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ARTICLE

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

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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.
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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*

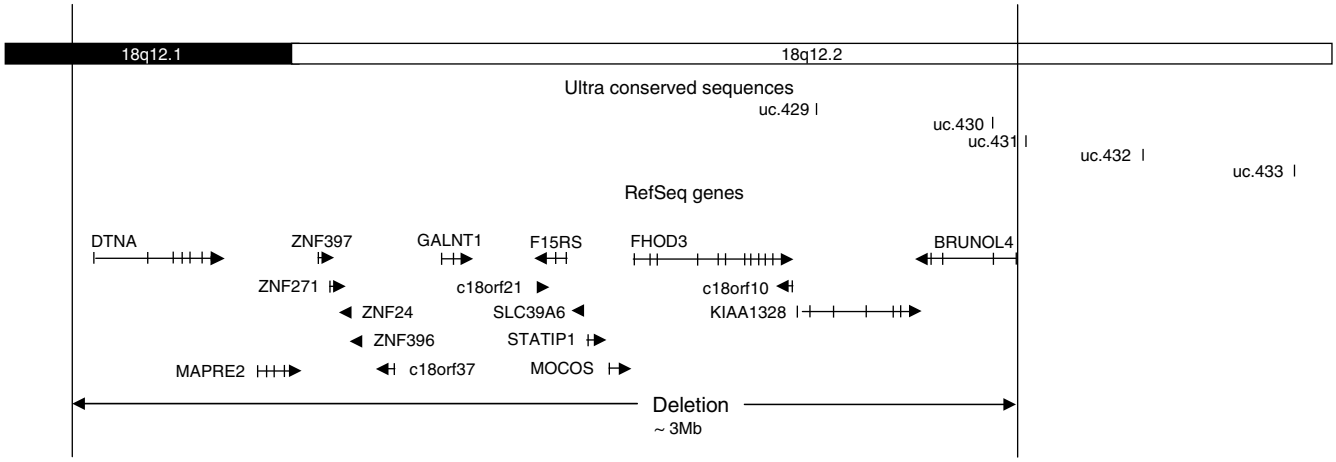

Figure 1 The 3.2 Mb deletion involving 17 genes on chromosome 18q12 in the translocation patient. Five ultraconserved non-coding sequences (UCSs) are located close to *BRUNOL4* within a region spanning from 343 kb downstream within intron 5 of *KIAA1328* to 918 kb upstream within the gene dessert. RefSeq 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]

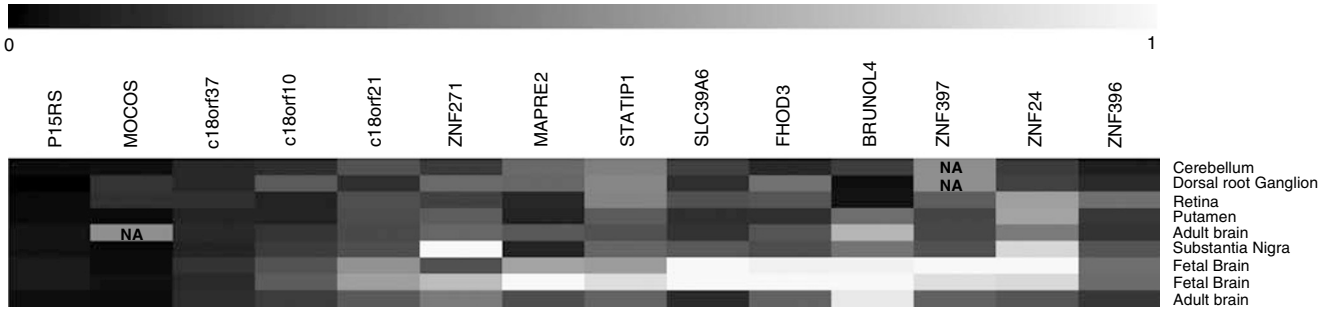

Figure 2 Cluster analysis of brain-specific, normalized Q-PCR data for 14 of 17 genes within the deletion of our translocation patient. Yellow is high expression, black is low expression, and NA (not a number) refers to expression outside of the standard curve. Multiple Array Viewer has been used to 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

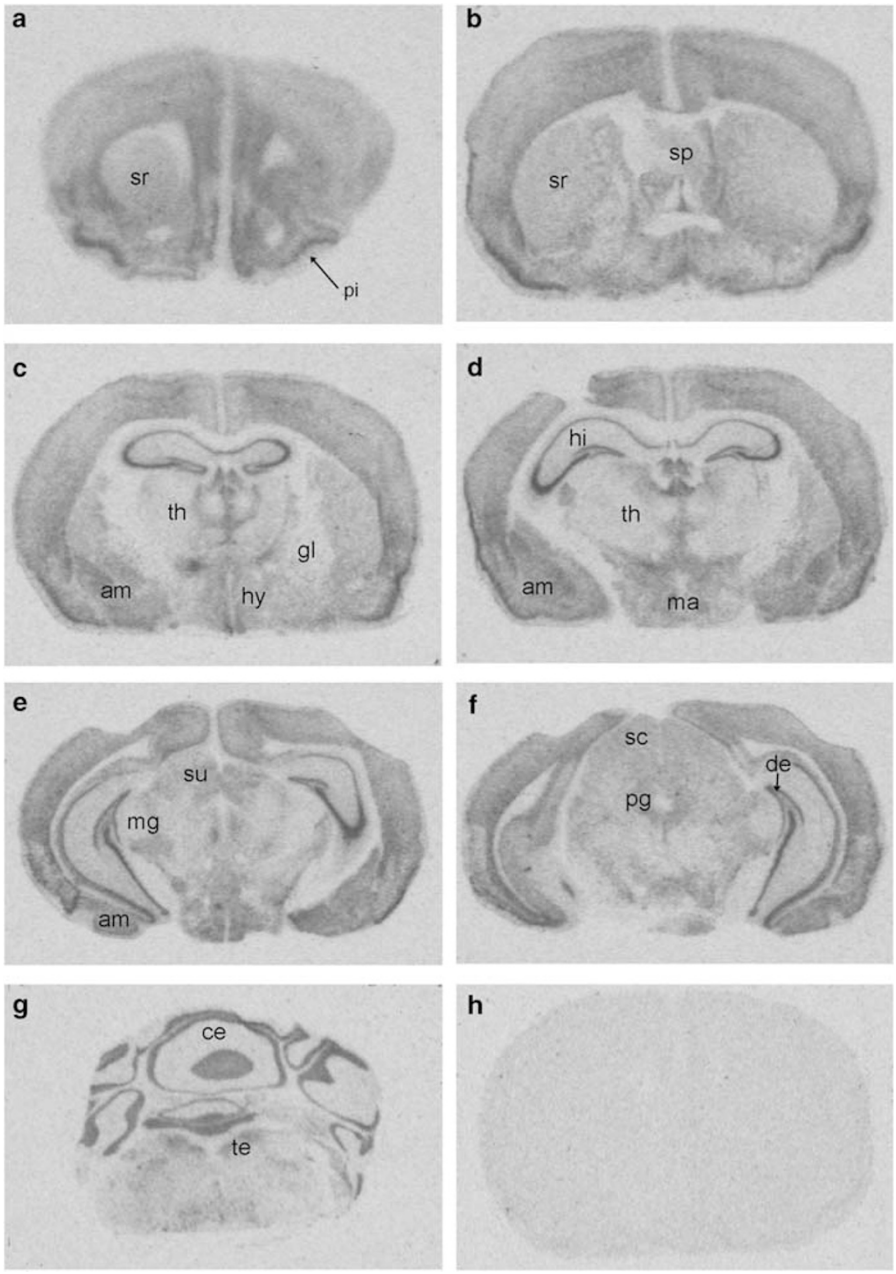

Figure 3 *In situ* hybridization for mRNA transcript of *Brunol4* on coronal sections of mouse brain. The montage shows images on X-ray films of hybridized coronal sections from rostral to caudal levels (**a**–**g**). A coronal section of the forebrain, hybridized with a sense probe, is seen in **h**. 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.

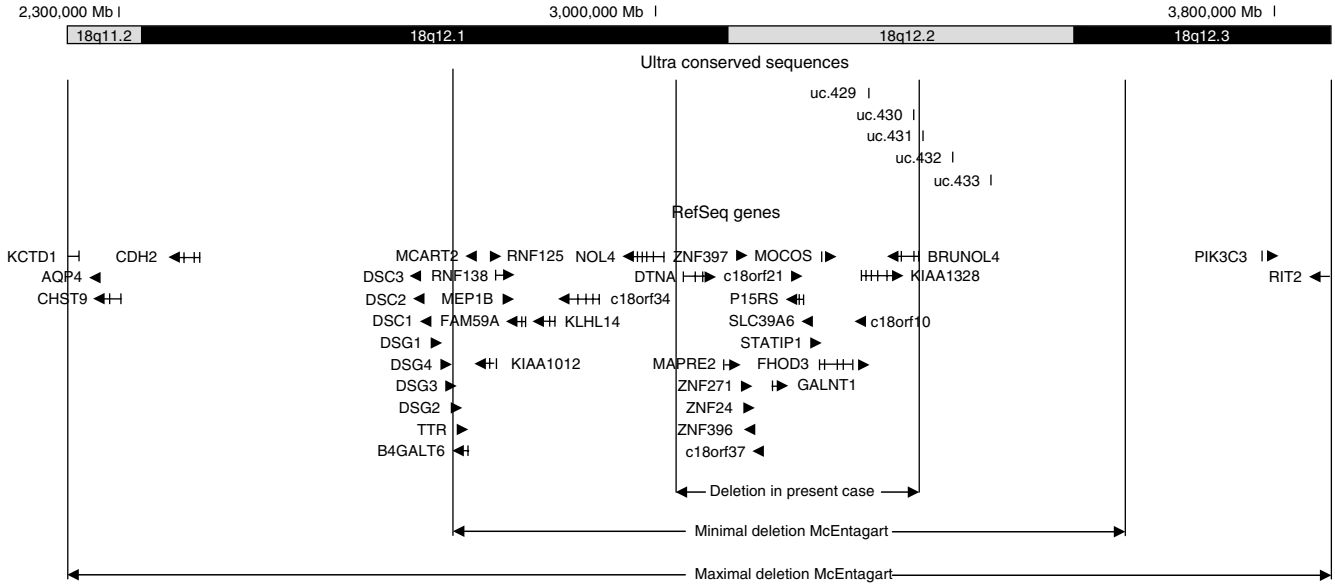

Figure 4 Deletion in the present case is depicted together with the minimal and maximal deleted region in the subject described by McEntagart. RefSeq genes are positioned in accordance with the UCSC genome browser, March 2006 assembly.

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.

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