Supporting Information for the article:

Berryer et al. Mutations in *SYNGAP1* cause intellectual disability, autism and a specific form of epilepsy by inducing haploinsufficiency

Supp. Patient Description

Patient-1 (c.283dupC [p.H95PfsX5])

This female patient, aged now 16 yrs, is the single child of unrelated Danish parents. Her mother is healthy with no history of learning or psychiatric problems. The father, however, has learning difficulties but without epilepsy or ID. He attended regular elementary school but always needed additional help and eventually dropped out of school at the age of 15 due to learning difficulties. He suffers from recurrent depression (treated with sertraline hydrochloride) and currently lives on disability aid.

His affected daughter was delivered at 42 weeks of gestation (birth weight 3.7 Kg, height 55 cm) after an unremarkable pregnancy. Her motor development was normal. She sat at 6 months of age and started to walk at 10.5 months of age. Language was initially normal with first words appearing at 12-to-18 months, but she was not able to form sentences until the age of 4 years, and her language remains delayed. Her current WISC-III scores indicate moderate ID (Full scale IQ = 40). Evaluation with the Beck Youth Inventory Test showed very high scores for recurrent Fall-and-Winter depressions but the scores were normal for anxiety and self-esteem.

Onset of seizures was at 18 months of age. Her seizures were characterized by myoclonic absences (EMA). Control was difficult until 12 years of age despite the administration of combinations of anticonvulsants, including clonazepam, clobazam, valproate and leviteracetam. Initial treatment with oxcarbazepine led to an increase in her seizure frequency. She was stable for several years on a combination of leviteracetam and valproate. She is currently on ethosuximide and valproate and has been seizure-free for two years. She was briefly put on atomoxetine for behavioural problems, but this was not helpful. Her EEG was recorded at different dates and showed generalized paroxysms of spike-wave discharges at 3½-5 Hz predominating over the posterior quadrant, sometimes lateralizing to the right sometimes to the left.

At 14y 5m of age, patient-1 weighed 46.2 kg (50^{th} centile) and measured 155.5 cm (15-50^{th} centile). Her latest head circumference was 50 cm (5^{th} centile) when she was 10 y 7 m. She

does not show any specific dysmorphic features. Neurological examination was unremarkable. Her karyotype, urine metabolic screening, and brain MRI were all normal. High-resolution SNP genotyping array (Affymetrix Genome-Wide Human SNP Array 6.0) and mutation analysis of *GLUT1* did not show any abnormality.

Patient-2 (c.1084T>C [p.W362R])

Patient-2, a male aged 3 years and 6 months, is the only child of unrelated European parents. Family history is unremarkable. He was delivered spontaneously at term after an unremarkable pregnancy. The APGAR score was 9^{1} and 9^{5}. At birth, his weight was 3.95 kg (75^{th} centile) and his head circumference was 37 cm (75^{th} centile). Motor development was characterized by some hypotonia. He started to walk at 2 years, 6 months of age. Currently, he does not say any words. Developmental assessments were performed at 4 years and 3 months of age with the Mullen Scales of Early Learning (MSEL) and the Vineland Adaptive Behaviour Scale (VABS-II), completed by the mother. The latter indicates a low level of adaptive functioning. Communication abilities are poor for both receptive and expressive language (age equivalence: 10 months and 5 months ,respectively). Socialization skills are poor for interpersonal relations, play, leisure time and coping skills (age equivalence: 1 year 10 months). A delay in terms of daily living skills such as personal care, domestic tasks and life in society is also observed (age equivalence: 3 months to 1 year, 1 month). Finally, motor skills, including gross and fine abilities, appear to be better developed compared to the other domains (age equivalence: 1 year, 10 months). During the administration of the Mullen Scales of Early Learning (MSEL), patient 3 had four epileptic episodes that lasted a few seconds each. He performed poorly on all the MSEL tasks compared to children of his age (1st percentile). Specifically, both gross and fine motor skills are poor (18 and 11 months, respectively). Concerning the visual reception domain, he shows little interest in the assigned tasks and performs at an age equivalent of 10 months. Both receptive and expressive language skills are poorly developed based on the MSEL, with performances equivalent to what would be expected of a 10- and 8-month-old respectively. Overall, the cognitive assessment showed a moderate intellectual disability. Assessment with the Autism Diagnostic Observation Schedule General (ADOS-G) performed at 4 years of age confirmed this diagnosis.

He had his first seizure at age 2.5 years, which was an atonic episode. From the onset, the seizures occurred in cluster of drop attacks every 5 minutes up to 10 times per day intermingled with brief absence seizures which led to an increment in medication. Soon after, multiple daily episodes of loss of contact were also observed. He was first started on levetiracetam, followed by clobazam, valproic acid, ethosuximide, clonazepam with no success except for less absence seizures with ethosuximide. The patient was started on a ketogenic diet in March 2012. EEGs demonstrated generalized spike-wave discharges with a posterior predominance.

At 2 years and 6 months of age, patient-2 weighed 15 kg (50-75th centile) and measured 89.5 cm (10-25th centile). His head circumference was 50.5 cm (50th centile). At the last physical examination, at 3 years and 6 months, no specific dysmorphic features were noticed. Eye contact was poor. The neurologic examination revealed hypotonia, normal reflexes and no focalizing or lateralizing sign.

Karyotyping (at a resolution of 450 bands), whole-genome comparative hybridization with an array containing 135000 oligonucleotides, and molecular testing for the triple repeat expansion associated with the Fragile X syndrome did not show any abnormality. A comprehensive metabolic work-up, including blood lactate and urine creatine and guanidinoacetate measurements, plasma amino-acid and urine organic acid chromatography, as well as evaluation of immunoreactive forms of transferrin after isoelectric focusing in polyacrylamide gels, was unremarkable. Brain MRI performed at 4 years of age was normal.

Patient-3 (c.1685C>T [p.P562L])

Patient-3, a female aged 4 years and 3 months, is the third child of unrelated European parents. Family history is unremarkable. She was delivered spontaneously at 35 weeks of gestation after an unremarkable pregnancy. The APGAR score was 1^{1}, 9^{5} and 9^{10}. She was initially hyporeactive but recovered within a few minutes after birth. Her weight was 4.0 kg (85-97th centile) and her head circumference was 34 cm (50th centile). She showed transient mild respiratory distress and was discharged at 4 days of life.

Patient-3 showed global developmental delay. She first walked at 15 months of age. She was saying 3 words at 2 years and 5 months of age but was not using signs to communicate. She understood simple commands and could designate parts of her body when asked. She started to use a spoon around that age. At four years of age, she was using 50 words but was not putting

them together. She could not undress herself and had no interest for drawing. Assessments were performed at 4 years and 3 months of age with the Mullen Scales of Early Learning (MSEL) and the Vineland Adaptive Behavioural Scale (VABS). The Vineland Adaptive Behaviour Scale (VABS-II), filled out by the mother, shows a low level of adaptive functioning. Communication abilities are heterogeneous, with receptive language skills in the average range (age equivalence: 3 years, 7 months), while expressive language skills are less developed (age equivalence: 1 year, 9 months). Concerning the socialization domain, Patient-3 appears to have poor abilities in terms of interpersonal relations, play and leisure time, and coping skills (age equivalence: 1 year, 5 months and 1 year, 4 months, respectively). A delay in terms of daily activities such as personal care, domestic tasks and life in society is observed (age equivalence 1 year, 5 months and 1 year, 4 months, respectively). Gross motor skills appear to be in the moderate to low range (age equivalence: 2 years, 2 months) while fine motor skills are poor (1 year, 5 months). The first attempt to administer the MSEL was impossible because the patient refused to complete the tasks. During the second assessment, she was more willing to participate but her mother had to use food rewards to motivate her. Visual reception and fine motor skills are below average (4th percentile) and are equivalent to the abilities of a 34-month-old child. Both receptive and expressive language skills are poor (1st percentile) and are equivalent to what is expected of a 31- month-old child. Overall, the cognitive assessment showed a mild intellectual disability

Non-verbal interactions were described as poor at 22 months of life, leading to a diagnosis of autism. Assessment with the Autism Diagnostic Observation Schedule performed at 4 years of age confirmed this diagnosis. During the second year of life, she had difficulty sleeping and she showed episodes of irritability with auto-mutilation. Both the sleeping difficulties and the irritability improved with the administration of valproate and omeprazole. There is no history of seizures.

At 2 years and 10 months of age, patient-3 weighed 18.9 kg (> 97th centile) and measured 94 cm (50-75th centile). At 3 years and 8 months of age, her head circumference was 51.8 cm (75-90th centile). On physical examination, no specific dysmorphic features were noticed. Neurological examination was unremarkable.

Karyotyping (at a resolution of 450 bands), whole-genome comparative hybridization with an array containing 135000 oligonucleotides, mutation analysis of the *MECP2* gene, and molecular testing for the triple repeat expansion associated with the Fragile X syndrome did not

show any abnormality. Plasma amino-acid and urine organic acid chromatography were unremarkable. Electroencephalogram performed at 26 months of age was normal whereas another study performed at 3 years of age showed intermittent and slow dysfunction in the occipital regions.

Patient-4 (c.2212_2213del [p.S738X])

Patient-4, a male aged 30 months, is the single child of unrelated European parents. His mother has a history of petit mal seizures starting at the age of 7. She was well controlled with the medication and seizures stopped at 13 years of age. The mother also shows macrocephaly. Patient-4 was delivered spontaneously at term after an unremarkable pregnancy. The APGAR score was 9^{1} and 9^{5}. His weight was 2.918 kg (10-25th centile) and his head circumference was 32 cm (2nd centile). Motor development was delayed. He sat at 9 months and walked at 21 months of age. He is currently unable to say words and does not use signs to communicate. He has moderate ID. Non-verbal social interactions are unremarkable. He is easily frustrated and bangs things with his hands or bangs his head. There is no history of seizures.

Patient-4 has difficulty feeding since the newborn period. He had trouble latching to the breast and as a result milk production was not established and he had to be weaned off by 1 month of age. He also had some difficulties feeding on formulas. At 10 months of age, a G-tube was placed for supplemental feeding. Currently, he gets about 30% of calories from the G-tube and receives a full diet orally but he shows an aversion to solid foods, crunchy foods and liquids and only eats food with a pureed consistency. He also had constipation from a young age.

At 30 months of age, patient-4 weighs 11.88 kg (15th centile) and measures 88.3 cm (15th centile). Serial measurements of head circumference showed acquired microcephaly.At 25 months of age, his head circumference was 45.7 cm (10th centile). Physical examination showed mild dysmorphic features, including thickened helices, crimped outer helix and bilateral 5th finger clinodactyly. Neurological examination revealed generalized hypotonia. He is unsteady on his feet but not ataxic.

Patient-4 started to show absence seizures at 36 months of age. EEG showed occasional bursts of generalized spike and slow wave discharges with a
bioccipital predominance. The patient is currently being treated with Keppra. The impact of this treatment is currently under evaluation.

Exhaustive metabolic work-up including blood lactate, ammonia, amino acids, acylcarnitines, and 7-dehydrocholesterol measurements, as well as urine organic acids chromatography was normal. Array genomic hybridization, methylation study of the region associated with Angelman syndrome and molecular testing for the triple repeat expansion associated with the Fragile X syndrome did not show any abnormality. Brain MRI and spectroscopy performed at age 20 months was normal.

Patient-5 (c.2184del [p.N729TfsX31])

Patient-5, aged 9 years and 4 months, is the daughter of unrelated European parents. Family history is unremarkable. She was delivered at 39 weeks of gestation after an uneventful pregnancy. The APGAR score was 9^{1} and 10^{5}. Her birth weight was 3.85 kg and head circumference at birth was 36 cm (55^{th} centile). She first walked at the age of 26 months, and said her first words at the age of ~ 5 years. Currently, she is able to speak with short sentences. Assessment by a developmental pediatrician led to diagnoses of moderate-to-severe global developmental delay and autism.

She started to present drop attacks and absence seizures at 3 years and 2 months of age. These seizures were well controlled by the administration of valproic acid. EEG was initially normal but a subsequent study showed abnormal bursts, in the right vertex region, of poorly formed waves during sleep that were potentially epileptogenic, mainly.

At 9 years and 4 months of age, her weight, height and head circumference all corresponded to the 75^{th} centile. On physical examination, no specific dysmorphic features were noticed. Neurological examination was normal aside from increased tone in the legs and an ataxic gait with truncal titubation.

Exhaustive metabolic work-up including blood amino acids and urine organic acids chromatographies as well as measurement of the activity of respiratory chain complexes in the muscle was normal. Array genomic hybridization and molecular testing for the triple repeat expansion associated with the Fragile X syndrome did not show any abnormality. Brain MRI was normal

Supp. Table S1. Paternity and maternity testing for patients 2-5 done using 6 informative polymorphic unlinked microsatellite markers

Trio	D3S1754		D4S3351		D8S1179		D15S659		D14S587		D19S215	
Patient-2	7	9	5	7	5	6	2	7	1	3	3	6
Father	7	8	5	6	5	7	2	3	2	3	2	3
Mother	7	9	5	7	8	6	3	7	1	6	6	6
Patient-3	6	8	2	6	5	6	6	7	6	7	1	8
Mother	6	7	5	6	5	6	6	6	6	7	6	8
Father	8	8	2	7	6	8	2	7	6	6	1	6
Patient-4	6	8	5	6	7	7	2	6	3	6	6	10
Father	6	8	6	6	6	7	2	2	6	7	2	6
Mother	1	8	5	13	7	5	6	7	3	3	6	10
Patient-5	7	7	4	6	2	7	3	7	5	9	1	6
Father	7	8	4	6	7	8	4	7	5	6	2	6
Mother	7	8	4	5	2	5	3	3	5	9	1	6

PCR was done using primers flanking each marker in the presence of specific fluorescent dyes and then analyzed on a 3730 DNA analyzer. Shown are the genotypes observed in each member of the 4 trios. Results are consistent with paternity and maternity for each trio.