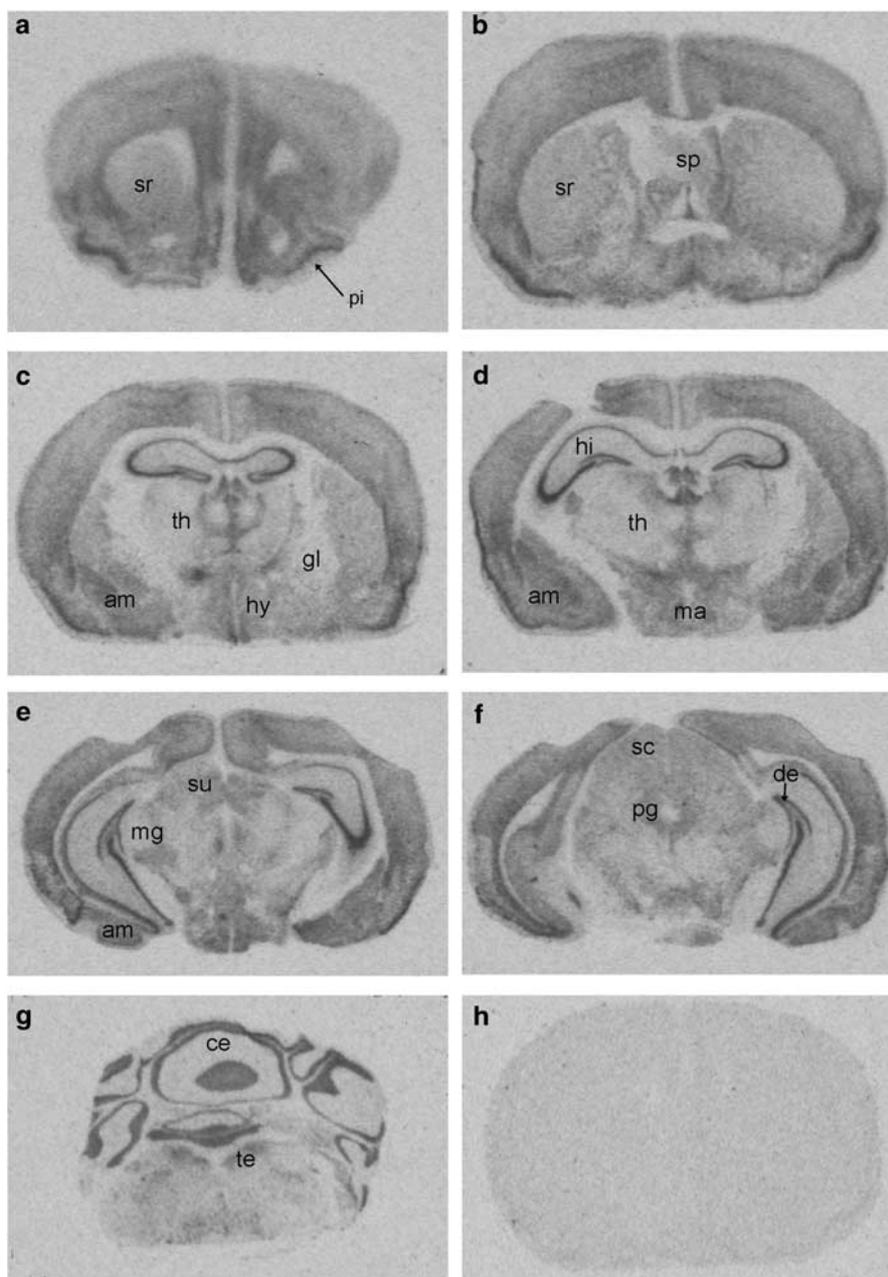
Figure 3 *In situ* hybridization for mRNA transcript of *Brunol4* on coronal sections of mouse brain. The montage shows images on X-ray films of hybridized coronal sections from rostral to caudal levels (**a–g**). A coronal section of the forebrain, hybridized with a sense probe, is seen in **h**. am = amygdala; ce = cerebellum; de = dentate gyrus; hy = hypothalamus; hi = hippocampus; ma = mammillary nuclei; mg = medial geniculate body; pi = piriform cortex; sp = septum; sr = striatum; su = superior colliculus; te = tegmental area; th = thalamus.

subject has a duplication of the gene desert 3′ to *MTNR1A* and *FAT* (chr4:187 636–187 797 Mb, NCBI35; HG17),[25] whereas two different normal controls have duplications (chr4:188 251–188 282 Mb, NCBI35; HG17)[26] and (chr4:188 353–189 810 Mb, NCBI35; HG17), and one has a deletion of the gene desert 5′ to *MTNR1A* and *FAT* (chr4:188 260–188 262 Mb, NCBI35; HG17).[27] Deletion of the *MTNR1A* and *FAT* genes is not reported in normal control subjects, but in a patient with an unknown phenotype, a deletion including *MTNR1A*, *FAT*, and six other genes was reported during screening of large-scale variations in the human genome.[28] Moreover, in a patient with schizoaffective disorder a 4q deletion possibly containing the *FAT* gene has been published.[29] The *FAT* gene is expressed in the eye and CNS in addition to other tissues, and encodes a protein of the cadherin superfamily of cell

adhesion molecules that is involved in cell migration, cell–cell contact, and establishment of cell polarity.[30] It has recently been suggested that FAT and its protein partners might be components of a molecular pathway involved in susceptibility to bipolar disorder.[31] Several lines of evidence suggest that neuropsychiatric disorders such as ASDs, schizophrenia, and bipolar disorder have common susceptibility genes[32–34] and *FAT* might be one of these. *MTNR1A* encodes a melatonin receptor in the brain that is mainly involved in transmitting the effect of melatonin on circadian rhythm.[35] Since biological rhythm disturbances are often reported in patients with mood disorders and a low melatonin level has been reported in individuals with ASDs, it is possible that haploinsufficiency of *MTNR1A* confer susceptibility to ASD.[35,36]

The deletion on chromosome 18 is at chromosome position chr18:30 197–33 392 Mb (NCBI35; HG17). A large number of deletions of varying sizes and locations on the long arm of chromosome 18 have already been published.[37–41] However, most of the deletions that apparently overlap with the present deletion have not been fine mapped, which complicates genotype/phenotype correlations. The most common features of the 18q12 deletion patients described in the literature are very mild dysmorphic features hardly disclosed at birth, psychomotor delay, hypotonia, ataxia, some degree of mental retardation, and behavioural abnormalities.[39] These features indicate that one or more genes within this region are crucial for development and normal function of the brain. McEntagart *et al*[42] have recently reported a patient with del(18)(q11.2q12.2). Even though the precise break points

of this deletion are unknown, it apparently includes the same 17 known genes identified in the present case (Figure 4). In this region, there is a 4.4 Mb large evolutionary stable gene desert[43] and 5 UCSs. UCSs are defined as sequences ≥ 200 bp with 100% identity in the human, mouse, and rat genome,[20,44] and some UCSs have been shown to posses enhancer activity,[45] suggesting that they are involved in gene regulation and development.[20,44] The presence of a stable gene desert as well as five UCSs in this region suggests that one or more of the deleted genes are developmentally important.

The phenotypic similarities of the present case and the patient reported by McEntagart and colleagues (Table 1), suggest that haploinsufficiency of one or more of the 17 deleted genes may lead to the common features. Tissue

Table 1 Phenotype comparison of the present case to the patient reported by McEntagart *et al*[42]

Phenotypes	Present case	McEntagart
Hypotonia	No	Yes
Febrile convulsions	No	Yes
Cerebral paresis	Yes	No
Delayed psychomotor development	**Yes**	**Yes**
Delayed language development	**Yes**	**Yes**
Behavioural difficulties	**Yes**	**Yes**
IQ	85	61
Autism	Yes	NR
Myopia	Yes	NR

NR, not reported.
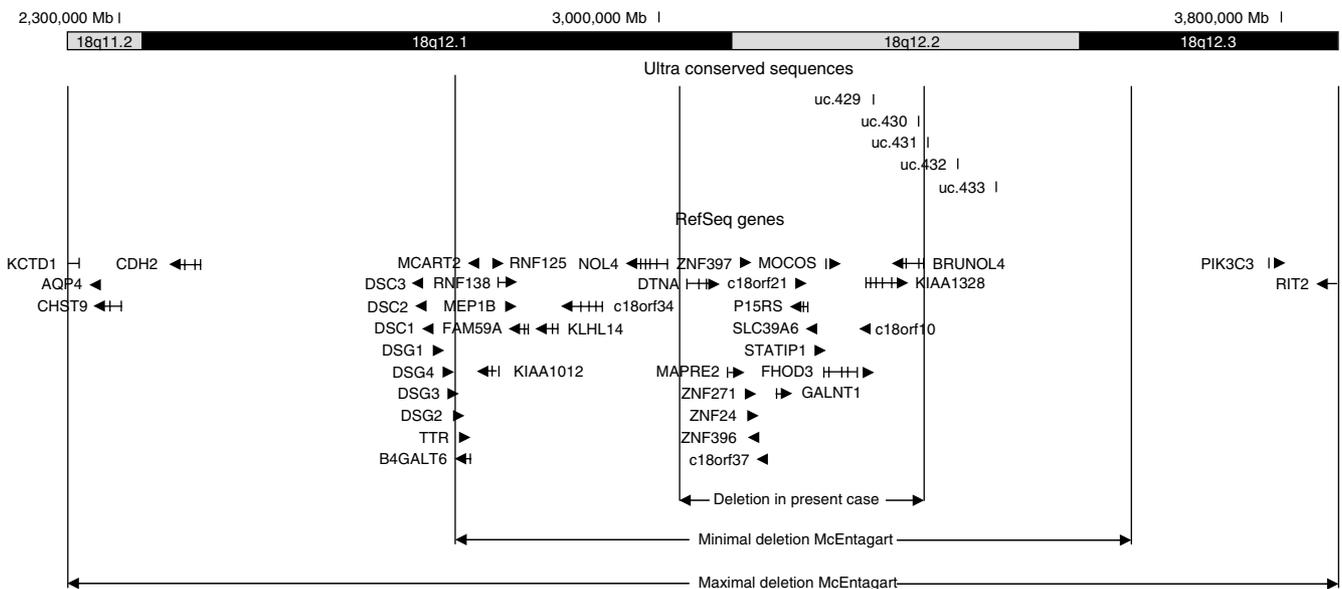Identical traits are written in bold.

Figure 4 Deletion in the present case is depicted together with the minimal and maximal deleted region in the subject described by McEntagart. RefSeq genes are positioned in accordance with the UCSC genome browser, March 2006 assembly.

expression profiles of 14 of these genes showed that 11 of them were expressed in the brain (Figure 2). Furthermore, these genes code for proteins, which may be involved in the normal functioning of the central nervous system; a zinc transporter (SLC39A6) that assures cofactors for hundreds of cellular enzymes;[46] four zinc finger transcription factors (ZNF397, ZNF396, ZNF271, ZNF24); a scaffolding protein (STATIP1) of the JAK-STAT signalling pathway suggested to be involved in neuronal and glial cell proliferation, survival, and differentiation;[47–49] an *O*-glycosylating enzyme (GALNT1) that might enable cells to adhere, differentiate, and migrate;[50] a microtubule associated protein (MAPRE2) that is possibly involved in the development of neuronal processes,[51] and an RNA-binding protein (BRUNOL4) that is most likely involved in mRNA splicing, regulation of translation, and rate of mRNA turnover.[21,52] The properties of these genes therefore suggest that each of them may have contributed to the ASD phenotype of the patient. Further studies should therefore be carried out to assess the involvement of these genes in ASDs using large patient and control cohorts.

We investigated one of the genes, *BRUNOL4*, further as this gene might be of interest with regards to the combined myopia and childhood autism phenotype observed in the present case, since it is expressed in the developing eye[21,22] as well as in the brain areas most consistently found to be affected in neuropathological investigations of autism (the limbic system, cerebellum, and cerebral cortex[53]) (Supplementary Table S1A and S1B; Figure 3). Moreover, *BRUNOL4* expression was significantly higher in both fetal and adult brain than any other gene residing in the deleted region. In addition, this gene belongs to the bruno-like elav (**e**mbryonic **l**ethal **a**bnormal **v**isual system) family of genes,[21,52] which result in abnormal eye and brain development in *Drosophila* when mutated.[54–56] We therefore sequenced the coding region of *BRUNOL4* in 157 ASD patients and 32 high-grade myopia patients, and identified 5 silent nucleotide substitutions that are most likely not involved in the development of myopia and/or ASDs. However, further studies including larger patient and control cohorts are necessary to investigate involvement of *BRUNOL4* in the aetiology of autism and/or myopia.

In conclusion, we have identified deletion of 19 genes in a patient with myopia and childhood autism, and one or more of these genes might have contributed to the development of these features. In addition, positional effects of the deletions and the translocation break points, and the asphyxia at birth may have contributed to the phenotype observed in this patient. Identification of multiple genetic changes in a patient with childhood autism is in line with the most frequently suggested genetic model of ASDs as complex, polygenic disorders.

References

1 Fombonne E: Epidemiological surveys of autism and other pervasive developmental disorders: an update. *J Autism Dev Disord* 2003; **33**: 365–382.
2 Baird G, Simonoff E, Pickles A *et al*: Prevalence of disorders of the autism spectrum in a population cohort of children in South Thames: the Special Needs and Autism Project (SNAP). *Lancet* 2006; **368**: 210–215.
3 Rutter M: Incidence of autism spectrum disorders: changes over time and their meaning. *Acta Paediatr* 2005; **94**: 2–15.
4 Folstein SE, Rosen-Sheidley B: Genetics of autism: complex aetiology for a heterogeneous disorder. *Nat Rev Genet* 2001; **2**: 943–955.
5 Rutter M: Genetic studies of autism: from the 1970s into the millennium. *J Abnorm Child Psychol* 2000; **28**: 3–14.
6 Risch N, Spiker D, Lotspeich L *et al*: A genomic screen of autism: evidence for a multilocus etiology. *Am J Hum Genet* 1999; **65**: 493–507.
7 Millar JK, Wilson-Annan JC, Anderson S *et al*: Disruption of two novel genes by a translocation co-segregating with schizophrenia. *Hum Mol Genet* 2000; **9**: 1415–1423.
8 Alkuraya FS, Saadi I, Lund JJ, Turbe-Doan A, Morton CC, Maas RL: SUMO1 haploinsufficiency leads to cleft lip and palate. *Science* 2006; **313**: 1751.
9 Petrij F, Giles RH, Dauwerse HG *et al*: Rubinstein–Taybi syndrome caused by mutations in the transcriptional co-activator CBP. *Nature* 1995; **376**: 348–351.
10 Lord C, Risi S, Lambrecht L *et al*: The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. *J Autism Dev Disord* 2000; **30**: 205–223.
11 Lord C, Rutter M, Le CA: Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *J Autism Dev Disord* 1994; **24**: 659–685.
12 Lauritsen MB, Borglum AD, Betancur C *et al*: Investigation of two variants in the DOPA decarboxylase gene in patients with autism. *Am J Med Genet* 2002; **114**: 466–470.
13 IMGSAC: A full genome screen for autism with evidence for linkage to a region on chromosome 7q. International Molecular Genetic Study of Autism Consortium. *Hum Mol Genet* 1998; **7**: 571–578.
14 American Psychiatric Association: *Diagnostic and Statistical Manual of Mental Disorders*. Washington, DC: American Psychiatric Association, 1994.
15 World Health Organization: *The ICD-10 Classification of Mental and Behavioural Disorders. Diagnostic Criteria for Research*. Geneva: World Health Organization, 1993.
16 Schopler E, Reichler RJ, DeVellis RF, Daly K: Toward objective classification of childhood autism: Childhood Autism Rating Scale (CARS). *J Autism Dev Disord* 1980; **10**: 91–103.

17 Erdogan F, Chen W, Kirchhoff M et al: Impact of low copy repeats on the generation of balanced and unbalanced chromosomal aberrations in mental retardation. Cytogenet Genome Res 2006; 115: 247–253.

18 Pfaffl MW, Tichopad A, Prgomet C, Neuvians TP: Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper – Excel-based tool using pair-wise correlations. Biotechnol Lett 2004; 26: 509–515.

19 Moller M, Phansuwan-Pujito P, Morgan KC, Badiu C: Localization and diurnal expression of mRNA encoding the beta1-adrenoceptor in the rat pineal gland: an in situ hybridization study. Cell Tissue Res 1997; 288: 279–284.

20 Bejerano G, Pheasant M, Makunin I et al: Ultraconserved elements in the human genome. Science 2004; 304: 1321–1325.

21 Meins M, Schlickum S, Wilhelm C et al: Identification and characterization of murine Brunol4, a new member of the elav/bruno family. Cytogenet Genome Res 2002; 97: 254–260.

22 Robinow S, White K: Characterization and spatial distribution of the ELAV protein during Drosophila melanogaster development. J Neurobiol 1991; 22: 443–461.

23 Menten B, Maas N, Thienpont B et al: Emerging patterns of cryptic chromosomal imbalance in patients with idiopathic mental retardation and multiple congenital anomalies: a new series of 140 patients and review of published reports. J Med Genet 2006; 43: 625–633.

24 Zogopoulos G, Ha KC, Nagib F et al: Germ-line DNA copy number variation frequencies in a large North American population. Hum Genet 2007; 122: 345–353.

25 Redon R, Ishikawa S, Fitch KR et al: Global variation in copy number in the human genome. Nature 2006; 444: 444–454.

26 Tuzun E, Sharp AJ, Bailey JA et al: Fine-scale structural variation of the human genome. Nat Genet 2005; 37: 727–732.

27 Mills RE, Luttig CT, Larkins CE et al: An initial map of insertion and deletion (INDEL) variation in the human genome. Genome Res 2006; 16: 1182–1190.

28 Iafrate AJ, Feuk L, Rivera MN et al: Detection of large-scale variation in the human genome. Nat Genet 2004; 36: 949–951.

29 Pickard BS, Hollox EJ, Malloy MP et al: A 4q35.2 subtelomeric deletion identified in a screen of patients with co-morbid psychiatric illness and mental retardation. BMC Med Genet 2004; 5: 21.

30 Down M, Power M, Smith SI et al: Cloning and expression of the large zebrafish protocadherin gene, fat. Gene Expr Patterns 2005; 5: 483–490.

31 Blair IP, Chetcuti AF, Badenhop RF et al: Positional cloning, association analysis and expression studies provide convergent evidence that the cadherin gene FAT contains a bipolar disorder susceptibility allele. Mol Psychiatry 2006; 11: 372–383.

32 Stahlberg O, Soderstrom H, Rastam M, Gillberg C: Bipolar disorder, schizophrenia, and other psychotic disorders in adults with childhood onset AD/HD and/or autism spectrum disorders. J Neural Transm 2004; 111: 891–902.

33 DeLong GR, Dwyer JT: Correlation of family history with specific autistic subgroups: Asperger's syndrome and bipolar affective disease. J Autism Dev Disord 1988; 18: 593–600.

34 Ghaziuddin M: A family history study of Asperger syndrome. J Autism Dev Disord 2005; 35: 177–182.

35 Melke J, Goubran BH, Chaste P et al: Abnormal melatonin synthesis in autism spectrum disorders. Mol Psychiatry 2007; 13: 90–98.

36 Wirz-Justice A: Biological rhythm disturbances in mood disorders. Int Clin Psychopharmacol 2006; 21 (Suppl 1): S11–S15.

37 Linnankivi T, Tienari P, Somer M et al: 18q deletions: clinical, molecular, and brain MRI findings of 14 individuals. Am J Med Genet A 2006; 140: 331–339.

38 Kline AD, White ME, Wapner R et al: Molecular analysis of the 18q- syndrome – and correlation with phenotype. Am J Hum Genet 1993; 52: 895–906.

39 Poissonnier M, Turleau C, Olivier-Martin M et al: Interstitial deletion of the proximal region of the long arm of chromosome 18, del(18q12) a distinct clinical entity? A report of two new cases. Ann Genet 1992; 35: 146–151.

40 Wilson MG, Towner JW, Forsman I, Siris E: Syndromes associated with deletion of the long arm of chromosome 18[del(18q)]. Am J Med Genet 1979; 3: 155–174.

41 Strathdee G, Zackai EH, Shapiro R, Kamholz J, Overhauser J: Analysis of clinical variation seen in patients with 18q terminal deletions. Am J Med Genet 1995; 59: 476–483.

42 McEntagart M, Carey A, Breen C et al: Molecular characterisation of a proximal chromosome 18q deletion. J Med Genet 2001; 38: 128–129.

43 Ovcharenko I, Loots GG, Nobrega MA, Hardison RC, Miller W, Stubbs L: Evolution and functional classification of vertebrate gene deserts. Genome Res 2005; 15: 137–145.

44 Sandelin A, Bailey P, Bruce S et al: Arrays of ultraconserved non-coding regions span the loci of key developmental genes in vertebrate genomes. BMC Genomics 2004; 5: 99.

45 Woolfe A, Goodson M, Goode DK et al: Highly conserved non-coding sequences are associated with vertebrate development. PLoS Biol 2005; 3: e7.

46 Taylor KM, Nicholson RI: The LZT proteins; the LIV-1 subfamily of zinc transporters. Biochim Biophys Acta 2003; 1611: 16–30.

47 Collum RG, Brutsaert S, Lee G, Schindler C: A Stat3-interacting protein (StIP1) regulates cytokine signal transduction. Proc Natl Acad Sci USA 2000; 97: 10120–10125.

48 Cattaneo E, Conti L, De-Fraja C: Signalling through the JAK-STAT pathway in the developing brain. Trends Neurosci 1999; 22: 365–369.

49 De-Fraja C, Conti L, Govoni S, Battaini F, Cattaneo E: STAT signalling in the mature and aging brain. Int J Dev Neurosci 2000; 18: 439–446.

50 Kingsley PD, Hagen KG, Maltby KM, Zara J, Tabak LA: Diverse spatial expression patterns of UDP-GalNAc:polypeptide N-acetyl-galactosaminyl-transferase family member mRNAs during mouse development. Glycobiology 2000; 10: 1317–1323.

51 Morrison EE, Moncur PM, Askham JM: EB1 identifies sites of microtubule polymerisation during neurite development. Brain Res Mol Brain Res 2002; 98: 145–152.

52 Antic D, Keene JD: Embryonic lethal abnormal visual RNA-binding proteins involved in growth, differentiation, and posttranscriptional gene expression. Am J Hum Genet 1997; 61: 273–278.

53 Palmen SJ, van EH, Hof PR, Schmitz C: Neuropathological findings in autism. Brain 2004; 127: 2572–2583.

54 Homyk Jr T, Isono K, Pak WL: Developmental and physiological analysis of a conditional mutation affecting photoreceptor and optic lobe development in Drosophila melanogaster. J Neurogenet 1985; 2: 309–324.

55 Campos AR, Grossman D, White K: Mutant alleles at the locus elav in Drosophila melanogaster lead to nervous system defects. A developmental-genetic analysis. J Neurogenet 1985; 2: 197–218.

56 Jimenez F, Campos-Ortega JA: Genes in subdivision 1B of the Drosophila melanogaster X-chromosome and their influence on neural development. J Neurogenet 1987; 4: 179–200.

Supplementary Information accompanies the paper on European Journal of Human Genetics website (http://www.nature.com/ejhg)