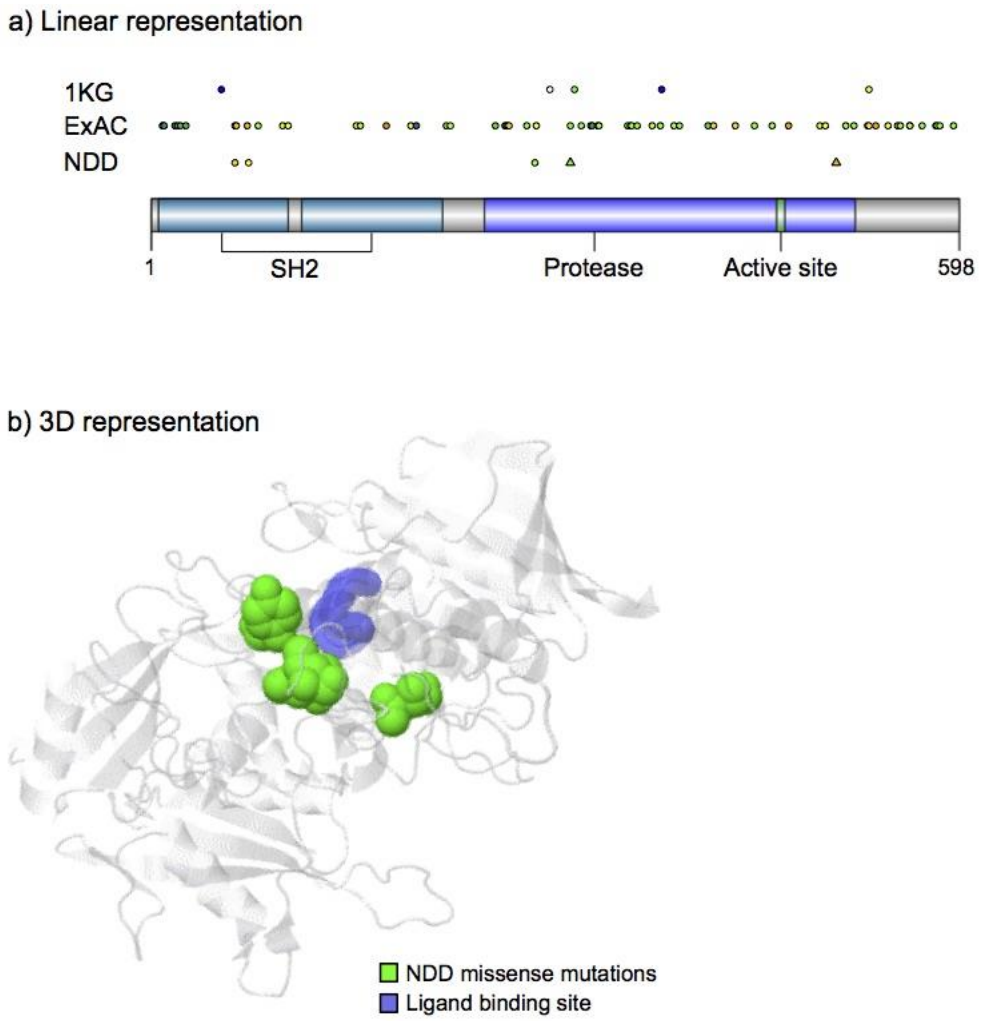

Supplementary Figure 1

Missense mutation clustering in PTPN11.

a) Linear representation of the protein (NP_002825.3) with known functional domains annotated. The top two lines show mutations in controls from the 1000 Genomes Project (1KG)[79] and the Exome Aggregation Consortium (ExAC)[18]. The third line shows *de novo* missense mutations in cases in denovo-db[64] v.1.2. The case mutations fall in three small clusters. **b)** 3D representation of the PTPN11 protein shows that the three clusters of mutations that are far apart in the linear plot are in close proximity to each other and the ligand binding site after folding[34].

Supplementary Materials for Manuscript: Hotspots of missense mutation identify novel neurodevelopmental disorder genes and functional domains

Supplementary Tables

See Excel document for Supplementary Tables 2-10.

Supplementary Table 1. Discovery cohorts

Study diagnosis	Cohorts	Individuals	Trios*	Quads**	*De novo* missense mutations†	*De novo* LGD mutations	Studies
Autism spectrum disorders (ASD)	8	4197	2120	1956	3185	677	De Rubeis 2014[14] (ASD1), Hashimoto 2015[48] (ASD2), Jiang 2013[51] (ASD3), Lee 2014[54] (ASD4), Michaelson 2012[57] (ASD5), Simons Simplex Collection[1,13,53,58] (ASD6), Tavassoli 2014[32] (ASD7), Yuen 2015[62] (ASD8)
Congenital heart disease (CHD)	2	775	775	0	308	152	Homsy 2015[50] (CHD1), Zaidi 2013[63] (CHD2)
Developmental delay (DD)	4	2104	2104	0	1545	483	de Ligt 2012[24] (DD1), Lelieveld 2016[55] (DD2), Hurles 2014[44] (DD3), Rauch 2012[59] (DD4)
Epilepsy (EPI)	6	602	601	1	267	76	Barcia 2012[43] (EPI1), Dimassi 2015[45] (EPI2), epi4k 2013[33] (EPI3), Helbig 2016[49] (EPI4), Veeramah 2012[60] (EPI5), Veeramah 2013[61] (EPI6)
Schizophrenia (SCZ)	4	799	715	84	502	87	Fromer 2014[46] (SCZ1), Gulsuner 2013[47] (SCZ2), Kranz 2015[52] (SCZ3), McCarthy 2014[56] (SCZ4)
TOTAL CASES	24	8477	7115	2041	5807	1478	
Unaffected	4	2178	270	1908	1475	237	GoNL 2014[65], Gulsuner 2013[47], Rauch[59], Simons Simplex Collection[1]

*Family with unaffected parents and one affected child

**Family with unaffected parents, one affected child, and at least one unaffected child

†Missense mutations with minor allele frequency (MAF) < 0.1% in ESP (N = 6503)

Supplementary Table 11. Versions of denovo-db

Version	Number of papers					Cases	*De novo* missense mutations
	ASD	CHD	DD/ID	EPI	SCZ		
v.0.8	11*	2	3	4	4	7332	5047
v.0.9	12*	2	4	6	4	8477	5807
v.1.2**	12†	0	4	5	0	9997	8917

*Includes 4 papers published on SSC cohort

**Papers used for CLUMP analysis

†Includes 5 papers published on SSC cohort

Supplementary Clinical Case Reports

ALG13 (N107S, inheritance unknown): Patient Troina 2679

This individual was last seen in 2006 at 23 months of age. She has severe ID and epilepsy. Apneic seizures first appeared at 3 months of age and the EEG showed high-voltage spikes and wave complexes on the occipital areas of the left hemisphere. Phenobarbital treatments were started. At age 4.5 months, tonic-clonic seizures with ocular globe revulsion and upper limb flexion began multiple times a day. Spasms began to appear and were treated with hydrocortisone. While her psychomotor development was regular during the first months of life, it then began to regress. Her OFC was in the 25th percentile at age 4.5 months but was slightly over the 3rd percentile at 23 months. Pregnancy was complicated by 1st trimester threatened abortion and ten cigarettes per day. Her mother had two previous abortions, at the 6th and 5th months. Delivery was natural at 37 weeks. The umbilical cord was short but she had no asphyxia or jaundice. She was infected by cytomegalovirus but the onset is not known. Epilepsy is present on the maternal side, in a cousin and grandfather. An aunt, also on the maternal side, died at age 18 months for unknown reasons. Genetic tests for PWS/AS, MECP2 and CDKL5 were normal. Parental DNA is not currently available for testing.

GRIA1 (A636T, *de novo*): Patient Stockholm 5015-11D

This individual was noted to have delayed motor and language abilities in his first year of life. He could sit at the age of 10 months and walked independently at 18-19 months. At the age of 4, he could speak 20 words. Over time his speech improved but he retains difficulties with pronunciation and vocabulary and has been diagnosed with an expressive language disorder. His cognition is in the low-normal range and he has been diagnosed with ASD. His brain MRI was normal but spine MRI showed cervical stenosis at the C2 level which required surgery at age 4. He was delivered by Cesarean section at 35 weeks due to maternal preeclampsia. During infancy, he had postural plagiocephaly, torticollis, and gastroesophageal reflux, all of which have improved remarkably. He was born to healthy non-consanguineous parents and has a healthy twin brother (most likely dizygotic). Sanger sequencing confirmed the variant in the individual and its absence in both of his parents.

GRIA1 (A636T, inheritance unknown): Patient Stockholm 2688-10D

This individual, who had unremarkable pregnancy and delivery, was referred at age 2 when language delays were noted. Speech improved over time but she retains difficulties with pronunciation and vocabulary although she has not formally been diagnosed with a speech disorder. Her cognition is in the low-normal range and she has been diagnosed with ASD and possibly ADHD. She also has motor tics involving her tongue and making sounds. At the age of 2 she began to have recurrent seizures and EEG during sleep showed occipital epileptic activity. They ceased at age 5 (EEG normal) and anti-epileptic drugs were stopped. CT and MRI of her brain were normal. She has experienced abdominal discomfort and recurrent otitis. Her parents are healthy and non-consanguineous. She also has a healthy younger sister. The variant is not present in her mother; paternal DNA is not available.

GRIA1 (A636T, inheritance unknown): Patient Stockholm 1947-12D
This individual has moderate ID and ASD. She has also been diagnosed with ADHD and OCD. Her MRI is normal. The variant is not present in her mother; paternal DNA is not available.

GRIA1 (I627T, *de novo*): Patient Adelaide 25431
This individual has DD and ID. He also has behavioral problems and regular migraine headaches. He has a bicuspid aortic valve and dysmorphisms including full lips, flushed cheeks and ears, and a high arched palate. He also carries maternally inherited 18p11.32 duplication (distal breakpoint 148,993; proximal breakpoints 776,937-825-118) and Xq26.1 duplication (distal breakpoints 130,280,328-130,358,536; proximal breakpoints 130,950,214-131,029,554). Both of his sisters and his mother also have learning difficulties. The sister with more severe learning difficulties also has the 18p duplication, and the one with milder learning difficulties has the Xq duplication. His face appears similar to the more severely affected sister. Sanger sequencing confirmed the presence of the *GRIA1* mutation in him and its absence in both of his parents and sisters.

GRIA1 (V640L, paternally inherited): Patient ACGC HEN0073.p1
This individual has ASD. The variant is present in him and his father.

PACS1 (R203W, *de novo*): Patient Stockholm 1658-13D
This individual (male) has ID. He has a distinct facial phenotype with fleshy upslanting ear lobes, wide mouth with widely spaced teeth, a thin upper lip, and upslanting palpebral fissures. In addition, he has a tethered spinal cord. Sanger sequencing confirmed the presence of this mutation in the individual and its absence in both of his parents.

PACS1 (R203W, *de novo*): Patient Troina 1190
This individual has severe ID, microcephaly, and cerebellar vermis atrophy. He shares this phenotype with his twin brother. This mutation is in both brothers but not their parents.

PACS1 (R203W, inheritance unknown): Patient Adelaide 36721
This individual has severe ID. She was seen at age 1 year for feeding difficulties, significant gastro-esophageal reflux, and laryngomalacia, all of which improved with time. She was diagnosed with global DD and there was concern about cerebral palsy or spastic diplegia. When she was last seen at age 12 years, she was described as having very slow movements but there was no evidence for cerebral palsy or upper motor neuron dysfunction. Her speech was also slow and limited, but intelligible. She did not have any growth abnormalities (height 50^{th} percentile and weight 75^{th} percentile at age 12 years). She also did not have any specific facial dysmorphologies. No syndromic diagnosis could be made from her features. There is no family history of ID. Parental DNA is not available.

PACS1 (A187V, paternally inherited): Patient TASC 220-9746-201
This individual has ASD. Sanger sequencing confirmed the presence of this variant in the individual and his father.

SATB2 (S395F, *de novo*): Patient Antwerp 90946

This individual has severe DD including ID, severe speech and language delay, and motor delays. She has both gross and fine motor delay (walked independently at 2 years) and balance problems. At the age of 3 years, formal testing using Bayley Scales of Infant and Toddler Development (3rd edition) showed a developmental age of 17.5 months. She also presented with a symmetric growth delay, although skeletal age was normal. At age 3, her height was 83 cm (-3.5 SD), weight 10.1 kg (-2 SD), and head circumference was 46.5 cm (-2 SD). She could speak only ~30 words and could not form a sentence. She also has facial dysmorphisms (low hairline and thick eyebrows) that were especially apparent as a toddler. She does not have hearing impairments. In the past, she had two surgical interventions for strabismus. She has also been noted to have hypersalivation. She does not have any behavioral problems. No structural abnormalities were detected on brain MRI. Sanger sequencing confirmed the presence of this mutation in the individual and its absence in both of her parents.

SATB2 (R405Q, inheritance unknown): Patient Adelaide 26352

This individual has DD including ID and imprecise speech articulation. He has a submucous cleft palate and several facial dysmorphisms, including asymmetrical nose, lack of velar elevation, and narrow maxillary arch. He also has congenital heart disease. Parental DNA is not currently available for testing.

SATB2 (R405Q, inheritance unknown): Patient Adelaide 28975

This individual (male) has intellectual disability. Parental DNA is not currently available for testing.

SATB2 (D370V, inheritance unknown): Patient CHOP 1451747261

No information available.

SMAD4 (I500T, *de novo*): Patient Antwerp 87305

This individual has DD including mild ID, dysmorphic features and hearing loss. The pregnancy was characterized by intrauterine growth retardation. Amniocentesis for advanced maternal age (38 years) showed no abnormalities. The girl was born after 35 weeks with a delivery by caesarian section for fetal bradycardia. Birth weight was 1,016g, length 36cm and head circumference 28cm. She presented with a unilateral preaxial polydactyly, which was surgically corrected. Facial dysmorphisms were observed consisting of short palpebral fissures, mid-facial hypoplasia, short philtrum, prognathism, narrow mouth and small ears. She has hypermetropia and has been diagnosed with bilateral moderate sensorineural hearing loss for which she has hearing aids. Cardiac evaluation was normal. Bone age is delayed. At the age of 12, she is being treated with growth hormone for short stature (length -5SD, weight -0.5SD). She is also being treated for early puberty (since age 9 years). She has frequent upper airway infections, sleep problems and dyspraxia. She receives physical therapy for a high muscle tone, stiff joints and tiptoe walking. Sanger sequencing confirmed the presence of the mutation in her and its absence in both of her parents.

SMAD4 (I500V, *de novo*): Patient Leuven 243139

This individual has ASD and borderline ID (verbal IQ 75, performance IQ 81, total IQ 75). She also has facial dysmorphisms, small stature, brachydactyly, hearing loss, and joint limitations. Sanger sequencing confirmed the presence of the mutation in her and its absence in both of her parents.

SMAD4 (R496C, inheritance unknown): Patient Adelaide 23883

The individual was referred at age 4 for global developmental delay. Early pregnancy was uneventful but labor was induced at 38 weeks due to poor growth in later pregnancy. She underwent normal vaginal delivery and was well at birth, although she required gavage feeds initially. She had gastroesophageal reflux, frequent ear and chest infections, and blocked nasolacrimal ducts. She also had strabismus which required eye patching. Her growth was small but symmetrical. She began toe-walking at age 15 months. At age 2, she was assessed for global developmental delay and was diagnosed with mild intellectual disability and ASD. She attends a special school. Additionally, she has severe behavioral issues including defiance and aggression for which she has ongoing psychiatric care. At age 14, her height is in the 3rd percentile, her weight is in the 10th percentile, and her head circumference is in the 10th percentile. She has a lean and muscular build, but no joint limitation or thickened skin, and skeletal survey was normal. She also has dysmorphic facial features – slightly downslanting and short palpebral fissures, bulbous nasal tip, midface hypoplasia, short philtrum, prognathism, small mouth with thin upper lip, and small ears. She also has a deep husky voice. Her mother has spina bifida and is confined to a wheelchair. Her father (deceased at age 69) had short stature, mild ID, and a facial appearance that was similar to the individual. She also had premature arthritis, diabetes (Type II), and required a quadruple bypass surgery. Parental DNA is not currently available for testing.

SMAD4 (R496C, inheritance unknown): Patient Stockholm 2807-11D

This individual was referred at the age of 5 years due to abnormal behavior in school. Formal assessment resulted in a diagnosis of ASD with cognitive level in the low-normal range. MRI of the brain showed slight enlargement of the corpus callosum as the only possibly abnormal finding. She has bilateral cataracts, strabismus, and hyperopia. Cataract surgery was performed at the age of 11 years. Parental DNA is not currently available for testing.

SMAD4 (S483R, inheritance unknown): Patient Adelaide 15996

This individual has developmental delay. She has a family history of similar problems, notably in her sister and two maternal uncles. Parental DNA is not currently available for testing.

SMAD4 (R496C, inheritance unknown): Patient Adelaide 30845

This individual (male) has both ASD and DD. Parental DNA is not currently available for testing.