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ARTICLE

Position effect leading to haploinsufficiency in a mosaic ring chromosome 14 in a boy with autism

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We describe an individual with autism and a coloboma of the eye carrying a mosaicism for a ring chromosome consisting of an inverted duplication of proximal chromosome 14. Of interest, the ring formation was associated with silencing of the *amisyn* gene present in two copies on the ring chromosome and located at 300 kb from the breakpoint. This observation lends further support for a locus for autism on proximal chromosome 14. Moreover, this case suggests that position effects need to be taken into account, when analyzing genotype–phenotype correlations based on chromosomal imbalances.
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Introduction

Chromosomal aberrations are a frequent cause of congenital malformations and developmental anomalies, and it is therefore evident that the characterization of chromosomal aberrations has been particularly contributive in the identification of genes causing specific phenotypes. Typically, a chromosomal aberration alters gene copy numbers, resulting in an increased gene dosage or in haploinsufficiency, and the precise delineation of the aberration can aid in the identification of candidate genes.[1] However, the interpretation may be more complicated when the imbalances are large, when different chromosomal regions are implicated (often with both an increased and a reduced dosage of different genes) or when the aberration is present in a mosaic state. Moreover, so called position effects, where the expression of genes located at a distance from the imbalance are affected, may further complicate the interpretation.[2,3]

One area where chromosomal aberrations have been instrumental in the identification of candidate genes and chromosomal regions is autism. Visible cytogenetic abnormalities are detected in >5% of affected children, involving many different loci on all chromosomes.[1] More recently, high-resolution techniques relying on array comparative genome hybridization (aCGH) have detected *de novo* submicroscopic chromosomal aberrations in a significant number of individuals with unexplained autism.[4,5]

We describe here the detailed molecular genetic analysis of a chromosomal aberration in a boy with autism carrying a *de novo* translocation involving chromosome 14q12 and a mosaic marker chromosome comprising an inverted duplication of the region proximal to the breakpoint at 14q12 and associated with a silencing of a gene located 300 kb proximal to the translocation breakpoint.

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Materials and methods

Molecular analysis

Positional cloning of the translocation breakpoints was performed as described before.[6] aCGH and real-time quantitative PCR (RTQ-PCR) analyses were performed as described.[3,7] Specific primers used for RTQ-PCR are listed as supplementary data.

Study of gene expression using single nucleotide polymorphisms (SNPs)

SNPs were used to study the effect of a breakpoint on the expression of several genes nearby the breakpoint at 14q12, for example, *amisyn*, *GZMH*, *CTSG*, *CMA1*, *KIAA1305*, and *NOVA1*. First, by sequencing the genomic DNA (gDNA) corresponding to coding exons and 5′ and 3′ untranslated regions (UTR) of these genes, we showed that the subject was heterozygous for reported SNPs rs1052484 (*amisyn*) and rs8017377 (*KIAA1305*). For the remaining genes, no reported or unreported SNPs were identified in the transcribed sequences. Next, for both heterozygous SNPs identified, the presence/absence of the polymorphism in the patient's mRNA was analyzed by sequencing the corresponding cDNA fragments, and for *amisyn*, biallelic expression was tested in four heterozygous controls. For obtaining DNA/RNA, Ebstein–Barr virus (EBV)-transformed lymphocytes of both patient and controls were grown in parallel to log phase. Isolation of mRNA and gDNA (RNeasy and DNeasy Mini kits, Invitrogen), reverse transcription of mRNA to cDNA (DNaseI treatment and Superscript III, Invitrogen), and gDNA/cDNA SNP analysis by subsequent PCR amplification and sequencing were performed as described.[3] Specific primers used for amplification and sequencing of the regions of interest are listed as supplementary data.

Results

Case report

The proband is a 16-year-old boy with a diagnosis of autism and borderline intelligence. Physical examination is normal with the exception of a unilateral coloboma of the eye. A more detailed case description can be found online as supplementary data. A first, chromosomal analysis suggested that he carried a *de novo* apparently balanced translocation with karyotype 46,XY,t(14;16)(q12;q24.3). However, successive molecular analysis revealed that the chromosomal aberration was more complex.

Molecular analysis

FISH analysis showed an intact subtelomeric region of der16q, with the presence of interstitial telomeric sequences at the translocation breakpoint and absence of subtelomeric 16q sequences on derivative chromosome 14 (Figure 1c), indicative of a non-reciprocal translocation. BAC CTD-2149C7 (NCBI AL132827) and cosmid 85f1 spanned the breakpoint on chromosome 14q12 (Figure 1a and b). aCGH revealed the presence of a duplication of the chromosomal region 14 proximal to the breakpoint on 14q12. The duplicated region included clones RP11-89K22 (NCBI AL161666), located near the breakpoint on 14q12, and RP11-84C10 (NCBI AL355922), more proximal on chromosome 14q11.2 (Figure 2a). RTQ-PCR on genomic DNA of the patient corresponding with this region (Figure 2a) confirmed the presence of an increased copy number (Figure 2b). FISH, using a centromere 14 probe showed one signal on the marker, indicating a single centromere (Figure 2c). FISH with a PNA telomere probe revealed the absence of telomeric sequences on the marker chromosome (Figure 2c), compatible with the cytogenetic appearance of the marker as a ring chromosome. To determine the orientation of the chromosomal fragments, FISH using BACs AL355922 (14q11.2) and AL161666 (14q12) was performed (see Figure 2a for the probes used). This indicated that the 14q12 regions were flanking and thus the proximal 14q regions have an inverted position with respect to each other (Figure 2c). Quantitative analysis of FISH metaphases revealed that the small derivative marker chromosome 14 is absent in approximately one-third of cells (22 out of 60 metaphases).

Taken together, this successive molecular analysis revealed that the proband carried a *de novo* apparently balanced translocation with karyotype 46,XY,der(16)(16pter→16qter::14q12→14qter)der(14)r(14;14)(::p11→q12::q12→p11::)[38]/45,XY,der(16)(16pter→16qter::14q12→14qter)[22]. This might explain the relative low values for the duplicated region in both the aCGH (<0.58) and RTQ-PCR (<3 alleles) analyses. In conclusion, this person carries a mosaicism for a partial trisomy for proximal chromosome 14 (2/3 of cells) and a partial monosomy of proximal 14 (1/3 of cells).

This patient has autism with borderline intelligence and we therefore searched for candidate genes for autism at the breakpoint region (Figure 1a). *Amisyn*, the closest gene located 300 kb centromeric to the breakpoint (Figure 1a), is mainly expressed in tissues with specialized cell types in which regulated secretory pathways are present, for example, brain and pancreas (Figure 1e and Scales *et al*[8]). It codes for a protein involved in the regulation of SNARE complex formation and thus in the regulated secretion of granules.[8,9] Given the evidence that genes implicated in synaptic processes, such as synaptogenesis and regulated secretion in neurons, may be involved in autism;[6,10–14] we investigated the expression of *amisyn* in the patient. The patient was heterozygous for SNP rs1052484, in the 3′ UTR (Figure 1d). *Amisyn* is expressed monoallelic in the patient, as at the mRNA level, only the G-allele could be detected in an EBV cell line from the patient (Figure 1d). The possibility of monoallelic expression due to genomic imprinting[15] was excluded by analysis of four heterozygous controls who showed biallelic *amisyn* expression (Figure 1d). Moreover, this clearly shows that *amisyn* is

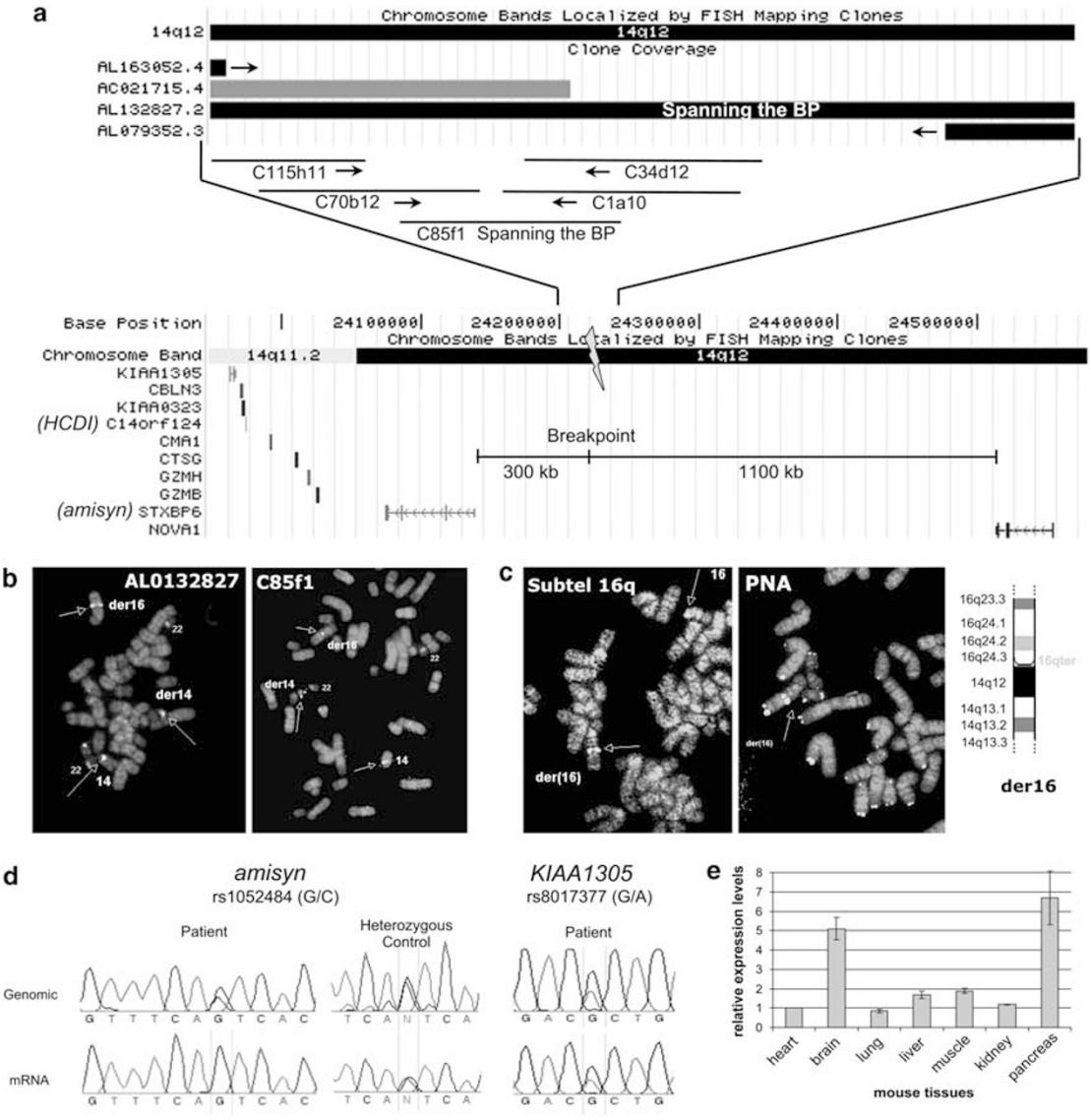

Figure 1 Identification of the translocation breakpoint on chromosome 14q: positional cloning and candidate gene analysis. (**a**) Physical map of 14q12 (UCSC Genome Browser, July 2003 version – centromere on the left). BAC AL132827 and cosmid 85f1 were shown to span the breakpoint on chromosome 14. Arrows indicate the position of the translocation breakpoint with regard to the genomic clone (→, distal; ←, proximal). Also shown the breakpoint position on 14q12 (a 'red lightning') and the nearest genes to this breakpoint (*amisyn*, 300 kb upstream and *NOVA1*, 1.1 Mb downstream). (**b** and **c**) FISH analysis on metaphase spread of the patient. (**b**) BAC AL0132827 (CTD-2149C7 – left) and cosmid 85f1 (right) span the breakpoint on chromosome 14. (**c**) FISH analysis on metaphase spread of the patient and schematic representation of breakpoint region on derivative chromosome 16. Hybridization with the subtelomere probe of 16q (green signal – left) showed the absence of subtelomere sequence on der14. Using the telomere probe (PNA – right), we showed the presence of an interstitial telomere on der16. (**d**) Expression analysis of *amisyn* and *KIAA1305* in the patient. Sequence analysis showed heterozygosity for rs1052484 (G/C, *amisyn*) and rs8017377 (G/A, *KIAA1305*) in the patient. cDNA analysis revealed the expression of the G-allele only for *amisyn*, compared to biallelic expression in a control. Both G- and A-alleles are expressed for *KIAA1305* in the patient mRNA. (**e**) Multiple tissue Northern blot (heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas) for human *amisyn*.

expressed in a normal chromosomal context, suggesting that the G-allele detected at the mRNA level originates from the *amisyn* gene located on the unaffected chromosome 14 (Figure 1d). In contrast, the C-allele is clearly undetectable at the mRNA level, suggesting that there is no expression from the *amisyn* alleles from the marker. To study expression of other flanking genes *GZMB*, *GZMH*, *CTSG*, *CMA1*, *KIAA1305* and *NOVA1* (Figure 1a), SNPs in the coding sequences of these genes were analyzed in the patient. However, only for *KIAA1305*, located at 920 kb proximal to the breakpoint and thus on the marker chromosome, the patient was heterozygous for an SNP (rs8017377, Figure 1d), but both G- and A-alleles were detected at the mRNA level (Figure 1d).

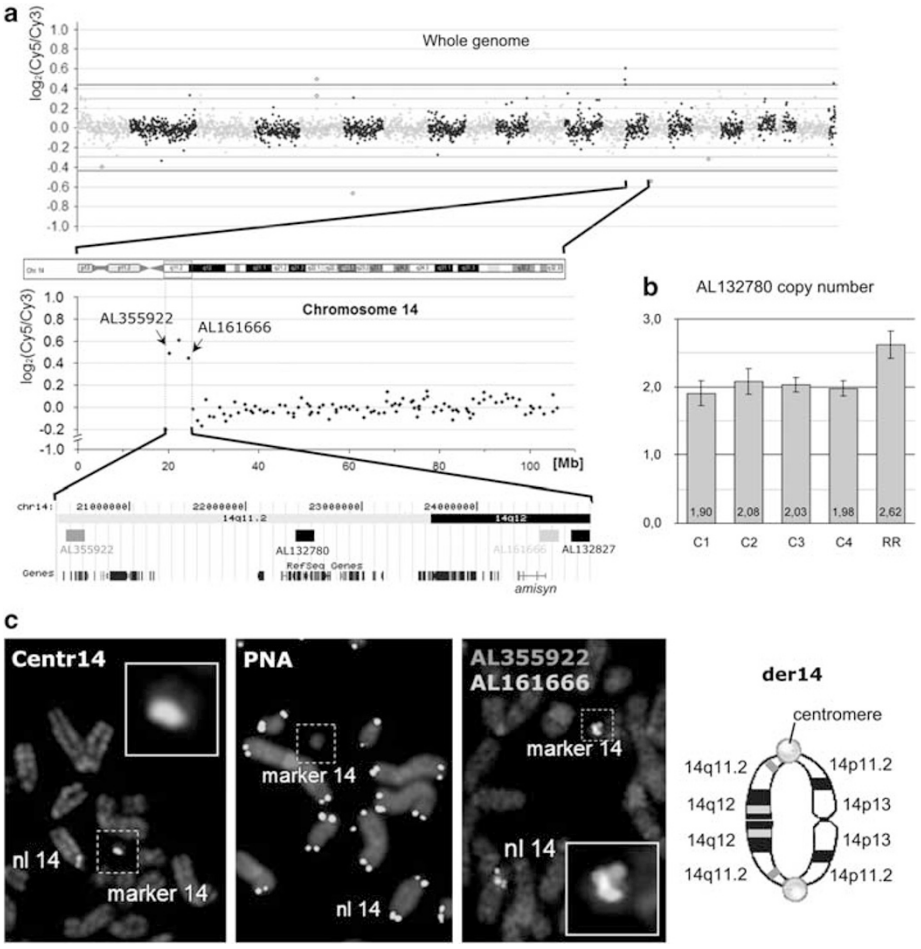

Figure 2 Mosaicism of a ring chromosome consisting of an inverted duplication of proximal chromosome 14q. (**a**) aCGH results of the patient for the whole genome and chromosome 14 at 1 Mb. X-axis: log2-transformed intensity ratios, Y-axis of lower panel: position of aCGH probes (Mb). Position of the BAC clones determining the duplicated region and used for FISH (panel c) are indicated. (**b**) RTQ-PCR results for the duplicated region (in AL132780 at 14q11.2): four independent controls and patient RR. Numbers at the base of the bars indicate the average relative measured copy number. The 95% confidence intervals are indicated. (**c**) FISH analysis on metaphase spread of the patient and schematic representation of the circular marker chromosome 14. BACs used as probes for FISH (right panel) are indicated (proximal = red and distal = green; eg, panel a). Hybridization with the centromeric probe of 14 (green signal – left) showed only one signal at the marker, the telomere probe (PNA – middle) showed the absence of telomeric sequences at the marker, and hybridization of both BACs determining the duplication showed an inverted position with respect to each other.

Taken together, despite the presence of a marker containing two copies of the *amisyn* gene in two-third of cells, this individual has haploinsufficiency for *amisyn* in all cells. For the genes proximal to the breakpoint, for example *KIAA1305*, we showed that all alleles present on the genomic level have a normal expression.

To further evaluate the expression of *amisyn*, we performed RTQ-PCR on cDNA from EBV-transformed lymphocytes of the patient and controls using two non-overlapping primersets. The observed expression levels were compared with the levels of *KIAA0323*, a gene located proximal to the breakpoint on 14q12 (Figure 1a). *KIAA0323* expression was stable between control individuals and showed a slightly elevated expression in the patient (1.21 ± 0.04 *versus* controls mean 1.00 ± 0.06), which is compatible with the observation of a mosaic duplication on the level of genomic DNA of the patient. In contrast, expression of *amisyn* in EBV-transformed cell lines from controls was found to be variable between individuals, with relative expression values in the broad range of 0.09–2.58. As a consequence, the relative expression level of *amisyn* in the patients cell line cannot be used to study a position effect.

To further evaluate *amisyn* as a candidate gene for autism, we performed DHPLC and subsequent sequencing analysis on all coding exons and exon–intron boundaries of the gene. However, in a cohort of 227 persons with autism, no mutations were detected.

Discussion

We present a boy with autism and an eye malformation carrying a non-reciprocal translocation of the larger part of the long arm of chromosome 14 to the telomere of chromosome 16q. The remaining untranslocated chromosome 14 fragment was found to form a ring chromosome consisting of an inverted duplication of this region. This ring chromosome was lost in a approximately 35% of cells, resulting in a mosaicism. Interestingly, the breakpoint on chromosome 14q was associated with silencing of the *amisyn* gene present in two copies on the ring chromosome and located at 300 kb from the breakpoint. Therefore, in both cells with and without the ring chromosome, monoallelic *amisyn* expression was observed. As a result, genotype–phenotype correlation in the present patient is complicated, as the phenotype could be the result of three different genetic mechanisms: (1) haploinsufficiency for *amisyn* in all cells, (2) and/or mosaic trisomy for other genes located in proximal chromosome 14 in approximately 65% of cells and (3) and/or mosaic monosomy for these genes in the other cells.

AMISYN as a candidate gene for autism

In the last years, several autism candidate proteins have been reported with a role in the overall process of neuronal signal transmission, such as synaptic scaffolding proteins, cell adhesion molecules, proteins involved in second messenger systems, secreted proteins, receptors and transporters.[10,16] AMISYN is a brain-enriched syntaxin-binding protein (STXBP6) interacting with t-SNAREs syntaxin-1 and SNAP-25 through its C-terminal VAMP-like coiled-coil domain.[8] As a consequence, AMISYN and v-SNARE VAMP-2 are competitive in their binding to the t-SNARE complex syntaxin-1/SNAP-25,[8] which plays an important role in vesicle fusion. However, since the unique N-terminus of AMISYN lacks the hydrophobic stretch that may serve as a transmembrane anchor to the vesicle,[8] AMISYN functions as an inhibitory SNARE (i-SNARE).[17,18] Therefore, AMISYN may be involved in fine-tuning vesicle fusion and secretion of vesicle content upon receipt of the appropriate stimulus,[8,19] making it a functional candidate for autism. However, in a cohort of 227 persons with autism, we were unable to detect mutations in the *amisyn* gene.

Proximal 14q as a candidate locus for autism

The main phenotypic manifestation in the present patient was autism and coloboma of the eye. The latter feature has not been reported in association with proximal 14q aberrations. Except for one study, no suggestive linkage for the proximal chromosome 14q has been reported.[20] However, several deletions in this part of 14q have been detected in patients with autism and/or mental retardation.[21,22] First, a small 700 kb deletion in 14q11.2, together with a gain of proximal 15q (resulting from a cryptic reciprocal translocation) segregated with autism and intellectual disabilities in three members of a family.[21] Although the gene-rich region at 14q did not contain any genes that appear to be involved in neurogenesis and/or neurodevelopment, the authors state that position effects cannot be ruled out. Second, small submicroscopic deletions of 14q11.2 have been reported in three unrelated children with similar dysmorphic features and developmental delay.[22] These deletions were located approximately 3.5 Mb centromeric from *amisyn*. Interestingly, autistic features were described in one of them (case DECIPHER 126). Taken together, the described chromosomal aberrations suggest the presence of an autism susceptibility gene in the region of chromosome 14q11.2–q12.

Translocation position effect

Chromosomal breakpoints altering the expression of adjacent genes, referred to as position effects, has been repeatedly demonstrated as a mechanism causing human genetic disease, with breakpoints up to a distance of 1 Mb, for example in campomelic dysplasia (*SOX9* gene[23]). In the present case, the breakpoint where ring fusion occurred is located at 300 kb from the 5′ end of both copies of *amisyn*. A number of different mechanisms could account for the observed changes in gene expression (reviewed by Kleinjan and van Heyningen[24]). The most likely mechanism causing absent *amisyn* expression from the rearranged chromosome 14 is the loss of a distant enhancer. This is suggested by the location of the breakpoint at the 5′ position of the gene, as is observed in most instances where position effects are encountered.[24] Moreover, the finding of a normal expression of another gene located in the vicinity of the *amisyn* gene, argues against a more general silencing mechanism imposed on the entire region. The alteration of the chromatin structure is postulated to be accompanied by altered DNA methylation and post-translational histone modifications.[25] However, methylation studies of the promoter region of the *amisyn* gene did not reveal any alterations (data not shown), which is another argument in favor of a disruption of an *amisyn* enhancer element rather than a general silencing effect due to the unusual chromosomal context in the ring chromosome.

To the best of our knowledge, no position effects have been shown in ring chromosomes, although they have been hypothesized to play a role in the phenotypic expression of ring chromosome 20 without the loss of subtelomeric regions or telomeric sequences.[26] It may also offer an explanation for the different phenotypic findings in individuals with terminal deletions of chromosome 14q and ring chromosome 14, despite similar breakpoints.[27]

In conclusion, this case illustrates that genotype–phenotype correlations derived from the physical mapping of chromosomal aberrations need to be taken with caution, given the possibility of position effects.

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References

1 Vorstman JA, Staal WG, van Daalen E, van Engeland H, Hochstenbach PF, Franke L: Identification of novel autism candidate regions through analysis of reported cytogenetic abnormalities associated with autism. *Mol Psychiatry* 2006; **11**: 1, 18–28.
2 Kleinjan DA, van Heyningen V: Long-range control of gene expression: emerging mechanisms and disruption in disease. *Am J Hum Genet* 2005; **76**: 8–32.
3 Castermans D, Vermeesch JR, Fryns JP *et al*: Identification and characterization of the TRIP8 and REEP3 genes on chromosome 10q21.3 as novel candidate genes for autism. *Eur J Hum Genet* 2007; **15**: 422–431.
4 Sebat J, Lakshmi B, Malhotra D *et al*: Strong association of *de novo* copy number mutations with autism. *Science* 2007; **316**: 445–449.
5 Marshall CR, Noor A, Vincent JB *et al*: Structural variation of chromosomes in autism spectrum disorder. *Am J Hum Genet* 2008; **82**: 477–488.
6 Castermans D, Wilquet V, Parthoens E *et al*: The neurobeachin gene is disrupted by a translocation in a patient with idiopathic autism. *J Med Genet* 2003; **40**: 352–356.
7 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.
8 Scales SJ, Hesser BA, Masuda ES, Scheller RH: Amisyn, a novel syntaxin-binding protein that may regulate SNARE complex assembly. *J Biol Chem* 2002; **277**: 28271–28279.
9 Constable JR, Graham ME, Morgan A, Burgoyne RD: Amisyn regulates exocytosis and fusion pore stability by both syntaxin-dependent and syntaxin-independent mechanisms. *J Biol Chem* 2005; **280**: 31615–31623.
10 Persico AM, Bourgeron T: Searching for ways out of the autism maze: genetic, epigenetic and environmental clues. *Trends Neurosci* 2006; **29**: 349–358.
11 Sadakata T, Washida M, Iwayama Y *et al*: Autistic-like phenotypes in Cadps2-knockout mice and aberrant CADPS2 splicing in autistic patients. *J Clin Invest* 2007; **117**: 931–943.
12 Durand CM, Betancur C, Boeckers TM *et al*: Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are associated with autism spectrum disorders. *Nat Genet* 2007; **39**: 25–27.
13 Stephan DA: Unraveling autism. *Am J Hum Genet* 2008; **82**: 7–9.
14 Kim HG, Kishikawa S, Higgins AW *et al*: Disruption of neurexin 1 associated with autism spectrum disorder. *Am J Hum Genet* 2008; **82**: 199–207.
15 Kotzot D: Comparative analysis of isodisomic and heterodisomic segments in cases with maternal uniparental disomy 14 suggests more than one imprinted region. *Clin Genet* 2001; **60**: 226–231.
16 Garber K: Neuroscience. Autism's cause may reside in abnormalities at the synapse. *Science* 2007; **317**: 190–191.
17 Short B, Barr FA: Membrane fusion: caught in a trap. *Curr Biol* 2004; **14**: R187–R189.
18 Varlamov O, Volchuk A, Rahimian V *et al*: i-SNAREs: inhibitory SNAREs that fine-tune the specificity of membrane fusion. *J Cell Biol* 2004; **164**: 79–88.
19 Gerst JE: SNARE regulators: matchmakers and matchbreakers. *Biochim Biophys Acta* 2003; **1641**: 99–110.
20 Auranen M, Vanhala R, Varilo T *et al*: A genomewide screen for autism-spectrum disorders: evidence for a major susceptibility locus on chromosome 3q25–27. *Am J Hum Genet* 2002; **71**: 777–790.
21 Koochek M, Harvard C, Hildebrand MJ *et al*: 15q duplication associated with autism in a multiplex family with a familial cryptic translocation t(14;15)(q11.2;q13.3) detected using array-CGH. *Clin Genet* 2006; **69**: 124–134.
22 Zahir F, Firth H, Baross A *et al*: Novel deletions of 14q11.2 associated with mental retardation and similar minor anomalies in three children. *J Med Genet* 2007.
23 Pfeifer D, Kist R, Dewar K *et al*: Campomelic dysplasia translocation breakpoints are scattered over 1 Mb proximal to SOX9: evidence for an extended control region. *Am J Hum Genet* 1999; **65**: 111–124.
24 Kleinjan DJ, van Heyningen V: Position effect in human genetic disease. *Hum Mol Genet* 1998; **7**: 1611–1618.
25 Klein CB, Costa M: DNA methylation, heterochromatin and epigenetic carcinogens. *Mutat Res* 1997; **386**: 163–180.
26 Zou YS, Van Dyke DL, Thorland EC *et al*: Mosaic ring 20 with no detectable deletion by FISH analysis: characteristic seizure disorder and literature review. *Am J Med Genet A* 2006; **140**: 1696–1706.
27 van Karnebeek CD, Quik S, Sluijter S, Hulsbeek MM, Hoovers JM, Hennekam RC: Further delineation of the chromosome 14q terminal deletion syndrome. *Am J Med Genet* 2002; **110**: 65–72.

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