

Supplementary Note. Clinical cases

In *family ASD 1*, the proband is the only child of healthy unrelated parents. Family history is negative for mental retardation and ASD. He was born at 41 weeks after a pregnancy marked by bleedings. Birth growth parameters were large (weight 4850 g, length 54 cm, head circumference [HC] 36 cm). He exhibited neonatal hypotonia, poor suction and cried a lot as a baby. Early motor milestones were normal. He said a few words such as mama and dada but stopped using them around 30-36 months, when his parents noted a regression, both for language and play behavior. Recurrent otitis media was observed during early infancy and he had sleep difficulties. There was no history of seizures. Psychiatric evaluation at the age of 9 years showed total absence of language and moderate mental retardation. Non verbal communication was impaired, as well as social interaction, and he had motor stereotypies. The clinical diagnosis of autism was confirmed with the ADI-R. Physical examination at the age of 12 years showed no minor dysmorphic features except large ears and elbow extension limitation. Weight was 44 kg (+1.5 SD), height was 143 cm (-0.5 SD), and HC was 54.5 cm (average). Neurological examination was normal. A standard karyotype and fragile X testing were normal. Routine screening of the 22q13 region by FISH revealed a *de novo* terminal deletion. An MRI and EEG were performed and were normal.

In *family ASD 2*, the probands are the first and the third children of three. The unaffected brother meets DSM-IV criteria for ADHD but has no autism spectrum symptoms and no language delay. The mother meets DSM-IV criteria for social phobia. Family history is otherwise negative for mental retardation, language disorders, ASD and other psychiatric disorders. Both children were born from full term and uneventful pregnancies. Birth parameters were average. Neonatal history was limited to neonatal jaundice and no hypotonia was recorded. The eldest boy had a normal development during the first year but was very agitated. He started walking at 16 months. He spoke his first words at 5 years and first sentences at 6 years, but never acquired functional language. At the age of 20 years, after he was transferred to a new institution, a regression with severe outbursts was noted, he lost skills including cleanliness, and he developed anorexia and marked weight loss. At that time he had a seizure-induced aspiration, he was hospitalized and died a few days later. The second affected brother had normal measures at birth (weight 3420 g, length 50 cm, HC 35.5 cm) but he showed progressive macrocephaly until 9 months of age (+2 SD), followed by slowed growth (HC + 1 SD). He had limited eye contact since birth. He started walking at 18 months. He never acquired words, except mama and dada. At the age of 16 years he had an episode of aspiration with loss of consciousness, which necessitated hospitalization for a few days, and a second episode at the age of 20. Since then, he is fed a special diet with no liquids and soft food. When he was 16, after being transferred to the same institution where his brother had been, a regression was also noted, with loss of autonomy and cleanliness, and marked weight loss. He developed epilepsy at the age of 17 and was started on clonazepam. Physical examination at the age of 20 years showed growth parameters in the average (height 175 cm, HC 55.5 cm), strabismus and no dysmorphic features. Both brothers had severe language impairment, severe mental retardation and marked deficits in social interaction. The clinical diagnosis of autism was confirmed for both of them with the ADI-R.

In *family ASD 3*, the probands are the first and the third born of three children. The unaffected brother has no history of psychiatric disorder. The mother fulfills DSM-IV criteria for agoraphobia; the father used to have symptoms of ADHD as a child but doesn't fulfill the criteria as an adult. There

is no family history of mental retardation or ASD, but the paternal grandfather has numerous rituals and routines and is socially isolated. The eldest boy was born full term (41 weeks of gestation) after an uneventful pregnancy, with large birth parameters (weight: 4040 g, length: 53 cm, HC: 38 cm). He had normal early motor milestones, said his first words at 12 months, first sentences at 18 months and already knew the letters at that time. He learned to read at 5 years, before attending primary school. He has impairment in social interactions, is often awkward in social situations and is quite isolated at school. He doesn't like to be observed by teachers and has marked difficulties to speak in class. He also has difficulties to identify social cues and to understand others people's reactions. His interests are focused on mechanical sports and he has great knowledge about this. He has excellent memory for car plates and is very rapid for mental calculation. He also has routines, especially when taking a shower. He has impairments in the interpretation of implied meanings. He has a small repertoire of facial expressions and limited use of gestures. Direct eye gaze is rare. His voice is often too loud, lacking modulation. He is quite clumsy and awkward. He has mood swings, hypersensitivity to noise, and phobia of chimney fire. Clinical evaluation at the age of 14.5 years confirmed the diagnosis of Asperger syndrome and showed a normal IQ. Physical examination at this age showed increased height (184 cm) and marfanoid habitus, without significant dysmorphic features. The youngest sister was born at 38 weeks of gestation, after an uneventful pregnancy. Birth parameters were low (weight: 2050 g, height: 47.5 cm, HC: 33 cm). She had discrete neonatal hypotonia but had normal early motor milestones. Language development was severely delayed with few words around 4 years and development of very short sentences before regression. Around 5 years old, she presented echolalia. She had motor stereotypies and exhibited some routines and need for sameness. She is now 12 years old and uses few words, with pronunciation errors. She communicates with pictograms. She went to school until 7 years old and now attends a day hospital. An IQ evaluation at 12.9 years showed a performance IQ of 44 (developmental age 5.8 years), and a verbal IQ of 15 (developmental age 24 months). Her non verbal communication is impaired, as well as her social interaction. At present she initiates activities by herself. She especially enjoys puzzles, she is very fast at them and can spend the whole day on this activity. She also plays with construction games and has a tendency to align the pieces. She still shows insistence on sameness. The diagnosis of autism was confirmed with the ADI-R. She has some self-aggressive behaviors, hitting her head. She has no hypersensitivity to noise and her reaction to pain is normal. She presents nocturnal enuresis. Fine and global motor skills are impaired. Physical examination at 10 years showed average growth parameters, without dysmorphic features. A brain MRI at 5 years of age was normal. The terminal 22q deletion in the girl was diagnosed by FISH when she was 8 years old. Study of other family members revealed a translocation t(14;22)(p11.2;q13.33) in the father and a 22qter partial trisomy in her brother.

The proband of *family ASD 4* carries the R12C SHANK3 mutation. He is the third child of healthy unrelated parents. His two brothers are healthy. Medical and psychiatric history of the maternal family includes Parkinson disease in both maternal grandparents, depression in the grandmother and thyroid disease in the grandmother, mother and several aunts. The mother had a major episode of depression during pregnancy and after the birth of the proband. In the father's family, two brothers of the grandmother committed suicide and two paternal uncles have a history of alcohol abuse. There is no history of mental retardation, language disorder or ASD. The proband was born after a full term and uneventful pregnancy. Delivery was unremarkable and birth parameters were in the average (weight 3750 g, length 51 cm, HC 35 cm). He didn't establish eye contact and early

milestones were delayed: he sat at 18 months and started walking at 4 years. EEG and brain imaging by tomodensitometry (TDM) were performed during his first year and were both normal. Vision was checked and was not impaired. Physical examination at the age of 23 years showed a narrow face, large ears, long philtrum, saddle nose, flattened midface, thin superior lip, and prominent supraorbital ridge. Height was 160 cm (-2 SD), weight 60 kg, and HC 55.3 cm. Neurological examination was normal and there was no history of seizures. There was a high pain threshold and low noise threshold. Psychiatric examination showed total absence of language and severe mental retardation. Non verbal communication was impaired, as well as social interaction, and he had motor stereotypies. There was no sleep disorder or hyperactivity. The clinical diagnosis of autism was confirmed with the ADI-R.

The proband of *family ASD 5* carries the R300C *SHANK3* mutation, which he inherited from his mother. He is the first of two children from non-consanguineous healthy parents of Chinese origin. His family history is non-contributory. The patient was born at term, after an uncomplicated pregnancy. The first symptoms were noticed at 2 years, when he lost some words (he said mama, dada, bye-bye, and could name certain things he wanted) and exhibited unusual eye contact. From this time, he showed no interest in other children and preferred to play on his own. He had sleeping problems during childhood. He showed repetitive behaviors and circumscribed interests, opening and closing doors, lining up objects, finding similar objects, leafing through certain books, or letting sand sift through his fingers. When evaluated at 42 months, he had no expressive verbal language, but used different sounds and song melodies. He had some receptive language; he could point out some familiar objects and was able understand some familiar sentences. He fulfilled the criteria for autistic disorder according to the ADI-R and had a Childhood Autism Rating Scale (CARS) score of 37.5, indicating severe autism. His developmental age at that time, evaluated with the Psycho-Educational Profile-Revised (PEP-R) was 18-20 months. No somatic pathology was detected at that time. A standard karyotype, fragile X testing and metabolic screening were normal. A brain CT scan performed at this time was also normal. Three EEGs, performed at 3, 7 and 10 years, did not show significant pathology. He started to speak at 5-6 years, but his language problems persisted. At the age of 10 years, his language was stereotyped and repetitive; his adaptive language function was below mental age of 3 years according to the Vineland Adaptive Behavior Scales. A Wechsler Intelligence Scale for Children (WISC) performed at 10 years showed a total IQ of 67, a verbal IQ of 63, and a performance IQ of 81. On examination at 11 years 4 months, his weight was 38 kg, his height 144.5 cm, and HC 55 cm. Neurological exam showed generalized hypotonia and brisk reflexes throughout all extremities. He had long arms, with narrow hands and long fingers. He had a supernumerary upper incisor (mesiodens) and he still had his milk teeth in his upper jaw except for the two front teeth. No obvious dysmorphic features were observed. His parents noted that the patient often took off his clothes and complained about being warm (patients with 22 deletion syndrome have a tendency to become overheated due to reduced perspiration¹).

Reference

1. Phelan, M.C. et al. *Am J Med Genet* **101**, 91-9 (2001).