

# A *de novo* microdeletion in *NRXN1* in a Dutch patient with mild intellectual disability, microcephaly and gonadal dysgenesis

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## Summary

This report is regarding a Dutch female with microcephaly, mild intellectual disability (ID), gonadal dysgenesis and dysmorphic facial features with synophrys. Upon genotyping, an ~455 kb *de novo* deletion encompassing the first exon of *NRXN1* was found. Bidirectional sequencing of the coding exons of the *NRXN1* alpha isoform was subsequently performed to investigate the possibility of a pathogenic mutation on the other allele, but we could not find any other mutation. Previously, many heterozygous mutations as well as microdeletions in *NRXN1* were shown to be associated with ID, autism, schizophrenia, and other psychiatric and psychotic disorders. Our results are in agreement with other reports that show that *NRXN1* deletions can lead to ID, microcephaly and mild dysmorphic features. However, this is the first report of gonadal dysgenesis being associated with such deletions. It is not clear whether there is a causal relationship between the *NRXN1* deletion and gonadal dysgenesis, but it is of interest that the *FSHR* gene, which encodes the follicle-stimulating hormone receptor causative correlation that is mutated in ovarian dysgenesis, is located proximal to the *NRXN1* gene. Given that most of the females carrying *NRXN1* deletions have been diagnosed at a prepubertal age, gynecologic screening of female carriers of a *NRXN1* deletion is warranted.

## 1. Introduction

The aetiology of moderate to severe ID is highly heterogeneous. Mutations in over 450 genes have been implicated in ID, which only represents a minor part of all ID genes that are anticipated (Inlow & Restifo, 2004; Schuurs-Hoeijmakers *et al.*, 2011; van Bokhoven, 2011). The sheer number of ID genes makes identification of the primary genetic defect in individual families and isolated cases difficult. ID disorders show impressive phenotypic variability in their clinical presentation. A plethora of associated features can be seen in a range of ID syndromes. Other neurological features, such as autism, epilepsy, ADHD and

behavioural anomalies are particularly common (van Bokhoven, 2011), and for mutations in some genes, these features can be seen in various combinations or in isolation. Copy number variations (CNVs) predispose to a wide variety of syndromes associated with ID, neurodevelopmental disorders and psychiatric disturbances (Vissers *et al.*, 2003; Sebat *et al.*, 2007). Thus, microdeletions at 15q11-2, 15q13-3 and 16p13-11 can induce ideopathic generalized epilepsy (Helbig *et al.*, 2008), ID (Burnside *et al.*, 2011), schizophrenia (Stefansson *et al.*, 2008) and autism spectrum disorder (Pagnamenta *et al.*, 2009), but such microdeletions are also found in healthy individuals. There is a possibility that in different neurological disorders, the CNVs disturb the normal neurodevelopmental continuum by functioning to disrupt the homeostasis of neuronal development (Coe *et al.*, 2012).

The *NRXN1* gene is one of the largest human genes (1.1 Mb) with 24 exons, located on chromosome

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2p16-3 (Boucard *et al.*, 2005). NRXN1 was previously recognized as a synapse protein in vertebrates and studies in *Drosophila* established that NRXN1 is present at the synapse where it regulates the levels of active zone protein bruchpilot (Zeng *et al.*, 2007; Zweier *et al.*, 2009). Neurexins are presynaptic cell-surface proteins that link the pre- and postsynaptic compartments of synapses by binding extracellularly to postsynaptic cell adhesion molecules and intracellularly to presynaptic PDZ domains (Missler *et al.*, 2003; Etherton *et al.*, 2009). *Nrxn1*-knockout mice have defects in excitatory synaptic strength displayed in electrophysiological tests and show behavioural changes (Geppert *et al.*, 1998). Both point mutations and heterozygous microdeletions disrupting *NRXN1* have been associated with a broad spectrum of psychiatric and neuronal development disorders including schizophrenia and autism. Compound heterozygous mutations in *NRXN1* have been identified in syndromic ID in Pitt–Hopkins syndrome (Kirov *et al.*, 2008; Rujescu *et al.*, 2009; Ching *et al.*, 2010). In addition, compound heterozygous *NRXN1* mutations consisting of a combination of exon-disrupting microdeletions and nonsense or splice-site mutations have recently been described in patients with severe early-onset epilepsy and profound ID (Harrison *et al.*, 2011; Duong *et al.*, 2012). Comorbid epilepsy has been reported in almost half of these cases (Ching *et al.*, 2010; Gregor *et al.*, 2011).

In the current study we report a *de novo* microdeletion of ~455 kb affecting the *NRXN1* gene in a patient with an ID phenotype and primary amenorrhoea due to gonadal dysgenesis. This is the first report of gonadal dysgenesis being associated with a *NRXN1* deletion.

## 2. Materials and Methods

### (i) Participants

Patient D1 was ascertained at the Department of Human Genetics of the Radboud University Medical Centre in Nijmegen, the Netherlands. She was included in a larger cohort study among individuals with unexplained ID.

### (ii) Genotyping

Informed written consent was obtained prior to blood sampling from all members of affected families. Genomic DNA was extracted from the blood using a standard phenol–chloroform extraction method (Sambrook & Russell, 2006) and the samples were stored at -20 °C until further processing. The affected and unaffected members of the D1 Dutch family were subjected to SNP array analysis using the Affymetrix 2.7 M cytogenetics array platform to obtain CNV

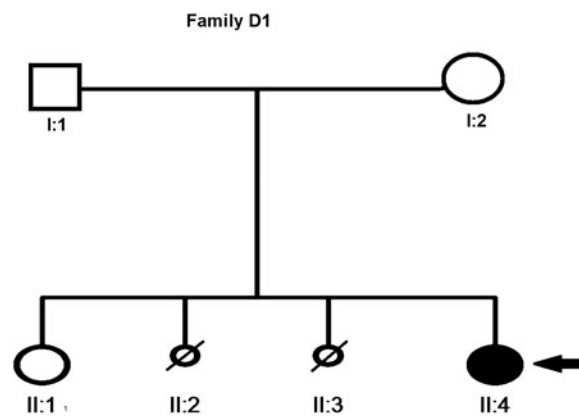


Fig. 1. Pedigree of family of patient D1. The small circles with diagonal lines represent the spontaneous abortion of female fetus while the filled circle shows the affected female.

data that revealed a heterozygous deletion of ~455 kb in patient D1. Subsequently the coding exons 2–24 of *NRXN1* were sequenced by bidirectional sequencing using ABI-PRISM 3730 (Life Technologies, Foster City, CA, USA) as described previously (Gregor *et al.*, 2011). For the affected female D1, sequencing of the *FSHR* gene was also performed because of gonadal dysgenesis.

## 3. Results

### (i) Patient report

Patient D1 (Fig. 1) is a female born to non-consanguineous Dutch parents. When referred for clinical genetic evaluation at age 28 years, mild ID was noted. The patient's mother had two abortions (both females) during early weeks of pregnancy. Patient D1 was born after a pregnancy of 42 weeks. The pregnancy was complicated by maternal hypertension, birth weight was low–normal (2980 g, 2-3th–5th percentile). Gross motor development was normal, but speech development was delayed, with the first single words being spoken after the age of 2 years. She went to a school for special education of children with developmental delay. Her behaviour was shy and introvert, and she was an anxious child. Medical problems included a primary amenorrhoea due to gonadal dysgenesis, diagnosed at the age of 16 years, which was treated with hormonal replacement therapy. Since the age of 16 years she has had recurrent episodes of loss of consciousness. Computed tomography of the brain and cardiologic evaluation did not reveal any abnormalities. Based on the clinical presentation of the events a probable psychogenic cause was considered. Upon physical examination she had a height of 173.5 cm (50th percentile), a weight of 70 kg (50th percentile) and a head circumference of 53.3 cm (39th percentile). She has minimal facial dysmorphic features including deeply set eyes

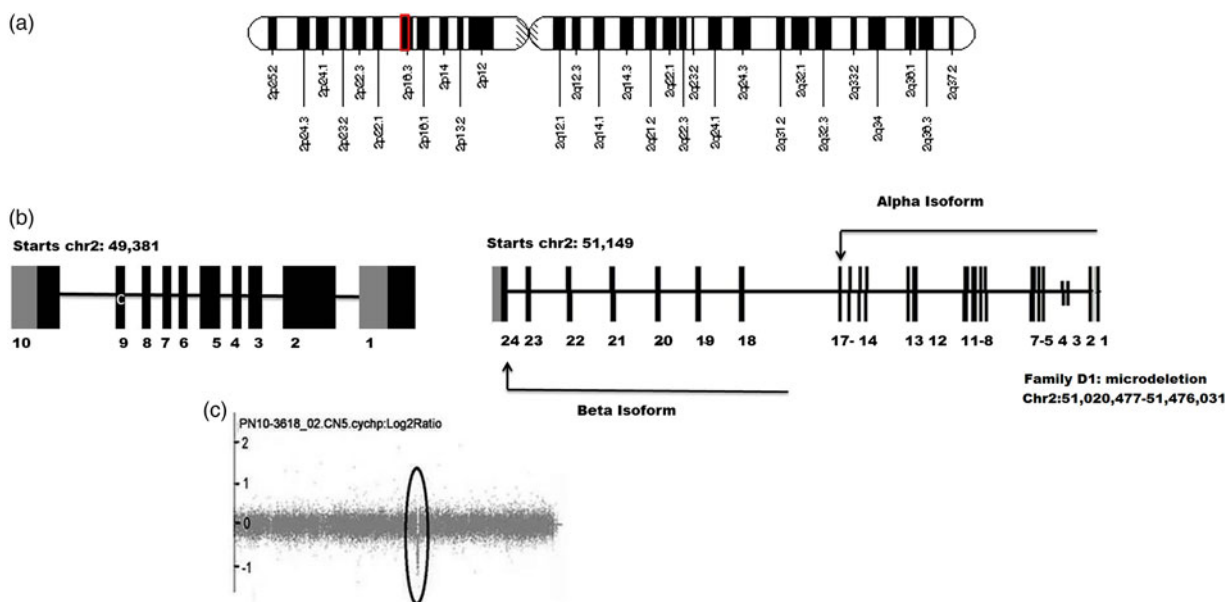


Fig. 2. (a) Original picture of chromosomal region 2p16.3 carrying both *NRXN1* and *FSHR* in close proximity. (b) Original data of CNV analysis derived from 2.7 Cytogenetic SNP array of patient D1 showing heterozygous deletion in the indicated chromosomal region 2p16.3 (chr2: 51 020 477–51 476 031 Mb, Hg19, built February 2009). (c) *NRXN1* exons from 1–24, with its two isoforms (alpha-isoform and beta-isoform) as well as the ~455 kb microdeletion in patient D1, which overlaps exon 1–7 encoding for the alpha-isoform.

and synophrys. Her feet were flat, long and narrow with long toes, and she also had long fingers. A metabolic screen could not explain the cause of her ID.

SNP array analysis on the DNA of the patient with the Affymetrix 2.7 M cytogenetic array revealed an ~455 kb loss in the chromosomal region 2p16.3 (chr2: 51 020 477–51 476 031 Mb, Hg19, built February 2009), encompassing the alpha-promoter of *NRXN1* including the first exon. Array analysis in both parents showed normal results, indicating that the deletion in this patient had occurred *de novo* (Fig. 2). Sequencing of the other allele of the *NRXN1* gene of the patient did not reveal another pathogenic mutation in patient D1. The deletion abolishes exons 1–7 of the *NRXN1* gene, similar to a large number of previously reported deletions. However, in contrast to the present patient, gonadal dysgenesis was not reported previously for any female deletion patients. Interestingly, the *FSHR* gene, which is associated with ovarian dysgenesis type I (OMIM 233300), is located proximal to the *NRXN1* gene (Fig. 2). We hypothesized that the deletion might affect the expression of the *FSHR* gene, which together with a mutation on the other allele might be causative for the gonadal dysgenesis. However, sequence analysis of the *FSHR* gene in affected female D1 revealed only known polymorphisms but no mutation that could explain the gonadal dysgenesis.

#### 4. Discussion

We report a heterozygous *de novo* deletion of 455 kb that encompasses the upstream promoter and exons

X–Y, encoding the alpha-isoform of *NRXN1*. A remarkable phenotypic variability has been observed for individuals with *NRXN1* deletion, ranging from apparently unaffected carriers to individuals with severe cognitive deficits such as autism, epilepsy and schizophrenia. Recently, in a study conducted by Moller *et al.* (2013) on a cohort of individuals with idiopathic generalized epilepsy, a number of exon disrupting deletions as well as five index cases with heterozygous *de novo* changes in *NRXN1* have been reported. Furthermore, two recent large studies by Dabell *et al.* (2013) and Bena *et al.* (2013) have reported an additional 34 and 25 pathogenic exon disrupting deletions in *NRXN1*, respectively. Recurrent clinical features associated with these deletions are ID, motor delay, autism, seizures, microcephaly and hypotonia. The patient from this study presents the main characteristic features that are observed in other deletion carriers. In addition, our patient shows gonadal dysgenesis. Amenorrhoea has not been reported previously in conjunction with *NRXN1* deletions and it is possible that the gonadal dysgenesis might have another aetiology. However, it is noteworthy that the *FSHR* gene is the nearest neighboring gene of *NRXN1* at the proximal side. Homozygous and compound heterozygous *FSHR* mutations are linked to ovarian dysgenesis (Aittomaki *et al.*, 1995). Sanger sequencing of the *FSHR* gene in our patient did not reveal any DNA variants, which in conjunction with a positional effect of the deletion could give rise to the gonadal

dysgenesis. Unfortunately, we were unable to test (bi-allelic) expression of *FSHR* in material from the patient and therefore it cannot be concluded whether close proximity of *FSHR* is coincidental nor exclude an involvement of the *FSHR* gene. The size and position of the *NRXN1* deletion may determine whether or not *FSHR* expression is affected. There are only a few older females who have been investigated in the study of Bena *et al.* (2013). There was a remarkable excess of males (19 males vs. six females) and most females were prepubertal. Also the patients investigated in detail by Dabell *et al.* (2013) showed a male excess (20 males vs. 14 females) and all the females were prepubertal.

The study has a certain limitation, as sequencing of the other already identified genes correlated with ovarian dysgenesis was not performed due to the direct 2-7 K cytogenetic array approach but this technique cannot rule out the involvement of other probable genes causing ovarian dysgenesis in patient D1. Future case report studies should keep this limitation in their approach so that other probable causes can be sorted out before the use of higher techniques such as microarray and many others. In conclusion, the occurrence of gonadal dysgenesis presented here warrants longitudinal investigation of females with a *NRXN1* deletion to establish a possible correlation with primary amenorrhoea.

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## Declaration of interest

None.

## References

- Aittomaki, K., Lucena, J. L., Pakarinen, P., Sistonen, P., Tapanainen, J., Gromoll, J., Kaskikari, R., Sankila, E. M., Lehväslaiho, H., Engel, A. R., Nieschlag, E., Huhtaniemi, I. & de la Chapelle, A. (1995). Mutation in the follicle-stimulating hormone receptor gene causes hereditary hypergonadotropic ovarian failure. *Cell* **82**, 959–968.
- Bena, F., Bruno, D. L., Eriksson, M., van Ravenswaaij-Arts, C., Stark, Z., Dijkhuizen, T., Gerkes, E., Gimelli, S., Ganesamoorthy, D., Thuresson, A. C., Labalme, A., Till, M., Bilan, F., Pasquier, L., Kitzis, A., Dubourg, C., Rossi, M., Bottani, A., Gagnebin, M., Sanlaville, D., Gilbert-Dussardier, B., Guipponi, M., van Haeringen, A., Kriek, M., Ruivenkamp, C., Antonarakis, S. E., Anderlid, B. M., Slater, H. R. & Schoumans, J. (2013). Molecular and clinical characterization of 25 individuals with exonic deletions of *NRXN1* and comprehensive review of the literature. *American Journal of Medical Genetics. Part, B., Neuropsychiatric Genetics* **162B**, 388–403.
- Boucard, A. A., Chubykin, A. A., Comoletti, D., Taylor, P. & Sudhof, T. C. (2005). A splice code for trans-synaptic cell adhesion mediated by binding of neuroligin 1 to alpha- and beta-neurexins. *Neuron* **48**, 229–236.
- Burnside, R. D., Pasion, R., Mikhail, F. M., Carroll, A. J., Robin, N. H., Youngs, E. L., Gadi, I. K., Keitges, E., Jaswaney, V. L., Papenhausen, P. R., Potluri, V. R., Risheg, H., Rush, B., Smith, J. L., Schwartz, S., Tepperberg, J. H. & Butler, M. G. (2011). Microdeletion/microduplication of proximal 15q11.2 between BP1 and BP2: a susceptibility region for neurological dysfunction including developmental and language delay. *Human Genetics* **130**, 517–528.
- Ching, M. S., Shen, Y., Tan, W. H., Jeste, S. S., Morrow, E. M., Chen, X., Mukaddes, N. M., Yoo, S. Y., Hanson, E., Hundley, R., Austin, C., Becker, R. E., Berry, G. T., Driscoll, K., Engle, E. C., Friedman, S., Gusella, J. F., Hisama, F. M., Irons, M. B., Lafiosca, T., LeClair, E., Miller, D. T., Neessen, M., Picker, J. D., Rappaport, L., Rooney, C. M., Sarco, D. P., Stoler, J. M., Walsh, C. A., Wolff, R. R., Zhang, T., Nasir, R. H., Wu, B. L. & Children's Hospital Boston Genotype Phenotype Study Group (2010). Deletions of *NRXN1* (neurexin-1) predispose to a wide spectrum of developmental disorders. *American Journal of Medical Genetics. Part, B., Neuropsychiatric Genetics* **153B**, 937–947.
- Coe, B. P., Girirajan, S. & Eichler, E. E. (2012). A genetic model for neurodevelopmental disease. *Current Opinion in Neurobiology* **22**, 829–836.
- Dabell, M. P., Rosenfeld, J. A., Bader, P., Escobar, L. F., El-Khechen, D., Vallee, S. E., Dinulos, M. B., Curry, C., Fisher, J., Tervo, R., Hannibal, M. C., Siefkas, K., Wyatt, P. R., Hughes, L., Smith, R., Ellingwood, S., Lacassie, Y., Stroud, T., Farrell, S. A., Sanchez-Lara, P. A., Randolph, L. M., Niyazov, D., Stevens, C. A., Schoonveld, C., Skidmore, D., MacKay, S., Miles, J. H., Moodley, M., Huillet, A., Neill, N. J., Ellison, J. W., Ballif, B. C. & Shaffer, L. G. (2013). Investigation of *NRXN1* deletions: clinical and molecular characterization. *American Journal of Medical Genetics. Part A* **161A**, 717–731.
- Duong, L., Klitten, L. L., Moller, R. S., Ingason, A., Jakobsen, K. D., Skjodt, C., Didriksen, M., Hjalgrim, H., Werge, T. & Tommerup, N. (2012). Mutations in *NRXN1* in a family multiply affected with brain disorders: *NRXN1* mutations and brain disorders. *American Journal of Medical Genetics. Part, B., Neuropsychiatric Genetics* **159B**, 354–358.
- Etherton, M. R., Blaiss, C. A., Powell, C. M. & Sudhof, T. C. (2009). Mouse neurexin-1alpha deletion causes correlated electrophysiological and behavioural changes consistent with cognitive impairments. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 17998–18003.
- Geppert, M., Khvotchev, M., Krasnoperov, V., Goda, Y., Missler, M., Hammer, R. E., Ichtchenko, K., Petrenko, A. G. & Südhof, T. C. (1998). Neurexin I alpha is a major alpha-latrotoxin receptor that cooperates in alpha-latrotoxin action. *The Journal of Biological Chemistry* **273**, 1705–1710.

- Gregor, A., Albrecht, B., Bader, I., Bijlsma, E. K., Ekici, A. B., Engels, H., Hackmann, K., Horn, D., Hoyer, J., Klapecki, J., Kohlhasse, J., Maystadt, I., Nagl, S., Prott, E., Tinschert, S., Ullmann, R., Wohlleber, E., Woods, G., Reis, A., Rauch, A. & Zweier, C. (2011). Expanding the clinical spectrum associated with defects in CNTNAP2 and NRXN1. *BMC Medical Genetics* **12**, 106.
- Harrison, V., Connell, L., Hayesmoore, J., McParland, J., Pike, M. G. & Blair, E. (2011). Compound heterozygous deletion of NRXN1 causing severe developmental delay with early onset epilepsy in two sisters. *American Journal of Medical Genetics. Part A* **155A**, 2826–2831.
- Helbig, I., Scheffer, I. E., Mulley, J. C. & Berkovic, S. F. (2008). Navigating the channels and beyond: unravelling the genetics of the epilepsies. *The Lancet. Neurology* **7**, 231–245.
- Inlow, J. K. & Restifo, L. L. (2004). Molecular and comparative genetics of mental retardation. *Genetics* **166**, 835–881.
- Kirov, G., Gumus, D., Chen, W., Norton, N., Georgieva, L., Sari, M., O'Donovan, M. C., Erdogan, F., Owen, M. J., Ropers, H. H. & Ullmann, R. (2008). Comparative genome hybridization suggests a role for NRXN1 and APBA2 in schizophrenia. *Human Molecular Genetics* **17**, 458–465.
- Missler, M., Zhang, W., Rohlmann, A., Kattenstroth, G., Hammer, R. E., Gottmann, K. & Südhof, T. C. (2003). Alpha-neurexins couple Ca<sup>2+</sup> channels to synaptic vesicle exocytosis. *Nature* **423**, 939–948.
- Moller, R. S., Weber, Y. G., Klitten, L. L., Trucks, H., Muhle, H., Kunz, W. S., Mefford, H. C., Franke, A., Kautza, M., Wolf, P., Dennig, D., Schreiber, S., Rückert, I. M., Wichmann, H. E., Ernst, J. P., Schurmann, C., Grabe, H. J., Tommerup, N., Stephani, U., Lerche, H., Hjalgrim, H., Helbig, I., Sander, T. & EPICURE Consortium (2013). Exon-disrupting deletions of NRXN1 in idiopathic generalized epilepsy. *Epilepsia* **54**, 256–264.
- Pagnamenta, A. T., Wing, K., Sadighi Akha, E., Knight, S. J., Bolte, S., Schmotzer, G., Duketis, E., Poustka, F., Klauck, S. M., Poustka, A., Ragoussis, J., Bailey, A. J., Monaco, A. P. & International Molecular Genetic Study of Autism Consortium (2009). A 15q13.3 microdeletion segregating with autism. *European Journal of Human Genetics* **17**, 687–692.
- Rujescu, D., Ingason, A., Cichon, S., Pietiläinen, O. P., Barnes, M. R., Touloupoulou, T., Picchioni, M., Vassos, E., Ettinger, U., Bramon, E., Murray, R., Ruggeri, M., Tosato, S., Bonetto, C., Steinberg, S., Sigurdsson, E., Sigmundsson, T., Petursson, H., Gylfason, A., Olason, P. I., Hardarsson, G., Jonsdottir, G. A., Gustafsson, O., Fossdal, R., Giegling, I., Möller, H. J., Hartmann, A. M., Hoffmann, P., Crombie, C., Fraser, G., Walker, N., Lonnqvist, J., Suvisaari, J., Tuulio-Henriksson, A., Djurovic, S., Melle, I., Andreassen, O. A., Hansen, T., Werge, T., Kiemenev, L. A., Franke, B., Veltman, J., Buizer-Voskamp, J. E., GROUP Investigators, Sabatti, C., Ophoff, R. A., Rietschel, M., Nöthen, M. M., Stefansson, K., Peltonen, L., St Clair, D., Stefansson, H. & Collier, D. A. (2009). Disruption of the neurexin 1 gene is associated with schizophrenia. *Human Molecular Genetics* **18**, 988–996.
- Sambrook, J. & Russell, D. W. (2006). *The Condensed Protocols From Molecular Cloning: a Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
- Schuurs-Hoeijmakers, J. H., Hehir-Kwa, J. Y., Pfundt, R., van Bon, B. W., de Leeuw, N., Kleefstra, T., Willemsen, M. A., van Kessel, A. G., Brunner, H. G., Veltman, J. A., van Bokhoven, H., de Brouwer, A. P. & de Vries, B. B. (2011). Homozygosity mapping in outbred families with mental retardation. *European Journal of Human Genetics* **19**, 597–601.
- Sebat, J., Lakshmi, B., Malhotra, D., Troge, J., Lese-Martin, C., Walsh, T., Yamrom, B., Yoon, S., Krasnitz, A., Kendall, J., Leotta, A., Pai, D., Zhang, R., Lee, Y. H., Hicks, J., Spence, S. J., Lee, A. T., Puura, K., Lehtimäki, T., Ledbetter, D., Gregersen, P. K., Bregman, J., Sutcliffe, J. S., Jobanputra, V., Chung, W., Warburton, D., King, M. C., Skuse, D., Geschwind, D. H., Gilliam, T. C., Ye, K. & Wigler, M. (2007). Strong association of de novo copy number mutations with autism. *Science* **316**, 445–449.
- Stefansson, H., Rujescu, D., Cichon, S., Pietiläinen, O. P., Ingason, A., Steinberg, S., Fossdal, R., Sigurdsson, E., Sigmundsson, T., Buizer-Voskamp, J. E., Hansen, T., Jakobsen, K. D., Muglia, P., Francks, C., Matthews, P. M., Gylfason, A., Halldorsson, B. V., Gudbjartsson, D., Thorgeirsson, T. E., Sigurdsson, A., Jonasdottir, A., Jonasdottir, A., Bjornsson, A., Mattiasdottir, S., Blondal, T., Haraldsson, M., Magnusdottir, B. B., Giegling, I., Möller, H. J., Hartmann, A., Shianna, K. V., Ge, D., Need, A. C., Crombie, C., Fraser, G., Walker, N., Lonnqvist, J., Suvisaari, J., Tuulio-Henriksson, A., Paunio, T., Touloupoulou, T., Bramon, E., Di Forti, M., Murray, R., Ruggeri, M., Vassos, E., Tosato, S., Walshe, M., Li, T., Vasilescu, C., Mühleisen, T. W., Wang, A. G., Ullum, H., Djurovic, S., Melle, I., Olesen, J., Kiemenev, L. A., Franke, B., GROUP, Sabatti, C., Freimer, N. B., Gulcher, J. R., Thorsteinsdottir, U., Kong, A., Andreassen, O. A., Ophoff, R. A., Georgi, A., Rietschel, M., Werge, T., Petursson, H., Goldstein, D. B., Nöthen, M. M., Peltonen, L., Collier, D. A., St Clair, D. & Stefansson, K. (2008). Large recurrent microdeletions associated with schizophrenia. *Nature* **7210**, 232–236.
- van Bokhoven, H. (2011). Genetic and epigenetic networks in intellectual disabilities. *Annual Review of Genetics* **45**, 81–104.
- Vissers, L. E., de Vries, B. B., Osoegawa, K., Janssen, I. M., Feuth, T., Choy, C. O., Straatman, H., van der Vliet, W., Huys, E. H., van Rijk, A., Smeets, D., van Ravenswaaij-Arts, C. M., Knoers, N. V., van der Burgt, I., de Jong, P. J., Brunner, H. G., van Kessel, A. G., Schoenmakers, E. F. & Veltman, J. A. (2003). Array-based comparative genomic hybridization for the genome wide detection of submicroscopic chromosomal abnormalities. *American Journal of Human Genetics* **73**, 1261–1270.
- Zeng, X., Sun, M., Liu, L., Chen, F., Wei, L. & Xie, W. (2007). Neurexin-1 is required for synapse formation and larvae associative learning in *Drosophila*. *FEBS Letters* **581**, 2509–2516.
- Zweier, C., de Jong, E. K., Zweier, M., Orrico, A., Ousager, L. B., Collins, A. L., Bijlsma, E. K., Oortveld, M. A., Ekici, A. B., Reis, A., Schenck, A. & Rauch, A. (2009). CNTNAP2 and NRXN1 are mutated in autosomal-recessive Pitt-Hopkins-like mental retardation and determine the level of a common synaptic protein in *Drosophila*. *American Journal of Human Genetics* **85**, 655–666.